A comprehensive chemical examination of methylamphetamine produced from *pseudo*ephedrine extracted from cold medication

By

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Abstract

This research evaluates the ability of gas chromatography mass spectrometry (GCMS), isotope ratio mass spectrometry (IRMS) and inductively coupled plasma mass spectrometry (ICPMS) to characterize methylamphetamine hydrochloride synthesised from precursors extracted from proprietary cold medication using three different extraction solvents. Two clandestine routes were utilized in the synthetic phase of the research, (i) Moscow route and (ii) Hypophoshorous route (Hypo). Repetitive batches of samples were prepared and analysed by each analytical technique to provide a robust sample set for data interpretation.

Organic impurity analysis was undertaken using a developed and validated GCMS impurity profiling method. The GCMS method discriminated the samples by synthetic route based on the presence of specific target impurities. Carbon, nitrogen and hydrogen stable isotope ratios facilitated the differentiation of samples by route, and precursor source with nitrogen and hydrogen isotopes providing the best results. Inorganic impurities present in the samples were analysed using inductively coupled plasma mass spectrometry (ICPMS). This technique provided meaningful discrimination according to the route and precursor utilized in the synthetic phase.

Pattern recognition techniques were applied to the generated data (raw and pre processed) from each of the analytical technique both individually and in combination. Pearson's correlation coefficient, hierarchical cluster analysis, principal component analysis and artificial neural networks (self organizing feature maps) were used to investigate the separation of samples to the individual routes and precursor extracted from the individual solvent systems. The mathematical tools demonstrated that methylamphetamine profiling linking precursors sourced from proprietary grade materials extracted from different solvent systems and synthetic route employed was achievable.

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Oral and Poster Presentations

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S.Jayaram, N.Nic Daeid, W.J.Kerr, Influence of precursor chemicals of the chemical profiling of methylamphetamine (Poster presentation). *University of Strathclyde, Research Day 2010,* 10-17th July, 2010 Glasgow, United Kingdom.

S.Jayaram, N.Nic Daeid, W.J.Kerr, W.Meier-Augenstein, H.Kemp, IRMS analysis of methylamphetamine synthesized via the Hypophosphorous and Moscow routes using cold medication as a precursor source (Poster presentation). *European Geosciences Union General Assembly 2012*, 22-27th April, 2012, Vienna, Austria.

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S.Jayaram,N.NicDaeid,W.J.Kerr,W.Meier-Augenstein,H.Kemp,Profiling methylamphetamine synthesized using precursors extracted from proprietary cold medication via the "Hypo and Moscow" route – part 2 Isotope ratio mass spectroscopy (Poster presentation). *European Academy of Forensic Sciences(EAFS),* 20-24th August, The Hague, Netherlands.

S.Jayaram, W.J.Kerr, N.NicDaeid, Profiling methylamphetamine synthesized using precursors extracted from proprietary cold medication via the "Hypo and Moscow" route – part 3 Inductively coupled plasma mass spectrometry (Poster presentation). *European Academy of Forensic Sciences(EAFS),* 20-24th August, The Hague, Netherlands

D.Ismail, S.Jayaram, N.NicDaeid, Unsupervised pattern recognition analysis of methylamphetamine synthesised from precursors extracted from proprietary cold medication via “Hypo and Moscow” routes using three different analytical techniques (Poster presentation). *European Academy of Forensic Sciences(EAFS),* 20-24th August, The Hague, Netherlands.

List of abbreviations

BMK	Benzyl methyl ketone
BSIA	Bulk Stable Isotope Analysis
CHM	Commission on Human Medicines
CDTA	Chemical Diversion and Trafficking Act
CHAMP	Collaborative Harmonisation of Amphetamine Method Profiling
CI	Chemical Ionisation
CSIA	Compound Specific Isotope Analysis
DCDA	Domestic Chemical Diversion Act
DI IRMS	Dual Inlet Isotope Ratio Mass Spectrometry
DWG	Drugs Working Group
EA/IRMS	Elemental Analyser Isotope Ratio Mass Spectrometry
EI	Electron Impact
ENFSI	European Network of Forensic Sciences
EtOH	Ethanol
FTIR	Fourier Transform infra Red
GCMS	Gas Chromatograph Mass Spectrometry
HCA	Hierarchical Cluster Analysis
Hypo	Hypophosphorous
ICPMS	Inductively Coupled Plasma Mass Spectrometry
IRMS	Isotope Ratio Mass Spectrometry
LSD	Lysergic Acid Diethylamide
MCA	Methylamphetamine Control act
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
MDR	Misuse of Drugs regulations
mg	miligram
mL	millilitres
MtOH	Methanol
NMR	Nuclear Magnetic Resonance
PCA	Principal Component Analysis
P2P	Phenyl-2-Propanone
RSD	Relative standard Deviation
SOFM	Self Organising Feature Maps
Std dev	Standard Deviation
TC/EA IRMS	Temperature Conversion/Elemental Analyser Isotope ratio Mass spectrometer
UNODC	United Nations Office on Drugs and Crime

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Research objectives

Many studies in organic impurity profiling of methylamphetamine have focused on samples synthesised from laboratory grade precursor chemicals via known methods. This provides valuable information relating to the determination of route specific markers, however, does not fully mimic the true clandestine nature of the synthesis where, in many cases proprietary over the counter medications and household items may be used as precursor sources.

In this work, repetitive batches of methylamphetamine were synthesised using the Moscow and Hypophosphorous ('Hypo') synthetic routes following the clandestine and published literature. The starting compound in each case was *pseudo*ephedrine extracted from 'Sudafed' cold medication tablets using a variety of different solvents. Red phosphorous and iodine were also extracted from locally sourced matchbooks and tinctures respectively. The same synthesis was preformed repetitively using laboratory grade starting compounds as control samples.

*Pseudo*ephedrine hydrochloride was extracted from cold medication using existing clandestine extraction methods. In total, three different solvents were used in the extraction process, ethanol, ethanol/methanol and commercial methylated spirits. 24 samples were prepared by each synthetic route, 18 of which were produced by precursor extracted from cold medication purchased in Glasgow. In addition 5 batches of methylamphetamine were synthesised from cold medication purchased in Kuala Lumpur, Malaysia. Following the synthesis of the methylamphetamine samples the objectives of this work were as follows:

- Using an existing GCMS profiling method, a full exploration of the range of route specific impurities produced in the synthesis of methylamphetamine was undertaken using precursors extracted from cold medication. The effect of the specific extracting solvents was also explored.

- The comparison of known provenance methylamphetamine samples using IRMS analysis to investigate linkage to precursors extracted using the three solvent systems. The sample set produced would allow the inter-synthesis variation (batch to batch variation) to also be assessed.

- An investigation of the ability of IRMS to identify methylamphetamine samples prepared from precursors from two different regions, United Kingdom and Malaysia.

- Elemental analysis of known provenance methylamphetamine samples and precursor samples extracted from cold medication using ICPMS.

- The final objective of this work was to assess the ability of different data analysis techniques to provide meaningful discrimination of the methylamphetamine data sets generated by the analytical techniques employed. Hierarchical cluster analysis, principal component analysis and self organizing feature maps (SOFM) were applied to the GCMS data on its own, the IRMS data on its own, ICPMS data on its own and combinations of data from all of the techniques. Various data pretreatment methods were also investigated.

Thesis Overview

The analytical techniques used in the identification of synthesised methylamphetamine and extracted *pseudo*ephedrine are discussed in chapter 2. Nuclear magnetic resonance (NMR), Fourier transform infra red spectroscopy (FTIR), melting point and CHN analysis which were used for the confirmation of extracted and synthesised products are addressed briefly. GCMS, IRMS and ICPMS are discussed in greater detail as they were the main analytical techniques used in the project.

The synthesis of methylamphetamine by both synthetic routes and the development of an efficient precursor/essential chemical (i.e. iodine and red phosphorous) extraction methodology is described in chapter 3. A discussion of the mechanism and chemistry which contributes to the final methylamphetamine molecule is explained in this chapter.

Chapters 4 and 5 outline in detail the GCMS profiling work. The method validation is presented in chapter 4. This includes preliminary work used to test column performance to ensure repeatability of the chromatography and method validation for the extraction procedure and GCMS conditions. The results of the analysis of the synthesised methylamphetamine are discussed and a list of target impurities is presented in chapter 5. The results were analysed using Pearson correlation matrix to evaluate the degree of similarity between samples based on the types and quantity of impurities present in each batch.

In Chapter 6, the results of ICPMS analysis of the synthesised methylamphetamine and *pseudo*ephedrine extracted from the proprietary cold medication samples are presented. The concentration of each inorganic element is assessed and evaluated within the context of the synthetic route used.

In Chapter 7, the results of the IRMS analysis of the 53 batches of synthesised methylamphetamine samples and batches of *pseudo*ephedrine extracted from the three different solvent systems are presented and discussed. The discrimination by precursor

source and synthetic route by one, two and three-dimensional plots of the isotope data is addressed.

Multivariate and artificial neural network analysis of the analytical data sets produced from the various techniques employed is discussed in chapter 8. This data were subjected to HCA, PCA and SOFM in order to assess which data analysis techniques including preprocessing techniques afford meaningful discrimination of the samples.

The overall conclusions of this work are presented in Chapter 9, together with the suggestions for future work in order to advance the field of methylamphetamine profiling.

Chapter 1 Introduction

1.1 Historical Overview of Amphetamine type stimulants

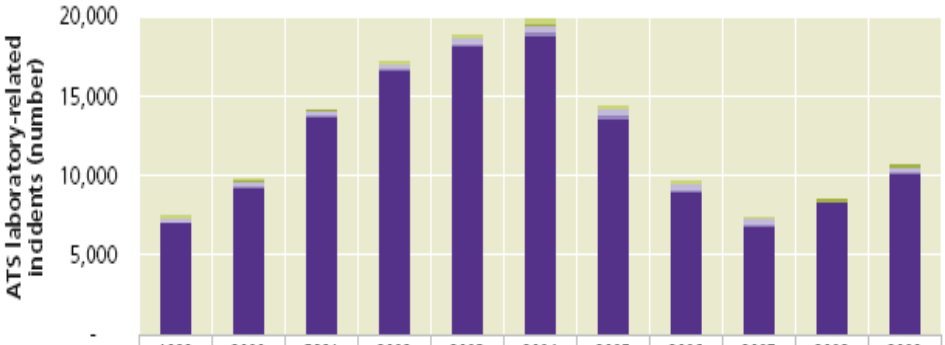
Records indicate that since the 1st century AD, Chinese herbalists have been prescribing "Ma Huang" to treat asthma, a material obtained from the dried stem of the *Ephedra Vulgaris* plant [1-8]. The plant has the ability to dilate bronchial passages and provides relief for mild asthmatic attack, bronchial congestion and catarrh. In the 20th century, a moderately potent stimulant, ephedrine, was extracted from *Ephedra Vulgaris* and used for these ailments [1-9]. As it was difficult to obtain the plant, pharmaceutical companies sought to produce a synthetic substitute, and in 1887 the German chemist called Leuckart first synthesised amphetamine [9]. In 1919, Nagai, a Japanese chemist synthesised methylamphetamine from ephedrine using hydroidic acid and red phosphorous [10]. In 1927, Alles, an American chemist, suggested that amphetamines could be used as a cheap alternative to ephedrine [10].

Amphetamines were used to treat various disorders such as narcolepsy, attention deficit disorder, depression and obesity. However, amphetamines were also found to have undesirable long term effects such as hypertension, depression, dependence and psychiatric disturbance. One of earliest non medical use of amphetamines was reported when students at the University of Minnesota used Benzedrine inhalers as a performance aid to study for examinations [10].

During World War II it has been estimated that over 200 million amphetamine and methylamphetamine tablets were distributed to American troops and over 27 million similar tablets given to the British service men as performance enhancers [10]. From 1942 onwards, similar tablets were also given to the Japanese forces. Post war misuse of amphetamine and methylamphetamine was observed by several countries, including the United States, Japan and Sweden a factor of which was attributed to the growing dependence on the drug by ex-servicemen [10]. Stringent controls were put in place in these countries to restrict the sale of amphetamine and methylamphetamine. However

such controls on the availability of the compounds contributed to a developing black market created to meet the growing demand for the drugs [11].

The United Nations Office on Drugs and Crime, (UNODC), World Drug Report 2011 estimates that between 149 and 272 million people or 3.3% to 6.1% of the Global population aged 15-64 had abused illicit substances at least once in the previous year [1]. In terms of drugs of abuse, cannabis is by far the most widely abused illicit drug type followed by the amphetamine-type stimulants (ATS), mainly methylamphetamine, amphetamine and 3,4-methylenedioxymethylamphetamine (generally referred to as ecstasy), the opioids (including opium, diamorphine (heroin) and prescription opioids) and finally cocaine [1]. The World Drug Report 2011 further concluded that due to lack of information regarding the illicit use of ATS, particularly in China and India but also in emerging markets in Africa, there was considerable uncertainty when estimating the global number of users [1]. Seizures of ATS have seen an increase between 2008 and 2009 of 26% to 10,600, though this figure was still 46% lower than in the peak year of 2004 as illustrated in Figure 1.1 [1].



	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
ATS lab total	7,412	9,726	14,138	17,093	18,783	19,799	14,312	9,611	7,398	8,415	10,598
Other synthetic stimulants	68	19	5	24	70	282	92	51	35	1	7
Ecstasy group	62	64	70	59	49	87	63	56	72	53	52
Combined ATS	204	221	272	244	314	330	370	361	344	15	300
Amphetamine	59	115	85	132	161	322	159	154	109	44	44
Methamphetamine	7,019	9,307	13,706	16,634	18,189	18,778	13,628	8,989	6,838	8,302	10,195

Figure 1.1. Total number of ATS laboratory incidents, 1999-2009 by World Drug Report 2011 [1].

In North America, Asia and Oceania methylamphetamine and ecstasy production dominate within the ATS drug classification as illustrated in Figure 1.2.

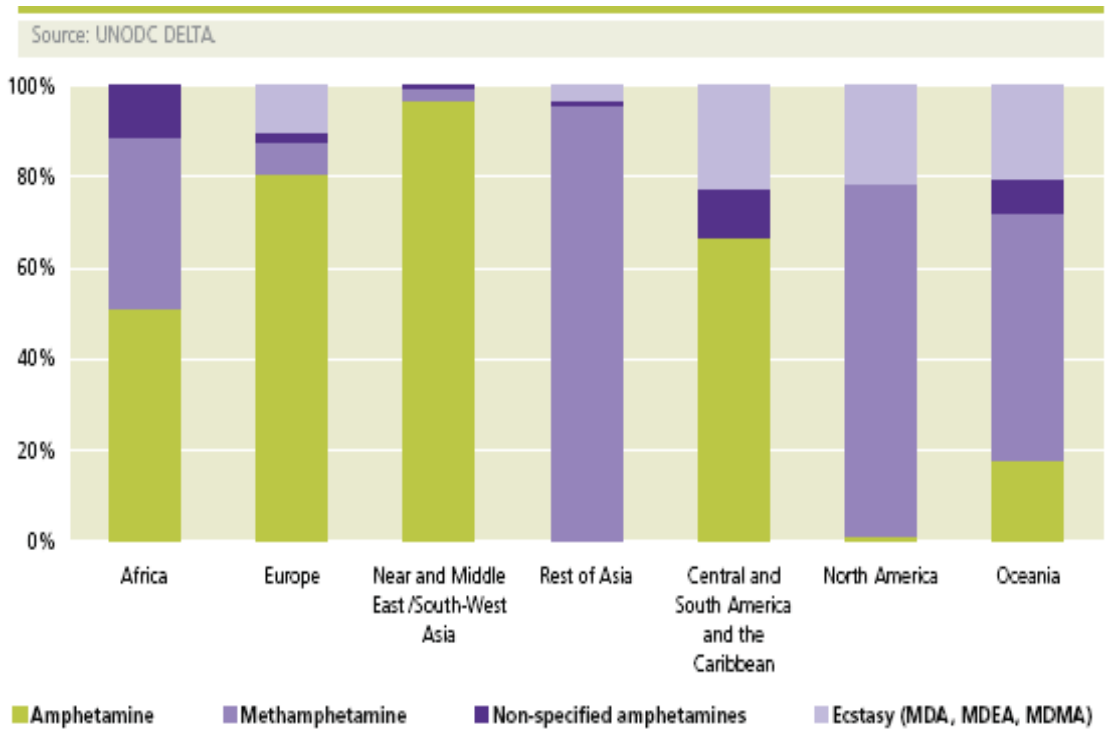


Figure 1.2. Distribution of ATS seizures by region by World Drug Report 2011 [1].

In relative terms the esctasy seizures remained important in Central, South America and the Carribean although majority of the reported seizures consisted of amphetamines [1]. The seizures in Oceania remained diversied with majority of the seizures reported were methylamphetamine and the other types of ATS [1]. In the African continent methylamphetamine seizures have been reported in Nigeria and South Africa. Notable locations of manufacture and main trafficking routes of ATS is shown in the figure 1.3 shown below [1].

In Europe, including the United Kingdom, 3,4-methylenedioxymethylamphetamine (MDMA) remains the most popular illicit synthetic drug [1] where as in Japan and the United States and more recently Australia, methylamphetamine is much more prevalent. On a global scale, methylamphetamine is the most popular produced illicit synthetic drug. It is firmly established in a number of countries such as United States, Australia, Japan, Thailand and a majority of nations in the East and Far East [12].

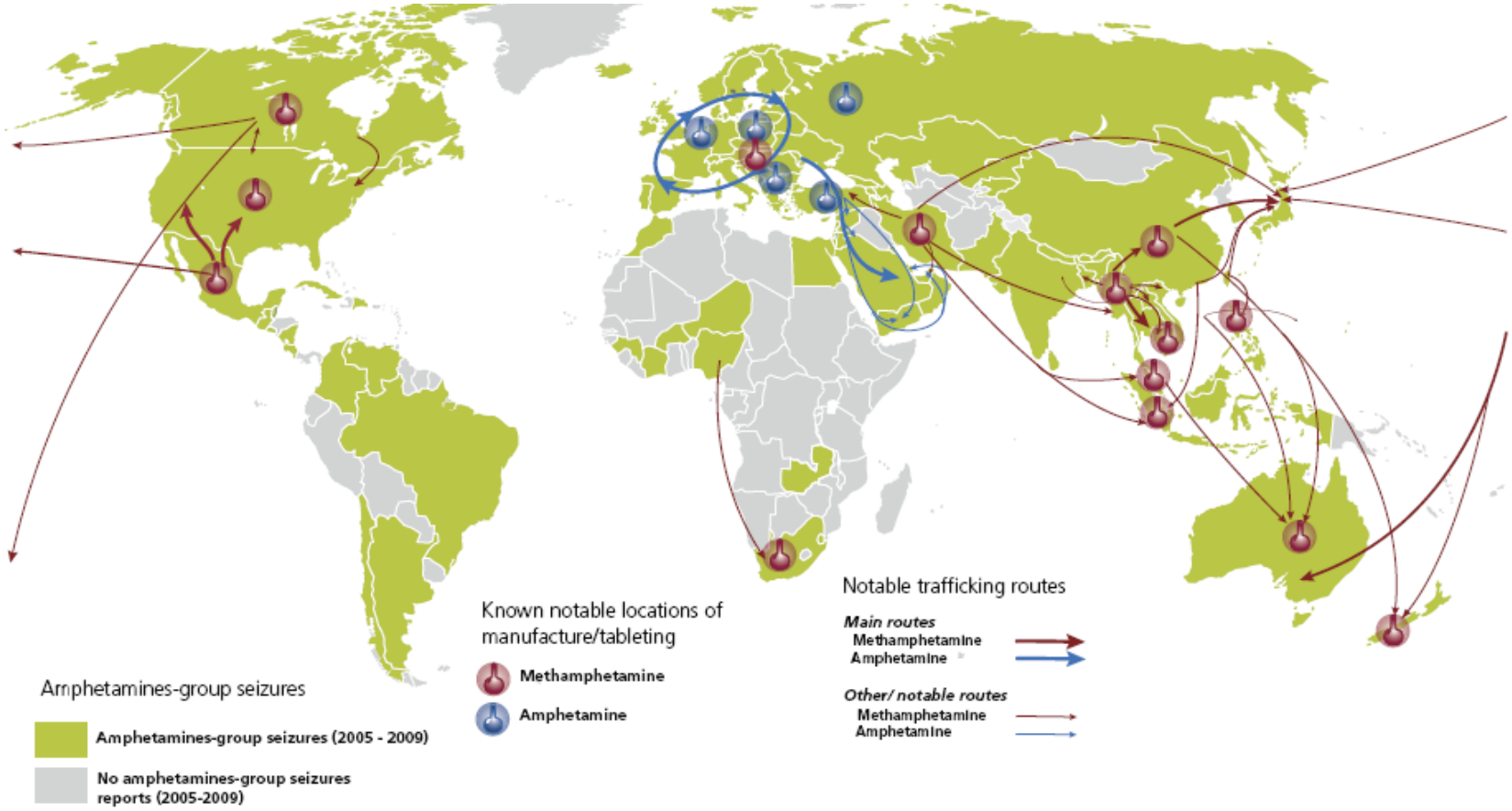


Figure 1.3. Notable locations of manufacture and main trafficking routes of ATS [1].

1.2 The global methylamphetamine situation

On a global scale, the consumption of amphetamine type of stimulants (ATS) has been reported as being stabilised in some parts of the world (North America and the European Union) but worsened in others such as China, the Middle East region and East, South East and Middle East Asia [13]. Currently, these countries largely lack the facilities to combat the increase in ATS abuse due to various factors such as ineffective information gathering and a poor regulatory framework coupled with corrupt law enforcement officials [1]. According to the UNODC, the number of people who regularly abuse ATS drugs at least once in the previous 12 month period now exceed those taking cocaine and heroin combined [13].

Amphetamine type of stimulants produce a high profit return for major illicit drug syndicates and can be synthesised from readily available precursors and pre-precursors using a variety of methods. There are no geographical limitations (as there are with plant based drugs such as heroin and cocaine) and clandestine laboratories can be situated both in close proximity and remotely from areas of consumption. The manufacture of the drugs does not require advanced knowledge of chemistry [13].

Since 2001 a number of specific developments in the synthesis of ATS drugs occurred which can be summarised as follows [13]:

(a) Regional shifts and a rapid spread to new markets: supply driven increases in the Near and Middle East and demand driven increases in MDMA manufacture in North America and South East Asia.

(b) Increases in size and sophistication of clandestine operations: The emergence of very large scale ATS clandestine laboratories;

- Zamboanga City (Philippines): monthly production capacity of 1 metric tonnes of crystalline methylamphetamine.
- Klang (Malaysia): 60 kg batch of crystalline methylamphetamine.

- Kulim (Malaysia): Theoretical production cycle of 1.4-1.7 metric tonnes of crystalline methylamphetamine (laboratory did not operate at full capacity).
- Semenyih (Malaysia): estimated output 1 metric tonne of crystalline methylamphetamine.
- Cikande, Indonesia : 75 kg of crystalline methylamphetamine.

(c) Diversification of ATS products to contain an increasing variety of substances both controlled and non-controlled depending on the region were the compounds are being abused.

(d) Vulnerability within developing countries to cope with the sudden increase of ATS manufacture ,trafficking and use within and across their national boundaries.

1.3 Global methylamphetamine and precursor seizures and trends

Ephedrine and *pseudo*ephedrine are the main precursors employed in the manufacture of methylamphetamine and both substances are controlled by the 1988 United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances. Seizures of these precursors can provide some indications about manufacturing trends [1]. In 2009, 41.9 metric tonnes of ephedrine and 7.2 metric tonnes of *pseudo*ephedrine were seized, shown in Figure 1.4. In 2008, 18.2 metric tonnes of ephedrine and 5.1 metric tonnes of *pseudo*ephedrine were seized. It was reported in the world drug report 2011 that there has been a shift from bulk substances to pharmacuetical preparations used in the illicit manufacture of methylamphetamine [1].

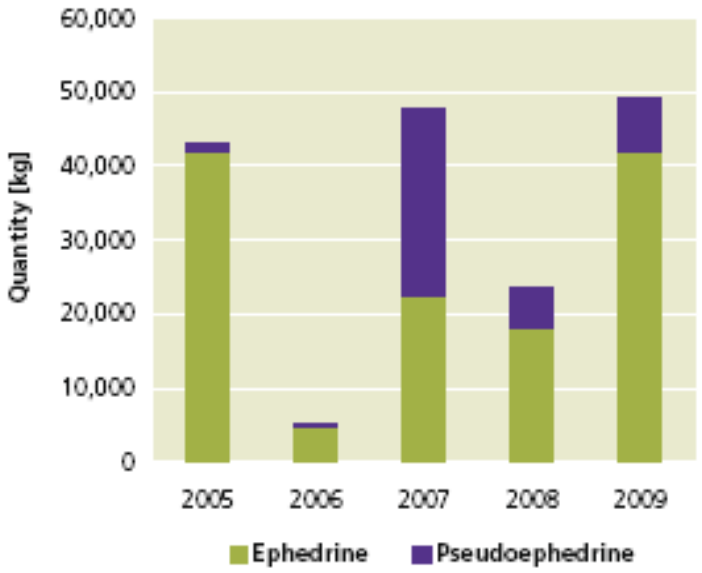


Figure 1.4. Global seizures of ephedrine and *pseudo*ephedrine, 2005-2009 [1].

1.4 Amphetamine and methylamphetamine and MDMA use in the United Kingdom – A brief overview

Amphetamine was first prescribed in the United Kingdom in the 1920's and 40's under the brand name Drinamyl and Dexdrine and sold under prescription. A surge of black market supply of amphetamine in the 60's forced the government to ban the importation and legally controlled the possession of the drug [14]. Prior to 1968, there were very few reported cases of methylamphetamine use in London. In 1968, the intravenous abuse of methylamphetamine increased dramatically and incidents of methylamphetamine related psychosis were reported at medical and social gatherings [14]. This increase was also due in part to private doctors prescribing methylamphetamine ("Methdrine ampoules") as substitutes to cocaine addicts [14]. This increase was short lived and in late 1968 a voluntary agreement between the manufacturers, the Ministry of Health and the Council of the Pharmaceutical Society immediately stopped the sale of methylamphetamine to retail pharmacists. After 1968, the only source of methylamphetamine was from limited supplies which were diverted or stolen from hospitals or pharmacies [14].

From 1970 to 1980, there is limited information available relating to the abuse of amphetamine and methylamphetamine in the United Kingdom as long term research efforts focused on other drugs such as heroin and opiates [14]. During the 1980s and 1990s an increase in MDMA and methylamphetamine abuse was associated with the developments of ‘rave culture’ and nightclub scenes at punk rock and ‘northern soul’ dance events [14]. Most of the ATS drugs which were imported into the United Kingdom were manufactured in Belgium and the Netherlands and manifested as amphetamine sulfate powder or MDMA tablets [14].

1.5 Law and legislation

In the early twentieth century, the Chinese opium epidemic spurred various international responses in particular, the 1961 Single Convention on Narcotic Drugs, the 1971 Convention on Psychotropic Substances and the 1988 Convention against the Illicit Traffic in Narcotic Drugs and Psychotropic Substances [2].

The 1961 Single Convention on Narcotic Drugs was created to combine legislation emerging from different nations into a primary instrument. Its sole purpose is to limit manufacture, possession and use of drugs to scientific study and medicine. Signatory countries of the convention provide annual statistical data and estimates for the use, and need for drugs covered by this convention to the United Nations Office on Drugs and Crime (UNODC) [2].

The 1971 Convention on Psychotropic Substances was the second major treaty created to combat the widening range of drugs of abuse. The sole purpose of this convention is to control the use of psychotropic substances which includes many synthetic drugs [3].

Finally, the 1988 Convention Against the Illicit Traffic in Narcotic Drugs and Psychotropic Substances was developed to deal with the growing problem in drug trafficking. The main aim of this treaty is to provide extradition of drug traffickers and it offers provision against money laundering and the divergence of precursors [4].

1.5.1 United Kingdom Legislation

The two main pieces of legislation regulate the use of controlled drugs in the United Kingdom. The Misuse of Drugs Act 1971 (MDA 1971) follows the United Nations conventions and lists what is prohibited in terms of the use of listed substances. The Misuse of Drugs Regulations 2001 (MDR 2001) explains what can be done with the listed substances [5].

1.5.2 The Misuse Of Drugs Act 1971

This piece of legislation covers drugs and categorises these into three classes, A,B and C, in order of known toxicity where class A drugs are the most harmful. The legistlation controls possession, supply and manufacture of the listed substances as well as extablishing the Misuse of Drugs Advisory Board [6].

1.5.3 The Misuse Of Drugs Regulations 2001

The Misuse of Drugs Regulations 2001 defines permitted use of the substances controlled under the misuse of drugs act 1971. Substances are divided into five schedules according to their medicinal value as follows [6]:

Schedule 1: includes drugs such as cannabis that are not, conventionally, used for medical purposes. Possession and supply are prohibited without specific Home Office approval.

Schedule 2: includes morphine and diamorphine, amphetamine and methylamphetamine that, because of their potential harm, are subject to special requirements relating to their safe custody, prescription, and the need to maintain registers relating to their acquisition and use.

Schedule 3: includes the barbiturates and are subject to special prescription, though not safe custody, requirements.

Schedule 4: includes the benzodiazepines and are subject to special prescription, though not safe custody, requirements.

Schedule 5: includes preparations that, because of their strength, are exempt from most of the controlled drug requirements.

1.5.4 Legislation relating to precursors

In the United Kingdom, the manufacture and the placing on the market of the precursor chemicals is regulated by the Controlled Drugs (Substances Useful for Manufacture) (Intra Community Trade) Regulations 1993. This legislation monitors precusors and requires that all import, exports and intermediary activities involving drug precusors are clearly defined and documented [7].

The scheduled precusors are defined into 3 categories. Category 1 contains true precusors which form the core structure of a controlled substance. Category 2 includes essential chemicals for the synthesis of the controlled substance and secondary precusors which can be modified to produce Category 1 precusors. Category 3 precusors include acids, bases and solvents required for final product synthesis [7].

1.6 Types of Methylamphetamine

1.6.1 Physical morphology

Illicit methylamphetamine is commonly encountered in one of four forms; tablet, powder, free base and crystal. Tablets normally contain methylamphetamine hydrochloride and caffeine. This formulation is more commonly known as ‘yaba’ which means ‘crazy day’ in Thai [12, 14]. In the United States, methylamphetamine tablets are encountered in different colours and shapes which can be flavoured, scented and stamped with various logos in a similar fashion to conventional ecstasy tablets. The tablets can be taken orally or smoked (commonly be enhaling the crushed tablet heated in foil) [12, 14].

The powder form of methylamphetamine is commonly known as ‘ice’, ‘crank’, ‘crystal’, ‘nazi crystal’ [12, 14]. It is bitter to taste and water soluble. The colour of the powder ranges from off white to reddish brown depending on the chemicals used in the manufacturing process. It can be taken orally, snorted and injected intravenously [12, 14].

A more uncommon form of methylamphetamine is the free base. This has a waxy or oily apperance and has been reported in Australia where it is usually ingested orally or injected intarvenously. This is thought to be made by clandestine chemists who are not skilled in their training to convert the methylamphetamine base to its hydrchloride salt [12, 14].

Crystalline methylamphetamine is usually obtained by re-crystallizing the hydrochloride powder using isopropyl alcohol or water. Crystalline methylamphetamine is usually smoked or snorted using a variety of pipes or smoking devices. This form of methylamphetamine has the highest purity compared to other forms [12, 14].

1.6.2 Chemical structure

Methylamphetamine has two stereoisomers, the S (*d*- or (+) methylamphetamine) shown in
Figure 1.5 and R (*l*- or (-) methylamphetamine) shown in Figure 1.6.

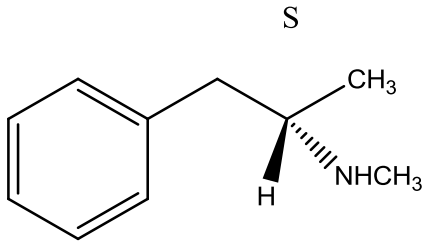


Figure 1.5. S(+)Methylamphetamine

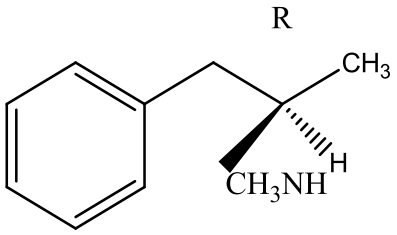


Figure 1.6. R (-) Methylamphetamine

There are three types of methylamphetamine chemical mixtures obtained through synthesis, each of which is identified by the balance of the *d*- or *l*- isomers, and are given below [14]:

- *l*-Methylamphetamine (levo-methylamphetamine). In terms of central nervous system activity, *l*-methylamphetamine is the least potent type of methylamphetamine. It is primarily active in the periphery systems (e.g. the cardiovascular system) [14].
- *dl*-methylamphetamine (dextro-levo-methamphetylamine) is the racemate (an equimolar mixture of the two enantiomeric isomers) form of methylamphetamine containing equal proportions of (*l*-) and (*d*-) stereoisomer [14].
- *d*-methylamphetamine (dextro-methylamphetamine). In terms of central nervous system activity *d*-methylamphetamine is the most potent, and widely abused form, of methylamphetamine [14].

1.7 Illicit Manufacture of Methylamphetamine

There are eight synthetic methods generally used in the illicit manufacture of methylamphetamine hydrochloride and these are presented in Figure 1.7. These methods are easily accessible through the Internet, the scientific literature, patents and books.

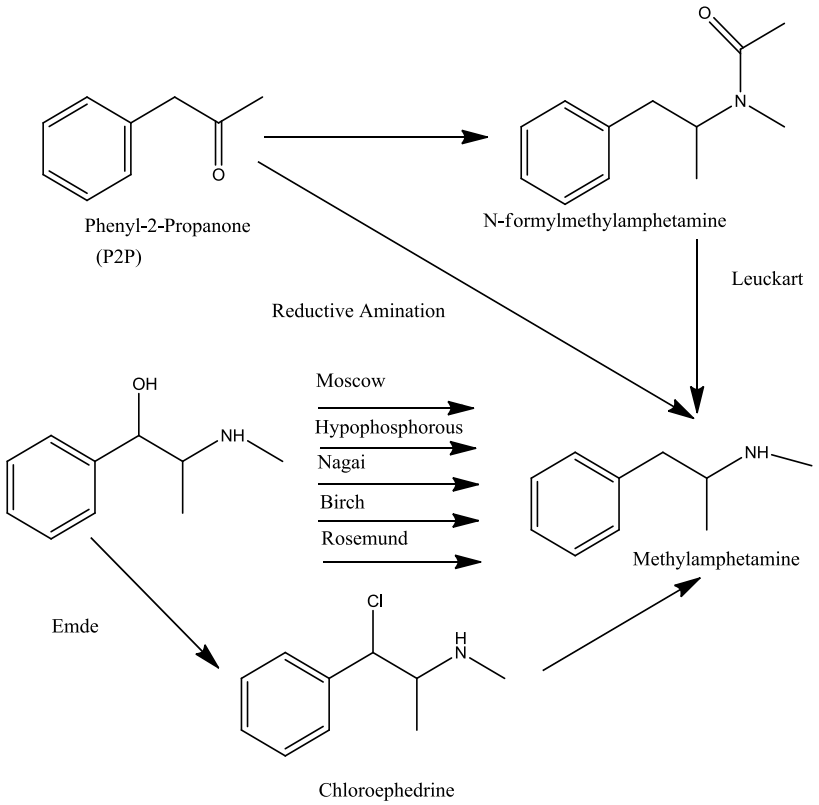


Figure 1.7. Outline of the synthetic routes used in the manufacture of methylamphetamine.

The method used to synthesize methylamphetamine depends on various factors such as availability of precursors, essential chemicals, complexity of the process, equipment and the chemical hazards presented [14]. With increasing internet usage, many clandestine chemists can purchase the required supplies using internet auction sites such as eBay [15].

Three categories of chemicals are used in the synthesis of methylamphetamine; precursors, reagents and general purpose chemicals. Precursors are essentially the 'building blocks' or basic chemicals needed in the synthesis of methylamphetamine. The molecular structure of the precursor is generally quite similar to that of the final product. The purpose of reagents in the synthesis is to chemically modify or combine precursors in a chemical reaction. General purpose chemicals are used to facilitate the reaction and isolate the products [14] of which examples include solvents, alkalis and acids. In many

countries, including the United Kingdom, the sale of these chemicals are placed under strict control and regulated by the individual legislation of the countries involved. Besides chemicals, another important tool required in the clandestine manufacture of methylamphetamine is appropriate glassware and these can be obtained either from chemical supply companies or made out of household items [14].

In general, methylamphetamine is synthesized from one of two different types of known precursors; *l*-ephedrine or *d-pseudo*ephedrine and phenyl-2-propanone (P2P) also known as benzylmethylketone (BMK), phenylacetone and phenylpropanone. In the United Kingdom, these precursor chemicals are placed under the Controlled Drugs (Substances Useful for Manufacture) (Intra-Community Trade) Regulations 1993 and the exportation to other European Union Countries is regulated by Controlled Drugs (Substances Useful for Manufacture) Regulations 1991 and Amendment 1992 [14]. These regulations do not apply to medical or pharmaceutical products. The precursor chemical phenyl-2-propanone is not readily available in the United Kingdom but can be imported or manufactured from other less restricted chemicals which are not controlled under the regulations mentioned. Ephedrine and *pseudo*ephedrine are used commonly in cold medication and sold as over the counter medication in the United Kingdom and elsewhere [14]. To combat the isolation of ephedrine and *pseudo*ephedrine from medication, manufacturers increasingly add various inhibitors such as aminoalkyl methacrylate copolymer (Eudragit-E), ferrous gluconate, lactose, ethylcellulose and hydroxypropyl cellulose which interferes with the conversion of ephedrines or *pseudo*ephedrines to methylamphetamine [14].

Besides extracting ephedrine and *pseudo*ephedrine from cold medication, clandestine chemists also isolate the compounds from *Ephedra* plant based products. The stem and leaves of the Asiatic and Mediterranean species of *Ephedra* contain ephedrine and *pseudo*ephedrine as well as other alkaloids such as norephedrine, nor*pseudo*ephedrine, methylephedrine, and methyl*pseudo*ephedrine [15]. An in-depth explanation of the reaction mechanisms for the precursor and final product synthesis is presented in Chapter 3.

In the United States, the earliest detection of clandestine methylamphetamine manufacturing was reported in the late 1960s. The total number of clandestine laboratories detected in 1969 was 21. Since then, there has been a dramatic increase of clandestine laboratories manufacturing methylamphetamine and in 2011 a total of 9,153 laboratories were reported according to the Drug Enforcement Agency [14]. The clandestine laboratories seized in the United States have varied in sophistication from well equipped laboratories to those with very crude operations. The laboratories seized were mainly small scale with ounce or multi ounce production capacity. The bulk of methylamphetamine sold are produced by laboratories which have a production cycle of in the order of 4 or 5 kilograms, so called 'super labs'. A total of 142 'super labs' were seized in the United States in 2009 [1, 14].

In February 1980, phenyl-2-propanone was reclassified as a Schedule II controlled substance in the United States. Most clandestine chemists in the United States started to synthesise phenyl-2-propanone from phenylacetic acid and also began synthesising methylamphetamine from other precursors such as ephedrine and benzylchloride. The preferred route to manufacture methylamphetamine was the reduction of ephedrine using hydroiodic acid and red phosphorous. In 1989 the Birch route was reported as the synthetic route most encountered in the clandestine manufacture of methylamphetamine, involving the reduction of ephedrine using lithium in ammonia. That year, a total of 652 clandestine laboratories were detected and 53% of these laboratories employed an ephedrine reduction method using hydroiodic acid and red phosphorous. To combat the sale of the precursors involved, the US Drug Enforcement Agency embarked on a broad chemical control program based on the Chemical Diversion and Trafficking Act (CDTA) of 1988. As a result of this, the supply of ephedrine and phenyl-2-propanone became more difficult. Clandestine operators begun using over the counter pharmaceutical preparations as alternatives to ephedrine or pseudoephedrine which were exempted from CDTA act [10, 14].

In 1993, the Drug Enforcement Agency seized 193 clandestine laboratories, 81% of the laboratories were manufacturing methylamphetamine using the ephedrine reduction method and the rest were utilising phenyl-2-propanone as the precursor. The domestic chemical control act was further strengthened with an enactment of Domestic Chemical Diversion Control Act (DCDCA), which added a registration requirement for List I chemical handlers and established record keeping and reporting requirements for transactions in single-entity ephedrine products. In the same year, hydroiodic acid was placed under List 1 chemicals and became virtually unavailable in the United States. Reduction of ephedrine with hydrogen iodide (hydriodic acid) or with hydrochloric acid and zinc or tinfoil was also reported. The next most popular method (less than 10%) involved the reaction of benzylchloride with magnesium to produce a Gringard reagent which, was then reacted with the product of an acetaldehyde/methylamine reaction [14]. Due to stricter controls on hydroiodic acid, clandestine chemists resorted to manufacture their own hydroiodic acid from in-situ reactions using red phosphorous, iodine and water. Another recipe emerged from Australia where the hydroiodic acid could be produced from reacting hypophosphorous acid with iodine [10, 14].

When stricter control of ephedrine was regulated under the DCDCA, clandestine chemists turned to the ephedrine's stereoisomer *pseudo*ephedrine. There were also reports of using phenylpropanolamine to produce amphetamine rather than methylamphetamine using the red phosphorus method. In 1995 an increase from 1.5% to 38% was reported in the usage of *pseudo*ephedrine as the main precursor in clandestine manufacture of methylamphetamine [14].

The Comprehensive Methamphetamine Control Act of 1996 (MCA) was introduced and expanded regulatory control of lawfully marketed drug products containing ephedrine, *pseudo*ephedrine and phenylpropanolamine. Iodine also became a List II Chemical, with a 400 gram threshold [14, 15]. From 1997 to 2004, due to stricter regulatory control on the sale of hydroiodic acid, red phosphorous and iodine, there was a reported increase of the usage of hypophosphorous acid in the clandestine manufacture of

methylamphetamine in California [14]. The DEA also reported several cases where phosphorous acid has been used as an alternative to hypophosphorous acid [15].

Between 2004 and 2009, North America accounted for the 44% of global seizures of methylamphetamine. A sharp increase in methylamphetamine seizures in Mexico which reached 6.1 metric tonnes was also reported in 2009 [1]. According to the UNODC, in recent years the availability of methylamphetamine seems to be directly linked to large scale methylamphetamine production in Mexico [1]. The increase reported is due to the number of small scale laboratories which has shifted their production from Mexico to the United States particularly in California. In recent years, stricter controls over the availability of ephedrine and *pseudo*ephedrine has lead to an increase in the use of P2P or its pre-precursor phenylacetic acid (PAA) in methylamphetamine production, particularly in the United States [1].

1.7.1 Trends in Illicit manufacture of Methylamphetamine in the United Kingdom

The first reported methylamphetamine laboratory in the UK was in 1981, where the route employed was an ephedrine reduction using hydroiodic acid with red phosphorous. Between 1981 and 1989 there was a steady increase of reported clandestine manufacture of methylamphetamines but towards the end of 1989, the drug was disappearing from the United Kingdom market [14]. Since the early 1990s a total of 15 laboratories were seized in the UK [14]. Most of the laboratories seized involved the illicit manufacture of amphetamines using phenyl-2-propanone and ammonium formate. Between 1992 and 1999 there was a general decline in clandestine amphetamine laboratories and most laboratories seized in that period were mainly small-scale with a manufacturing capability of less than 100 kilograms. In 2002, a large illicit amphetamine laboratory was detected in a specially converted outbuilding on a farm in East Essex. The pre-precursor, benzyl cyanide was being converted to phenyl-2-propanone via an intermediate α-acetyl-phenylacetonitrile. Phenyl-2-propanone was then being converted to amphetamine via the Leuckart method. The potential yield of the laboratory was

calculated to be 200 kilograms of high purity amphetamine sulfate, equivalent to 2 tonnes of 'street level' amphetamine powder at 10% purity [14]. Another illicit laboratory was reported in 2004, used for the illicit manufacture of tryptamines and ring substituted phenethylamines related to 'ecstasy' [14].

1.7.2 Trends in Illicit manufacture of Methylamphetamine in other parts of the world

Over the last decade notably in 2009, Mexico has become an important manufacturing location. In 2009, Mexican authorities reported dismantled 191 laboratories the upward trend has been continuing and another 63 laboratories has been dismantled since May 2010 [1]. The Mexican laboratory operators tend to manufacture large quantities of end products compared to their North American counterparts who appear to manufacture the substance on a much smaller scale [1]. The Mexican clandestine operators tend to use the P2P method to prepare the less potent racemic *d/l* methylamphetamine [1].

A significant number of clandestine methylamphetamine laboratories have been dismantled over recent years in East and South East Asia. China reported 391 seizures of clandestine synthetic drug laboratories in 2009 mainly in the Guangdong, Sichuan and Hubei provinces and these were primarily for the manufacture of crystalline methylamphetamine [1]. Since 2008, a total of 244 laboratories have been dismantled in China [1].

Indonesian authorities seized 35 clandestine methylamphetamine laboratories since 2009 including 25 large scale and 10 small scale laboratories [1]. Since 2010, 11 clandestine laboratories manufacturing methylamphetamine have been seized in Malaysia in various parts of the country including Kuala Lumpur and Southern Malaysia. The manufacturing of crystalline methylamphetamine has also been reported in the Philippines with 9 manufacturing laboratories detected in 2010 [1].

The Islamic republic of Iran has reported increased seizures of quantities of methylamphetamine since 2005 [1]. In the first nine months of 2010 Iranian authorities

reported seizures of 883 kilograms of methylamphetamine compared to 271 in 2009 [1]. The precursor used in the clandestine synthesis in the Middle, East and South East Asia has been the precursor *pseudo*ephedrine [1]. Methylamphetamine manufacture in Africa is rare and was reported only in South Africa and in Egypt [1].

In Oceania, methylamphetamine manufacture has been reported in Australia and New Zealand and the Australian authorities reported dismantling 316 clandestine laboratories since 2009 where most of the laboratories were involved in the manufacture of amphetamine and methylamphetamine [1]. New Zealand authorities reported a total of 135 laboratories having been dismantled in 2010 [1].

Compared to the other parts of the world, illicit manufacture of methylamphetamine in Europe is fairly low [1]. Until recently, methylamphetamine manufacture in this region has been mainly restricted to the Czech Republic where 300-400 kitchen laboratories have been dismantled since 2009 [1]. Seizures also have been reported in neighboring countries such as Slovakia, Germany, Poland and Austria. An emerging second region for the clandestine synthesis of methylamphetamine is in the Baltic countries particularly Lithuania and Estonia [1].

1.8 Drug Profiling

Recognising an urgent need for the development of drug profiling strategies and its importance in the field of drug analysis, the Commission on Narcotic Drugs, in resolution 1 (XXXIX) of 24 April 1996, requested the Executive Director of the United Nations International Drug Control Programme (UNDCP) to develop standard methods for the profiling/signature analysis of key narcotic drugs and psychotropic substances. This was an important tool in developing harmonised methods for the characterisation and impurity profiling of illicit drugs [1, 18]. The United Nations Office on Drugs and Crime (UNODC) impurity profiling methods which have been used extensively in Thailand, Japan, Australia, United States and the Philippines [1, 18].

Due to the prevalence of the clandestine manufacture of synthetic drugs, trafficking and involvement of organised criminal groups in drug related activity, the ability to provide information related to synthesis and supply chains is of interest to law enforcement communities. Clandestine manufacture, trafficking and abuse of methylamphetamine appear to be increasing in different parts of the world, particularly in the East and South East Asia and the Middle East. Law enforcement authorities are faced with challenges to identify the sources of supply and establish trafficking routes and distribution patterns for these materials. A potential means of achieving this is through chemical characterisation of the illicit drugs [1]. The characterisation of synthetic reaction impurities and by products is increasing in its role as a valuable component in clandestine drug investigation and it is suggested that such derived data can potentially provide information relating to the following [1, 18]:

- chemical links between samples
- origin of samples
- identification of trafficking patterns and distribution networks
- output from illicit laboratories
- common methods of synthesis
- precursor trends

Organic impurities extracted from illicit methylamphetamine have been studied and profiled by researchers around the world based on seized illicit street samples. Detailed studies of impurity profiling of methylamphetamine have been reported on samples seized in countries such as Japan, Thailand, China and the Philippines where methylamphetamine abuse poses serious potential for harm [18]. The most important tool to date that aids researchers in the impurity profiling of methylamphetamine is gas chromatography mass spectrometry (GCMS), although the chromatographic results, rather than the mass spectral data is predominantly used.

In 2005, collaboration between seven laboratories in Europe funded through the European Union (SMT-CT98-2277) resulted in a ‘harmonised’ GCMS amphetamine

profiling method [19] and resultant published literature suggested that harmonized methods would allow the exchange of data and intelligence information [19]. This project was extended into the "Collaborative Harmonisation of Methods for Profiling of Amphetamine Type Stimulants (CHAMP)" and funded by the sixth framework programme of the European Commission in 2008 [19]. In 2006, an agreed definition for drug profiling has been presented by the European Network of Forensic Science Institutes (ENFSI) drug working group as :

"*The use of methods to define the chemical and/or physical properties of a drug seizure for comparing seizures for intelligence and evidential purposes"* [16].

Besides GCMS profiling, Isotope Ratio Mass Spectrometry (IRMS) and Inductively Coupled Plasma Mass Spectrometry (ICPMS) both offer potential as additional techniques for methylamphetamine profiling. Research conducted and developments in this area are discussed in detail in sections 1.8.3 and 1.8.4.

1.8.1 Methylamphetamine profiling with gas chromatography mass spectrometry

Methylamphetamine is synthesised predominantly using the eight synthetic routes previously mentioned and the derived drug profiles can contain impurities which arise due to a number of factors. These include the nature of the precursor and precursor synthesis/extraction, incomplete reactions, inadequate purification of intermediates, and reagents/solvents that are carried over to the final product. Knowledge of impurities formed is useful for several reasons [20-23]. The impurities detected may reveal the synthetic method employed and essential chemicals used in the manufacture. Gas chromatography mass spectrometry (GCMS) is an important tool that can provide information relating to the identification of the molecules contained within a reaction mixture [22, 23]. A typical GCMS impurity profile is presented in Figure 1.8.

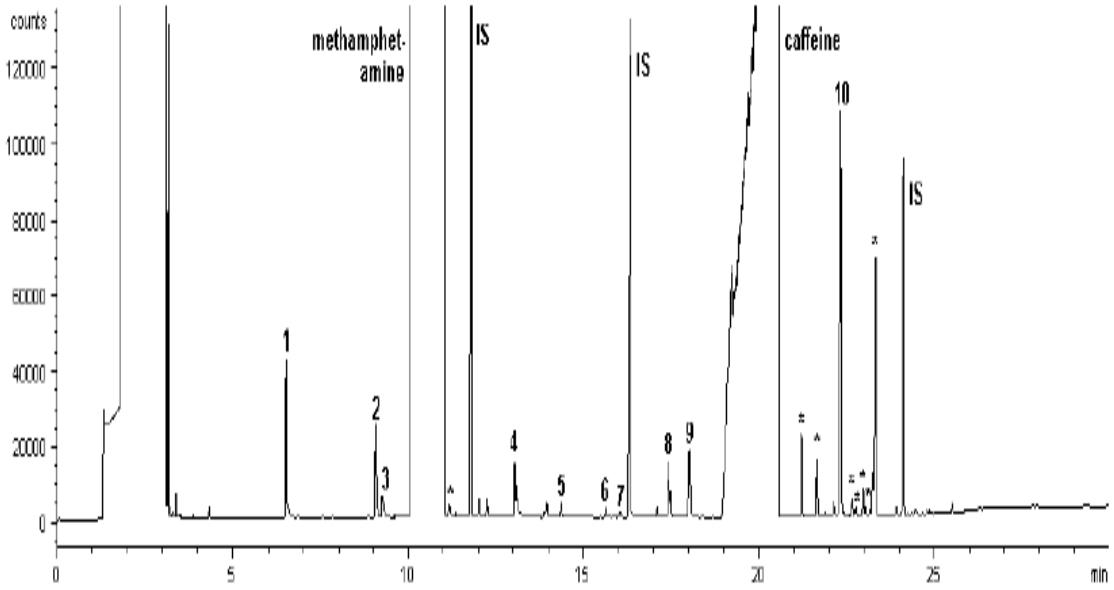


Figure 1.8. Gas chromatogram of a impurity profile of methylamphetamine tablet [18]. Selected impurities: (1) benzaldehyde, (2) cis-1,2-dimethyl-3-phenylazridne, (3) amphetamine, (4) 3,4-dimethyl-5-phenyl-oxazolidine, (5) ethyl vanillin (colouring agent), (6) N-methyl-ephedrine, (7) N-formylmethamphetamine, (8) N-acetylmethamphetamine, (9) N-acetylephedrine and (10) methylamphetamine dimer [18].

Normally, only the synthetic byproducts or impurities are utilised in subsequent data comparisons [22-34]. Various factors need to be considered before the synthetic route can be attributed and these include the frequency of occurrence of molecules in multiple samples, the presence of artifacts or co-eluting peaks as a consequence of the GCMS method and the repeatability of both the extraction method and analytical method [24]. The extraction method and analytical column used may affect both the nature and amount of impurities presented in the final chromatogram. Acidic or basic pH buffers are commonly used in liquid-liquid extractions of the sample. Impurities may be preferentially extracted at each pH and some analytical columns may have better specificity and selectivity for these molecules. The balance between extraction pH and analytical column can thus alter the final appearance of the impurity profile [23, 24].

1.8.2 Route specific impurities in methylamphetamine samples

The clandestine synthesis of methylamphetamine generally involves either phenyl-2-propanone or ephedrine/*pseudo*ephedrine as the precursor chemicals [14, 18]. These precursors themselves can be extracted from other innocuous materials such as over the counter medications or increasingly produced clandestinely [14, 18]. When the materials are utilised in the various synthetic reactions, they produce a collection of reaction and partial reaction impurities which may identify the type of precursor molecule used as well as present a series of molecules which may be considered as specific to the particular synthetic route.

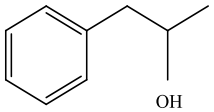
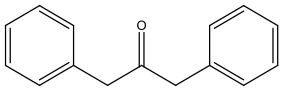
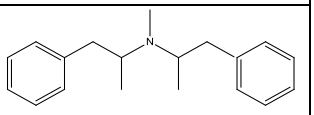
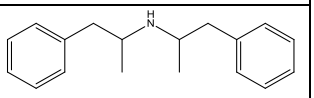
To date the most comprehensive known study of route specific impurities of methylamphetamine samples was conducted by Kunalan [32]. Here, she repetitively synthesised over 20 batches of methylamphetamine via seven of the eight synthetic routes and characterized all of the route specific impurities within each route. This has either confirmed or clarified much of the previous literature in the area.

Methylamphetamine produced from P-2-P: Leuckart and reductive amination routes

In the 70's scientific literature began to report route specific impurities arising from the Leuckart synthesis of methylamphetamine using phenyl-2-propanone as the precursor. Kram and Kruegel [35] suggested that methylamphetamine synthesized via this route might be contaminated by methylamine or methylformamide and identified α-benzyl-N-methylpheneethylamine, dibenzylketone, and N-methyldipheneethylamine as possible route specific impurities. Barron *et al*. [36] also identified α,α'-dimethyldiphenethylamine and N,α,α'-trimethyldiphenethylamine as impurities present in the Leuckart route. Barron *et al*. and Bailey *et al*. [36, 37] reported that N-formylmethylamphetamine as a route specific impurity of methylamphetamine synthesized via Leuckart route. However Qi *et a*l. [34] suggested that this molecule was also present in samples believed to be manufactured via the ephedrine based routes [34]. Kunalan confirmed α,α'-dimethyldiphenethylamine and N,α,α'-trimethyldiphenethylamine as route specific impurities for the Leuckart route.

Verweij suggested that 1-phenyl-2-propanol was a route specific impurity of methylamphetamine synthesized via the reductive amination route of synthesis [38] and this was confirmed by Kunalan *et al.* [38] who suggested it was the only route specific impurity for this synthesis [38]. These route specific impurities are summarised in Table 1.1.

Table 1.1. Route specific impurities synthesised via reductive amination and Leuckart route.

Name	Major Ions	Origin	Chemical Structure
1-Phenyl-2-Propanol	92,91,45	Intermediate (reductive amination route)	
dibenzylketone	91,65,119	Route specific impurity of the leuckart route	
N,α,α'-trimethyldiphenethylamine	176,91,58	Route specific impurity of the leuckart route	
α,α'-dimethyldiphenethylamine	91,162,119	Route specific impurity of the leuckart route	

Methylamphetamine produced from ephedrine or pseudoephedrine: Emde, Rosenmund, Birch, Nagai and Moscow routes

As with the Leuckart and reductive amination synthesis, a number of studies have been undertaken purporting to identify route specific impurities for the ephedrine/*pseudo*ephedrine routes. In many cases confusion arose within the literature until the clarifying work of Kunalan resolved a number of conflicting reports [29].

Emde Synthesis

Salouros *et al.* [33] isolated and identified N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine (two stereoisomers) from Emde synthesised samples while N-methyl-1-{4-[2-(methylamino)propyl]}-1-phenylpropan-2-amine was observed in Emde, Moscow and Hypophosphorous samples and is thus not route specific [33]. Both of these impurities had been previously listed as unknown compounds. Qi *et al*. [34] reported various compounds within the profile of Emde samples all or which had also been observed in Leuckart synthesised samples. One impurity however was route specific to the Emde method and that was the presence of chloroephedrine. Listed in the Table 1.2 below are full the list impurities identified by studies conducted by Kunalan [32]. Impurity highlighted in italics is the route specific impurity of the Emde route.

Table 1.2. Impurities identified from methylamphetamine synthesised via emde route [32].

Name	**Peak m/z (base peak in bold)**
Cis-1,2-dimethyl-3-phenylazridine	**146,** 105, 132, 91
N-(1-methyl-2-phenylethylidene)methenamine	**56**, 91, 65, 39, 77
Dimethylamphetamine(DMA)	**72**, 91, 56, 42
Ephedrone	**58**, 77, 105
Chloroephedrine	**58**, 77, 91, 146, 166
Ephedrine	**58,** 77, 117, 132, 146
3,4-Dimethyl-5-phenyloxazolidine	**71**, 56, 91
N-formylmethamphetamine	**86**, 58, 118
N-acetylmethamphetamine	**58**, 100
Benzylmethamphetamine	**91**, 160, 119, 65, 77, 207
N-β-(phenylisopropyl)benzylmethylketimine	**91**, 160, 119, 65, 77, 207
3,4-Dimethyl-2,5-diphenyl-oxazolidine	**146**, 147, 105, 132
Methylamphetamine dimer	**238**, 91, 120, 148

Rosenmund synthesis

Studies conducted by Kunalan [32] identified three route specific impurities, ethylamphetamine and two unknown compounds which were present in the batches of samples synthesized via the Rosenmund route [32]. A full list of impurities synthesised by methylamphetamine via the Rosenmund route identified by Kunalan is shown in the Table 1.3 below. Impurities highlighted in italics are the route specific impurities of the Rosenmund route.

Table 1.3. Impurities identified in methylamphetamine synthesised via Rosenmund route [32].

Name	**Peak m/z (base peak in bold)**
Dimethylamphetamine(DMA)	**72**, 91, 56, 42
Ephedrone	**58**, 77, 105
Ephedrine	**58**, 77, 117, 132, 148
Ethylamphetamine	**72**, 44, 58, 91
N-formylamphetamine	**44**, 118, 72, 91, 58
N-formylmethylamphetamine	**86**, 58, 118
N-acetylmethylamphetamine	**58**, 100
Cis 3,4-Diphenyl-3-buten-2-one	**179**, 178, 222, 221
Trans 3,4-Diphenyl-3-buten-2-one	**179**, 178, 222, 221
Unknown 1	**58**, 91, 118, 239
Unknown 2	**58,** 263, 248

Birch reduction

Research conducted by Person *et al.* [31] reported 1-(1,4-cyclohexadienyl)-2-methylaminopropane (CMP) as a route specific impurity for the Birch reduction route [31]. This was confirmed by Kunalan [32], a full list of impurities synthesised by methylamphetamine via the birch route identified by Kunalan is shown in the Table 1.4 below [32]. Impurities highlighted in bold is the route specific impurity of the birch route.

Table 1.4. Impurities identified in methylamphetamine synthesised via birch route [32].

Name	**Peak m/z (base peak in bold)**
Benzaldehyde	**105**, 107, 77, 51
CMP	**58**, 56, 91, 77, 152
Dimethylamphetamine (DMA)	**72**, 91, 56, 42
Ephedrone	**58**, 77, 105
Ephedrine	**58**, 77, 117, 132, 148
Unknown 1	**58**, 77
N-formylmethamphetamine	**86**, 58, 118
N-acetylmethamphetamine	**58**, 100
N-formylephedrine	**86**, 87, 58, 77, 100
N-acetylephedrine	**58,** 77, 100
3,4-Dimethyl-2,5-diphenyl-oxazolidine	**146**, 147, 105, 132

Nagai synthesis

Cis or trans azridines presented within impurity profiles associated with the Nagai route may undergo a ring opening process producing phenyl-2-propanone (P2P) [23]. Two molecules of P2P can undergo a self-condensation process followed by dehydration to form 1-benzyl-3-methyl-naphtalene and 1,3-dimethyl-2-phenylnaphtalene impurities [25]. Windahl *et al*. [25] reported that the azridine species may not always be present in Nagai samples (and thus could not be a route specific impurity), and carried out a systematic study of reaction times concluding that the reaction time was key factor in determining the amount of azridines and napthalenes present in the final product. As the reaction time increased, the aziridines decreased and naphthalenes increased. This study also suggested two new impurities, N-methyl-N-(α-methylphenethyl)amino-1-phenyl-2-propanone and (Z)-N-methyl-N-(α-methylphenethyl)-3-phenylpropenamide [25]. Other researchers reported the presence of a methylamphetamine dimer [26] formed via condensation of 1,2-dimethyl-3-phenylazridine and methylamphetamine. This was demonstrated by the successful synthesis of the methylamphetamine dimer with the two

mentioned compounds. They also reported the presence of aziridines impurity as a route specific impurity of the ephedrine route [26]. Analysis of seized samples of Nagai synthesised samples reported the presence of all three specific impurities [27, 28]. The literature was finally clarified by Kunalan *et al.* [29] and the formation of all specific impurities clarified as a function of synthetic conditions and are presented in Table 1.5.

Table 1.5. Impurities found by Windahl *et al.* [25], Tanaka *et al*. [26] and Kunalan *et al. [*29] in their synthesis of methylamphetamine by the Nagai route.

Impurities	Windahl	Tanaka	Kunalan
Cis-1,2-dimethyl-3-phenylaziridine	√		√ (1/2, 2, 4 hr)
Trans-1,2-dimethyl-3-phenylaziridine	√		√ (1/2, 2, 4 hr)
Methylamphetamine dimer		√	√ (1/2, 2 hr)
1,3-dimethyl-2-phenylnapthalene	√		√ (2, 4, 24 hr)
Isomers of N-methyl-N-(α-methylphenylethyl)amino-1-phenyl-2-propanone	√		√ (2, 4, 24 hr)
(z)-N-methyl-N-(α-methylphenethyl)-3-phenylpropenamide	√		√ (2, 4, 24 hr)

Moscow synthesis

Studies conducted by Kunalan [32] identified three route specific impurities which were identified in the Nagai samples were also observed in the samples synthesised via the Moscow route [32]. A full list of impurities synthesised by methylamphetamine via the Moscow route identified by Kunalan is shown in Table 1.6 below. Impurities highlighted in italics are the route specific impurity of the Moscow route.

Table 1.6. Impurities identified from methylamphetamine synthesised via Moscow route [32].

Name	**Peak m/z (base peak in bold)**
N-(1-methyl-2-phenylethylidene)methenanmine	**56,** 91, 65, 39, 77
Dimethylamphetamine(DMA)	**72**, 91, 56, 42
Z(1-phenylpropan-2-one oxime)	**91**, 149, 116, 131
E((1-phenylpropan-2-one oxime)	**91**, 131, 116, 149
3,4-Dimethyl-5-phenyloxazolidine	**71**, 56, 91
3,4-Dimethyl-5-phenyloxazolidine	**71**, 56, 91
N-formylamphetamine	**118**, 72, 44, 91
Bibenzyl	**91**, 182
N-formylmethylamphetamine	**86**, 58, 118
N-acetylmethylamphetamine	**58**, 100
Cis-3,4-Diphenyl-3-buten-2-one	**179**, 178, 222, 221
Trans-3,4-Diphenyl-3-buten-2-one	**179**, 178, 222, 221
Benzylmethamphetamine	**91,** 148, 65, 105
N-β-(phenylisopropyl)benzylmethylketimine	**91**, 160, 119, 65, 77, 207
Dimethylphenylnapthalene	**232**, 217, 202, 77
N-benzoylamphetamine	**105**, 77, 148, 91, 118
Benzylmethnapthalene	**232**, 217, 202, 58
N-methyl-N-(α-methylphenethyl)amino-1-phenyl-2-propanone	**238**, 91, 105, 190, 120
N-benzoylmethylamphetamine	**105**, 62, 77, 91
N,N-di-(β-phenylisopropyl)formamide	**190**, 91, 58, 119, 77, 105
(Z)-N-methyl-N-(α-methylphenethyl)-3-phenylpropenamide	**131**, 91, 58, 103, 188, 77

1.8.3 Methylamphetamine profiling using isotope ratio mass spectrometry

The application of Isotope Ratio Mass Spectrometry (IRMS) in determining the geographic origin, source and/or discriminating between batches of various types of drug compounds has been applied over the years [39-43]. Kurashima *et al.* [43] investigated the possibility of determining the geographic origin of ephedrine on the basis of $\delta^{13}C$ and $\delta^{15}N$ values. Ephedrine is one of the precursors of methylamphetamine and it can be manufactured by three routes shown in Figure 1.9 below, where (a) full chemical synthesis; (b) semi-synthesis from sugar; and (c) extraction from the ephedra plant [43]. Kurashima *et al*. [43] reported that combining $\delta^{13}C$ and $\delta^{15}N$ values obtained allowed the discrimination of all three forms of ephedrine shown in the figure below, confirming the importance of nitrogen isotope values for greater sample discrimination compared to carbon alone.

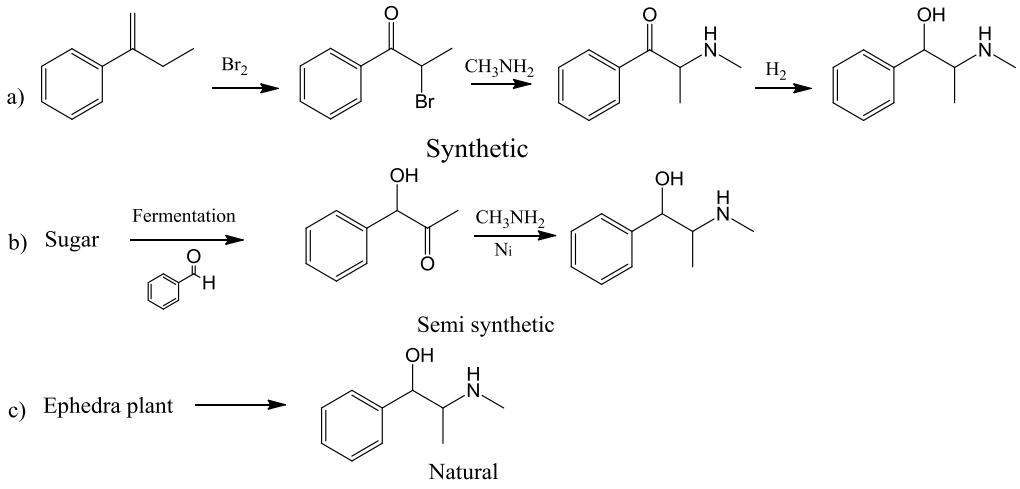


Figure 1.9. Production schemes of ephedrine [43].

Studies conducted by Makino *et al*. [44] in 2005 confirmed the work of Kurashima *et al*. [43] and suggested the potential for IRMS to reveal the geographic origin that had been used as a precursor [44]. The authors investigated the isotopic ratios for carbon and nitrogen for ephedrine samples produced from the three different synthetic methods

shown in Figure 1.9 and together with pseudoephedrine of known origin sourced from six different manufacturers shown in Figure 1.10 [44].

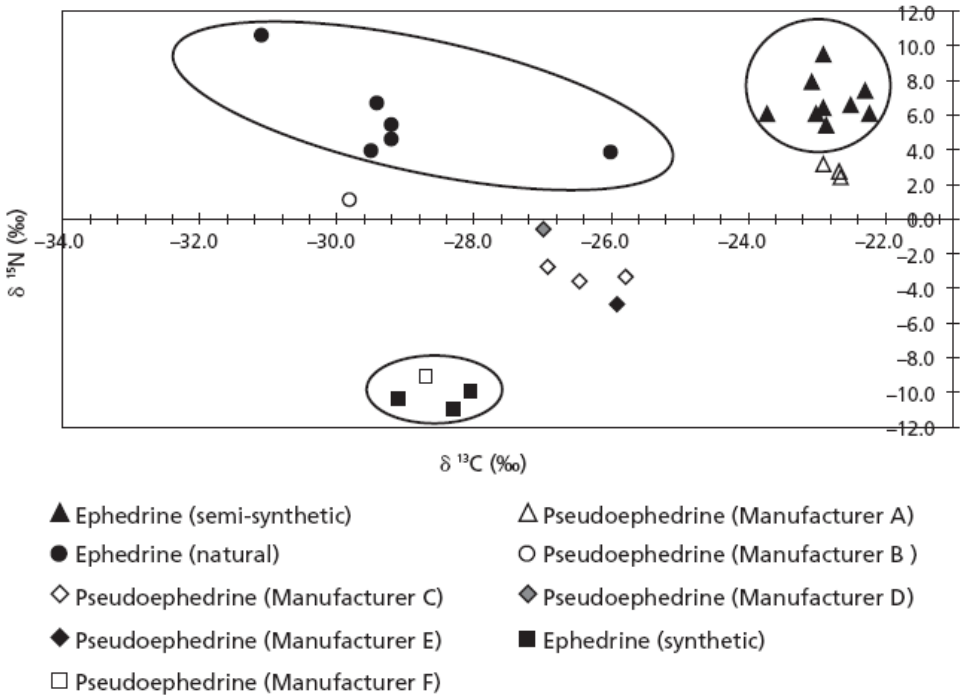


Figure 1.10. Two dimensional plot of carbon and nitrogen isotope ratios of ephedrine and pseudoephedrine samples [44].

The authors concluded that the lower ($\delta^{15}N$) value for d-*pseudo*ephedrine was a direct result of nitrogen isotope fractionation during the isomerization process. The ($\delta^{15}N$) and ($\delta^{13}C$) values shown for manufacturer C were much lower compared to semi-synthetic ephedrine [44]. The information provided to the authors from manufacturer C suggested that it was imported from Europe. The lower ($\delta^{13}C$) values were attributed to manufacturer C using sugar beet (C_3) instead of sugar cane (C_4) is a possible explanation of lower ($\delta^{13}C$) value seen in the figure above [44]. The lower ($\delta^{15}N$) values obtained were probably due to nitrogen fractionation during the isomerization process. The authors of this study also reported that *pseudo*ephedrine from manufacturer B was synthesized from natural ephedrine and from manufacturer F was synthesized chemically [44]. The *pseudo*ephedrine from manufacturers D and E were reagent grade.

The authors also suggested that *pseudo*ephedrine from manufacturer C, D and E were all manufactured from European semi-synthetic ephedrine because of their proximity [44].

In 2009, Kurashima *et al*. [45] analysed 27 precursor samples and discussed identified that semi-synthetic ephedrines synthesized from pyruvic acid could be discriminated from natural and synthetic ephedrines based on δ^2H values. The authors noted that using δ^{13}C and δ^{15}N values alone did not discriminate ephedrine synthesised from the three different manufacturing processes. Figure 1.11 illustrates a two dimensional plot of δ^{15}N values versus δ^{13}C values of l-ephedrine and d-*pseudo*ephedrine samples manufactured from synthetic, semi-synthethic (molasses and pyruvic acid) and biosynthetic processes [45].

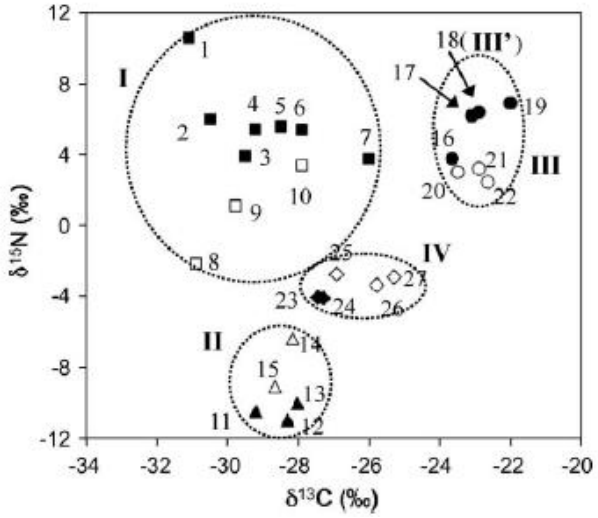


Figure 1.11. Graphical two dimensional plot of carbon and nitrogen isotope ratios of l-ephedrine and d-*pseudo*ephedrine samples: biosynthetic, synthetic, semi-synthetic from molasses and semi-synthetic from pyruvic acid. Open symbols indicate d-*pseudo*ephedrine samples. The biosynthetic group is indicated as I, the synthetic group as 'II', the semi-synthetic group from molasses as 'III' and the semi-synthetic group from pyruvic acid as 'IV' [45].

Kurashima *et al.* [45] suggested that δ^2H values of naturally synthesized ephedrine (^{2}H: -193% to -151%) facilitated clear discrimination from synthetic ephedrines (^{2}H: -73% to -30%), semi-synthetic ephedrines derived from pyruvic acid (^{2}H: +75% to +148%) and semi-synthetic ephedrines derived from molasses (^{2}H: -74% to +243%) [45]. Furthermore, the authors suggested that using δ^{13}C and δ^{15}N values it was possible to distinguish apparently similar methylamphetamine samples seized on different occasions. Their results are illustrated in Figure 1.12.

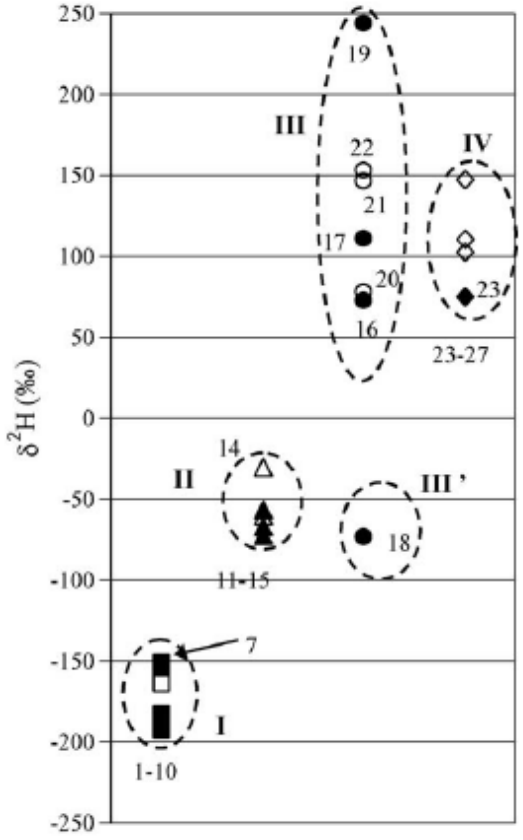


Figure 1.12. 2H values of l-ephedrine and d-*pseudo*ephedrine samples: biosynthetic, synthetic, semi-synthetic from molasses and semi synthetic from pyruvic acid. Open symbols indicate d-*pseudo*ephedrine samples. I-IV are the same as Figure 1.11 [45].

Kurashima *et al.* [43] noted that the origins of ephedrine could be discriminated by $\delta^{13}C$ and $\delta^{15}N$ values and these were similar in value to methylamphetamine samples synthesized using these specific precursors and the authors demonstrated a relationship between ephedrine and methylamphetamine using $\delta^{13}C$ and $\delta^{15}N$ values shown below in Table 1.7 [43].

Table 1.7. The $\delta^{13}C$ and $\delta^{15}N$ values of ephedrine used as precursors of methylamphetamine synthesis and methylamphetamine synthesized from the ephedrine [43].

compound	synthetic pathway	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
methamphetamine	Nagai	−29.2	−11.1
ephedrine (synthetic)		−29.2	−10.5
methamphetamine	Nagai	−23.0	9.5
ephedrine (semisynthetic)		−22.9	9.5
methamphetamine	Nagai	−23.1	5.8
ephedrine (semisynthetic)		−23.1	6.2
methamphetamine	Emde	−26.1	4.4
ephedrine (natural)		−26.0	3.8
methamphetamine	Nagai	−29.8	3.7
ephedrine (natural)		−29.5	3.9
methamphetamine	Nagai	−31.4	10.9
ephedrine (natural)		−31.1	10.6
methamphetamine	Nagai	−29.5	3.9
ephedrine (natural)		−29.2	4.2

In the second part of their study, authors demonstrated the ability of IRMS to distinguish between methylamphetamine prepared from natural, semi synthetic or synthetic ephedrine the results of which are shown in Table 1.7 where Figure 1.9 illustrates the reaction pathways used to derive the various precursors [43]. The data obtained indicates that the IRMS is indeed a useful instrumental technique to link precursors to illicit substance [43]. This is shown in Table 1.8 and Figure 1.13.

Table 1.8. Comparisons of δ ^{13}C and δ ^{15} N of precursor and methamphetamine [43].

SAMPLE/PRECURSOR	δ ^{13} C ($^{0}/_{00}$)	δ ^{15} N($^{0}/_{00}$)
Natural ephedrine	-29.2	+4.2
Methamphetamine from natural ephedrine	-29.5	+3.9
Semi-synthetic ephedrine	-23.1	+6.2
Methamphetamine from semi-synthetic ephedrine	-23.1	+5.8
Synthetic ephedrine	-29.2	-10.5
Methamphetamine from synthetic ephedrine	-29.2	-11.1

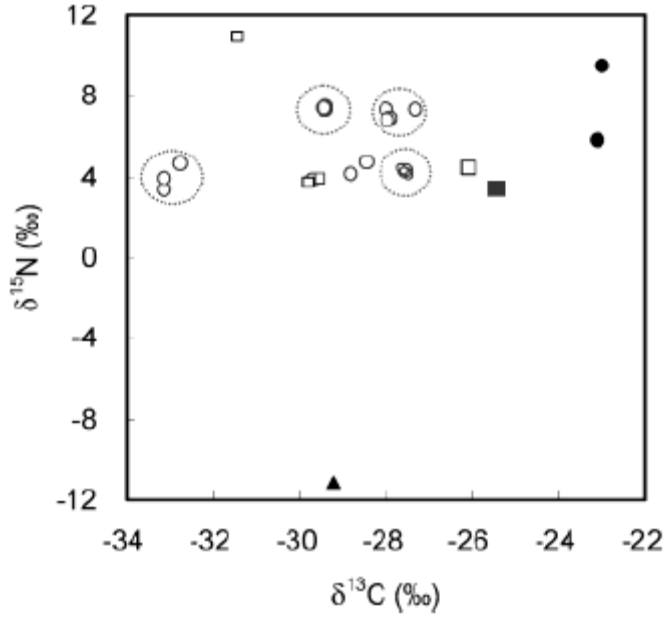


Figure 1.13. Carbon and nitrogen isotope ratios of methylamphetamine samples: seized, synthesized from natural ephedrine, synthesized from synthetic ephedrine, synthesized from semi-synthetic ephedrine and commercial methylamphetamine. Dotted circles indicate samples that were seized on the same occasion [43].

In a similar study conducted in 2008, Collins *et al.* [46] synthesised methylamphetamine using ephedrine and *pseudo*ephedrine of different synthetic provenance (synthetic, semi-synthetic and natural) via four different clandestine routes (Nagai, Hypophosphorous, Emde and Moscow). This is presented in Table 1.9.

Table 1.9. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values for ephedrine and *pseudo*ephedrine of known provenance (values reported as the mean of three consecutive measurements) [46].

Precursor source	**$\delta^{13}C_{VPDB}(^{o}/_{oo})$**	**$\delta^{15}N_{air}(^{o}/_{oo})$**	**$\delta^{2}H_{VSMOW}(^{o}/_{oo})$**
Pseudoephedrine (I) *Sigma Aldrich (USA)/semi synthetic origin*	-23.3	+5.1	+168
Ephedrine (II) *Fluka product*	-26.8	+6.8	-135
Pseudoephedrine (III) *Sigma Aldrich/semi synthetic origin*	-26.2	+3.6	+159
Pseudoephedrine (IV) *Extracted from Sudafed tablets/Wellcome*	-26.5	+0.6	+78
Ephedrine (V) *Sigma Aldrich Product(India)/semi synthetic*	-25.6	-0.1	+171

It was observed that methylamphetamine synthesized from semi-synthetic ephedrine and *pseudo*ephedrine had positive $\delta^{2}H$ values and methylamphetamine synthesized prepared from natural material had negative $\delta^{2}H$ values [46]. The authors concluded from their findings that the positive values of $\delta^{2}H$ ephedrine and *pseudo*ephedrine from semi-synthetic source was attributed to the synthetic benzaldehyde used as a starting material in the manufacture of these precursors shown in Table 1.10. The study demonstrated that besides using IRMS analysis of $\delta^{13}C$ and $\delta^{15}N$ values, the $\delta^{2}H$ value also provided a viable option to distinguish batches of methylamphetamine synthesized from different routes to its precursor from different origins [46].

Table 1.10. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values for methylamphetamine synthesised from ephedrine and *pseudo*ephedrine of known provenance (values reported as the mean of three consecutive measurements) [46].

Methylamphetamine product					
Precursor	**Reaction**	**Purity($^{0}/_{oo}$)**	**$\delta^{13}C_{VPDB}$($^{o}/_{oo}$)**	**$\delta^{15}N_{air}$($^{o}/_{oo}$)**	**$\delta^{2}H_{VSMOW}$($^{o}/_{oo}$)**
Pseudo ephedrine (I)	Nagai(1)	96	-23.2	+5.2	+134
	Nagai(2)	95	-23.1	+5.3	+111
	Nagai(1)	93	-22.9	+5.2	+131
	Nagai(2)	94	-23.0	+5.1	+128
	Hypo (1)	93	-23.8	+4.1	+124
	Hypo (2)	93	-23.2	+4.3	+120
	Hypo (3)	90	-23.1	+4.7	+114
	Emde	89	-22.8	+5.6	+145
	Moscow	95	-23.4	+4.7	+127
Ephedrine (II)	Emde(1)	94	-27.2	+6.6	-134
	Emde(2)	94	-27.4	+6.5	-133
	Emde(3)	93	-27.3	+6.5	-131
	Hypo (1)	93	-27.7	+6.3	-132
Pseudo ephedrine (III)	Nagai	94	-26.2	+4.3	+120
Pseudo ephedrine(IV)	Nagai(1)	90	-25.9	+2.7	+65
	Nagai(2)	93	-26.1	+2.4	+65
	Emde(1)	95	-25.7	+1.7	+79
	Emde(2)	94	-25.9	+1.9	+81
	Emde(3)	90	-25.6	+1.6	+77
Ephedrine (V)	Emde	87	-25.5	-0.1	+166
	Nagai	95	-25.6	-0.4	+133
	Moscow	95	-25.4	-0.3	+142
	Hypo	94	-25.0	-0.2	+140

Kurashima *et al*. [45] observed a substitution of exchangeable hydrogen atoms during the synthetic process of seven batches of methylamphetamine synthesized by the Nagai or Emde routes. To study this substitution, the batches of methylamphetamine were synthesised using untreated (treated with water) and treated with Milli-Q-water [45]. The authors suggested the difference in $\delta^{2}H$ values of methylamphetamine obtained were due to various experimental conditions during the synthetic process including the drying process, solvent used and humidity [45].

Makino *et al.* [44] applied IRMS profiling to seized crystalline methylamphetamine samples where the reported source of methylamphetamine was (i) various locations of seizures of methylamphetamine in different parts of Japan, (ii) samples smuggled in from Malaysia, Hong Kong, Philippines, Canada and (iii) samples seized in different locations, Australia, Korea, United States [44]. Using carbon and nitrogen isotope ratios, the authors suggested that the precursor used in all of the seized methylamphetamine were natural or semi-synthetic and not synthetic ephedrine. They reported that from IRMS analysis (specifically $\delta^{13}C$ and $\delta^{15}N$ values) of samples from Canada and Malaysia suggested a semi-synthetic ephedrine precursor source. The methylamphetamine from Australia and United States were suggested to have been synthesised from semi-synthetic *pseudo*ephedrine. The samples found in different parts of Japan and from Philippines were suggested to have been synthesized from natural ephedrine as were the samples from Korea. The findings of the IRMS analysis are shown in Figure 1.14 [44].

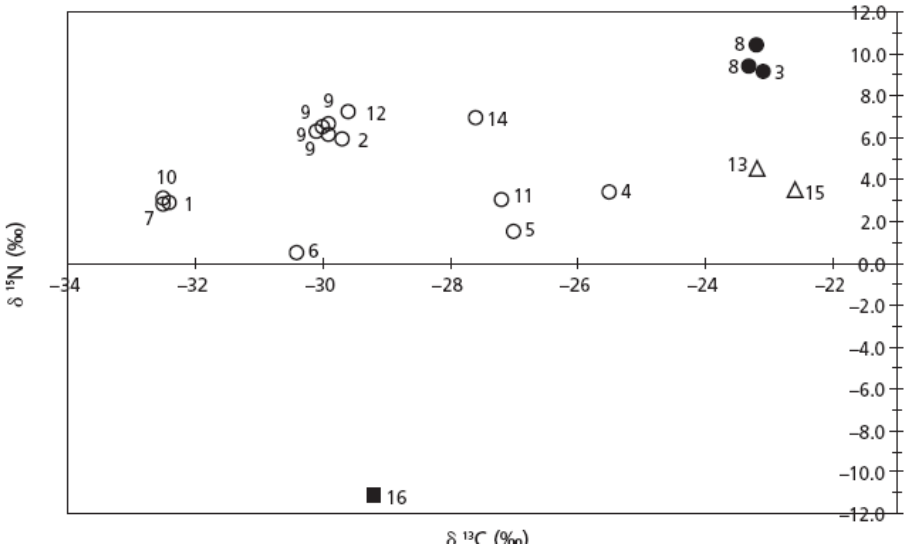


Figure 1.14. Methylamphetamine carbon and nitrogen isotope ratios [44].

Studies conducted by Kunalan *et al*. [32] reported stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and hydrogen ($\delta^{2}H$) of methylamphetamine prepared using seven synthetic routes using two different precursors, phenyl-2-propanone and ephedrine/*pseudo*ephedrine. The synthesized methylamphetamine batches could be discriminated using the isotopic data by precursor. The $\delta^{13}C$ values offered the best discrimination which was to be expected as all of the carbon atoms on the final methylamphetamine molecule are contributed by the precursor. It was reported also that $\delta^{2}H$ values could discriminate whether the methylamphetamine prepared was from either a phenyl-2-propanone (P2P) or an ephedrine *pseudo*ephedrine pathway and the $^{15}\delta N$ data appeared to be most sensitive to inadvertent differences in preparative methods [32].

In 2010, David *et al*. [47] reported in their study that $\delta^{2}H$ and $\delta^{15}N$ were found to fractionate during the precipitation of methylamphetamine as the hydrochloric salt. This was similar to $\delta^{15}N$ values during the fractionation of cocaine hydrochloride salt [47]. It was also reported that successive fractions of precipitate were found to be depleted in heavy isotopes with more negative δ values [47]. However mixing the different fractions of precipitate together and homogenizing them eventually minimized the effects of the

fractionation. In the authors opinion, in a clandestine situation samples would not be fully precipitated or mixed this fractionation could create greater than expected variation between illicit samples for δ^2H and $\delta^{15}N$ even when synthesized from the same batch of precursor [47].

1.8.4 Drug profiling with Inductively Coupled Plasma Mass Spectrometry

Few studies involving amphetamine type stimulants have been reported using Inductively coupled Plasma mass Spectrometry (ICPMS) in comparison with other techniques and, generally speaking, this may be as a result of the less specific nature of the results obtained in terms of route or precursor specificity.

Waddell *et al*. [49] reported on ICPMS analysis data of seized ecstasy tablets and their potential in seizure to seizure comparison. The study utilized chemometric methods and artificial neural networks to analyse the resultant data. The authors reported that principal component analysis (PCA) and hierarchical cluster analysis (HCA) failed to differentiate tablets from different seizures adequately [49]. The limitations were due to significant variation in the intensity of metals present within each seizure which still impacted the cluster analysis despite data pre processing. The authors also noted significant improvements in the classification of the tablets to the original seizures by applying neural network algorithms.

Marumo *et al*. [51] analysed seized samples of methylamphetamine using ICPMS and atomic absorption spectrometry (AAS). The authors identified seven elements (barium (Ba), antimony (Sb), palladium (Pd), strontium (Sr), bromine (Br), zinc (Zn) and copper (Cu)) and concluded a possible classification of the samples into five groups, however given the unknown provenance of the samples, no route identification was possible [51]. Suzuki *et al*. [52] reported a number of inorganic impurities identified in methylamphetamine samples using anion exchange ICPMS analysis. The authors reported inorganic impurities of seized crystalline methylamphetamine using ICPMS and ion chromatography. The authors also observed large variations of the target

elements within the same crystal and concluded that several parts of the crystal should be analysed.

Kishi *et al.* [48] reported sodium (Na), bromine (Br), palladium (Pd), barium (Ba) and iodine (I) in Emde synthesised samples and iodine, bromine and sodium apparent in Nagai synthesised methylamphetamine samples [48]. Similarly Suh *et al.* [50] analysed 51 seized methylamphetamine samples using ICPMS and identified iodine (I) in the majority of samples known to have been prepared via the Nagai route and palladium (Pd) and barium (ba) in samples known to have been prepared via the Emde route. Bromine (Br) was also detected in samples identified as being prepared via Nagai or Emde synthesis.

Kunalan [32] also used ICPMS to interrogate methylamphetamine samples prepared via seven different synthetic routes. This work highlighted the non specific nature of the technique in relation to sample of this type where clusters of elements were associated with more than one synthetic method: mercury (Hg) and lithium (Li) were associated with both the reductive amination and birch routes, phosphorous (P) and iodine (I) were associated with the Nagai and Moscow routes, paladium (Pd) and barium (Ba) were associated with the Rosenmund and Emde routes [32].

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Chapter 2 Analytical Techniques

2.1 Introduction

The identity of the extracted precursor, essential chemicals and target compound synthesised for this study was confirmed by a variety of analytical techniques and comparison of the results obtained was made against literature values. This chapter provides a brief description of each of these instruments together with a summary of the background theory upon which each is based.

Melting point analysis, optical rotation, Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) were used to confirm the synthetic products and extracted precursors. Microanalysis and X-ray powdered diffraction (XRD) were used to determine the purity of the extracted precursor from the proprietary medication. Gas chromatography mass spectroscopy (GCMS) was used to analyse the synthesised samples to indicating organic impurities present. Inorganic impurities (elemental profiles) were obtained for synthetic products, precursor, extracted essential chemicals and extracting solvents using inductively coupled plasma mass spectrometry (ICPMS). All of the synthesised samples and extracted precursors were subjected analysis using stable isotope ratio mass spectrometry (IRMS) at the Stable Isotope Unit, The James Hutton Institute, Invergowrie, Dundee, Scotland.

2.2 Melting point

The melting point of a solid is a temperature range over which the material changes state from a solid to a liquid. This range is shorter for pure solids and as such melting points measurements are often used as indicators for purity as well as identification of an organic compound [1]. Solid to liquid transition is precise for pure solids and melting point can be measured at 0.1^{o}C and a pure solid has a higher melting point compared to a less pure compound of the same material [1].

2.3 Optical rotation

A known number of solids, liquids, crystals and vapours rotate the electric vector of linearly polarized light that passes through them. This property is known as optical activity. The rotation is proportional to the thickness of the medium traversed where clockwise rotations are defined as dextrorotatory (positive) and anticlockwise rotations defined as laevorotatory (negative) [2]. The specific rotation of solid is defined as 'the optical rotation in degrees produced by a 1 mm thickness of the solid' and is given by the following equation:

$$[\alpha] = 10^4 \alpha / dc \qquad \textbf{Equation 2.1}$$

Where:

α is the measured rotation (degrees)

d the path of length in solution (mm)

c the concentration (g/100 cm^3)

2.4 Fourier Transform Infrared Spectroscopy (FTIR)

Infrared (IR) Spectroscopy is used as a means to confirm the molecular structure of pure compounds and has particular uses in the differentiation of stereoisomers such as ephedrine and *pseudo*ephedrine [9,10,11]. The basic components of an FTIR instrument include (i) a beam splitter (ii) a stationary mirror and (iii) a moveable mirror. Figure 2.1 represents a schematic diagram of the FTIR.

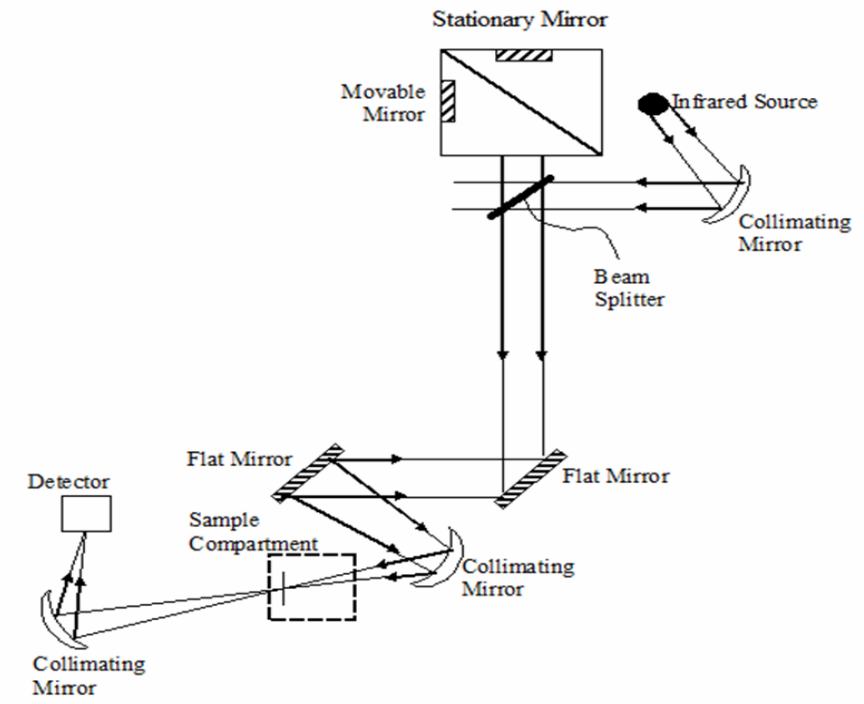


Figure 2.1. Schematic illustration of FTIR spectrophotometer [12].

Half of the infra red light is reflected from the beam splitter to the fixed mirror and back to the detector, while the other half is passed through to a moveable mirror and then back to the beam splitter, where the beam is again split and half of the light from the reflected beam is passed to the detector. Using an infrared source, the moveable mirror is moved so that the two light beams interact constructively. The detector records the sum of the sine waves of the light and hence all the wavelengths of interest are collected. A sample is introduced into a sample compartment where the light beam will absorb some wavelengths but not others. Hence some wavelengths will be detected more strongly than others and an interferogram is produced. These signals are transformed into an infra red (IR) spectrum using a mathematical function called a Fourier Transform [9-11].

A molecule absorbs infrared radiation when the vibration of the atoms in the molecule produces an oscillating electric field with the same frequency as the incident infrared light. This absorption of light causes an energy transition in the form of vibrational

excitation of bonds in the molecule. There are two types of molecular vibrations and these results in the stretching and the bending of bonds. After the light as passed through the sample, the frequencies which have been absorbed are detected and intensities are recorded in the resultant spectrum. Light of wavelength λ will only be absorbed if there is an energy transition, according to the following equation:

$$(E) = hc/\lambda \qquad \textbf{Equation 2.2}$$

Where:

h is Planck's constant (6.6 X 10^{-34} Js),

c is the speed of the light (3.0 X 10^{8} m/s),

λ is the wavelength of light in metres.[9,10,11].

Some typical wavelengths of functional groups are presented in Table 2.1.

Table 2.1. Typical wavelength of functional groups [13].

Organic Compounds	**Wavelength (cm^{-1})**
Carbonyl Compounds	1670-1780
Alkenes (non terminal)	1640-1680
Amines	3300-3500
(C-N)	1030-1250

The 1450-600 cm^{-1} is described as the 'fingerprint region' due to the complexity of the infrared spectra in that region [13]. Absorption bands in the 4000 to 1450 cm^{-1} region are usually due to stretching vibrations of diatomic units and this is sometimes called the group frequency region or functional group region [13].

2.5 Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy facilitates structural characterisation of molecules. The principle behind NMR analysis relies on the fact that nuclei (such as ^{1}H or ^{13}C) with an odd number of protons, neutrons or both, will have an intrinsic nuclear angular momentum or nuclear spin. When a nucleus with a non-zero spin is placed in a magnetic field, the nuclear spin can align in either the same direction or in the opposite direction to the external magnetic field. A nucleus that has its spin aligned with the external field will have a lower energy than when its spin is aligned in the opposite direction to the field [14]. The circulation of electrons about the proton itself which generates a magnetic field opposed to the applied field. The field felt by the proton itself is thus diminished and the proton is said to be shielded. If the induced field reinforces the applied field, then the field felt by the proton is enhanced the proton is said to be deshielded. The resonance frequencies of different nuclei in an atom are described by a relative shift (chemical shift, δ (ppm)) compared to the frequency of a standard which for ^{1}H and ^{13}C NMR spectroscopy the reference compound is tetramethylsilane, $Si(CH_3)$ or TMS [14].

2.6 Microanalysis (Elemental Microanalysis)

The samples to be analysed are held in a tin container and placed inside an autosampler drum [3]. Samples are dropped into a vertical quartz tube maintained in a furnace at high temperature under a continuous flow of helium. In the quartz tube, the helium stream is temporarily enriched with pure oxygen and both the sample and container melt (flash combustion). Quantitative combustion is achieved when the mixture of gases formed is passed over a layer of copper catalyst which removes oxygen and reduces nitrogen oxides to elemental nitrogen. The mixture is passed through a chromatographic column where it is separated into nitrogen (N_2), carbon dioxide (CO_2) and water (H_2O) using a thermal conductivity detector (TCD). A known standard is used to calibrate the

instrument [3, 4]. A general diagram of the instrumental set up is presented in Figure 2.2.

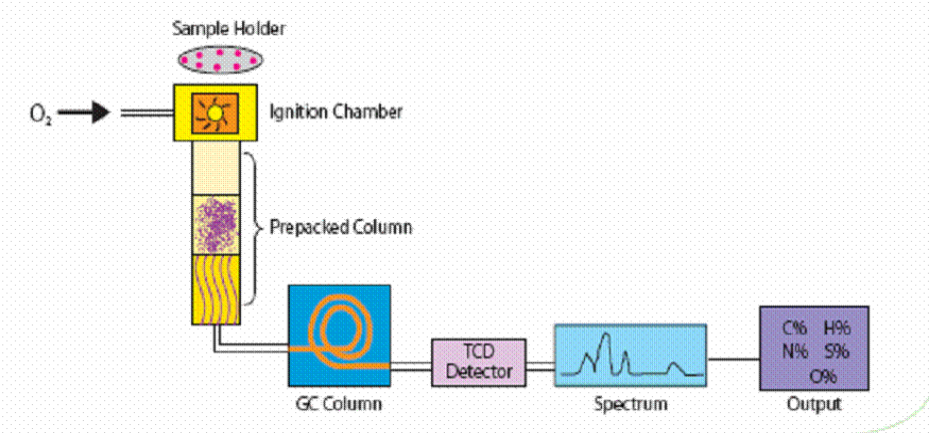
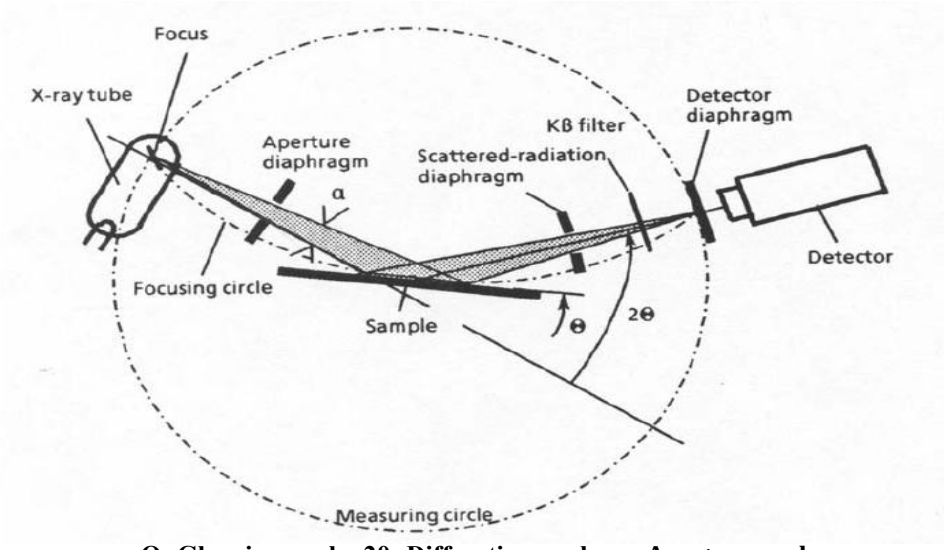


Figure 2.2. Schematic diagram of CHN analyser [5].

2.7 Powdered diffraction technique (XRD)

X-ray powder diffraction (XRD) is commonly used for identification of single phase materials such as minerals, chemical compounds, ceramics or other engineered products. This analytical technique is used extensively in the determination of crystal structures as well as recognition of amorphous materials in partially formed crystalline mixtures [6, 7]. Figure 2.3 illustrates a typical XRD instrument.



Θ: Glancing angle; 2θ: Diffraction angle; α: Aperture angle

Figure 2.3. Schematic diagram of XRD [8].

A coherent beam of monochromatic x-rays of a known wavelength are produced when an anode of a particular metal is struck with a beam of high energy electrons in a sealed vacuum tube. The X-ray generated is influenced by the choice of metal anodes and energy of the accelerated electrons. Usually copper (Cu), chromium (Cr), iron (Fe), cobalt (Co) or molybdenum (Mo) tubes are used. Radiation is produced as $K\alpha_1$, $K\alpha_2$ and K_β radiation. Kα is commonly used for X-ray diffraction analysis and other X-rays are removed by a filter or a monochromator [6, 7].

The X-ray is collimated and directed to the powdered sample. To create the parallel beams most diffractometers have a series of parallel plates that are arranged perpendicular to the diffractometer circle that are used to beam the X-rays to the samples. Filters are located at the generator or detector side of the diffractometer to remove the $K\alpha_2$ and K_β radiation [6, 7]. Recent instruments have optical systems in place that produce tightly controlled and focused incident beams of X-ray [6, 7].

The intensity of the deflected (diffracted) X-ray is recorded and sent to a microprocessor which converts the signal to a count rate. The distances between the planes of the atom are calculated using Bragg's Law [6, 7].

$$n\,\lambda = 2d\,sin\theta \qquad \textbf{Equation 2.3}$$

Where:

Integer *n,* is the order of the diffracted beam

λ is the wavelength of X-ray beam in nm

d is the distance between the adjacent planes of atoms (d-spacings)

θ is the angle of incidence of the X-ray beam in degrees

A typical X-ray scan provides a unique 'fingerprint' of the mineral or crystal lattice of the target and the interpretation process involves comparison to known standards or reference compounds [6, 7].

2.8 Chromatography

The International Union of Pure and Applied Chemistry (IUPAC) definition of chromatography is [15-17]:

"a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves in a definite direction. Elution chromatography is a procedure in which the mobile phase is continually passed through or along the chromatographic bed and the sample is fed into the system in a definite slug."

In chromatography, the separation of components in a mixture occurs as a result of their relative interaction with a mobile phase and a stationary phase. The components can be separated as a result of differences in molecular charge, size or mass, polarity, redox potential, ionization constants or structural differences such as isomeric structures or chirality [15]. The sample is generally dissolved or distributed within the mobile phase

(gas, liquid or supercritical liquid) and the mobile phase is forced through a stationary phase. The distribution of the sample between the mobile and stationary phases determines the rate at which it will travel through the stationary phase [15].

Migration rates of individual molecules differ due to partition differences with the mobile and stationary phases. The various components are hindered by interaction (sorption) with the stationary phase. The sorption-desorption process occurs many times as the analyte flows through the chromatographic system and the time required to reach the detector (retention time) is dependent on the retention of the individual analytes by the stationary phase. This type of chromatographic separation is called zonal or batch chromatography because the sample is applied to the chromatographic system in one narrow zone or band.

Besides adsorption, other factors that influence chromatographic separations include properties of the analytes that can influence the intermolecular forces which it can experience between the stationary and mobile phases. Some of these properties include, ionization potential, electron affinity, electronegativity, molar volume, ionic radius, ionic potential, dipole moment, dielectric constant, polarity, boiling point and solubility [15, 16].

2.8.1 Introduction to Gas Chromatography

2.8.1.1 Distribution of analytes between phases

An analyte is in equilibrium between two phases according to its equilibrium constant (partition constant), K_c presented in Equation 2.4 $\boldsymbol{Kc} = \frac{[\boldsymbol{A}]\boldsymbol{s}}{[\boldsymbol{A}]\boldsymbol{m}}$[15, 16]:

$$\boldsymbol{Kc} = \frac{[\boldsymbol{A}]\boldsymbol{s}}{[\boldsymbol{A}]\boldsymbol{m}} \qquad \textbf{Equation 2.4}$$

Where:

[A]s is the equilibrium concentration of analyte A in the stationary phase.

[A]m is the equilibrium concentration of analyte A in the mobile phase.

The equilibrium constant equation can also be used to express the relationship between retention, column diameter and stationary phase as shown in Equation 2.5 [16]:

$$Kc = k\,\beta = k[r/2df] \qquad \textbf{Equation 2.5}$$

Where:

k = retention factor

r = column radius (µm)

β = phase ratio

d_f = column film thickness (µm).

The phase ratio, β, is a unitless value that depends on the diameter and thickness of the column and reflects the impact of the column diameter and film thickness on retention.

The phase ratio is described in Equation 2.6 [15]:

$$\beta = r/2df \qquad \textbf{Equation 2.6}$$

The retention factor (*k*), also referred to as the partition ratio or capacity factor, can be calculated using Equation 2.7:

$$k = \frac{t_1 - t_m}{t_m} \qquad \textbf{Equation 2.7}$$

Where:

t_r = retention time (seconds).

t_m= retention time of a non-retained compound (peak) (seconds).

2.8.1.2 Theoretical Plate Model of Chromatography

The theoretical plate model of chromatography suggests that the chromatographic column consists of a large number of separate layers called 'theoretical plates' as shown in Figure 2.4.

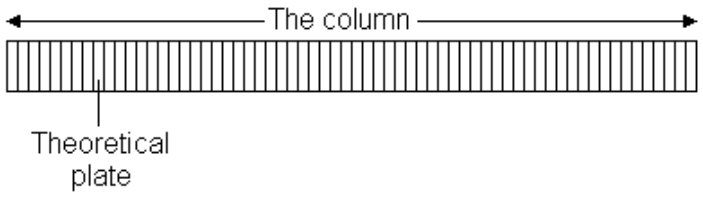


Figure 2.4. Theoretical plate model of chromatography

Equilibrium occurs separately at each individual plate between the stationary and mobile phase. This model can be used in the measurement of column efficiency by calculating the Height Equivalent to a Theoretical Plate (HETP), where N and H are inverse to each other and the number of theoretical plates in the column (N) using Equation 2.8 and Equation 2.9 respectively [16, 17].

$$H = L/N \qquad \textbf{Equation 2.8}$$

Where:

L is the total length of the column (cm or mm).

H is the length of a column that contains one plate.

N is the number of theoretical plates in the column.

$$N = \frac{5.55 t_R^2}{w_{1/2}{}^2} \qquad \textbf{Equation 2.9}$$

Where:

$w_{½}$ is peak width at half height (cm or mm).

t_R is the retention time (seconds).

The plate number provides a measure of the relative peak broadening ($w_{½}$) which has occurred when the analyte passes through the system in time t_R.

The plate theory explaining chromatographic separation is useful for providing some information in relation to column efficiency but has been largely replaced by rate theory concepts. This describes band broadening of the chromatographic peak using the Van Deemter plot, represented in Figure 2.5 and the Van Deemter equation [15-17].

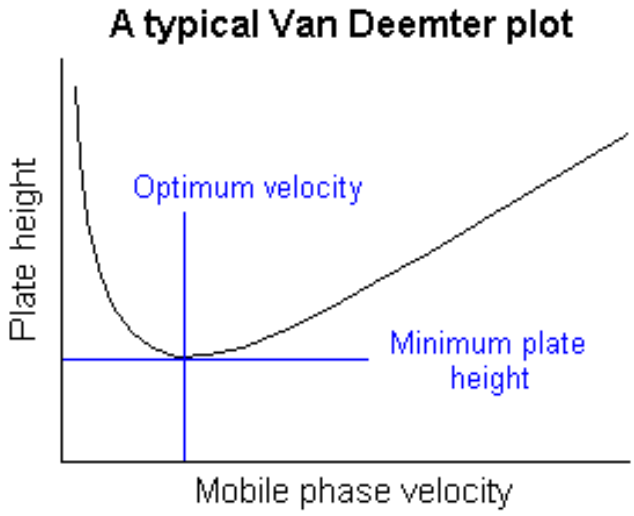


Figure 2.5. Van Deemter plot [15]

A Van Deemter plot is a representation of the plate height with respect to the average linear velocity of the mobile phase. The Van Deemter plot is used to determine the optimum mobile phase flow rate. The minimum point of the curve represents the optimum velocity that provides the highest efficiency and smallest plate height. The Van Deemter plot can be manipulated to obtain the best performance in a given analysis time [15-17].

Van Deemter and co workers identified three factors that effected band broadening in packed gas chromatography columns [15] which were expressed in the Van Deemter equation:

$$H = A + B/\mu + Cu \qquad \textbf{Equation 2.10}$$

Where:

A relates to eddy diffusion (metres, m).

B relates to longitudinal molecular diffusion (m^2s^{-1}).

C relates to the mass transfer in stationary liquid phase (seconds, sec).

H is the height of a theoretical plate (metres, m).

u is average gas velocity (cm/s).

Eddy Diffusion

The A term in the Van Deemter equation is given in Equation 2.11 [15, 16]:

$$A = 2\lambda d_p \qquad \textbf{Equation 2.11}$$

Where:

d_p , represents the diameter of the particles packed in the column (μm)

λ is the packing factor.

To minimize A, small tightly packed particles are used to form the matrix of the stationary phase to achieve maximum column efficiency.

Molecular Diffusion

The B term in the Van Deemter equation is given by Equation 2.12 [15, 16]:

$$B = 2\psi D_M \qquad \textbf{Equation 2.12}$$

Where:

D_M is the diffusion coefficient for the solute in the mobile phase.

ψ is the obstruction factor that allows for the nature of packed beds.

A low diffusion coefficient is desirable and is achieved in gas chromatography by using carrier gasses which have larger masses such as nitrogen and argon. Larger mobile phase velocities will minimize the B value by ensuring that the solute resides for shorter times in the column [15].

Mass Transfer

The C term in the Van Deemter equation is given in Equation 2.13 [15,16]:

$$C = \frac{8}{\pi^2}\frac{kd_f^2}{(1+k)^2 D_s}$$ **Equation 2.13**

Where:

d_f^2 is average film thickness of the stationary phase in µm.

Ds is the diffusion coefficient of the solute in the stationary phase.

k is the capacity factor

In order to minimize mass transfer resistance, the film thickness of the column needs to be small and the diffusion coefficient large [15, 16].

2.8.2 Chromatographic Separation in gas chromatography

Gas chromatographic separation relies on samples being volatile, thermally stable and that the separation of mixtures of analytes is affected by their relative size, shape and functionality which dictate their interaction with the chemical coating of the chromatographic column [15-17].

In the chromatographic column, the analyte molecules are repetitively distributed between the mobile phase (carrier gas) and the stationary phase, where molecules with a greater affinity for the stationary phase are retarded. In general small molecules travel through the column more quickly than larger molecules. The overall rate of movement of the molecule down the column depends on the distribution of the molecules in stationary and mobile phases respectively. The number of molecules in the mobile phase and the rate at which they travel through the column are reflected by the size of the

corresponding peak in the chromatogram and the retention time of the analyte respectively. Separation between two molecules occurs when their distribution between the mobile and stationary phases are different. If they are similar, co-elution occurs. The length, width, temperature of the column and thickness of the stationary phase also play an important role in the chromatographic separation [15-17]. Identification of chromatographic peaks is accomplished by a comparison with known standards.

2.8.3 The Basic Parts of a Gas Chromatograph

A gas chromatography instrument, presented in Figure 2.6, is comprised of six major components: the gas supply and flow controllers, injector, detector, oven, column and a data system [19].

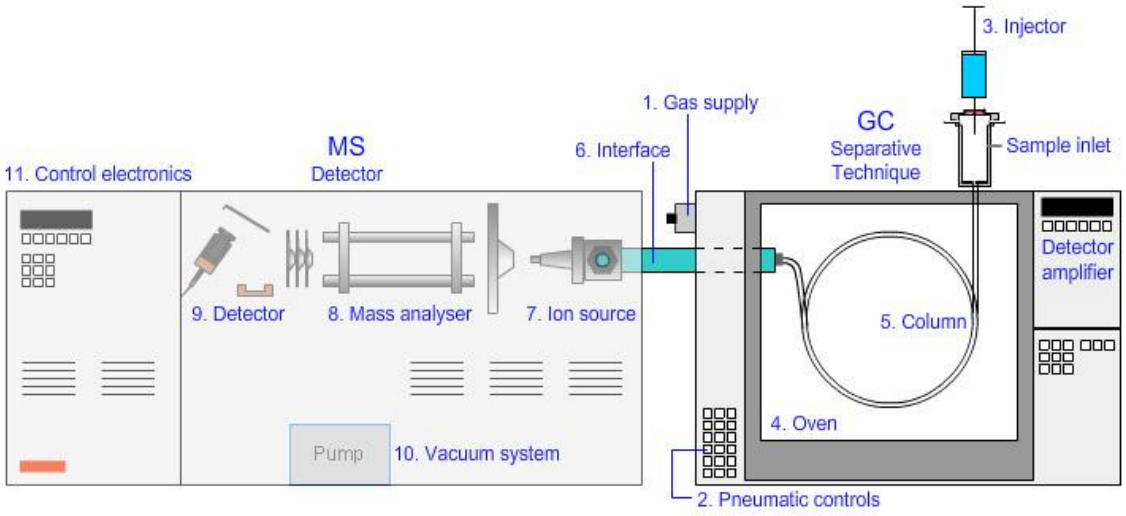


Figure 2.6. Diagram of gas chromatography mass spectrometry (GCMS) [18].

Gas Supply and Flow Controllers: High purity gases are regulated by pressure regulators which control the gas flow into the instrument. Further pressure regulators deliver the carrier gas to the injector at precisely controlled rates. The carrier gas flows through the injector to the column and exits through the detector [19, 20]. The main purpose of carrier gas is to carry the sample through the column [19, 20]. It acts as an inter mobile phase and provides a suitable matrix for the detector to measure sample

components [19, 20]. Helium is the most popular carried gas used particularly with GCMS instruments although it can be expensive. Hydrogen is also commonly used, though poses greater fire and explosion hazard and nitrogen provides slightly more sensitivity but a slower analysis time than helium [19, 20].

Injector: The function of the injector is to introduce the sample into the column. It is made from a hollow, metal cylinder normally containing a glass liner or insert. The column is inserted into the bottom of the injector so that the column end resides in the lower region of the glass liner. The usual temperature of the injector is between 100-300^{o}C, to ensure that any volatile sample is vaporised. The carrier gas is mixed with the vaporised sample and swept into the column [19, 20].

Capillary Column and Oven: The column is housed in an oven whose temperature can be accurately controlled. The interior wall of the column is coated with a thin film of polymeric material which forms the stationary phase. Interactions occur between the analytes and the stationary phase to impede the movement of different analytes to different degrees. Compound retention is affected by various factors such as, length and diameter of the column, the chemical structure and thickness of stationary phase and column temperature [19, 20].

Detector: When an analyte exits the column it enters the detector which records the analyte based on its physical and/or chemical properties. The detector response corresponds to the amount of the analyte present [19, 20].

Data System: The recording device, normally a computerized system, plots the detector signal against the time elapsed since the sample introduction into the injector to produce the chromatogram. Computer operated data systems are very versatile and offer various plotting, reporting and storage options [19, 20].

2.8.4 Mass spectrometry as a detection system in chromatographic analysis

The mass spectrometer is kept under vacuum of between 10^{-4} to 10^{-6} torr using either a diffusion or turbomolecular pump. The mass spectrometer consists of three zones, (i) Ionizer (ii) Mass Analyzer (iii) Detector. Ion production in the spectrometer occurs using one of two methods, chemical ionization (CI) or electron ionization (EI). In the case of chemical ionization, methane is ionized creating a radical which in turn ionizes the sample molecule to produce $(M + H)^+$ molecular ions. The CI process is a less energetic way of ionizing a molecule and less fragmentation occurs compared electron ionization [19, 20].

For electron ionization, a beam of electrons ionize the sample molecules emerging from the chromatographic column resulting in the loss of one electron. Usually the ionization source is heated to 150-300^{o}C, to prevent condensation of the sample. The electrons are supplied by a filament and enter through a small hole directed by a negatively charged shield behind the filament. The electrons are focused into a narrow beam by magnets. The energized electrons enter the source and collide with the molecules exiting from the column. This creates a molecular ion, represented by M^+ (a radical cation). From the collision, energy is released from the molecules and accumulates in a single bond or group of bonds which dissociate to produce smaller positive ionic fragments (daughter) ions with characteristic relative abundance that provide a 'fingerprint' for that molecular structure [19, 20].

2.8.5 Quadrupole mass analyzer

The mass analyzer separates the positively charged ions according to various mass related properties depending upon the analyser used. Several types of analyser exist: quadrupoles, ion traps, magnetic sector, time of flight, radio frequency and cyclotron resonance. [19, 20] A simplified schematic diagram of a quadrupole mass spectrometer is shown in Figure 2.7.

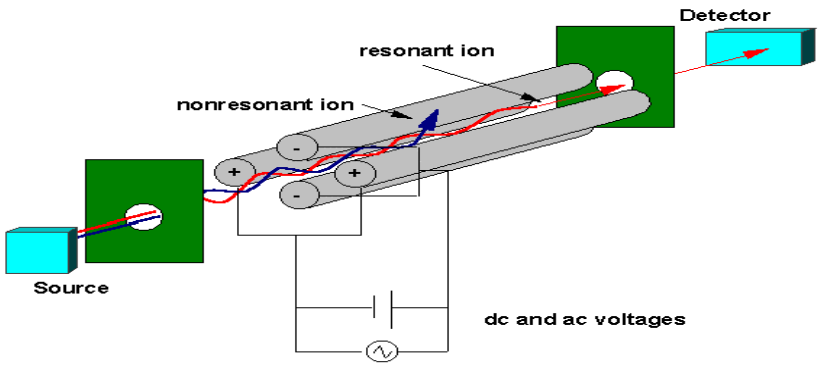


Figure 2.7. A Schematic diagram quadrupole mass spectrometer [21].

Positive ions are ejected from the source by a repeller held at a slightly positive potential. The ions produced move through a series of focusing plates which accelerate and focus the ions into a narrow beam before they pass into the quadrupole analyzer. A negatively charged detector at the other end of the quadrupole array attracts the ions. The quadrupole consists of four accurately machined rods set in a square array. In the array a complex field is generated by the electrical potentials supplied to the rods. The field is maintained at 2-4 scans per second, allowing the quadrupole to act as a mass filter. The ion fragments oscillate through the quadrupole array and ions with specific mass-to-charge (m/z) ratio pass completely through to the detector without colliding with any one of the four rods [19, 20]. The signal resulting from the impact of ions on the detector is amplified by an electron multiplier in the detector. The intensity or abundance of the ions detected are plotted against time to produce a total ion chromatogram (TIC) [19, 20].

2.9 Isotope Ratio Mass Spectrometry (IRMS)

Isotope ratio mass spectrometry provides an 'isotope fingerprint' of a chemical molecule which may facilitate the identification of a sample. Stable isotope ratio mass spectrometry (IRMS) has been used for the analysis of both licit and illicit drugs for the purpose of determining geographic origin and to determine the potential for discrimination between batches and manufacturing routes. The results obtained have been promising for the technique, however the data remains limited due to the difficulty in acquiring a sufficient number of samples of know origin and history, though continued research is addressing this issue [21-23].

2.9.1 Isotope ratios

Isotopes are defined as atoms of the one element that differ in the number of neutrons present in the nuclei, i.e. have different mass numbers. All but 12 elements exist as mixtures of isotopes [24, 25]. For example, hydrogen usually has one proton in its nucleus (written as ^{1}H), but an isotopic form exists in which the nucleus contains one proton and one neutron (^{2}H, deuterium). Each element has a dominant light isotope, for example ^{12}C (carbon), ^{14}N (nitrogen), ^{16}O (oxygen), ^{32}S (sulphur) and ^{1}H (hydrogen), and one or more heavy isotopes (e.g. ^{13}C, ^{15}N, ^{17}O, ^{18}O, ^{33}S, ^{34}S and ^{2}H) [25, 26]. Table 2.2 below illustrates the relative abundances of common naturally occurring isotopes [25, 26].

Table 2.2. Relative abundances of naturally occurring isotope elements analysed by the isotope ratio mass spectrometer [26].

Element	Isotope	Relative Abundance %
Hydrogen	^{1}H ^{2}H	99.984 0.0156
Carbon	^{12}C ^{13}C	98.892 1.108
Nitrogen	^{14}N ^{15}N	99.635 0.365
Oxygen	^{16}O ^{17}O ^{18}O	99.759 0.037 0.204
Sulfur	^{32}S ^{33}S ^{34}S ^{36}S	95.02 0.76 4.22 0.014

2.9.2 Delta notation

Data derived from IRMS analysis are generally quoted as delta values, δ which are calculated using the following formula [22-26]:

$$\delta 13C = 1000\left[\frac{R_{samp}}{R_{std}} - 1\right] \qquad \textbf{Equation 2.14}$$

Where:

R_{samp} is the ratio of the number of atoms of the heavy isotope to the number of atoms of the light isotope.

R_{std} is the equivalent ratio corresponding to a standard.

Because differences in isotope abundance ratios between the sample and standard are typically only 0.001-0.05%, δ values include a multiplication by 1000 for ease of discussion and therefore quoted 'per mill' ($^{0}/_{00}$) [27].

The isotope ratio is a relative measurement usually made against laboratory reference material. These working standards are calibrated against international standards arbitrarily set to $0^0/_{00}$. The International Atomic Energy Agency and the National Institute of Standards and Technology (NIST) both supply a range of standards [39]. A negative value delta indicates that the sample is light or depleted in the heavy isotope relative to the standard. A positive value indicates that the sample is enriched in the heavy isotope relative to the standard [22-27].

In natural abundance isotope analysis, the emphasis is on the relative difference between samples analysed under the same conditions, as opposed to exact values obtained. One of the main advantages of this relative measurement approach is higher precision [22-24].

2.9.3 Fractionation Effects

Isotope fractionation is a process that changes the relative abundance of stable isotopes of an element. The atmosphere and all living things, have various elements of stable isotopes which occur naturally. Lighter elements are more prone to natural variation of their isotopic composition and this variation is caused by fractionation effects. This results in the creation of specific isotope values that are characteristic of the origin and purity of the sample. In general, only the lighter element seems to be effected by isotope fractionation. Increased precision of the modern isotope ratio mass spectrometer has enabled researchers to identify natural variation as a result of isotopic fractionation in heavier elements also [22-24,].

Isotopic fractionation arises from chemical, physical and biological processes and occurs via two main mechanisms; (a) a kinetic isotope effect which is produced by differences in reaction rates and (b) a thermodynamic effect which relate to the energy state of the system [22-27].

Kinetic Isotope Effects

Kinetic isotope effects occur during reaction processes where different isotopes have different reaction rates in a bond due to differences in their bond strength. This effect represents changes in chemical bonding between the ground state and transition state of the reaction where, according to statistical models, the lighter isotopes in an element form a weaker bond. Primary isotopic effects occur when a bond which contains the atom or isotope is broken or formed during the rate determining step of the reaction. A secondary isotope effect occurs in reactions where the isotopic atom is situated next to the reactive bond [22-24].

Kinetic isotope effects can be defined as a ratio of rate of constants of compounds which contains light versus heavy isotopes at reactive sites, illustrated in [22-27]:

$$^{light}k/^{heavy}k \qquad \textbf{Equation 2.15}$$

If the ratio is greater than one, the isotope effect is referred to as normal, and occurs when light isotopes react faster and as a consequence the substrate becomes enriched by heavy isotopes. If the ratio is less than one, the isotope effect is inverted and the substrate becomes enriched by the light isotope. Fractionations that arise from chemical reactions such as evaporation and condensation produce substrates that are isotopically lighter (contains less heavy isotopes) than their starting material. These can be seen in lighter elements such as hydrogen and deuterium because their isotopes show larger differences in mass compared to heavier elements such as carbon or nitrogen [27, 28].

The selective incorporation of ^{12}C into organic matter during photosynthesis is another vital kinetic isotope effect. The amount of metabolic carbon available in the plants depends on numerous factors such as carbon fixation and the rate of diffusion of carbon dioxide in the plant. The composition of ambient carbon dioxide will also vary according to the temperature and photosynthetic process taking place (whether over land or ocean) [22-27].

Most plants consume carbon dioxide via a C_3 (Calvin), C_4 (Hatch-Slack) or CAM (Crassulacean Acid Metabolism) photosynthetic cycle. It is reported that 85% of plant species follow the C_3 pathway including wheat, rye and cotton whereas plants such as sugar cane, tropical grasses and desert plants follow the C_4 pathway [22-27]. The photosynthetic pathway is also dependent on the isotopic composition of the plant which is affected by environmental conditions including humidity, temperature, and the isotopic composition of the soil [22-24, 27].

Thermodynamic Isotope Effects

The second most common isotopic fractionation is the thermodynamic isotope effect due to free energy changes brought about when one atom in a compound is replaced by its isotope. A heavier isotope has a smaller reserve of free energy compared to the same molecule containing a lighter isotope. Differences in physical-chemical properties (such as infrared absorption, molar volume, vapour pressure, boiling point and melting point, all related to vibrational energy levels) are generally associated with thermodynamic isotopic effects [22, 23, 27].

2.9.4 Isotope ratio Mass Spectrometer

Isotope ratio mass spectrometers (IRMS) are specialised mass spectrometers that produce precise and accurate measurements of the variations in the natural isotopic abundance of light stable isotopes. IRMS instruments are different from conventional mass spectrometers, in that they do not scan a mass range of characteristic fragment ions in order to provide structural information on the sample being analysed [25, 26, 27]. The mass spectrometers used for isotopic analysis, illustrated in Figure 2.8, are divided into three main sections; (i) an ion source (IS), (ii) a mass analyser (EM) and (iii) an ion collection assembly (FCC) [27].

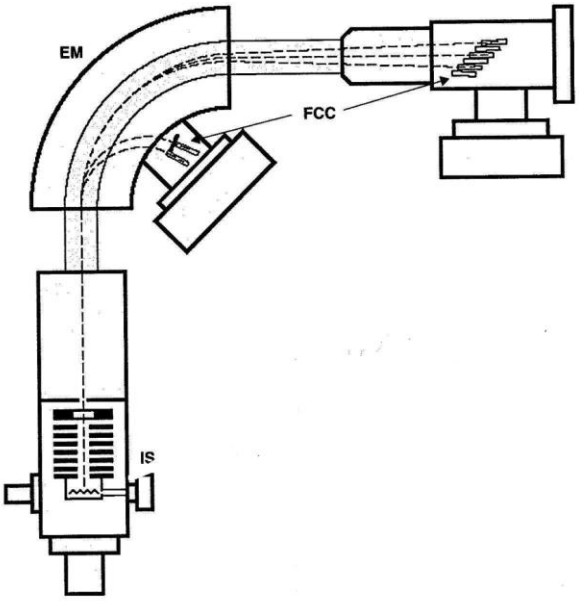


Figure 2.8. General layout of an Isotope Ratio Mass spectrometer [27].

Gaseous samples for analysis enter the ionization chamber of the mass spectrometer. A focused electron beam within a high vacuum environment interacts with the sample and results in the loss of electrons from molecules, producing positive ions [22, 27]. These ions are accelerated through a flight tube between the poles of an electromagnet, where they are separated according to their mass to charge ratios (m/z). The ions are collected by a collector array generally consisting of three (or sometimes up to eight) Faraday cup (FC) collectors [27]. The Faraday cups are positioned so that the major ion currents simultaneously strike at the middle of the entrance of the silt of the respective cups. Each incoming ion contributes one charge. No stray ions or electrons can enter the cup and no secondary particles formed from the impact with the inner walls of the cups exit the cup. The ion currents are continuously monitored and then amplified, digitised using a voltage to frequency converter (VFC) [27].

Sample size needed for routine, high precision isotope ratio analyses of an illicit drug is 1-2 mg. The sample is placed in a tin and combusted in an elemental analyzer coupled to the mass spectrometer. Oxidizing and reducing columns ensure that all carbon and nitrogen are converted to CO_2 and N_2 respectively. A gas chromatograph is positioned

following the elemental analyzer and before the mass spectrometer to separate CO_2 and N_2 gas. The carbon and nitrogen isotope ratios of a sample can be determined on a single analysis. Additionally, the C and N peaks are integrated as the gases pass through the GC, so that precise C/N ratio can be determined facilitating the calculation of sample purity [27]. The analysis of the isotopes must be performed on a simple gas which is isotopically representative of the sample. For instance, the isotope ratio measurement of $^{2}H/^{1}H$ is achieved on H_2 gas, $^{13}C/^{12}C$ on CO_2, $^{15}N/^{14}N$ on N_2 and $^{18}O/^{16}O$ on CO [27].

The continuous flow (CF) IRMS introduces the sample into the ion source of the IRMS via a helium carrier gas. This system is connected to a range of automated sample preparation devices such as (a) bulk stable isotope analysis (BSIA) and (b) compound specific isotope analysis (CSIA). CF-IRMS is commonly used in forensic science research due on-line sample preparation, smaller sample size needed, faster analysis time, increased cost effectiveness and the possibility of interfacing with GC and LC systems.

Bulk Stable Isotope Analysis (BSIA)

In bulk stable isotope analysis (BSIA) all of the sample interest is converted into simple gases for IRMS analysis producing values reflecting the isotopic composition of all the components in the sample. The setup of the instrument consists of an elemental analyser coupled to the isotope ratio mass spectrometer (EA/IRMS) [27, 28]. Sample preparation for BSIA involves weighing the samples into capsules, usually tin then loading these onto an autosampler. Samples are then purged with helium to prevent the introduction of water, oxygen and nitrogen. There are two instruments presented in Figure 2.9 and Figure 2.10, which are involved in the preparation of samples for bulk stable isotope analysis, (a) quantitative high temperature combustion and (b) quantitative high temperature conversion. The quantitative high temperature combustion for bulk samples is generally used for the analysis nitrogen and carbon [27].

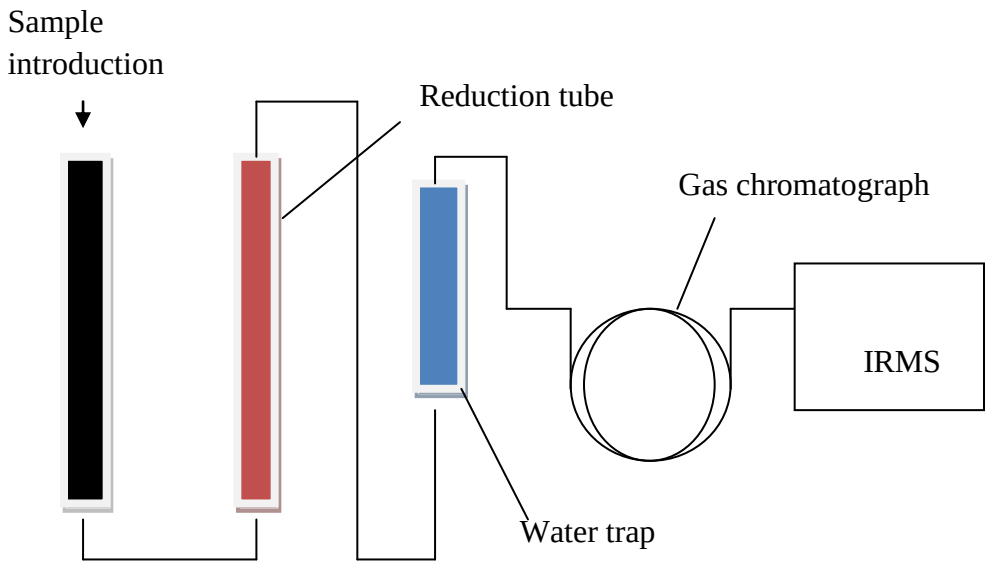


Figure 2.9. General layout of an EA-IRMS system for the measurement of carbon and nitrogen bulk stable isotope ratios. Diagram based on Benson *et al.* [28].

High temperature conversion elemental analysers (TC/EA) are used for the analysis of hydrogen and oxygen isotope ratios of bulk samples. The samples are converted to H_2 and CO gases, separated on a packed GC column and enter the IRMS via an open split interface. Hydrogen and oxygen isotope ratios are measured from a single analysis [27, 28].

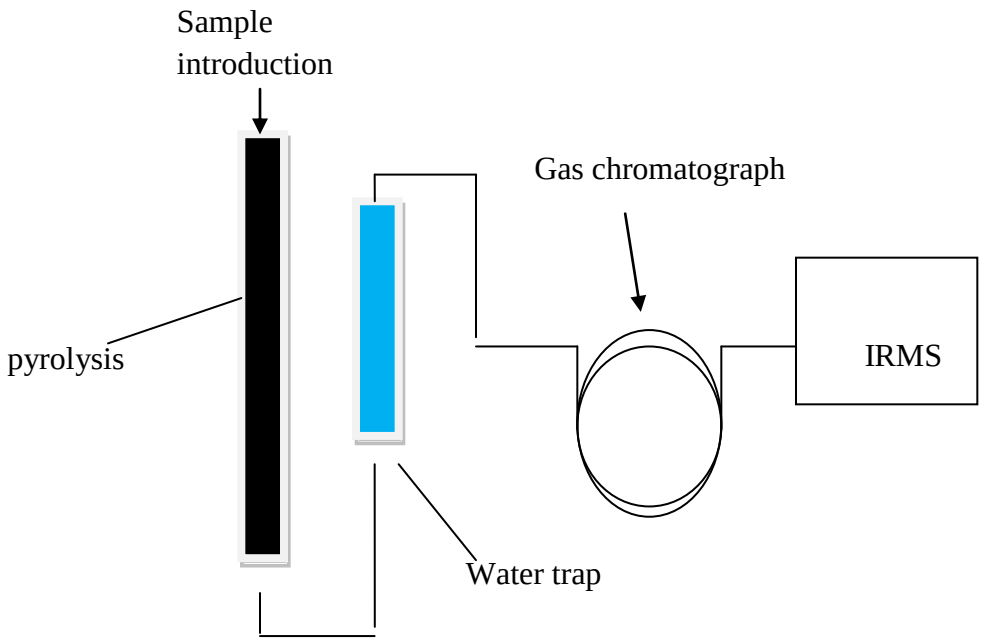


Figure 2.10. General layout of an TC/EA-IRMS system for the measurement of hydrogen and oxygen bulk stable isotope ratios. Diagram based on Benson *et al*. [28].

Compound Specific Isotope Analysis (CSIA)

A capillary gas chromatographic column is used to achieve baseline separation of complex mixtures of analytes prior to isotopic analysis. A splitter at the end of the gas chromatographic column diverts most of the sample to a combustion or pyrolysis tube. The remainder is sent to an optional flame ionization detector (FID), ion trap mass spectrometer or is vented to the atmosphere [27, 28]. Diagrams of the typical instrumental set up are presented in Figure 2.11 and Figure 2.12 [27, 28]. In order to determine nitrogen and carbon isotope ratios, the eluting compounds are passed into a combustion tube which contains an oxidation catalyst and other materials. The function of the tube is to quantify carbon dioxide (CO_2), water (H_2O) and nitrogen. After the combustion tube the products pass to a reduction tube to reduce NO_x to nitrogen (N_2) and remove excess oxygen (O_2). The sample finally passes through a trap to remove water (H_2O) and onwards to the IRMS [27, 28]. When nitrogen isotope ratios are

measured, the carbon dioxide formed is removed by cryogenic trapping as the ions formed will interfere with the nitrogen isotope measurements. Hydrogen and oxygen isotope ratios are quantitatively measures by converting the elements in the samples to hydrogen gas or carbon monoxide [27, 28].

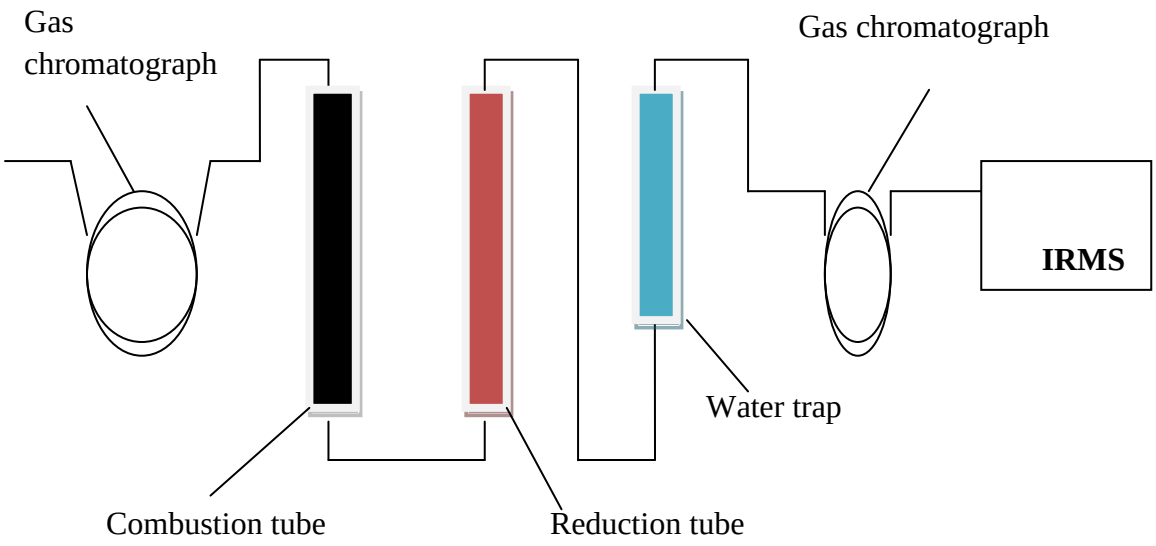


Figure 2.11. General GC-C/IRMS layout for the measurements of carbon and nitrogen isotope ratios by CSIA. Diagram based Benson *et al.* [28].

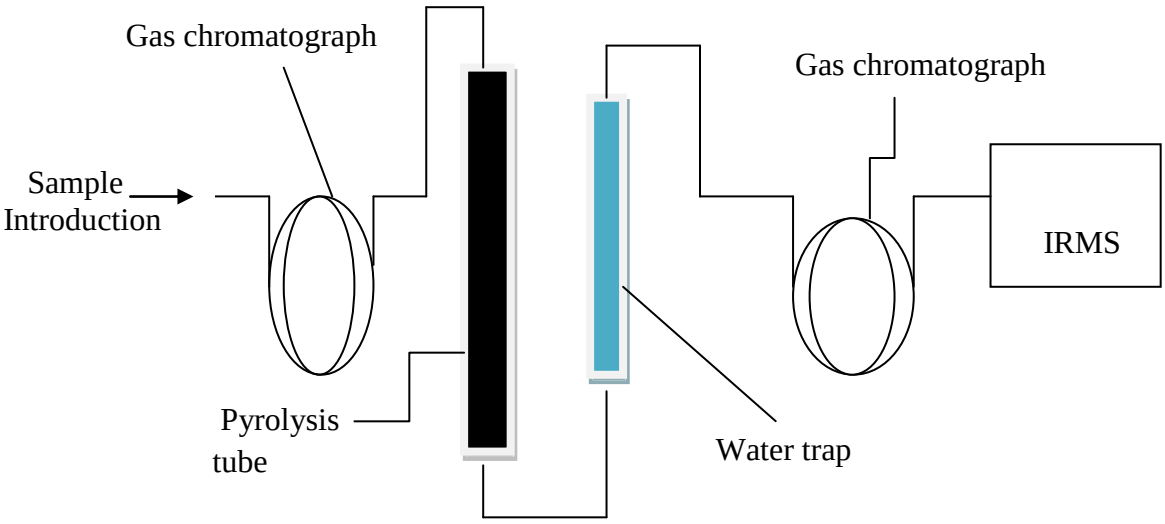


Figure 2.12. General GC-C/IRMS layout for the measurements of hydrogen and oxygen isotope ratios by CSIA. Diagram based on Benson *et al.* [28].

2.10 Inductively Coupled Plasma Mass Spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) is a combination of two analytical techniques, (a) inductively coupled plasma and (b) mass spectrometry [29]. A typical instrumental set up is illustrated in Figure 2.13.

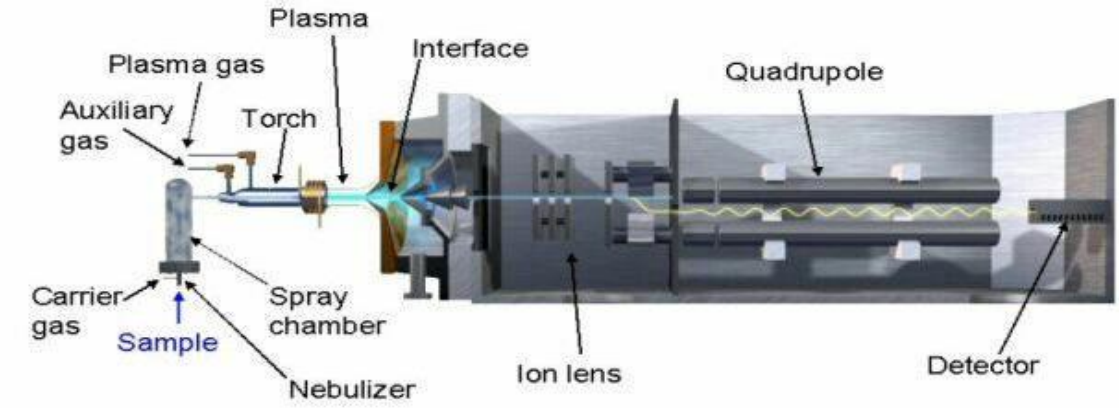


Figure 2.13. Schematic diagram of the inductively coupled plasma mass spectrometry [31].

General sample preparation for the ICP-MS analysis requires 1 or 2 mL of sample to be diluted with nitric acid (generally 2% w/v) [29, 30]. The minimum sample volume required for analysis is 5 mL with the total dissolved solids less than 0.1 wt % and no suspended solids. A peristaltic pump and a nebuliser are used to create a fine aerosol of sample within a spray chamber which is then transported into the sample injector of the plasma torch using a flow of argon gas. The aerosol is directed into the inductively coupled plasma (ICP) ion source. Plasma formation occurs as a result of the application of a high voltage spark to the flow of argon gas stripping electrons from the argon atoms [29, 30]. The electrons generated from this process are accelerated into a magnetic field formed by the application of a radio frequency (RF) energy applied to a coil surrounding the plasma torch. A chain reaction occurs between the newly formed electrons causing an ICP discharge due to collision induced ionization. The temperatures in the ICP reach between 6000 to 8000K [29, 30].

An interface consisting of an assembly of two cones (skimmer and sampler cone) is needed to maintain a series of pressured differentials to facilitate an efficient sampling of the plasma gases. This is because, the ions travelling in the argon stream which are at atmospheric pressure (1-2 torr) need to transition into the low pressure region of the mass spectrometer ($< 1 \text{ X } 10^{-5}$ torr). The sampler and skimmer cones are basically metal disks with a small hole in the centre. These cones sample the center portion of the ion beam coming from the plasma torch. The ions emerging from the ICP source via the cones are focused into the mass spectrometer (MS) by a series of positively charged electrostatic lenses or ion optics. The ions are then separated according to their mass to charge (*m/z*) ratios [29, 30].

2.11 Conclusion

Structural determination of the extracted and synthesised compounds was determined using nuclear magnetic resonance (NMR) and fourier transform infrared (FTIR). Determination of the identity and purity of the compounds were facilitated using melting point and optical rotation analysis. Microanalysis and XRD analysis was undertaken to determine the purity of the extracted and synthesised compounds.

Organic impurity profiling of the synthesised methylamphetamine was undertaken using gas chromatography mass spectrometry. The determination of inorganic profiling or elemental profile for the batches precursors extracted from proprietary cold medication, essential chemicals such as iodine and red phosphorous extracted from matchboxes and tinctures and methylamphetamine synthesised was performed using inductively coupled mass spectrometry (ICPMS). The investigation of isotopic variation of methylamphetamine and the precursors used in the synthetic phase were analysed using isotope ratio mass spectrometry (IRMS).

2.12 References

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Chapter 3 Clandestine Synthesis of Methylamphetamine

3.1 Introduction

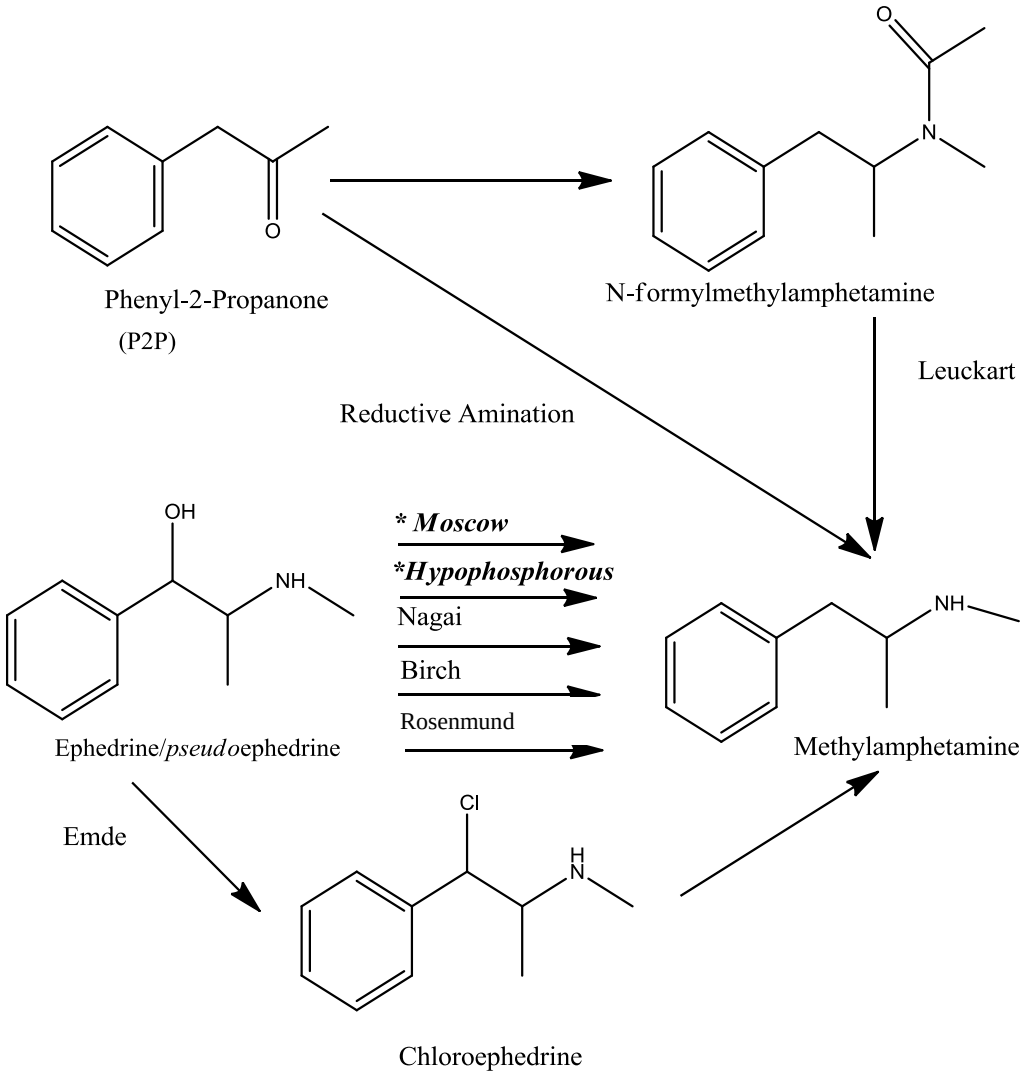
Illicit drugs manufactured from clandestine laboratories are often impure due to poor laboratory conditions, variations in synthesis and impure starting materials (precursors and essential chemicals) extracted either from common household products or pharmaceutical grade chemicals [1, 2, 3]. As a consequence, chemical impurity profiles can be derived from illicit drugs synthesized within these clandestine laboratories. Chemical analysis, in particular, using gas chromatography mass spectrometry (GCMS) can provide useful information on the materials, methods and synthetic route involved in manufacture of the drugs [1, 2, 3].

Information relating to the clandestine manufacture of methylamphetamine and amphetamine is widely available on the internet and various websites have been dedicated to the clandestine manufacture of these compounds [1, 2, 3]. One well known text widely available ('The Secrets of Methamphetamine Manufacture' by Uncle Fester) is designed for novices with little or no knowledge in organic chemistry [4]. The book gives details on using common household products to make methylamphetamine [4].

Many precursors and essential chemicals used in the clandestine manufacture of methylamphetamine can be purchased in pharmacies and local hardware stores. For example *pseudo*ephedrine hydrochloride, a known precursor for methylamphetamine can be easily extracted (using methylated spirits or other commonly available solvents) from cold medication widely available as over the counter pharmaceuticals [4]. Essential chemicals such as iodine and red phosphorous are also readily available and can be extracted from iodine tinctures and matchboxes respectively [4].

3.2 Synthetic Routes

Most clandestine laboratories produce methylamphetamine hydrochloride using one or other of eight synthetic pathways presented in Scheme 3.1.



Routes labelled* in bold are the routes investigated in this research

Scheme 3.1. Pathways of methylamphetamine manufacture.

The synthetic routes can be simply separated into two groups based on the precursor chemical. The Leuckart and reductive amination synthesis both use phenyl-2-propanone (P2P) and are popular in North American clandestine laboratories. All the other routes (Birch, Nagai, Rosenmund, Emde, Moscow and Hypophosphorous) use ephedrine or *pseudo*ephedrine base or hydrochloride as the starting material and are favoured elsewhere in the world mainly because of the precursor availability. In general the synthetic routes are named after the individual who first reported the reactions [5-11]. Methylamphetamine synthesized from phenyl-2-propanone yields a racemic mixture, regardless of the synthetic route. Manufacture via *d-pseudo*ephedrine or *l*-ephedrine yields the more potent *d*-methylamphetamine [9, 10, 11].

3.2.1 Commercial production of Ephedrine/pseudoephedrine

Ephedrine and *pseudo*ephedrine shown in Figure 3.1 and Figure 3.2, are used in many cough and nasal remedies to treat congestion caused by the common cold, sinusitis and hay fever.

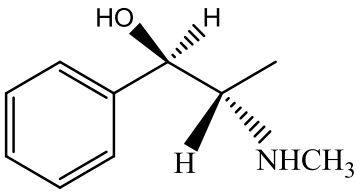


Figure 3.1. *l*-ephedrine

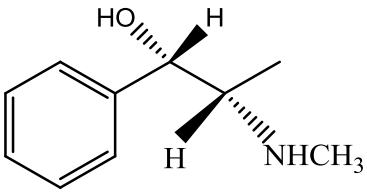
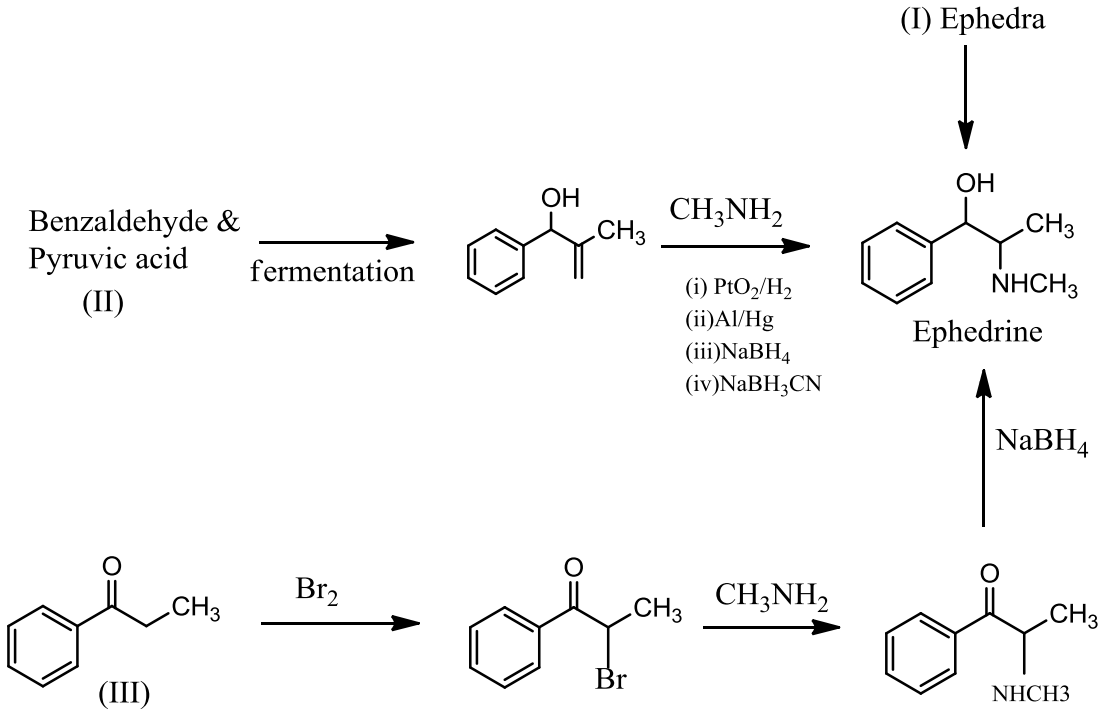


Figure 3.2. *d*-p*seudo*ephedrine

Both ephedrine and *pseudo*ephedrine are under international control in bulk form. Restrictions on the supply of ephedrine and *pseudo*ephedrine pharmaceutical products have been imposed by the Medicines and Healthcare products Regulatory Agency (MHRA) in the United Kingdom. However, medication containing both of these compounds (ephedrine and *pseudo*ephedrine) remain available in the UK and elsewhere as over the counter products. Due to concern that *pseudo*ephedrine and ephedrine can be extracted from cold medication, MHRA held a public consultation in 2007 on whether to reclassify *pseudo*ephedrine, ephedrine from OTC (sold over the counter) to POM

(prescription only medicines). Following the consultation, The Commission on Human Medicines (CHM), an independent body which gives advice to government ministers about safety and quality and efficacy of medicines, advised that number of measures should be introduced to control the supply of *pseudo*ephedrine and ephedrine. The measures include reducing the pack size for OTC containing ephedrine, *pseudo*ephedrine and a restriction on sale to one pack per transaction, which is the current situation [12, 13]. Various brands names for medication containing *pseudo*ephedrine hydrochloride include Sudafed (Johnson and Johnson formerly Pfizer), Actifed (Burroughs Wellcome) and Contac (GlaxoSmithKline) [12, 13].

Commercial ephedrine and *Pseudo*ephedrine are produced via the three processes (a) extraction from the *ephedra* plant (b) full chemical synthesis or (c) semi synthesis and these are presented in Scheme 3.2.



Scheme 3.2. (I) Preparation of ephedrine from the ephedra plant; (II)Semi-synthetic synthesis of ephedrine from fermentation of sugar and (III) fully synthetic route to ephedrine [14].

Ephedrine and *pseudo*ephedrine can be readily extracted from the *ephedra* plant. The stems and leaves of the Mediterranean and Asiatic species of the plant contain both ephedrine and *pseudo*ephedrine alkaloids **[**14, 15**]**. This process is typically employed for ephedrine manufacture in China. Preparation of Ephedra alkaloids from the crude plant material involves an acid/base extraction procedure. The powdered plant material is first made alkaline. The base is extracted using chloroform and distilled. The residue obtained is dissolved in dilute acid and filtered using decolorising carbon. The filtrate is alkalinised and the alkaloids obtained are re-extracted with diethyl ether or chloroform. Finally, the residue obtained is recrystallised from hot water and the solvent evaporated to yield pure ephedrine crystals.

The most economical and popular method for large scale production of pharmaceutical ephedrine is through the fermentation of a mixture of benzaldehyde and molasses followed by a reductive amination of the resulting carbinol. Ephedrine is crystallized as the hydrochloric salt. *Pseudo*ephedrine hydrochloride is also produced from ephedrine hydrochloride via an acetylation/deacetylation procedure [14, 15]. This process is known to be used in India.

The semisynthetic process ephedrine is produced by fermentation of sugar followed by amination. Acetaldehyde is generated by sugar fermentation is condensed with benzaldehyde to form phenylacetylcarbinol (PAC). PAC reacts with methylamine over a catalyst to produce ephedrine [14, 15, 16].

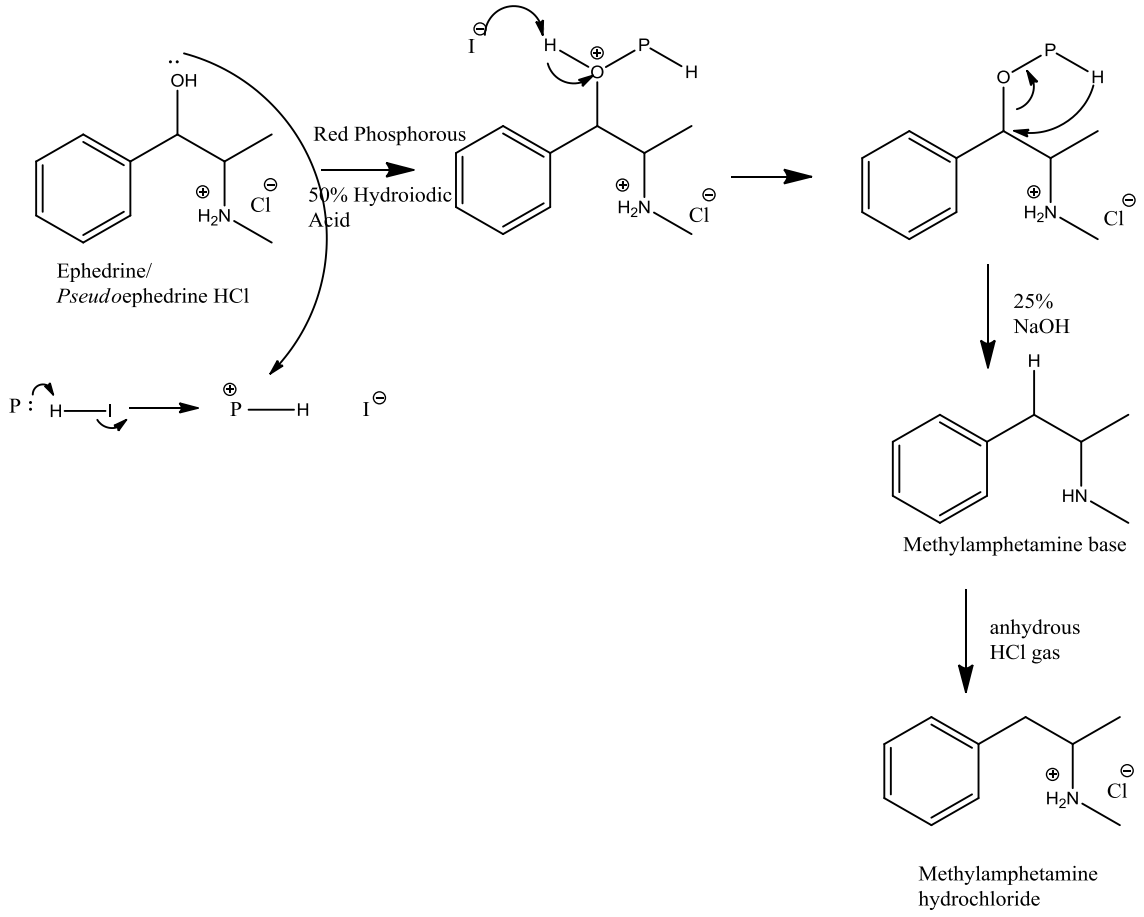
3.2.2 Methylamphetamine synthesis using Ephedrine/*Pseudo*ephedrine as the precursor chemical

Six synthetic routes are documented for the preparation of methylamphetamine using ephedrine or *pseudo*ephedrine as the starting material. The Moscow and Hypophosphorous routes are both variations of the Nagai route and all three are described in more detail in the next section.

3.2.2.1 Nagai Route

The clandestine manufacture of methylamphetamine using the Nagai route is a benzylic alcohol reduction with typical methylamphetamine hydrochloride yields of between 54% and 82% [10]. A mixture of ephedrine/*pseudo*ephedrine hydrochloride, red phosphorous and hydroiodic acid is heated, filtered and basified, extracted and crystallised as the hydrochloric salt from ether/acetone with hydrochloric acid or hydrogen chloride gas [10, 16]. The reaction involves a cyclic oxidation of the iodide anion to iodine and reduction of iodine back to the anion by the red phosphorous, the latter being converted to phosphorous or phosphoric acid. The diastereoisomers, (-) ephedrine and (+) *pseudo*ephedrine are reduced to (+) methylamphetamine and a (+,-) mixture of ephedrine reduces to racemic methylamphetamine. The enantiomer or diastereoisomer of ephedrine selected as precursor thus dictates which isomer of methylamphetamine will be produced.

The reaction mechanism for the reduction of ephedrine with hydroiodic acid/red phosphorus is presented in Scheme 3.3 and summarised as follows: ephedrine/*pseudo*ephedrine reacts with hydroiodic acid to form iodoephedrine which is predominantly reduced to methylamphetamine. Iodoephedrine can also undergo ring closure to form aziridines as a reaction by product [10].

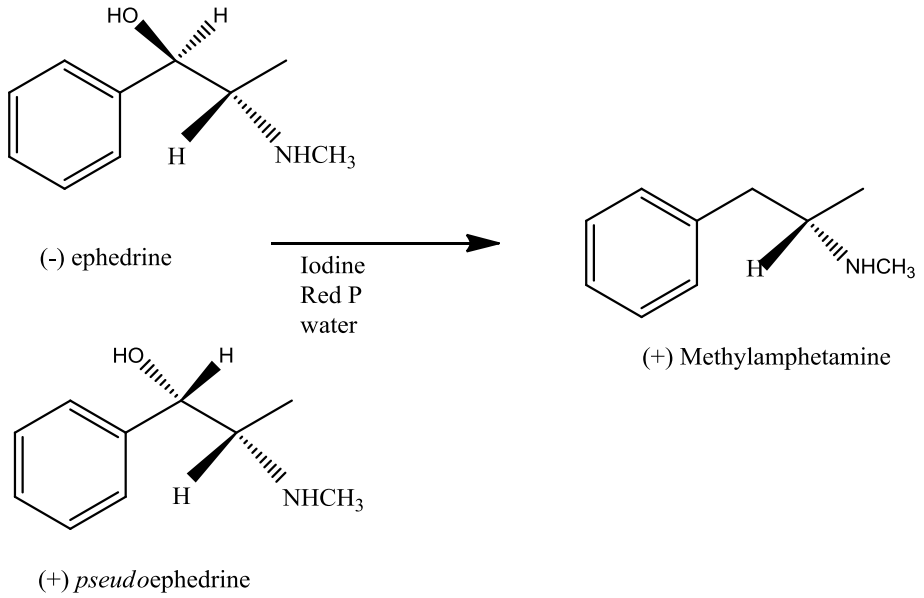


Scheme 3.3. Methylamphetamine manufacture using Nagai route [16].

This is a popular clandestine synthesis due to its simple process and ease of use in large scale production. There are two variations in the Nagai route, (a) the Moscow route and (b) the Hypophosphorous route (known as the 'Hypo route') [11]. In both of these methods, hydroiodic acid is made in situ during the reaction process. These modifications are most likely to have occurred as a result of the difficulty in obtaining hydroiodic acid regulated by the Controlled Drugs (Substances Useful for Manufacture) Regulations 1983 in the United Kingdon and is listed in List 1 of Domestic Chemical Diversion Control Act (DCDCA) in the United States **[**2, 3].

3.2.2.2 Moscow and Hypophosphorous routes

The Moscow route is presented in Scheme 3.4. Hydroiodic acid is formed in situ by adding red phosphorous, iodine and water together which is reacted with the precursor either, ephedrine or *pseudo*ephedrine hydrochloride.



Scheme 3.4. Moscow Route synthesis.

Within the Moscow synthesis, HI is produced by reacting red phosphorus and iodine in the presence of water. The catalytic cycle is presented in Figure 3.3 shown below. In anhydrous media the oxidation of red phosphorous by iodine allows the equilibrium to shift in favour of the generation of hydrogen by removal of iodine as P_2I_4 which decomposes in the presence of water to phosphoric acid and phosphonium iodide which affords under heating, the hydroiodic acid and phosphine PH_3. Oxidation of PH_3 by the liberated iodine in the presence of water gives H_3PO_2 [10, 17, 18, 19].

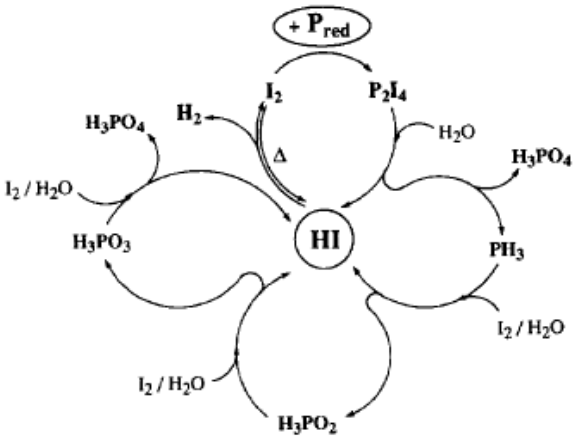
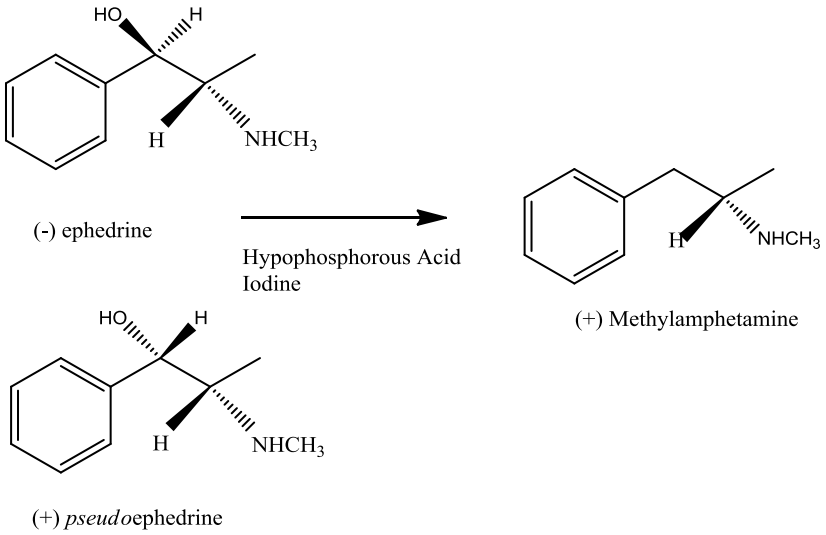


Figure 3.3. Red phosphorous involvement in a catalytic cycle for generation of hydroiodic acid in anhydrous media [19].

The Hypophosphorus (hypo) route has become an established synthetic route employed by clandestine laboratories [17] and differs from the Moscow route in terms of the phosphorus source. The Hypophosphorus approach uses commercially available hypophosphorus (phosphinic) acid, which, upon reacting with iodine, generates hydroiodic acid in-situ [18].

This method does not require any red phosphorous as the hypophosphorous acid (commercially available in 50%, 30-32% and 10%) acts as the reducing agent [11, 17, 18]. The reaction can effectively be carried out in one reaction vessel and is much faster than that of the Moscow route with typical yields of 70-80 %. The reaction is presented in Scheme 3.5.



Scheme 3.5. Methylamphetamine manufactured using the Hypo Method [11].

For the hypophosphorous route, the hydroiodic acid is generated from the reaction between hypophosphorous acid and iodine and is illustrated in Figure 3.4 [19].

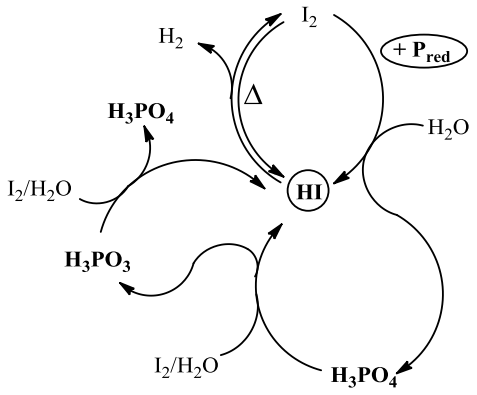
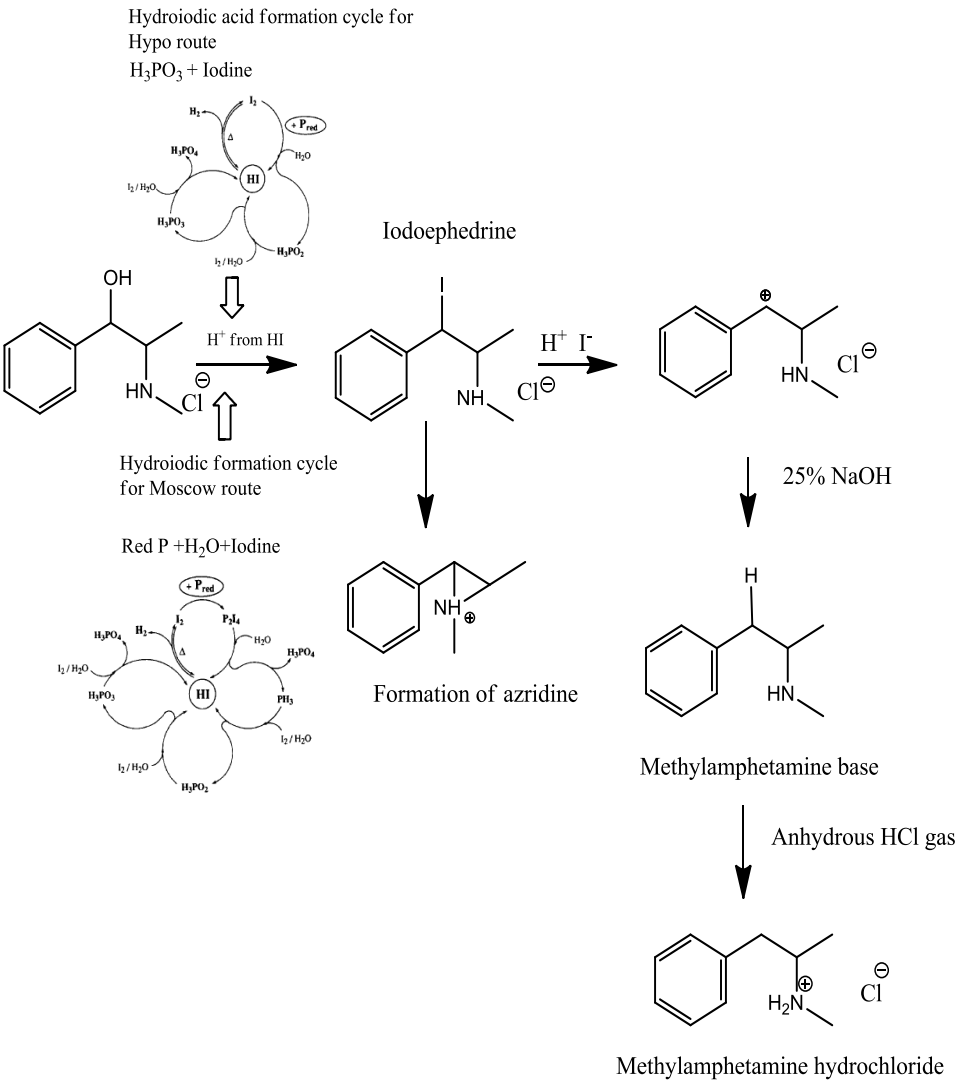


Figure 3.4. Hypophosphorous acid involvement in a catalytic cycle for generation of hydroiodic acid in aqueous media [19].

For both catalytic cycles, hydroiodic is regenerated using a dissociative mechanism which affords hydrogen and iodine. Both of these are consumed by the individual phosphorous source (red phosphorous for the Moscow route and hypophosphorous acid for the hypo route) [19]. The in-situ generated hydroiodic acid protonates the hydroxyl group of ephedrine or *pseudo*ephedrine, which is removed from the compound. This is most likely facilitated by the secondary amine which is capable of forming an aziridine intermediate. The introduction of iodide in place of the hydroxyl group is then followed by reduction of this new functionality to yield methylamphetamine [10, 19]. The reaction mechanism is presented in Scheme 3.6.



Scheme 3.6. Moscow and Hypophosphorous mechanism

3.2.3 Extraction and preparation of *pseudo*ephedrine from commercially available cold medication.

Initial studies optimised the extraction of the precursor *pseudo*ephedrine from pharmaceutical products and iodine and red phosphorous from commonly available materials. The extracted materials were then used to systematically synthesise methylamphetamine as the hydrochloride salt using both the Moscow and Hypophosphorous routes.

3.2.4 Materials and Methods

Toluene, diethyl ether, methanol, absolute ethanol and chloroform were all purchased from Fischer Scientific. Hydrochloric Acid (37%), sulfuric acid (95-97%) and hypophosphorous acid (40%) were purchased from Sigma Aldrich. Sodium chloride and sodium hydroxide pellets were purchased from GPR (Poole, England). *d-pseudo*ephedrine hydrochloride was purchased from Sigma Aldrich. Methylated Spirits was purchased from B&Q. Apparatus used were a Philips PW9421 pH meter, a Fisons Whirlmixer vortex, an American Beauty S/70 sonicator and an Edmund Buhler Swip KS-10 rotative shaker. Distilled water was obtained from an in house water purification system.

Sudafed, a non drowsy decongestant, containing *pseudo*ephedrine hydrochloride (60 mg per tablet), was purchased from pharmacies in Glasgow, Scotland. Other components of the tablets included lactose, microcrystalline cellulose, maize starch, silica, magnesium stearate, hypermellose, polyethylene glycol and red iron oxide (E172). Panadol and Allerpid tablets were purchased from various pharmacies in Kuala Lumpur, Malaysia. The Panadol brand tablets contained *pseudo*ephedrine hydrochloride (30 mg per tablet). Other active components included paracetamol (500 mg per tablet) and chlorphenramine (5 mg per tablet). The Allerpid brand tablets contained *pseudo*ephedrine hydrochloride (120 mg per tablet) and loratadine (5 mg per tablet).

3.2.5 Extraction of *pseudo*ephedrine from pharmaceutical tablets

Sudafed tablets: Initial studies were undertaken to determine the volume of solvent, time and method of agitation which facilitated the optimal conditions for the extraction of *pseudo*ephedrine from the Sudafed tablets. The tablets were coated with a red iron oxide (E172) and optimisation studies also included exploring whether this coating needed to be removed prior to extraction. The iron oxide coating was removed by repetitive washing with acetone (10 mL in total) until all the red colour had disappeared. The following general method of sample extraction was undertaken [4].

One Sudafed tablet was crushed using a mortar and pestle and the crushed tablet placed in a beaker. A measured volume of solvent was added to the beaker which was then covered with aluminium foil and agitated for a fixed period of time. The sample was left to settle for one hour at room temperature and then filtered using gravity filtration. The solvent was evaporated and the resultant solid collected and analysed using NMR and FTIR.

Panadol/Allerpid tablets: A different approach was required for the extraction of *pseudo*ephedrine from the Panadol and Allerpid tablets. This was because both tablets also contained relatively large amounts of paracetamol. The extraction methodology followed was originally suggested by the US Drug Enforcement Administration (DEA) [20]. 10 tablets (one strip) were crushed using a mortar and pestle and placed in a beaker (250 mL) together with 100 mL of hot water (90^{o}C) and stirred. The pH of the mixture was adjusted to pH 1 using hydrochloric acid (4% vol/vol). The mixture was washed three times using a total of 100 mL of diethyl ether. The pH of the mixture was further adjusted to pH 12 using sodium hydroxide (10%) and extracted three times using a total of 100 mL of diethyl ether and the washings retained. The collected diethyl ether washings were further washed with 50 mL of distilled water and the solvent was left to evaporate and resultant solid collected [20].

3.2.6 Extraction of essential chemicals

3.2.6.1 Iodine from iodine tinctures

Iodine tincture (7 mL of 2.5%) and distilled water (7 mL) were combined together and mixed with swirling. Concentrated hydrochloric acid (1 mL) was added drop wise with swirling, followed by hydrogen peroxide (7 mL, 6% 20 vols). The mixture was poured into a beaker containing distilled water (50 mL) and left to stand for 20 minutes [4]. The mixture was subsequently filtered using gravity filtration to reveal the iodine crystals. In total 214.6 g of iodine was produced.

3.2.6.2 Red phosphorous from matchboxes

Matchbook strikers from locally purchased K TWO safety matchboxes were cut off the boxes and soaked in acetone (10 mL). After 30 minutes the red phosphorous was scraped from the strikers and the paper discarded. The extracted red phosphorous was washed with distilled water and left to dry completely. The dry red phosphorous was placed in a beaker and sodium hydroxide solution (20% wt/vol, 20 mL) was added and placed on a low heat for 2 hours. The final product was filtered, washed with distilled water and left to dry [4]. In total, 23.4 g of red phosphorous was extracted from 360 matchboxes.

3.2.7 Synthesis of methylamphetamine using the Moscow Route

*Pseudo*ephedrine hydrochloride (2.0 g) was mixed in a round bottom flask (100 mL) together with red phosphorous (0.6 g), iodine (4.0 g) and distilled water (2 mL) and a condenser attached. The mixture was refluxed for 24 hours and then allowed to cool. Once cool, the mixture was diluted with an equal volume of water and the red phosphorus filtered out. A few grams of sodium thiosulfate were placed into a beaker, and sodium hydroxide solution (25% wt/vol, 8 mL) added. This was then added to the filtered reaction mixture, and swirled to reveal methylamphetamine free base as an oil which floated to the top of the aqueous solution. Toluene (20 mL) was added to extract the methylamphetamine free base. The toluene extract was clear to pale yellow.

Anhydrous hydrogen chloride gas was bubbled through to reveal a white precipitate, which was washed with toluene. The solid was dried under high vacuum. Structural conformation of the product was achieved using nuclear magnetic resonance (NMR) and typical values obtained were ^{1}H NMR (400 MHz, D_2O): δH 1.23 (d, 3H, *J*=8.0 Hz, CH_3), 2.66 (s, 3H, CH_3), 2.87(dd, 1H, *J*=24.0, 8.0 Hz, CH), 3.04 (dd, 1H, *J*=20.0, 8.0 Hz, CH), 3.48-3.53 (m, 1H, CH), 7.24-7.39 (m, 5H, C_6H_5). This data is confirmed by work published by Kram *et al*. [21]. A sample spectra is attached in Appendix A.

IR ν_{max} (KBr)/cm^{-1}: 3419(N-H), 2972, 2731, 2461(C-C), 1605(N-C). This is in agreement with published data for IR [22]. A sample spectra is attached in Appendix B.

The synthesis was repeated six times each for laboratory grade *pseudo*ephedrine and *pseudo*ephedrine extracted from Sudafed tablets using ethanol, ethanol:methanol (90:10% vol/vol) and commercial methylated spirits. In these cases essential chemicals (iodine and phosphorous) extracted from tinctures and matchboxes were also used. Two samples were prepared from the *pseudo*ephedrine extracted using Allerpid tablets extracted using the acid base extraction.

3.2.8 Synthesis of methylamphetamine using Hypophoshorous Route.

*Pseudo*ephedrine hydrochloride (2.0 g) was placed into a round bottom flask (100 mL) and mixed with iodine (4.0 g) and hypophosphorus acid (3.6 mL) and a condenser attached. The mixture was refluxed for 8 hours then allowed to cool. Once cool, the mixture was diluted with an equal volume of water. A few grams of thiosulfate was placed into a beaker, and 25% sodium hydroxide solution (24 mL) was added to extract the methylamphetamine free base. Extraction and precipitation of the salt was as previously described. Structural conformation of the product was achieved using nuclear magnetic resonance (NMR) and typical values obtained were ^{1}H NMR (400 MHz, D_2O): δH 1.20 (d, 3H, *J*=8.0 Hz, CH_3), 2.62 (s, 3H, CH_3), 2.85(dd, 1H, *J*=24.0, 8.0 Hz, CH), 3.01(dd, 1H, *J*=20.0, 8.0 Hz, CH), 3.42-3.50 (m, 1H, CH), 7.23-7.36 (m, 5H, C_6H_5). This data is confirmed by work published by Kram *et al*. [21]. A sample spectra is attached in Appendix C.

IR ν_{max} (KBr)/cm^{-1}: 3419(N-H), 2972, 2731, 2461(C-C), 1605(N-C). This is in agreement with published data for IR [22]. A sample spectra is attached in Appendix D.

The synthesis was repeated six times each for laboratory grade *pseudo*ephedrine and *pseudo*ephedrine extracted from Sudafed tablets using ethanol, ethanol:methanol (90:10% vol/vol) and commercial methylated spirits. In these cases essential chemicals (iodine and phosphorous) extracted from tinctures and matchboxes were also used. Three batches of methylamphetamine in total were also prepared from the *pseudo*ephedrine extracted from Panadol (1 batch) and Allerpid (2 batches) tablets extracted using the acid base extraction.

3.3 Result and discussions

3.3.1 Extraction of Sudafed tablets using various solvents

*Pseudo*ephedrine was extracted from commercially available Sudafed tablets. Each tablet was coated in red iron oxide (E172) and contained 60 mg of the target drug per tablet. Three solvents were chosen with reference to the literature (ethanol, ethanol:methanol (90:10 vol/vol) and methylated spirits). A systematic study was undertaken using ethanol as the extracting solvent to determine the optimum extraction conditions. The optimised conditions were subsequently used for each solvent to prepare the desired weight of precursor. During the optimisation experiments, three different agitation methods were examined where the volume of solvent and time of agitation was systematically varied. Both coated and non coated tablets were used. The yields of *pseudo*ephedrine recovered for each set of extracting conditions are presented in Table 3.1, Table 3.2 and Table 3.3 where the highest yielding method is highlighted in each case.

Table 3.1. Extraction of *pseudoe*phedrine from Sudafed tablets (with and without coating) with ethanol – shaking by hand.

Sample	Method	Coating	Time (mins)	Volume (mL)	Yield (%)
SC-1	Hand shaking	Yes	10	5	7.5
SC-2	Hand shaking	Yes	10	10	8.0
SC-3	Hand shaking	Yes	10	15	8.5
SC-4	Hand shaking	Yes	15	5	8.0
SC-5	**Hand shaking**	**Yes**	**15**	**10**	**14.0**
SC-7	Hand shaking	Yes	15	15	13.9
SC-8	Hand shaking	Yes	20	5	9.1
SC-9	Hand shaking	Yes	20	10	13.7
SC-10	Hand shaking	Yes	20	15	13.9
S-11	Hand shaking	No	10	5	2.1
S-12	Hand shaking	No	10	10	3.0
S-13	Hand shaking	No	10	15	5.8
S-14	Hand shaking	No	15	5	2.5
S-15	Hand shaking	No	15	10	3.8
S-16	Hand shaking	No	15	15	3.8
S-17	Hand shaking	No	20	5	4.0
S-18	Hand shaking	No	20	10	5.6
S-19	Hand shaking	No	20	15	7.0

Table 3.2. Extraction of *pseudoe*phedrine from Sudafed tablets (with and without coating) with ethanol – using a mechanical agitator.

Sample	Method	Coating	Time (mins) (mins)	Volume (mL)	Yield (%)
SC-20	Agitator	Yes	10	5	1.4
SC-21	Agitator	Yes	10	10	15.0
SC-22	Agitator	Yes	10	15	15.3
SC-23	Agitator	Yes	15	5	10.9
SC-24	Agitator	Yes	15	10	15.5
SC-25	**Agitator**	**Yes**	**15**	**15**	**18.9**
SC-26	Agitator	Yes	20	5	6.4
SC-27	Agitator	Yes	20	10	10.1
SC-28	Agitator	Yes	20	15	16.7
S-29	Agitator	No	10	5	3.6
S-30	Agitator	No	10	10	12.3
S-31	Agitator	No	10	15	3.7
S-32	Agitator	No	15	5	5.9
S-33	Agitator	No	15	10	3.3
S-34	Agitator	No	15	15	11.3
S-35	Agitator	No	20	5	3.2
S-36	Agitator	No	20	10	3.9
S-37	Agitator	No	20	15	6.2

Table 3.3. Extraction of *pseudoe*phedrine from Sudafed tablets (with and without coating) with ethanol – using a vortex.

Sample	Method	Coating	Time (mins) (mins)	Volume (mL)	Yield (%)
SC-38	Vortex	Yes	10	5	10.5
SC-39	Vortex	Yes	10	10	10.5
SC-40	Vortex	Yes	10	15	10.0
SC-41	Vortex	Yes	15	5	14.5
SC-42	Vortex	Yes	15	10	11.9
SC-43	Vortex	Yes	15	15	10.2
SC-44	Vortex	Yes	20	5	6.6
SC-45	Vortex	Yes	20	10	10.7
SC-46	Vortex	Yes	20	15	11.8
S-47	Vortex	No	10	5	4.6
S-48	**Vortex**	**No**	**10**	**10**	**14.9**
S-49	Vortex	No	10	15	6.0
S-50	Vortex	No	15	5	4.6
S-51	Vortex	No	15	10	11.5
S-52	Vortex	No	15	15	13.0
S-53	Vortex	No	20	5	12.1
S-54	Vortex	No	20	10	13.7
S-55	Vortex	No	20	15	14.0

The general trend observed in the data suggests that better yields are obtained with tablets where the coating hasn't been removed and the best results were obtained using 15 mL of ethanol with mechanical agitation for 15 minutes. Repeatability of this extraction method was explored for each solvent used (ethanol, ethanol:methanol (90:10% vol/vol) and commercial methylated spirits) the results of which are presented in Table 3.4. The total weight of precursor material extracted from Sudafed using each solvent is presented in Table 3.5.

Table 3.4. Repeatability of extraction using various solvents (15 mL) with mechanical agitation (15 minutes) SE = ethanol, SLMS = ethanol:methanol (90:10% vol/vol), SMS – commercial methylated spirits.

Sample	Weight (mg)	Yield (%)
Ethanol extraction		
SE 25C-1	254.8	18.70
SE 25C-2	254.7	18.90
SE 25C-3	256.4	18.70
SE 25C-4	256.6	18.50
SE 25C-5	255.3	18.40
SE 25C-6	256.6	17.90
%RSD		**1.88**
Ethanol:Methanol (90:10% vol/vol) extraction		
SLMS 25C-1	256.0	17.78
SLMS 25C-2	252.1	18.90
SLMS 25C-3	256.8	18.30
SLMS 25C-4	254.0	18.26
SLMS 25C-5	260.2	17.60
SLMS 25C-6	252.5	17.90
% RSD		**2.58**
Commercial methylated spirits extraction		
SMS 25C-1	254.6	16.78
SMS 25C-2	255.7	16.90
SMS 25C-3	253.5	16.30
SMS 25C-4	257.0	16.26
SMS 25C-5	254.1	16.60
SMS 25C-6	259.6	16.90
% RSD		**1.73**

Table 3.5. Summary of precursor yields obtained from Sudafed using each solvent.

Precursor extraction solvent	Yields (grams) from 840 tablets (70 boxes)	Average yield (%)
Ethanol	34.50	18.51
Ethanol:methanol (90:10) vol/vol	34.00	18.12
Commercial methylated spirits	32.00	16.62

3.3.2 Extraction of Panadol and Allerpid tablets using acid/base extraction

The availability of Panadol and Allerpid tablets was more limited as these were sourced from Malaysia. The yields of *pseudo*ephedrine hydrochloride obtained from the acid base extraction of these samples are provided in Table 3.6. The lower amounts of these materials restricted the number of repetitive synthesis of methylamphetamine possible with these precursors.

Table 3.6. Summary of precursor yields obtained from Panadol and Allerpid tablets using each acid/base extraction.

Precursor acid base extraction	Yield (grams)	Yield (%)
Panadol tablets	300pills - 3.00 g	5.80
Allerpid tablets	50 pills - 9.25	15.90

Confirmational analysis of each of the extracted *pseudo*ephedrine samples was undertaken using melting point, microanalysis, FTIR and X-ray powdered diffraction (XRD) and examples of the results presented in Table 3.7 and Figure 3.5Figure 3.6to Figure 3.13.

Table 3.7. CHN and melting point data of *pseudo*ephedrine HCl extracted from Sudafed tablets.

Sample	C %	H %	N %	Melting point (^{0}C)
Laboratory grade *pseudo*ephedrine	59.40	7.86	6.96	185-189
Sudafed extracted using Ethanol	58.74	7.81	6.50	185-188
Sudafed extracted using Ethanol:methanol (90:10 %vol/vol)	58.60	7.89	6.38	184-187
Sudafed extracted using commercial methylated spirits	59.11	8.07	5.99	185-186
Panadol tablets extracted using acid base extraction	58.40	7.90	6.90	185-187
Allerpid tablets extracted using acid base extraction	59.20	7.80	6.78	186-187
Theoretical value	**59.55**	**7.94**	**6.94**	**186-187**

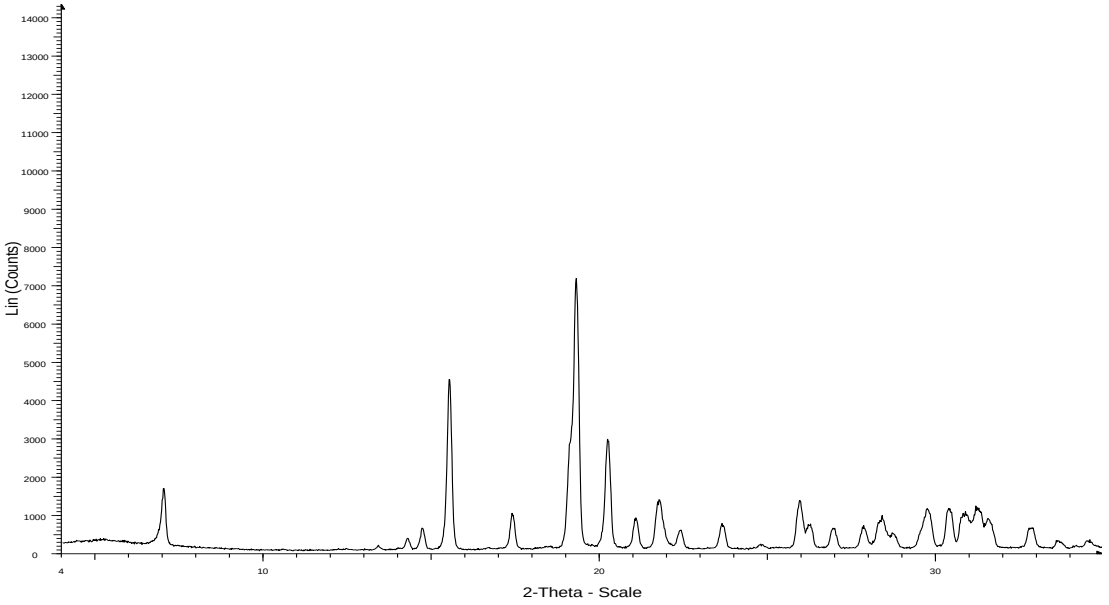


Figure 3.5. X ray Powder diffraction of *Pseudo*ephedrine HCl extracted from Sudafed using ethanol as extraction solvent.

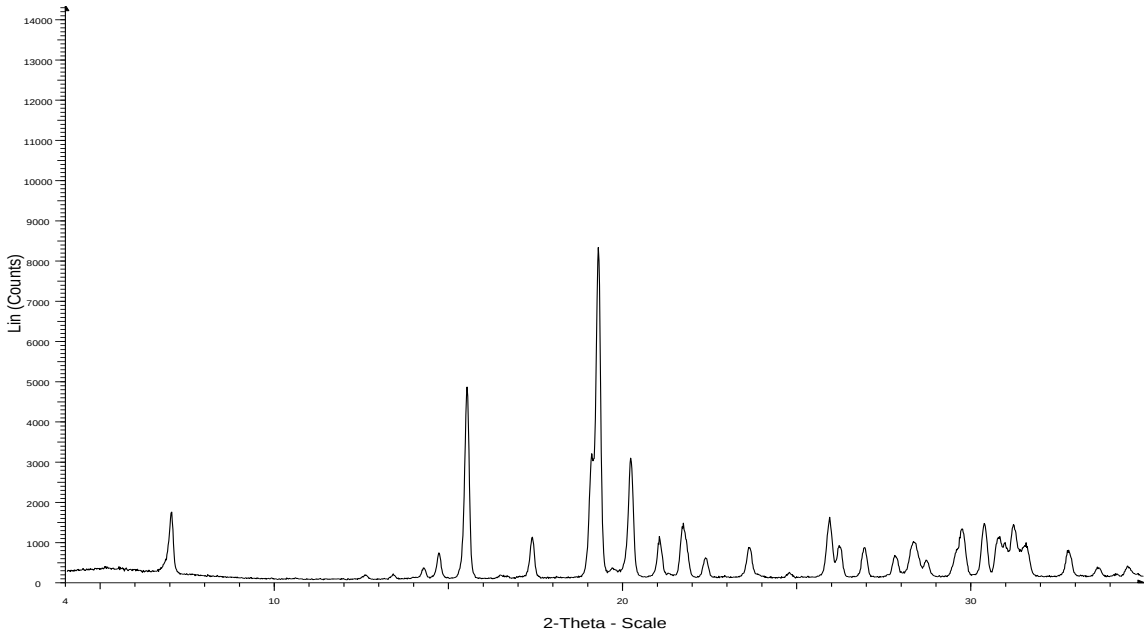


Figure 3.6. X ray Powder diffraction of *Pseudo*ephedrine HCl extracted from Sudafed using ethanol:methanol(90:10) vol/vol as extraction solvent

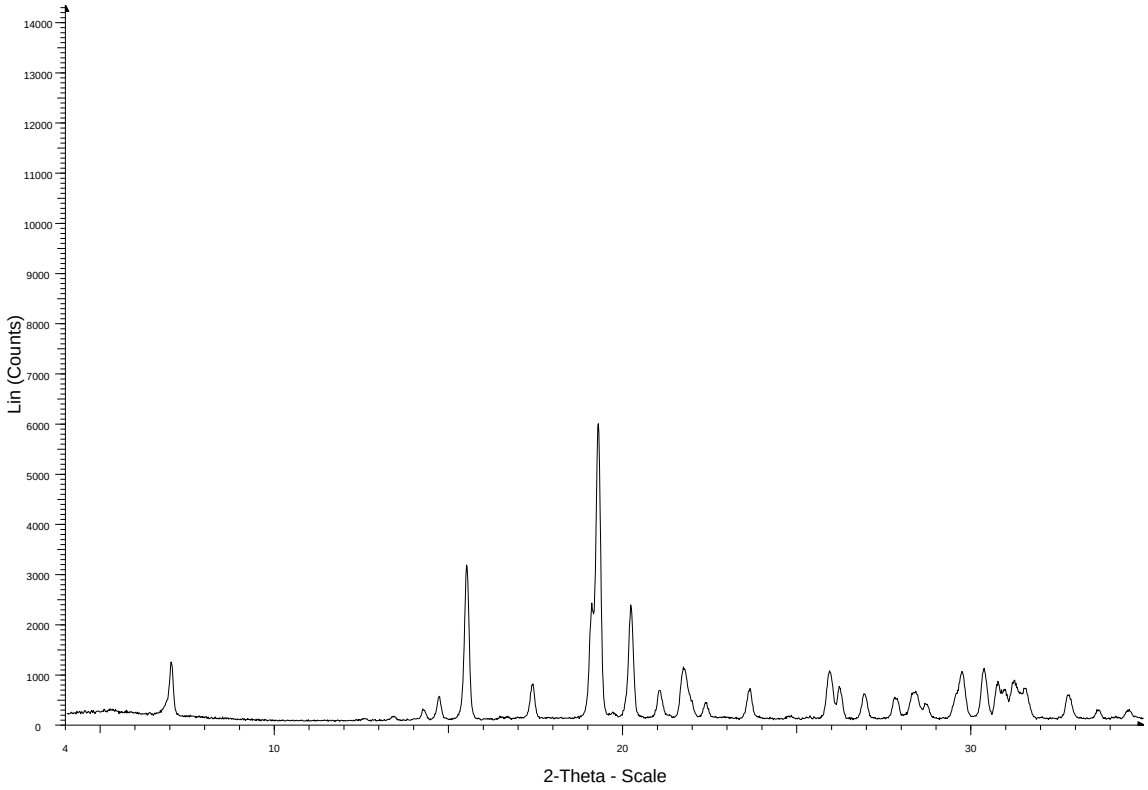


Figure 3.7. X ray Powder diffraction of *Pseudo*ephedrine HCl extracted from Sudafed using commercial methylated spirit as extraction solvent

X-ray powder diffraction analysis confirmed that *pseudo*ephedrine hydrochloride extracted from the three solvent systems were all of the same crystalline phase. The pattern is consistent with that of the single crystal structure of laboratory grade *pseudo*ephedrine hydrochloride presented in Figure 3.8 and was in agreement with previous studies [23, 24]. Interestingly, the powdered sample extracted from Sudafed using methylated spirits was purple, the colour deriving from the violet colour dye present in the solvent. The dye was not crystalline and did not contribute to the diffraction pattern.

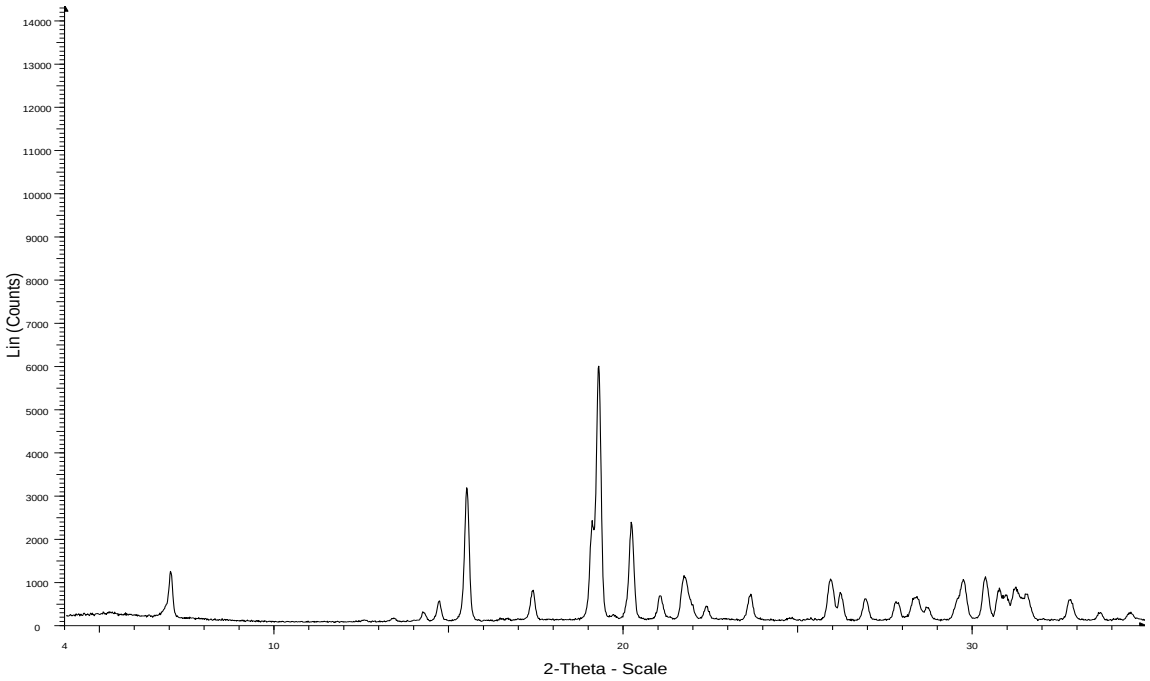


Figure 3.8. X ray Powder diffraction of Laboratory grade p*seudo*ephedrine HCl.

The FTIR spectra of the extracted precursor samples are presented in Figure 3.9 to Figure 3.13 and demonstrate consistency across all extracted samples.

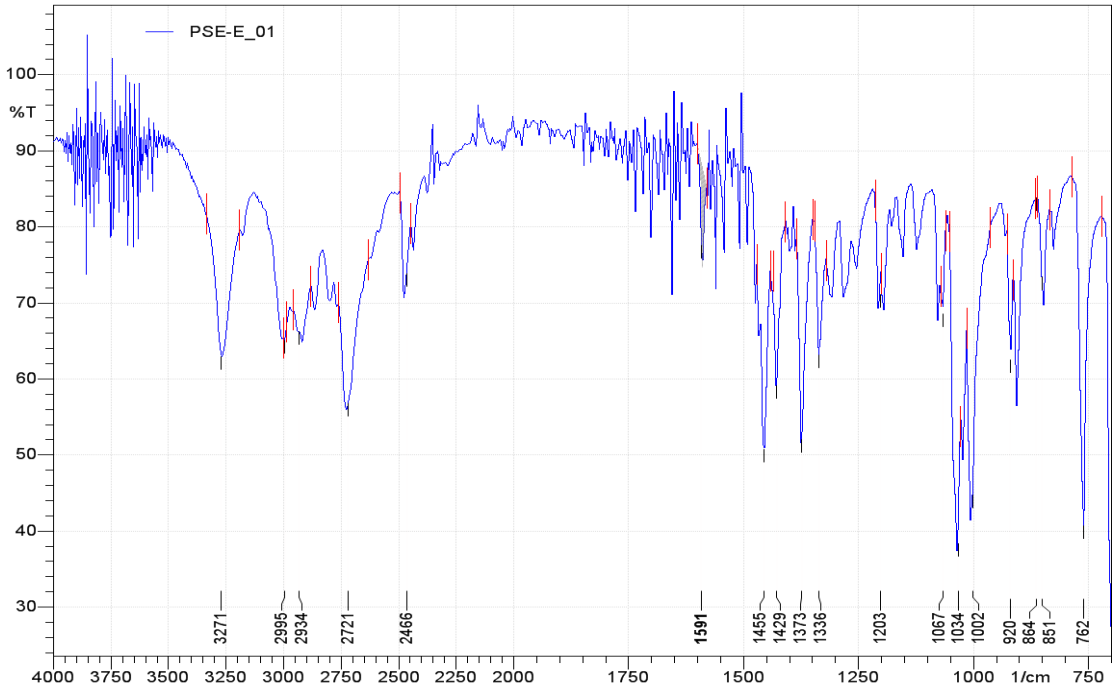


Figure 3.9. FTIR spectra of *Pseudo*ephedrine HCl extracted from Sudafed using ethanol as extraction solvent.

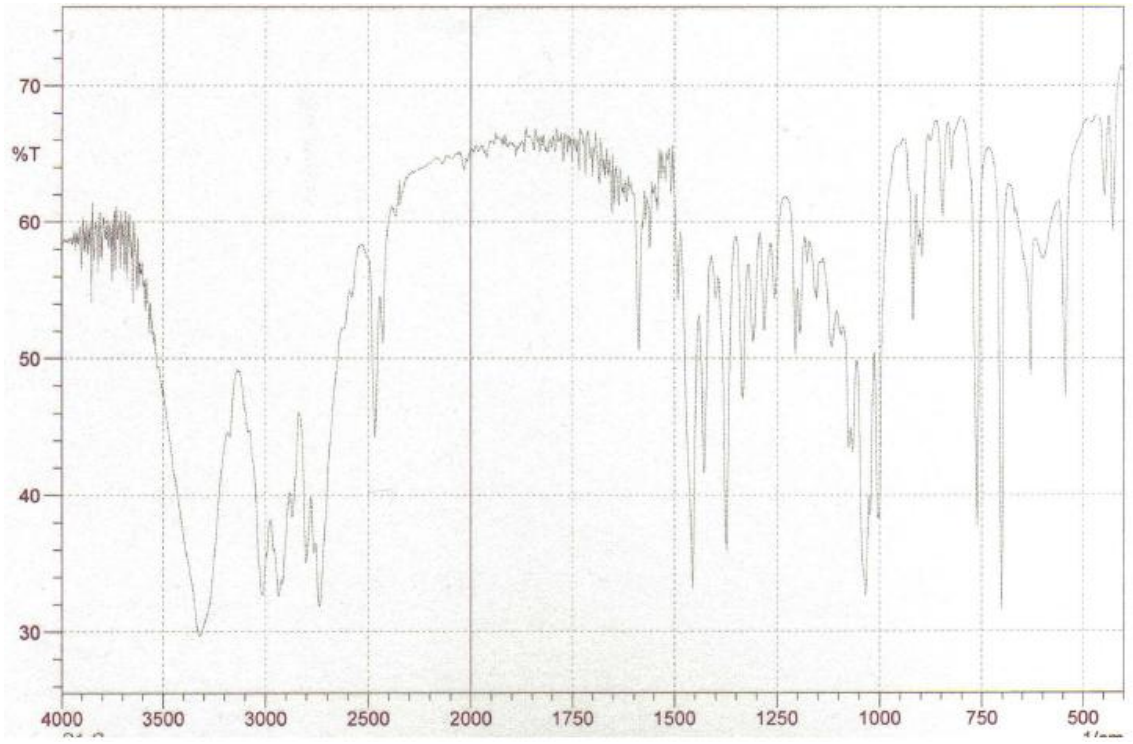


Figure 3.10. FTIR spectra of *Pseudo*ephedrine HCl extracted from Sudafed using ethanol:methanol(90:10) vol/vol as the extraction solvent

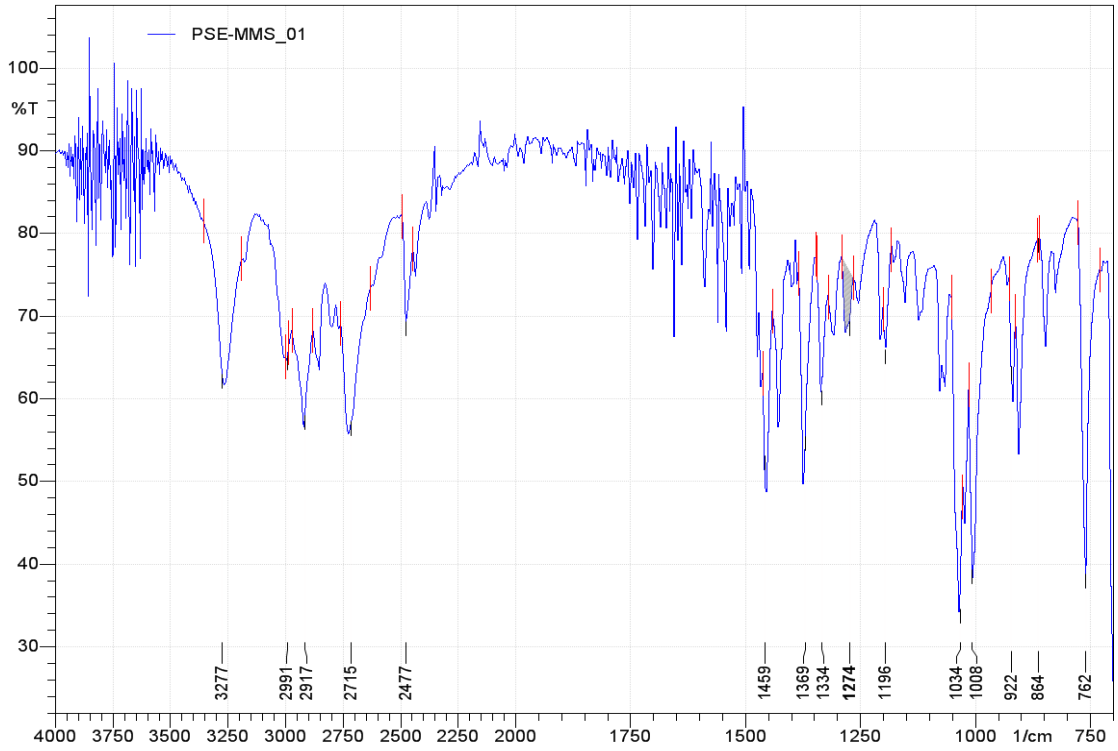


Figure 3.11. FTIR spectra of *Pseudo*ephedrine HCl extracted from Sudafed using commercial methylated spirits as the extraction solvent.

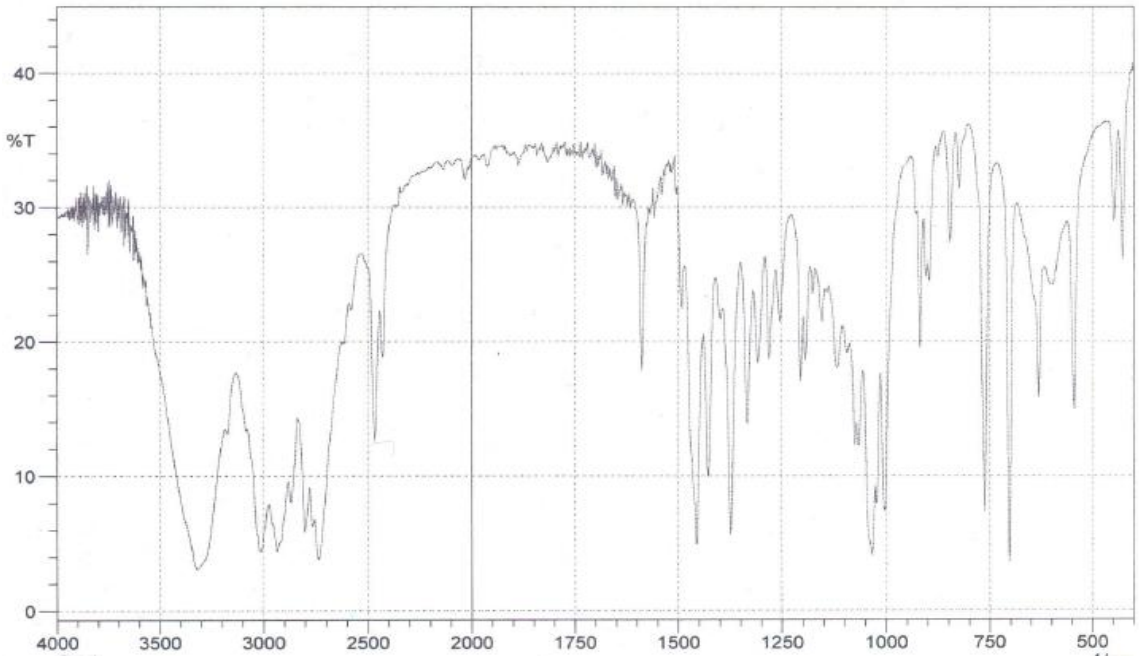


Figure 3.12. FTIR spectra of *Pseudo*ephedrine HCl extracted from Panadol using acid base extraction.

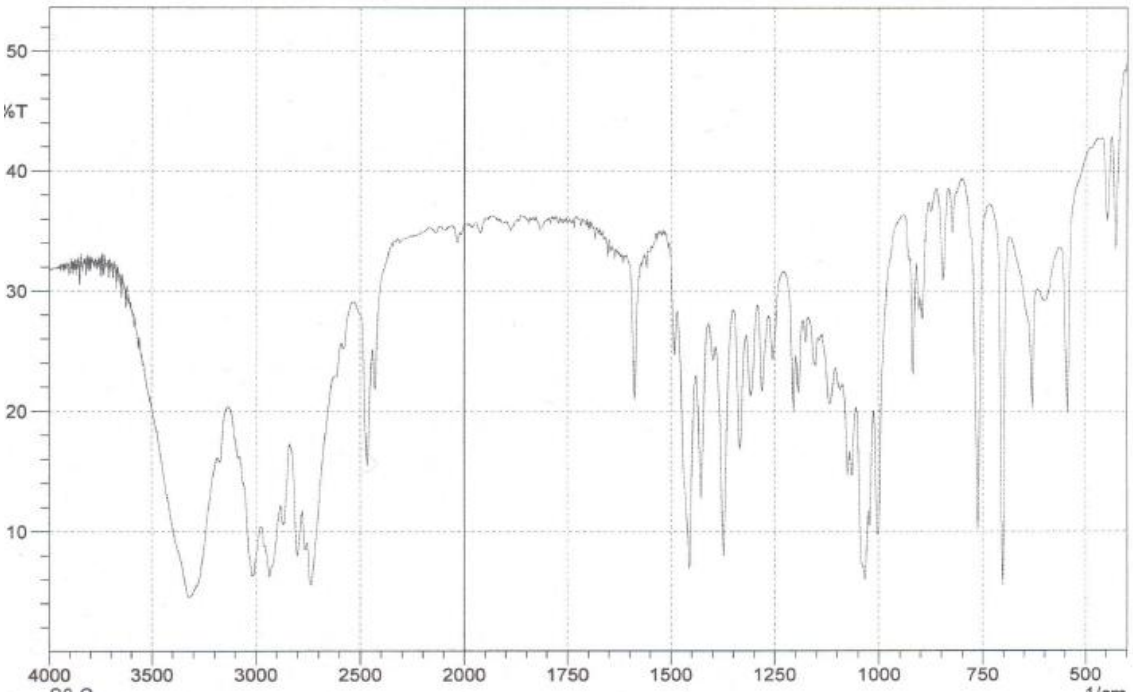


Figure 3.13. FTIR spectra of *Pseudo*ephedrine HCl extracted from Allerpid using acid base extraction.

3.3.3 Extraction of essential chemicals

Both iodine and red phosphorous are essential chemicals in the Moscow and Hypophosphorous synthetic routes. The extraction of these chemicals from household materials is straightforward and easily accomplished from iodine tinctures or matchboxes. Table 3.8 and 3.9 provide the yields of iodine and phosphorous respectively recovered using the methods described in section 3.2.3.

Table 3.8. Yield of iodine extracted from iodine tinctures.

Bottles of Tincture (250 mL)	Yield (g)
10 bottles	8.70
10 bottles	15.20
10 bottles	14.79
10 bottles	15.00
10 bottles	14.63
10 bottles	13.69
10 bottles	14.20
10 bottles	14.00
10 bottles	14.78
10 bottles	14.65
10 bottles	14.34
10 bottles	15.17
10 bottles	15.28
10 bottles	15.89
10 bottles	14.32
Overall yield obtained (gram)	**214.64**

Table 3.9. Yield of red phosphorous from matchboxes striker pads.

Amount of striker pads from matchboxes	Yield (g)
15 strips	2.07
15 strips	1.78
20 strips	2.66
10 strips	1.24
10 strips	1.25
10 strips	1.13
10 strips	2.67
15 strips	2.11
15 strips	1.94
10 strips	1.17
10 strips	1.78
15 strips	2.26
10 strips	1.34
Overall yield (grams)	**23.40**

3.3.4 Synthesis of Methylamphetamine using the Moscow and Hypophosphorous routes

Six samples of methylamphetamine hydrochloride were prepared using laboratory grade precursor and essential chemicals for both the Moscow and Hypophosphorous synthetic routes. These samples were prepared as a set of control samples.

Six repetitive samples of methylamphetamine hydrochloride were produced for both synthetic routes using the precursor extracted from Sudafed and the extracted iodine and red phosphorous. In total 36 samples were prepared with typical yields obtained of 22%-31% and 48%-86% for the Moscow and Hypophosphorous routes respectively

In the case of the Panadol and Allerpid tablets, an acid/base extraction was used to recover *pseudo*ephedrine from the tables which resulted in much lower yields of the precursor restricting the number of repeat samples of methylamphetamine which could be produced as a consequence. In total two repeat samples using each synthetic method were prepared from the precursor extracted from the Allerpid tablets with typical yields obtained of 11% and 13.5% for the Moscow and Hypophosphorous routes respectively. A single methylamphetamine sample was synthesised using the Hypophosphorous route from the precursor extracted from the Panadol tablets (yield 12.5%).

The yields of methylamphetamine obtained for each synthetic route are presented in Table 3.11 and Table 3.12. Confirmation of each methylamphetamine sample was undertaken using melting point, microanalysis, FTIR and examples of the results are presented in Table 3.12 and in Figure 3.14 to Figure 3.21.

Table 3.10. Methylamphetamine yields obtained from Moscow route synthesis.

Reagent grade chemicals		***Pseudo*ephedrine extracted with ethanol**		***Pseudo*ephedrine extracted with ethanol/methanol**		***Pseudo*ephedrine extracted with Methylated spirits**		***Pseudo*ephedrine extracted from Allerpid**	
Sample ID	Yield (g)	Sample ID	Yield (g)	Sample ID	Yield (g)	Sample ID	Yield (g)	Sample ID	Yield (g)
ML1	0.46	ME1	0.47	MD1	0.40	MMS1	0.32	Allep-M1	0.20
ML2	0.40	ME2	0.60	MD2	0.34	MMS2	0.55	Allep M2	0.25
ML3	0.51	ME3	0.62	MD3	0.38	MMS3	0.30		
ML4	0.50	ME4	0.31	MD4	0.37	MMS4	0.42		
ML5	0.42	ME5	0.41	MD5	0.70	MMS5	0.45		
ML6	0.38	ME6	0.43	MD6	0.40	MMS6	0.41		
Mean	*0.44*	*Mean*	*0.47*	*Mean*	*0.43*	*Mean*	*0.44*	*Mean*	0.22
RSD	*12.3%*	*RSD*	*25.02%*	*RSD*	*30.9%*	*RSD*	*22.3%*	*RSD*	*15.71%*

Table 3.11. Methylamphetamine yields obtained from Hypophosphorous route synthesis.

Reagent grade chemicals		***Pseudo*ephedrine extracted with ethanol**		***Pseudo*ephedrine extracted with ethanol/methanol**		***Pseudo*ephedrine extracted with Methylated spirits**		***Pseudo*ephedrine extracted from Panadol**		***Pseudo*ephedrine extracted from Allerpid**	
Sample ID	Yield (g)	Sample ID	Yield (g)	Sample ID	Yield (g)	Sample ID	Yield (g)	Sample	Yield (g)	Sample	Yield (g)
HL1	1.42	HE1	1.04	HD1	0.98	HMS1	1.20	PND-H	0.25	Allep-H1	0.30
HL2	1.30	HE2	0.85	HD2	1.15	HMS2	1.08			Allep-H2	0.25
HL3	1.73	HE3	1.11	HD3	0.85	HMS3	1.07				
ML4	1.72	HE4	1.02	HD4	0.70	HMS4	0.83				
HL5	1.50	HE5	1.30	HD5	0.91	HMS5	0.93				
HL6	1.57	HE6	1.40	HD6	1.24	HMS6	1.00				
Mean	*1.54*	*Mean*	*1.12*	*Mean*	*0.97*	*Mean*	*1.01*	*Mean*		*Mean*	*0.27*
RSD	*11.0%*	*RSD*	*17.92%*	*RSD*	*20.4%*	*RSD*	*12.7%*	*RSD*		*RSD*	*12.85%*

Table 3.12. Results of CHN analysis of methylamphetamine synthesized via Moscow and Hypophosphorous routes

Sample	**C %**	**H %**	**N %**	**Melting point(^{0}C)**
Theoretical / Literature Values	64.6	8.62	7.54	169-172
ML-methylamphetamine synthesized using laboratory grade *pseudo*ephedrine via Moscow route	63.60	8.55	7.68	170-172
ME-methylamphetamine synthesized using *pseudo*ephedrine via Moscow route extracted from Sudafed tablets using ethanol	62.87	8.45	7.60	170-172
MD-methylamphetamine synthesized using *pseudo*ephedrine via Moscow route extracted from Sudafed tablets using ethanol:methanol (90:10) % vol/vol	62.93	8.41	7.44	169-173
MMS-methylamphetamine synthesized using *pseudo*ephedrine via Moscow route extracted from Sudafed tablets using commercial methylated spirit	61.85	8.34	7.50	170-173
HL-methylamphetamine synthesized using laboratory grade *pseudo*ephedrine via Hypo route	64.40	8.58	7.52	170-172
HE-methylamphetamine synthesized using *pseudo*ephedrine via Hypo route extracted from Sudafed tablets using ethanol	63.57	8.45	7.44	169-172
HDA-methylamphetamine synthesized using *pseudo*ephedrine via Hypo route extracted from Sudafed tablets using ethanol:methanol (90:10) % vol/vols	63.22	8.45	7.36	168-170
HMS-methylamphetamine synthesized using *pseudo*ephedrine via Hypo route extracted from Sudafed tablets using commercial methylated spirit	61.93	8.24	7.30	170-173
Allep-Moscow- methylamphetamine synthesized using *pseudo*ephedrine via Moscow route extracted from Allerpid tablets using acid base extraction	64.22	8.45	7.31	169-172
Allep-Hypo- methylamphetamine synthesized using *pseudo*ephedrine via Hypo route extracted from Allepid tablets using acid base extraction	61.80	8.44	7.40	170-172
PND-Hypo- methylamphetamine synthesized using *pseudo*ephedrine via Hypo route extracted from Panadol tablets using acid base extraction	63.40	8.30	7.50	170-173

The carbon (C), hydrogen (H) and nitrogen (N) values obtained fro the synthesised samples were within ± 5% of the theoretical values obtained for methylamphetamine.

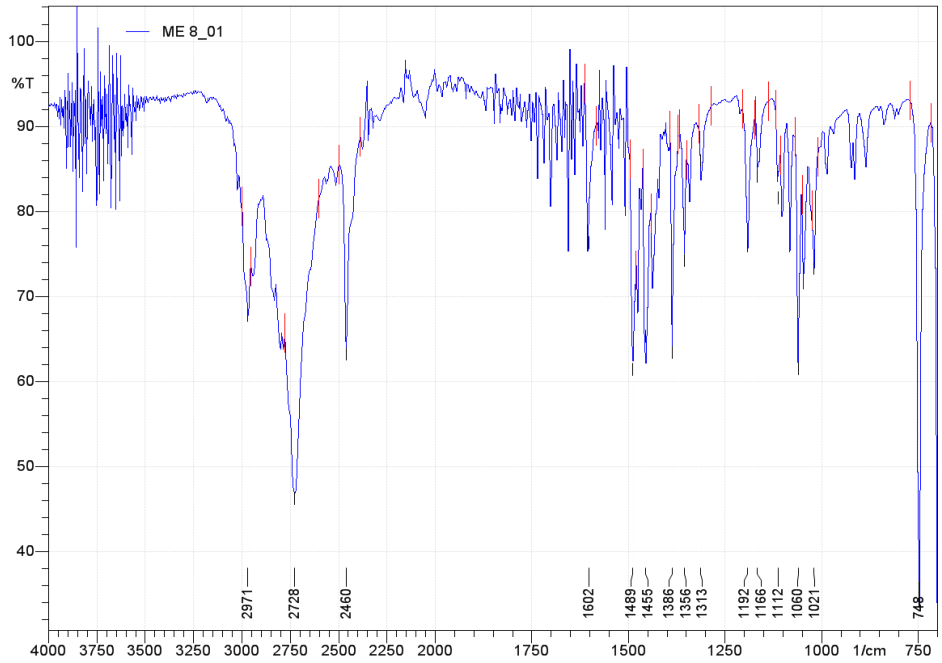


Figure 3.14. FTIR spectra of methylamphetamine synthesised via the Moscow route utilising *pseudo*ephedrine HCl extracted from Sudafed using ethanol as extraction solvent.

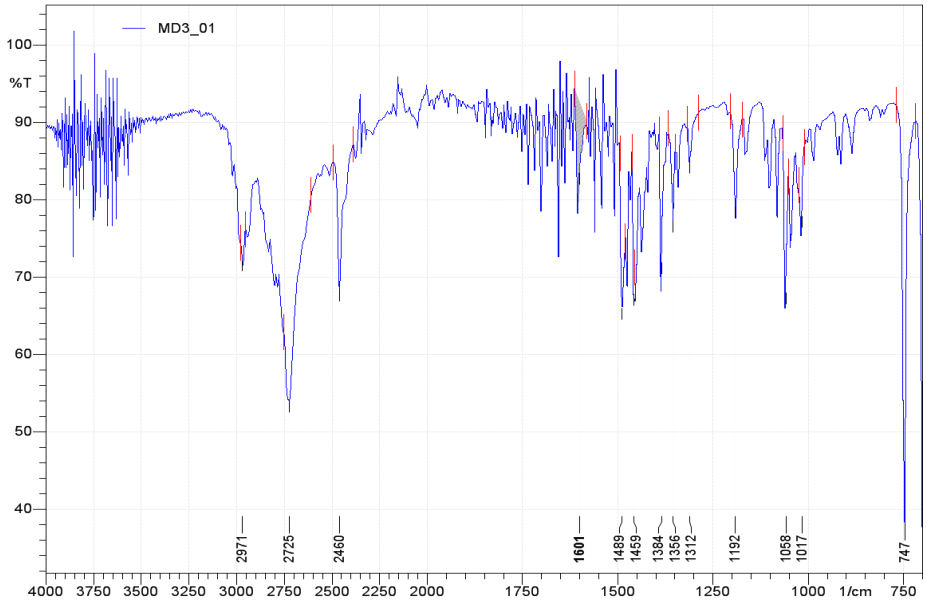


Figure 3.15. FTIR spectra of methylamphetamine synthesised via the Moscow route utlising *pseudo*ephedrine HCl extracted from Sudafed using ethanol/methanol(90:10)% vol/vol as extraction solvent.

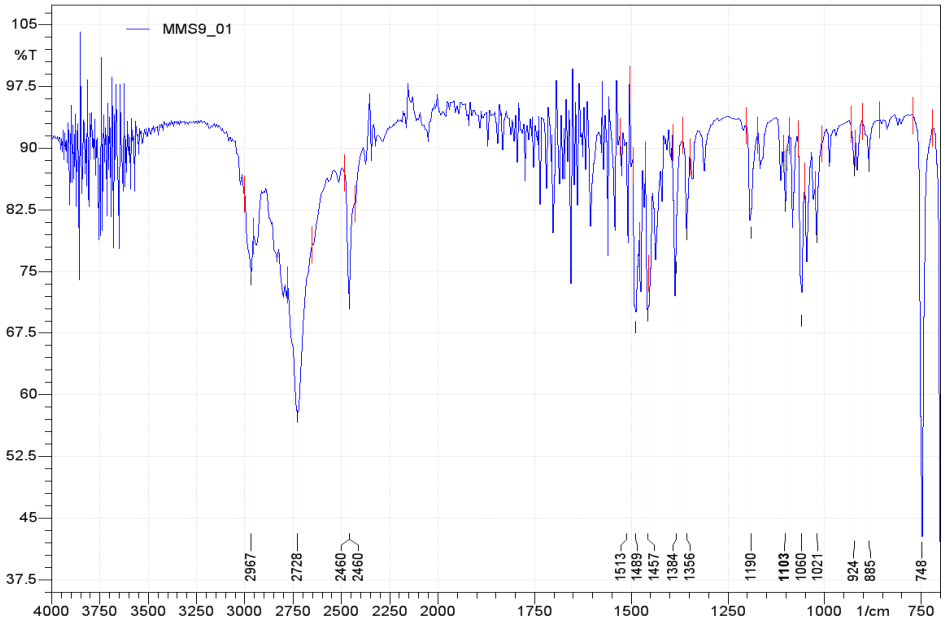


Figure 3.16. FTIR spectra of methylamphetamine synthesised via the Moscow route utlising *pseudo*ephedrine HCl extracted from Sudafed using commercial methylated spirit as extraction solvent.

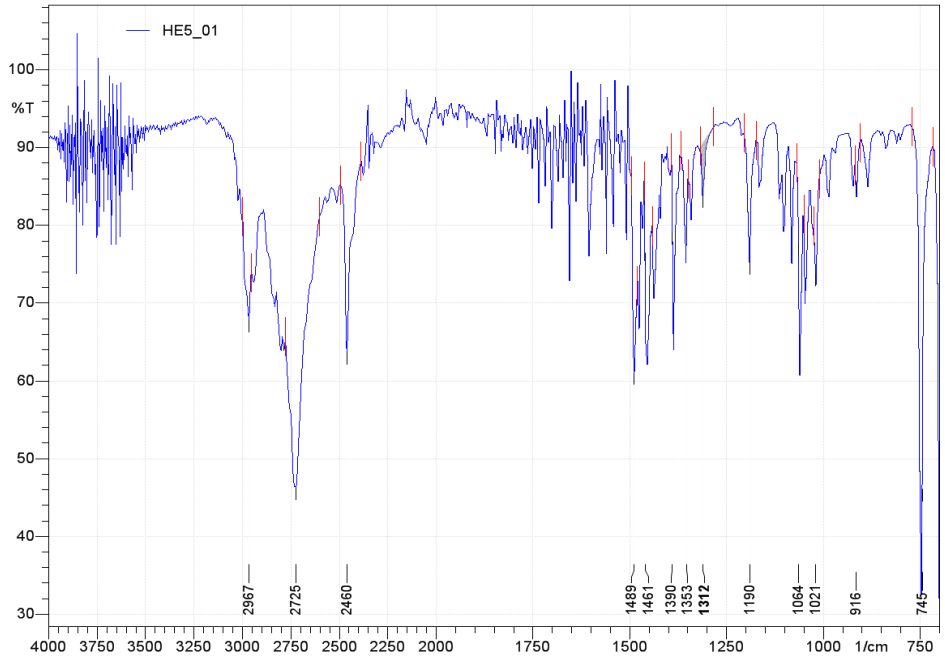


Figure 3.17. FTIR spectra of methylamphetamine synthesised via the Hypo route utlising *pseudo*ephedrine HCl extracted from Sudafed using ethanol as extraction solvent.

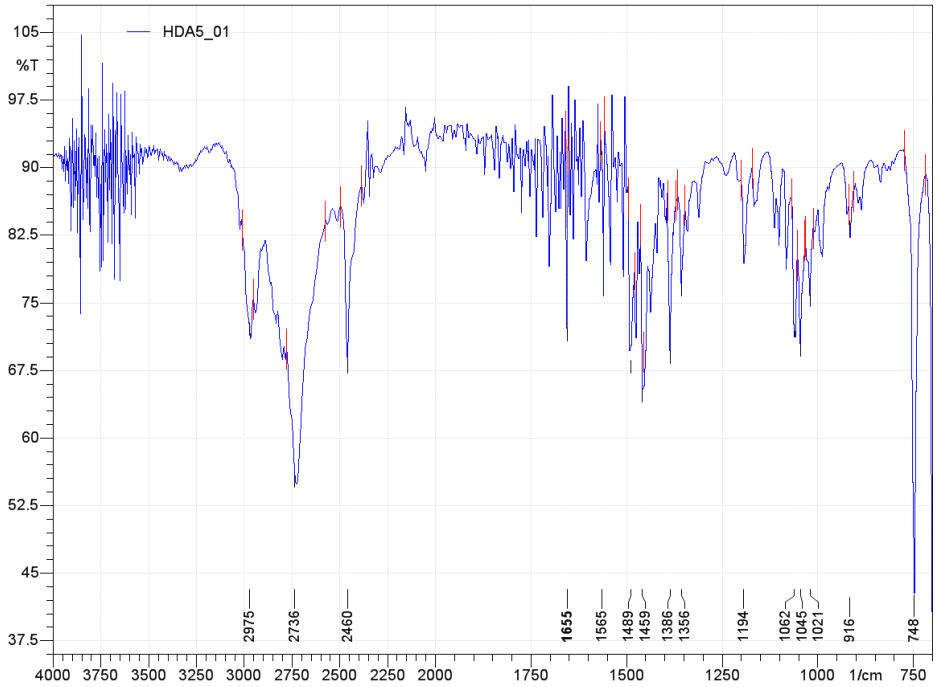


Figure 3.18. FTIR spectra of methylamphetamine synthesised via the Hypo route utlising *pseudo*ephedrine HCl extracted from Sudafed using ethanol/methanol(90:10)% vol/vol as extraction solvent.

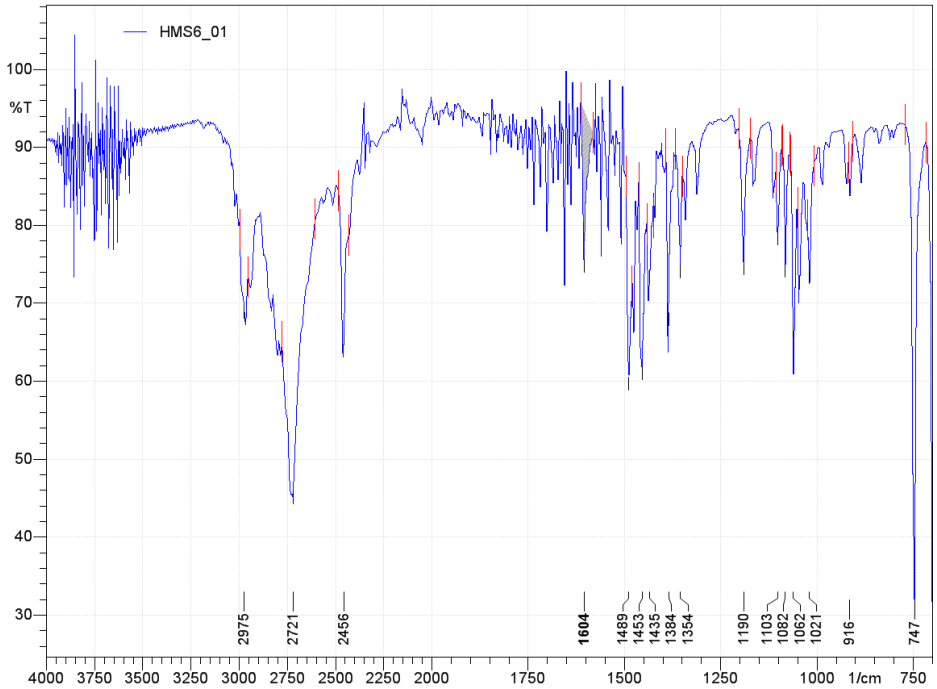


Figure 3.19. FTIR spectra of methylamphetamine synthesised via the Hypo route utlising *pseudo*ephedrine HCl extracted from Sudafed using commercial methylated spirit as extraction solvent.

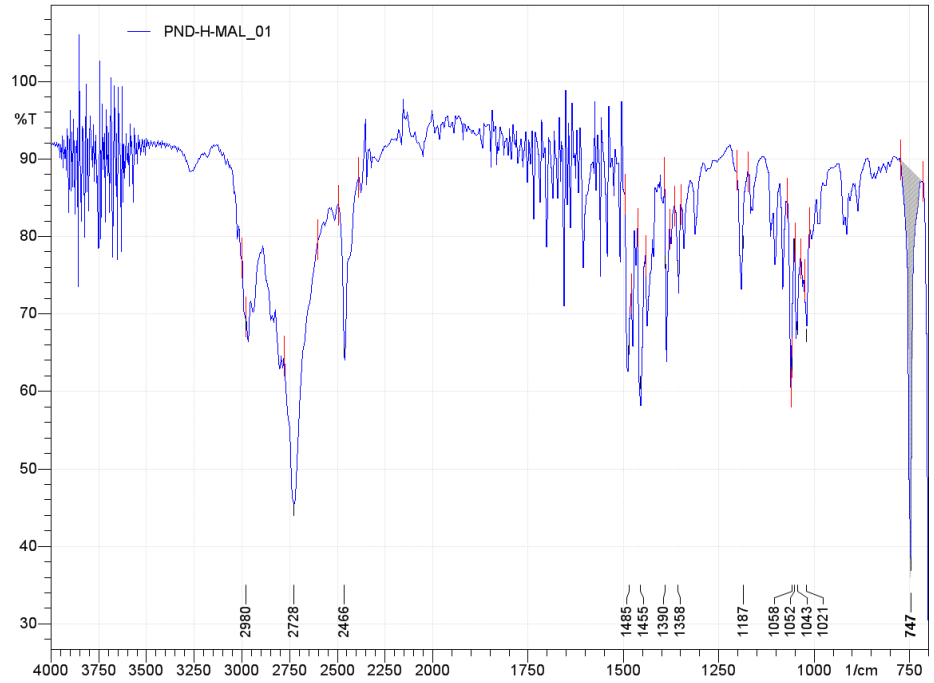


Figure 3.20. FTIR spectra of methylamphetamine synthesised via the Hypo route utlising *pseudo*ephedrine HCl extracted from Panadol using acid base as extraction.

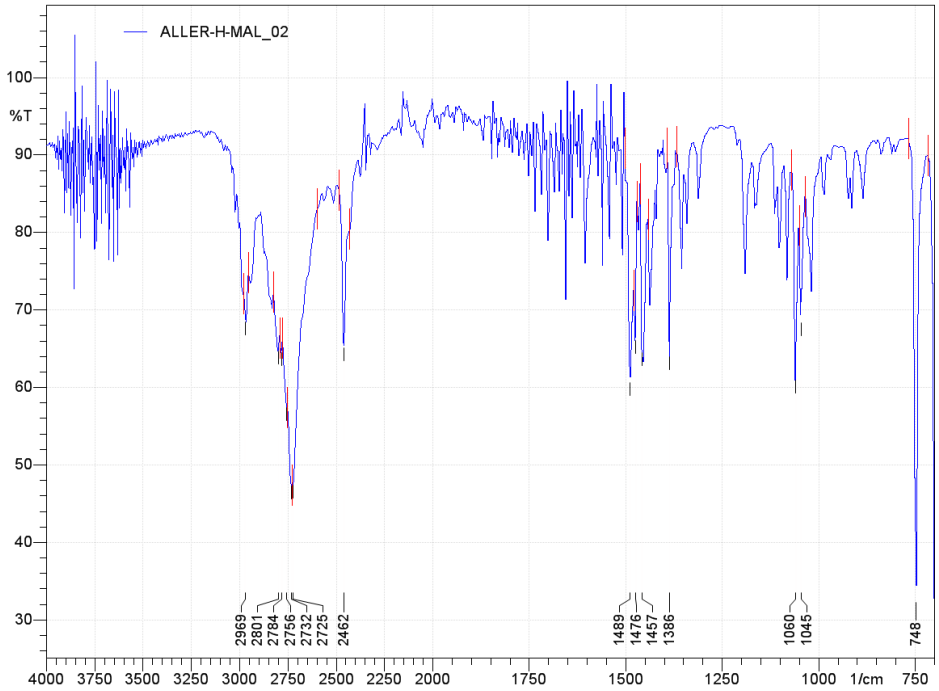


Figure 3.21. FTIR spectra of methylamphetamine synthesised via the Hypo route utlising *pseudo*ephedrine HCl extracted from Allerpid using acid base extraction.

3.4 Conclusion

*Pseudo*ephedrine hydrochloride was successfully extracted from commercially available medications using various solvents suggested in the clandestine literature (ethanol, ethanol:methanol and commercial methylated spirit). A total of 36 batches of methylamphetamine was synthesised from this precursor for each of the Hypophosphorous and Moscow synthetic routes using extracted iodine and red phosphorous. For comparison purposes, six batches of methylamphetamine were synthesised using each route using laboratory grade chemicals.

A total of five batches of methylamphetamine were prepared using *pseudo*ephedrine hydrochloride extracted from Panadol and Allerpid brand tablets from Malaysia and extracted iodine and red phosphorous. Three samples were prepared using the Hypophosphorous method (one from Panadol and two from Allerpid tablets) and the remaining two samples were prepared from Allerpid samples using the Moscow synthetic route.

3.5 References

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Chapter 4 Validation of organic impurity extraction and gas chromatography mass spectrometry (GCMS)

4.1 Introduction

Optimised impurity extraction and gas chromatography mass spectrometry (GCMS) methods are essential to the chromatographic profiling of route specific impurities inherent within clandestinely produced methylamphetamine samples. The aim is to identify a method that efficiently and reproducibly extracts the maximum number of impurities and GCMS conditions that will produce chromatograms with well resolved peaks.

Validation of extraction systems and chromatographic systems can be undertaken in a number of ways. These can include the development and validation of new methods suited for a particular purpose, or the adaptation of existing methods and assuring that they achieve the requirements for validation for a particular analysis. The latter approach is used here and partial validation of an existing GCMS analysis is described. Of importance is the identification of impurities rather than the quantification of samples and, as such, certain aspects normally associated with validation such as limit of detection or limit of quantification are not addressed.

Currently, two of the most common GCMS methods used in the organic impurity profiling of methylamphetamine are the CHAMP method and the method proposed by Tanaka *et al*. [1] and Inoue *et al*. [2] and confirmed by Kunalan *et al*. [3]. Studies conducted by Kunalan *et al.* [3] identified that all route specific impurities for methylamphetamine synthesized from seven different routes could be elucidated through a basic extraction of the synthesised sample followed by GCMS analysis using a DB-5 column, equivalent to a HP5-MS column. This was a considerable improvement on previous work which reported that both an acidic and basic extraction were required [4]. As a consequence the GCMS method used by Kunalan *et al*. [3] has been adopted for this work [3].

The quality and performance or deterioration of a GC column can be monitored during use using a Grob mixture [5, 6]. A mixture of acids, bases, alcohols, hydrocarbons and neutral compounds was suggested by Grob *et al*. [5] as a single test mixture for capillary columns.

4.2 Experimental Methods

The chemicals used were reagent grade unless stated otherwise. Manufacturers were as follows: hexane by Rathburn; methyl decanoate ester, 1-octanol, potassium phosphate monobasic (KH_2PO_4), sodium phosphate dibasic dehydrate ($Na_2HPO_4.2H_2O$) from Fluka; ethyl acetate, dicyclohexylamine, 2,6-dimethylaniline, 2,6-dimethylphenol, dodecanoate, eicosane, methyl undecanoate ester, tetracosane and tridecanoate from Sigma Aldrich; glacial acetic acid from Riedel de Haen. Tridecane was decanted from stock within the university and the manufacturer was not available.

Other apparatus used were a Philips PW9421 pH meter, a Fisons Whirlmixer vortex, an American Beauty S/70 sonicator, an Edmund Buhler Swip KS-10 rotative shaker, and a Jouan centrifuge. Distilled water was obtained from an in house water purification system.

Glassware was washed with Teepol and then rinsed with acetone and dried. Samples prepared for gas chromatography mass spectrometry analysis were transferred to 250 µL silanised microvial inserts (Agilent part no.5181-8872) inside non-deactivated vials with PFTE/silicone septa screw caps (Agilent part no:5183-4428).

4.3 Instrumental Parameters

The analysis was carried out using an Agilent 6850 gas chromatograph (GC) coupled to a 5975C VL MSD (Triple –Axis Detector). A HP-5MS column (30 m length x 0.25 mm internal diameter, 0.25 mm µm film thickness) was used. Analysis was undertaken with the oven temperature programmed as follows: 60^{o}C for 1 minute, 10^{o}C/minute to 300^{o}C and then held at 300^{o}C for 10 minutes. The injector and detector temperatures were set

at 250^{o}C and 300^{o}C respectively [3]. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/minute. Injection of 1µL of sample was made in the splitless mode (purge on time; 1.0 minutes). Data were acquired at a rate of 20 Hz and a peak width of 0.05 minutes. Hewlett Packard HP3365 Chemstation software was used for controlling the GCMS system, data acquisition and integration of the gas chromatograms.

The mass spectrometer (MS) was tuned weekly using the tuning compound Perfluorotributylamine (PFTBA) and an air and water check was performed daily, column performance was monitored using a Grob mixture over approximately six weekly cycles, and solvent blanks were run between sample injections (unless indicated otherwise). Peaks were integrated using the total ion chromatogram.

4.4 Preparation and Analysis of Grob Mixture

A modified Grob test mixture was prepared to test column performance using the following components:

1. 1-octanol
2. 2,6-dimethylphenol
3. 2,6-dimethylaniline
4. dodecane, C12
5. tridecane, C13
6. methyl decanoate ester
7. methyl undecanoate ester
8. dicyclohexylamine
9. eicosane, C20
10. tetracosane,C24

Each compound was weighed (40 mg) into clean 5 mL volumetric flasks and filled to the mark with hexane to give a stock solution of concentration 8 mg/mL. A volume of each stock solution (5 µL) was removed and combined in one 5 mL volumetric flask which was filled to the mark with hexane. This resulted in a Grob mixture of ten components, each at a concentration of 8 µg/mL. This concentration was used so that, theoretically, a

1 µL injection would result in 8 ng of analyte on column [5, 6]. Six repeat injections were performed to establish instrument repeatability and peak symmetry.

4.4.1 Preparation of phosphate buffer

0.1 M of phosphate buffer was prepared by combining KH_2PO_4 (1.36 g) and $Na_2HPO_4.{}_2H_2O$ (1.78 g) in a 100 mL volumetric flask and filling to the mark with distilled water. This solution was made to pH 10.5 by adding sodium carbonate [7].

4.4.2 Sample preparation

Varying amounts of homogenised and unhomogenised methylamphetamine hydrochloride (50 mg, 100 mg and 150 mg) were placed in separate centrifugation tubes and dissolved in phosphate buffer (2.0 mL). The mixture was sonicated for 5 minutes and vortexed for 1 minute. 400 µL of extraction solvent (ethyl acetate, hexane or toluene) containing eicosane as an internal standard (0.05 mg/mL) was added and the sample centrifuged for a further 5 minutes. The organic layer was transferred to a gas chromatograph vial insert for analysis [3, 4, 8]. The stability of the extracts in each of these solvents over a period of 0, 1 and 2 days were also investigated. Extracts were stored at room temperature and in the refrigerator (5^oC) and repetitively analysed to assess any alterations in peak area.

4.5 Results and Discussion

4.5.1 Peak symmetry and repeatability of analysis

The Grob sample was used to assess the repeatability of the GCMS in the analysis of similar samples to those expected in the target impurity profiles. This sample was also used to assess the peak symmetry. Peak symmetry is used in determining column efficiency. The asymmetry factor, A, can be calculated according to Equation 4.1:

$$A = b/a$$ **Equation 4.1**

Where:

a and *b* are the left and right halves of the peak width at 10% peak height.

If A is greater than one, the peak is said to be tailing, if this occurs, components are strongly retained on the stationary phase and lag behind the main body of the component band. When A is less than one, peak fronting occurs. This happens when components are retained to a lesser extent and elute before the main body of the component band.

A typical chromatogram of the Grob mixture is presented in Figure 4.1. The measurement for peak symmetry for the components in the Grob mixture was made by hand and are presented in Table 4.1.

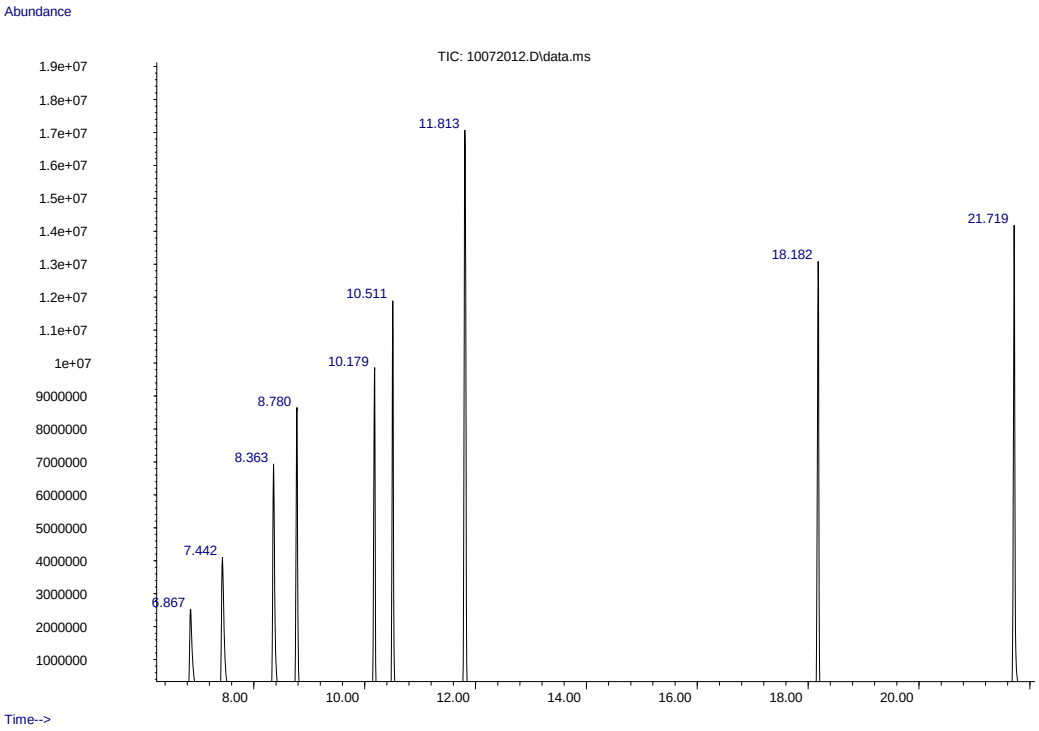

Figure 4.1. Chromatogram of the Grob mixture used.

Table 4.1. Peak Symmetry of the individual Grob mixture components.

Peak	Components	Asymmetry Factor
1	1-Octanol	1.33
2	2,6-dimethylphenol	1.00
3	2,6-dimethylaniline	1.00
4	Dodecane,C12	1.23
5	Tridecane, C13	1.11
6	Methyl decanoate ester	1.43
7	Methyl undecanoate ester	1.00
8	Dicyclohexylamine	1.00
9	Eicosane, C20	1.00
10	Tetracosane, C24	1.00

The analysis of the Grob mixture was also used to assess repeatability. The results obtained across six repetitive analysis of the same Grob sample were compared and the relative standard deviation (RSD) of the peak areas calculated. For repeatability, a value of less than 5% was chosen to indicate the reliability of the analysis. The results are provided in Table 4.2.

Table 4.2. Relative standard deviation (RSD) of the Grob Mixture over 6 repeat injections.

Peak	Components	RSD (Peak Area)
1	1-Octanol	3.31%
2	2,6-dimethylphenol	3.40%
3	2,6-dimethylaniline	3.59%
4	Dodecane,C12	3.54%
5	Tridecane, C13	2.63%
6	Methyl decanoate ester	3.74%
7	Methyl undecanoate ester	1.80%
8	Dicyclohexylamine	2.27%
9	Eicosane, C20	0.88%
10	Tetracosane, C24	2.89%

4.5.2 Impurity extraction

Sample extraction plays an important role in impurity profiling analysis and is pH dependent. pH buffers used are selected for their ability to 'push' the impurities of the methylamphetamine sample out of the aqueous phase and into an organic phase [3, 7**]**. Two common buffers, a phosphate buffer and an acetate buffer have been used for the majority of methylamphetamine profiling [1, 2, 3, 7]. In this study, the impurity extraction of methylamphetamine has been carried out using the phosphate buffer (pH 10.5) only, following the work of Kunalan *et al*. **[**3, 7**]**.

Three specific issues arise during impurity extraction. Firstly the ability of a given solvent to extract sufficient quantity of the impurities with good repeatability and reproducibility, secondly the weight of sample required and finally the nature of the sample (homogenised or unhomogenised). The pH used for this work was pH 10.5 as reported by Kunalan *et al*. [3]. Three solvents (ethyl acetate, toluene and hexane) and three weights of sample (50 mg, 100 mg and 150 mg) were evaluated based upon previous study. Exemplars of the chromatograms obtained for each solvent-weight combination are presented in Figure 4.2 to Figure 4.10. Table 4.3 to Table 4.6 indicate the impurity profiles produced in each case. The tables show the data for the first 10 peaks in the impurity profile and indicate the total number of peaks present in the overall profile.

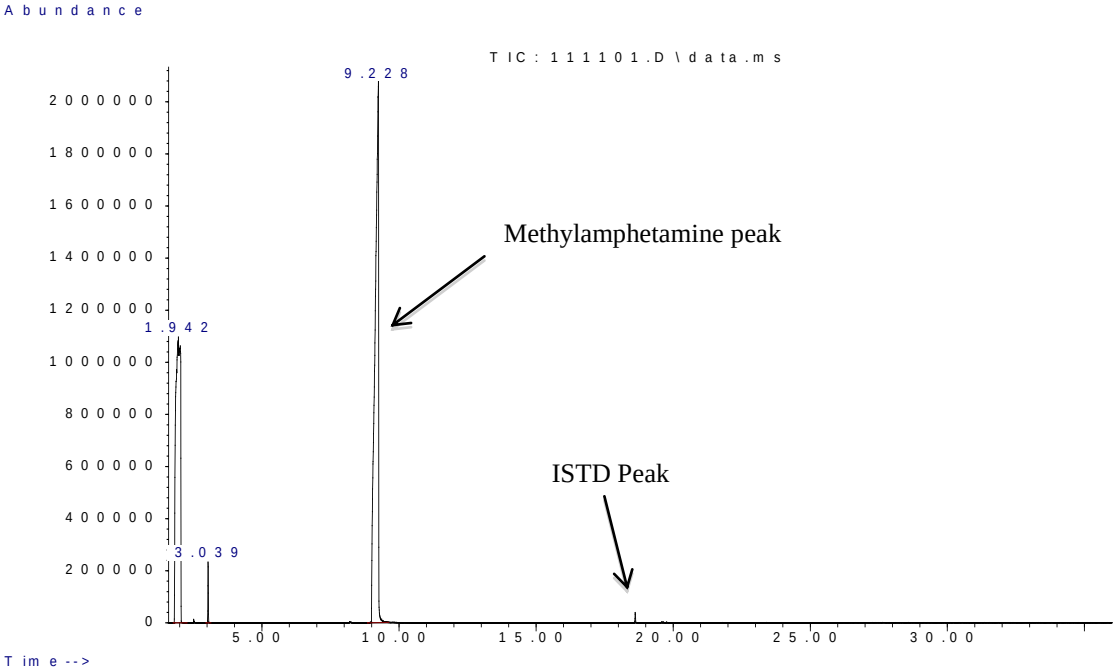

Figure 4.2. Impurity profile of 50 mg of methylamphetamine extracted at pH 10.5 with ethyl acetate as extraction solvent.

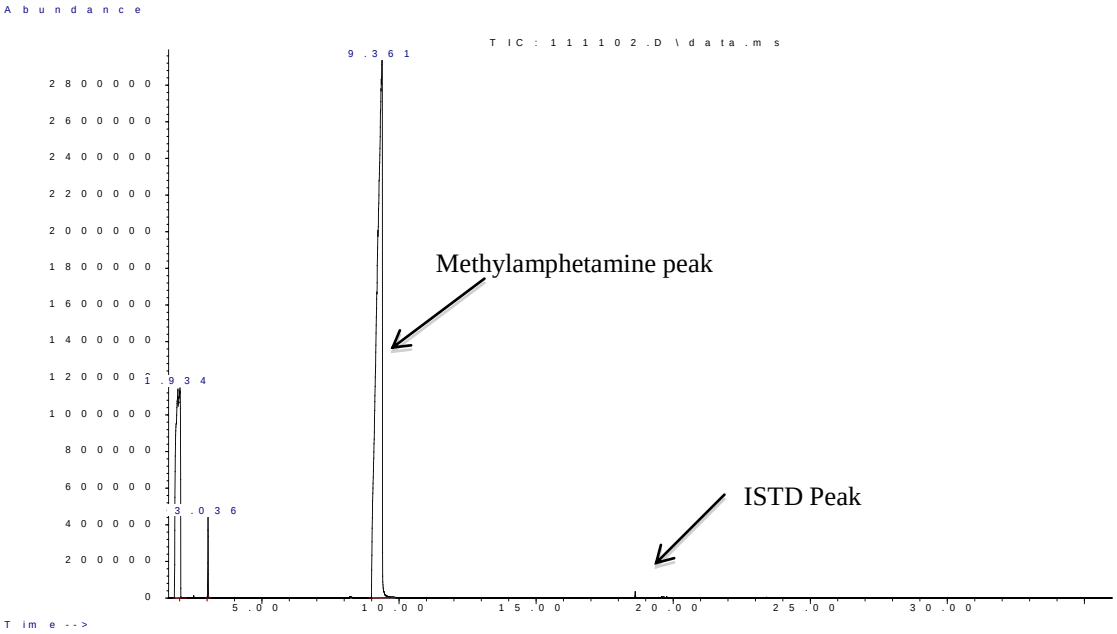

Figure 4.3. Impurity profile of 100 mg of methylamphetamine extracted at pH 10.5 with ethyl acetate as extraction solvent.

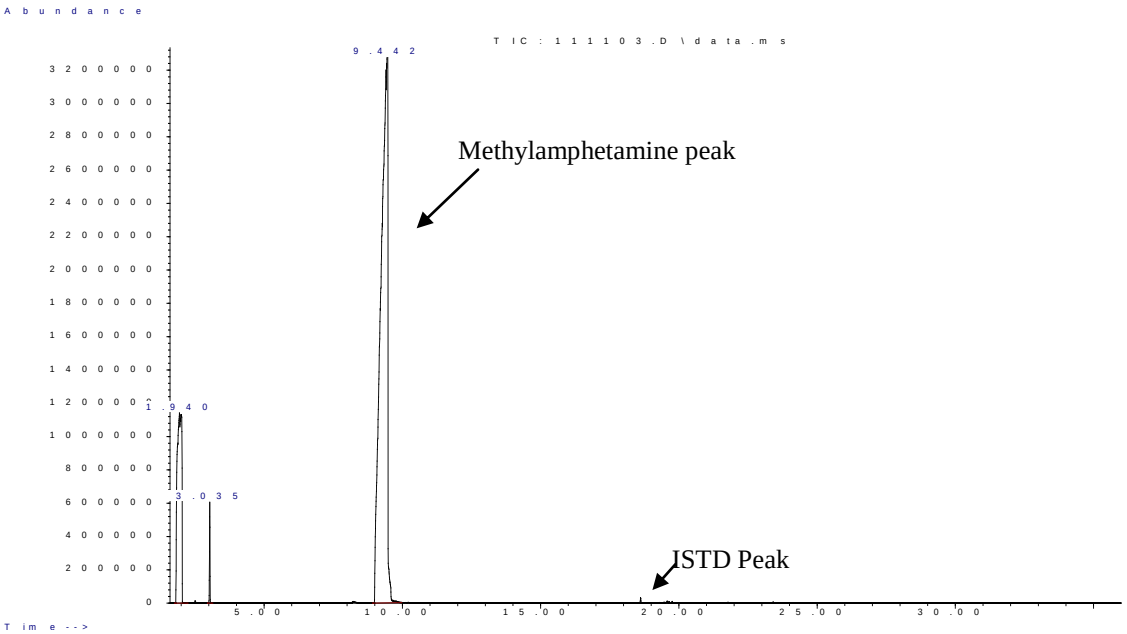

Figure 4.4. Impurity profile of 150 mg of methylamphetamine at pH 10.5 with ethyl acetate as extraction solvent.

Table 4.3. RSD and normalized (to the internal standard [IS] and total peak area [TPA]) RSD values of the varying mass of methylamphetamine for impurity profiling analysis using ethyl acetate as extraction solvent at pH 10.5.

	50 mg			100 mg			150 mg		
Peak	**% RSD**	**% RSD (IS)**	**% RSD (TPA)**	**% RSD**	**% RSD (IS)**	**% RSD (TPA)**	**% RSD**	**% RSD (IS)**	**% RSD (TPA)**
1	4.3	3.8	1.30	2.4	8.2	2.4	25.7	27.1	21.7
2	19.3	18.2	16.9	18.1	18.6	17.7	29.0	14.2	10.7
3	16.6	14.4	11.1	2.5	1.7	2.9	78.2	4.0	60.5
4	7.9	4.2	3.6	4.0	3.9	4.3	97.5	94.1	91.0
5	10.4	4.1	4.3	5.0	3.4	3.3	41.5	30.8	17.7
6	19.8	17.8	18.2	1.2	0.9	0.3	27.6	15.7	24.8
7	156.3	124.4	123.4	24.9	23.0	24.1	40.9	35.6	2.7
8	3.0	3.0	3.0	5.7	7.2	7.5	58.9	48.8	31.5
9	16.7	11.1	13.5	15.7	12.4	13.8	29.0	27.1	23.3
10	18.0	12.7	3.4	5.5	1.8	5.0	15.8	14.1	14.8
Ave	**27.2**	**3.8**	**19.9**	**8.5**	**8.1**	**8.1**	**44.4**	**37.2**	**29.9**
Total Peaks		**45**			**75**			**85**	

The 100 mg methylamphetamine sample was found to produce the best peak shape and the greatest number of detectable impurities with reasonable reproducibility based on the average RSD peak area values obtained compared to the other sample weights.

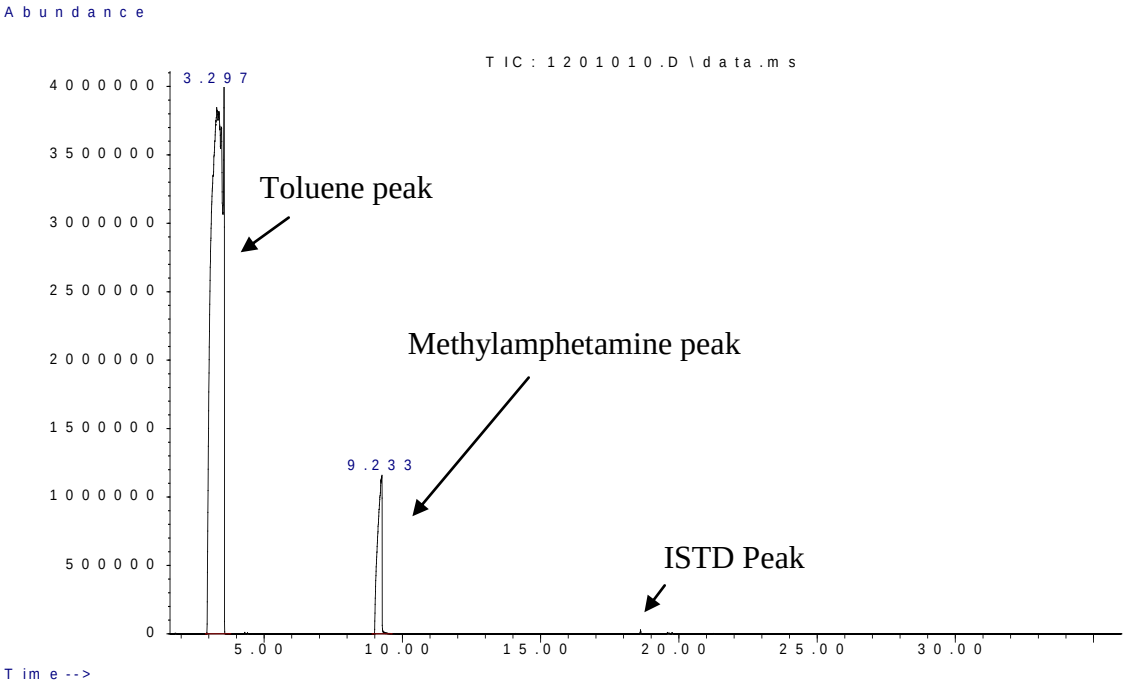

Figure 4.5. Impurity profile of 50 mg of methylamphetamine extracted at pH 10.5 with toluene as extraction solvent.

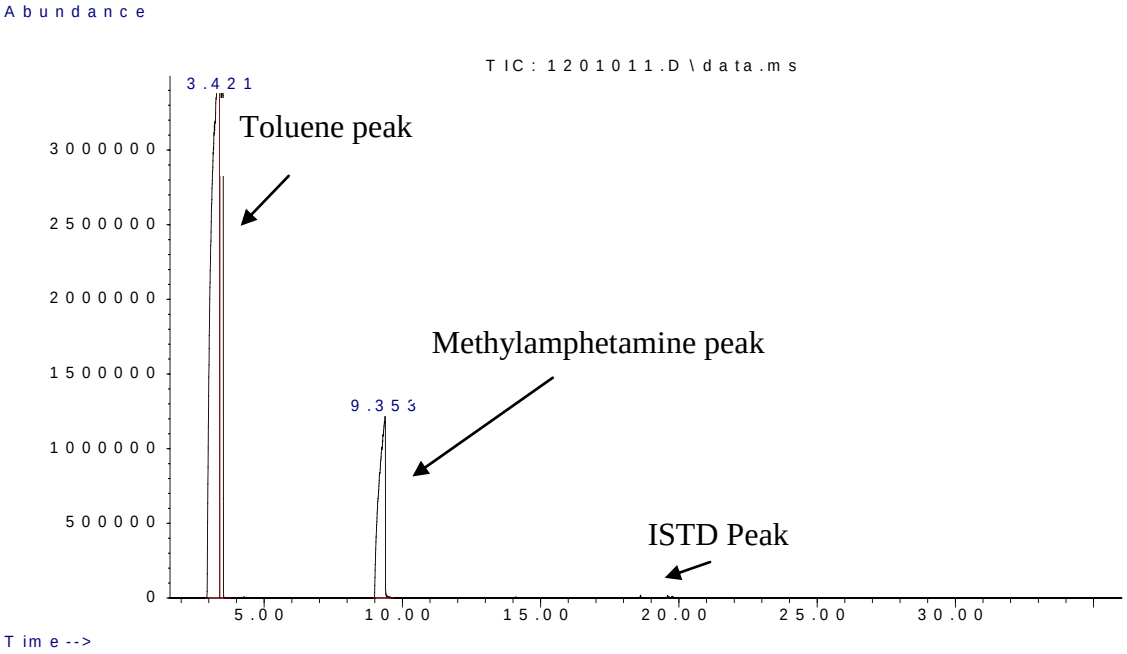

Figure 4.6. Impurity profile of 100 mg of methylamphetamine extracted at pH 10.5 with toluene as extraction solvent.

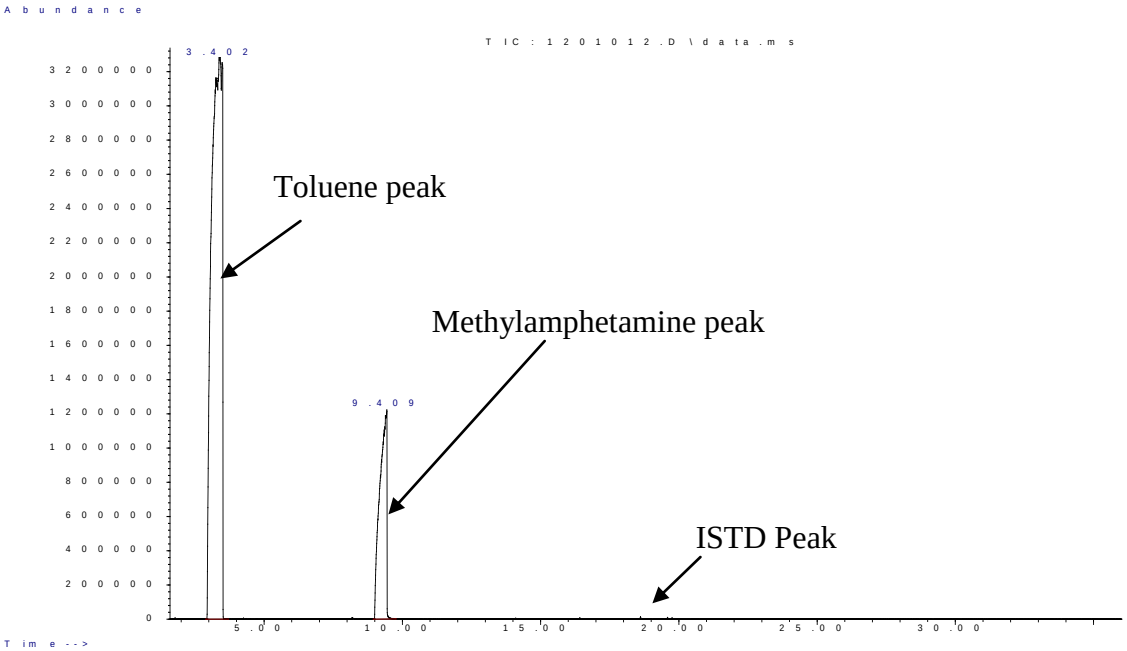

Figure 4.7. Impurity profile of 150 mg of methylamphetamine extracted at pH 10.5 with toluene as extraction solvent.

Table 4.4. RSD and normalized (to the internal standard[IS] and total peak area[TPA]) RSD values of the varying mass of methylamphetamine for impurity profiling analysis using toluene as extraction solvent at pH 10.5.

	50 mg			100 mg			150 mg		
Peak	**% RSD**	**% RSD (IS)**	**% RSD (TPA)**	**% RSD**	**% RSD (IS)**	**% RSD (TPA)**	**% RSD**	**% RSD (IS)**	**% RSD (TPA)**
1	24.9	24.1	38.0	112.5	111.5	110.4	143.3	125.1	123.5
2	48.9	46.2	36.3	175.4	145.5	145.5	110.3	110.7	141.9
3	82.1	73.4	60.1	116.2	112.4	113.8	16.7	14.8	14.7
4	44.9	31.6	28.9	167.2	13.6	12.2	103.0	100.1	116.2
5	41.2	40.0	32.9	15.2	7.9	9.8	244.9	240.7	236.7
6	10.7	7.05	6.3	24.9	24.2	23.5	104.2	94.9	92.8
7	78.9	68.8	60.9	156.5	124.4	122.5	211.6	193.7	184.1
8	5.6	5.0	4.8	44.9	42.6	39.9	9.3	2.6	3.3
9	17.8	16.7	15.7	14.5	12.5	14.9	5.9	7.4	5.7
10	25.7	21.8	20.5	44.7	42.2	43.3	5.3	4.9	6.1
Ave	**38.1**	**33.5**	**30.5**	**87.2**	**63.7**	**63.6**	**95.4**	**89.5**	**92.4**
Total Peaks		**25**			**60**			**55**	

The 50 mg methylamphetamine sample extracted with toluene was found to produce the best peak shape and the greatest number of detectable impurities with reasonable reproducibility.

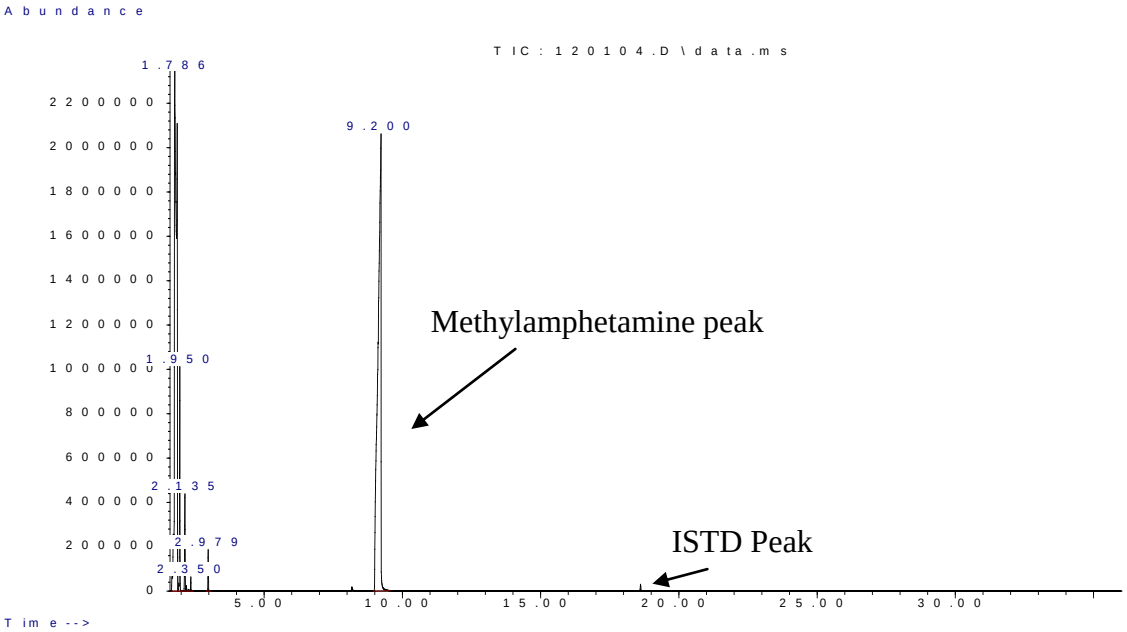

Figure 4.8. Impurity profile of 50 mg of methylamphetamine extracted at pH 10.5 with hexane as extraction solvent.

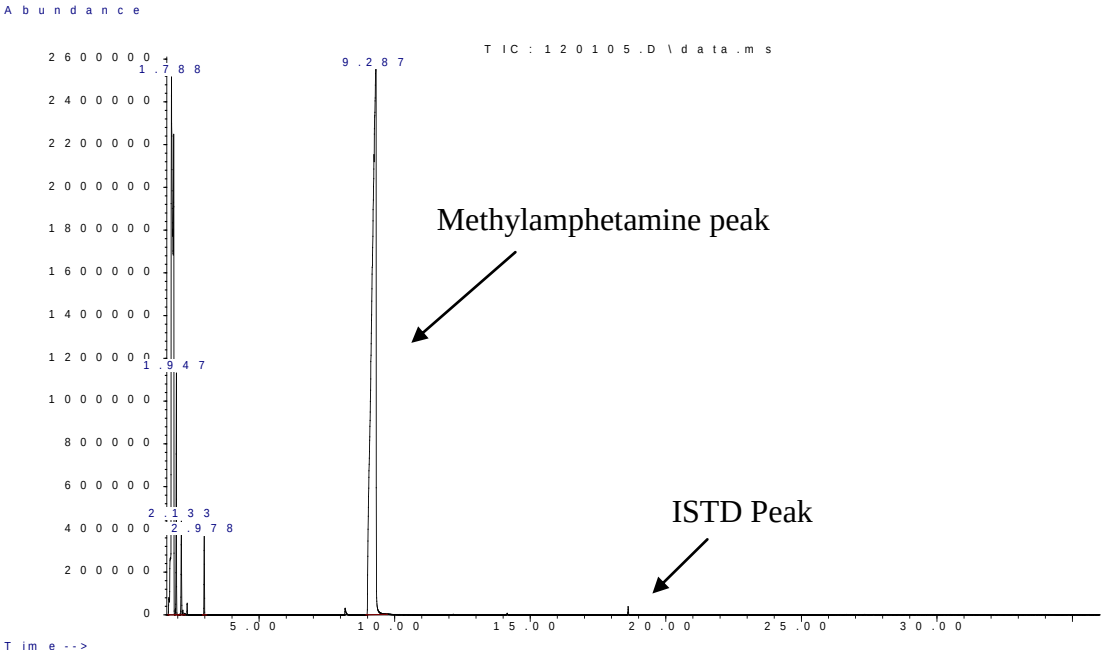

Figure 4.9. Impurity profile of 100 mg of methylamphetamine extracted at pH 10.5 with hexane as extraction solvent.

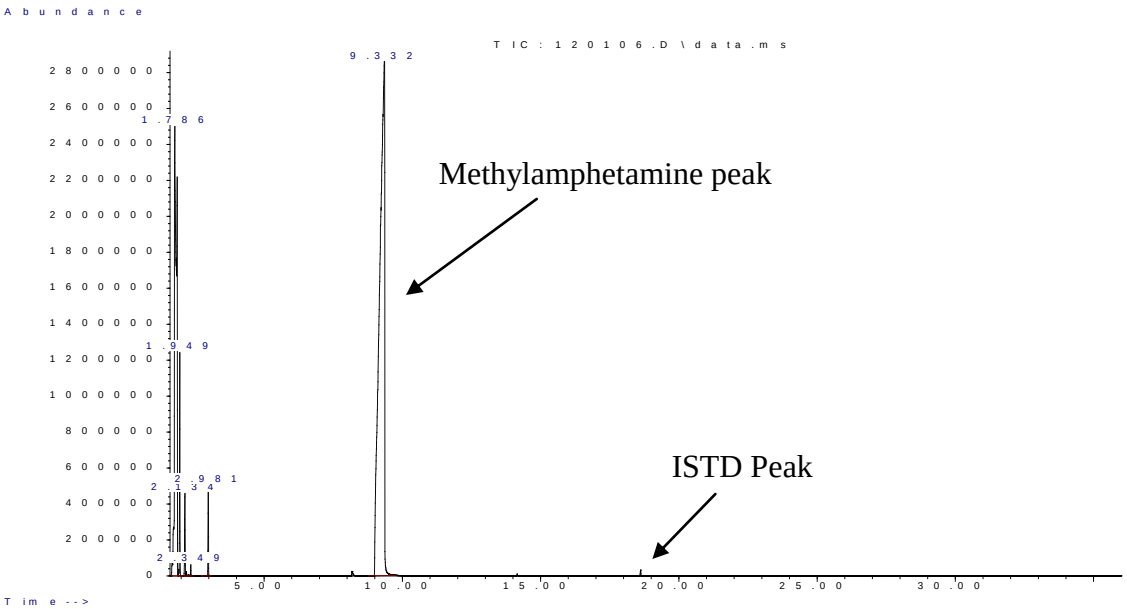

Figure 4.10. Impurity profile of 150 mg of methylamphetamine extracted at pH 10.5 with hexane as extraction solvent.

Table 4.5. RSD and normalized (to the internal standard[IS] and total peak area[TPA]) RSD values of the varying mass of methylamphetamine for impurity profiling analysis using hexane as extraction solvent at pH 10.5.

	50 mg			100 mg			150 mg		
Peak	**% RSD**	**% RSD (IS)**	**% RSD (TPA)**	**% RSD**	**% RSD (IS)**	**% RSD (TPA)**	**% RSD**	**% RSD (IS)**	**% RSD (TPA)**
1	28.0	18.1	16.3	19.6	19.1	12.9	27.2	26.8	24.2
2	25.5	22.4	23.8	16.8	9.7	8.7	45.1	43.3	44.4
3	35.3	31.4	32.2	25.8	15.5	13.0	56.1	54.5	51.5
4	45.7	40.2	43.3	11.6	13.5	18.8	7.5	4.1	3.4
5	19.0	17.2	9.7	17.3	11.2	14.2	8.4	8.5	6.8
6	29.9	28.5	24.8	25.2	17.7	9.9	13.2	12.7	11.6
7	29.3	27.0	16.2	23.6	10.9	18.2	29.2	26.5	23.2
8	27.6	27.4	10.8	21.5	24.5	31.5	8.0	5.3	4.8
9	28.8	22.8	22.4	18.7	9.4	10.6	27.4	28.1	24.3
10	17.3	13.6	13.9	24.0	16.7	14.8	38.4	35.6	32.3
Ave	**28.6**	**24.8**	**21.3**	**20.4**	**14.7**	**15.2**	**26.0**	**24.5**	**22.6**
Total Peaks		**30**			**60**			**80**	

The 100 mg methylamphetamine sample extracted in hexane was found to produce the best peak shape and the greatest number of detectable impurities with reasonable reproducibility.

From results obtained and shown in Table 4.3 to Table 4.6 the 100 mg methylamphetamine sample using ethyl acetate has effectively extracted a greatest number of impurity peaks, while maintaining a relatively low relative standard deviation (75 impurity peaks and %RSD of 8.5 for the first ten impurities).

4.5.3 Homogeneity of Samples studies

Homogenised and non homogenised 100 mg samples of methylamphetamine were extracted using ethyl acetate. The impurity profile data for the ten peaks in the chromatorgam is presented in Table 4.6 to Table 4.8 clearly demonstrates better relative standard deviations for the homogenized samples.

Table 4.6. Results of unnormalised values unhomogenised and homogenised batches of methylamphetamine.

Unhomogenised methylamphetamine		**Homogenised methylamphetamine**	
Peak	**RSD Area**	**Peak**	**RSD Area**
1	76.2	1	25.6
2	110.5	2	18.9
3	45.4	3	8.2
4	118.9	4	27.4
5	68.9	5	11.4
6	23.6	6	17.5
7	38.9	7	6.6
8	11.8	8	7.8
9	28.9	9	10.9
10	6.7	10	3.4
Average Values	**52.92**	**Average Values**	**13.82**

Table 4.7. Results of normalized values to internal standard(IS) of unhomogenised and homogenised batches of methylamphetamine.

Unhomogenised methylamphetamine		Homogenised methylamphetamine	
Peak	**RSD Area**	**Peak**	**RSD Area**
1	73.3	1	20.1
2	108.8	2	12.1
3	40.8	3	6.9
4	112.7	4	22.1
5	64.7	5	10.1
6	21.8	6	13.7
7	32.7	7	5.6
8	9.8	8	7.7
9	24.9	9	9.8
10	4.7	10	2.4
Average Values	**49.4**	**Average Values**	**11.9**

Table 4.8. Results of normalized to total peak area (TPA) values unhomogenised and homogenised batches of methylamphetamine.

Unhomogenised methylamphetamine		Homogenised methylamphetamine	
Peak	**RSD Area**	**Peak**	**RSD Area**
1	70.7	1	21.7
2	111.8	2	13.7
3	41.9	3	6.5
4	110.8	4	23.0
5	63.7	5	10.7
6	20.8	6	14.8
7	30.6	7	5.7
8	8.8	8	7.5
9	23.6	9	10.3
10	4.2	10	3.3
Average Values	**48.7**	**Average Values**	**11.7**

4.5.4 Reproducibility of the Extraction Analytical Method

4.5.4.1 Within Day Reproducibility

The within day reproducibility of the analysis was evaluated by preparing, in parallel, six separate extractions from six homogenized sub samples of 100 mg each from the same batch of methylamphetamine. Extractions and analysis were undertaken on the same day and the data is presented in Table 4.9 for 10 prominent impurity peaks within each sample.

Table 4.9. Results of within day reproducibility studies.

Peak	% RSD	% RSD (Normalised to int. standard)	% RSD (Normalised to total peak area)
1	21.2	21.4	21.6
2	26.4	26.7	27.2
3	16.2	12.9	14.7
4	20.9	19.3	16.8
5	25.4	25.4	25.3
6	4.5	3.1	5.0
7	12.9	11.7	11.1
8	24.4	20.0	21.6
9	18.9	**1**7.1	16.0
10	3.4	2.9	2.8
Ave Value	**17.4**	**16.1**	**16.2**

The data obtained for a within a day reproducibility takes into account the instrumental precision. The best average RSDs obtained for the within day reproducibility study of methylamphetamine was 16.1 and is in line with the published literature for similar samples [8, 9, 10].

4.5.4.2 Reproducibility of the Analysis over time

External environmental conditions such as humidity and temperature can have adverse effects on the extraction process of methylamphetamine samples, thus the reproducibility of the analysis over time was also investigated. The stability of the extract over three days was assessed by preparing two extracts from the same homogenised batch of methylamphetamine and analyzing the samples over a period of three days. The extracts were stored under different conditions (one at room temperature in the dark and a second at 5^{o}C) and the peak areas for the ten components were plotted against time. The results are shown in Figure 4.11 and Figure 4.12.

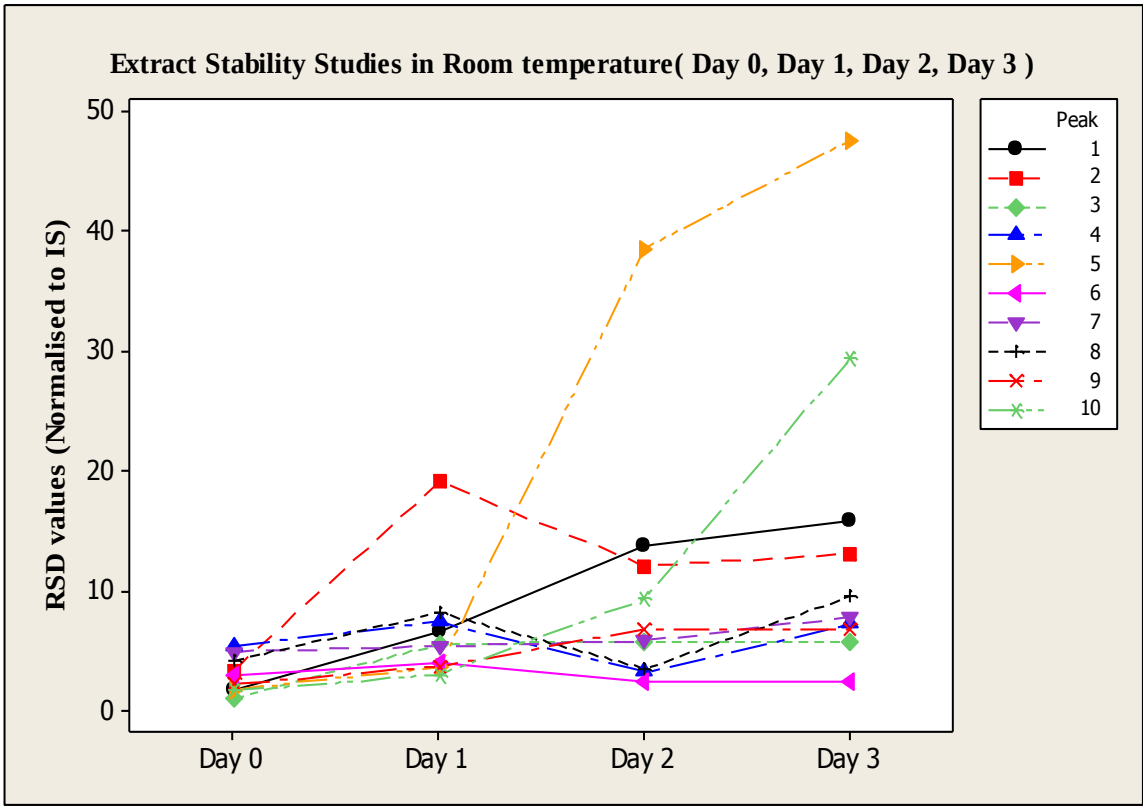

Figure 4.11. Methylamphetamine extract stability (pH 10.5) over three days stored at room temperature (20^{o}C).

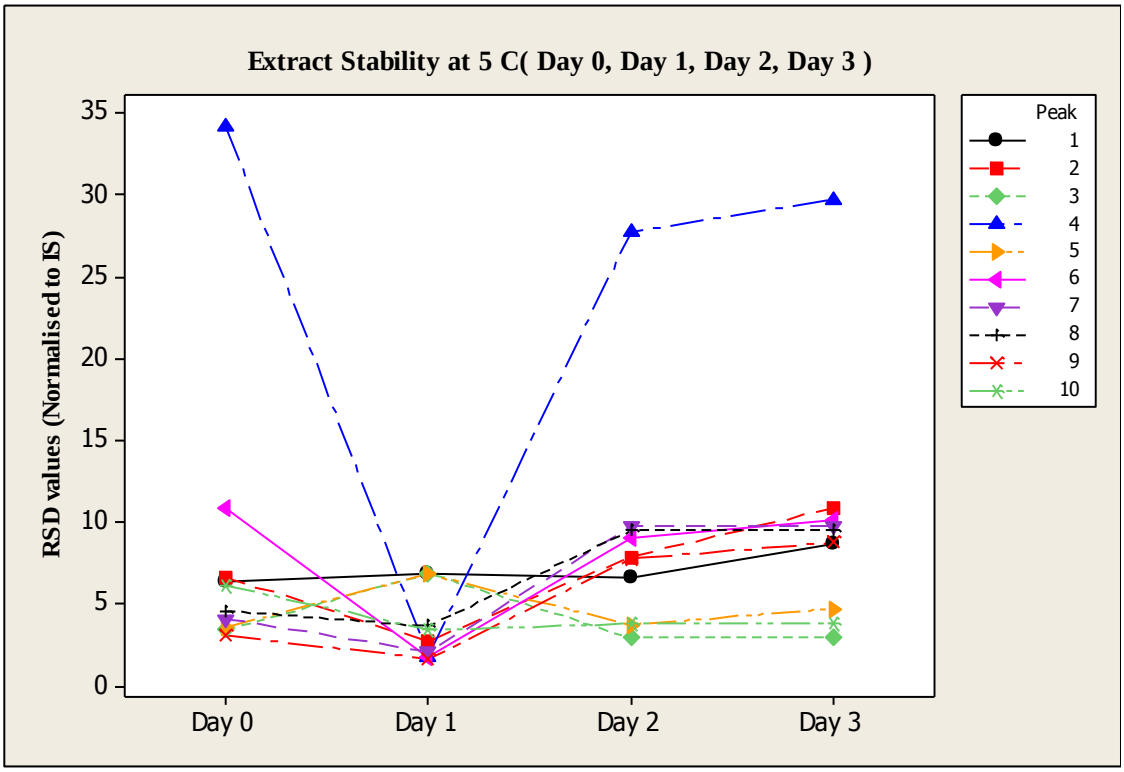

Figure 4.12. Methylamphetamine extract stability (pH 10.5) over three days stored at 5°C.

It can be seen that the concentrations of the selected components vary from each other even after one day. For instance, peaks 2, 5, 4 and 10 change dramatically over the time frame studied. There were also differences apparent within the sample extracts stored at 5°C. This further illustrates that storing the extract at a cooler temperature does not increase extract stability. As a consequence extracts were analysed on the same day of preparation.

4.6 Conclusions

The impurity extraction method was evaluated and best results were obtained using 100 mg of a homogenized sample extracted using a phosphate buffer (pH 10.5) with ethyl acetate as the extraction solvent. Samples were extracted and analysed on the same day using a HP5-MS column; the oven temperature started at 50^{o}C for 1 minute and then increased at 10^{o}C/min until 300^{o}C, where it was held for 10 minutes; the injector and detector temperatures were set at 250^{o}C and 300^{o}C respectively; helium was used as carrier gas at a constant flow rate of 1 mL/min; 1µL of extract was injected in the splitless mode.

4.7 References

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Chapter 5 Organic impurity profiling of methylamphetamine using gas chromatography mass spectrometry (GCMS)

5.1 Introduction

Both the Moscow and Hypophosphorous (Hypo) synthetic routes are linked to the Nagai route through the mechanism of synthesis. The difference between the three methods is the means by which the essential chemicals iodine and red phosphorous are introduced. As a consequence of this interconnectivity it is reasonable to assume that there will be some considerable overlap of the impurity profile generated from samples of each route. Some limited literature exists relating to the organic route specific impurities of both the Nagai and Moscow routes but no published papers relate to route specific impurities from the Hypophosphorous route.

Windahl *et al.* [1] reported that the length of time that the Nagai reaction proceeded,had an effect on the levels of aziridines and napthalenes present in the final synthesised methylamphetamine. The authors concluded that as the reaction time increased, the concentration of aziridines and napthalenes decreased [1]. Tanaka *et al*. [2] reported the presence of a methylamphetamine dimer formed via the condensation of methylamphetamine and aziridine, however neither the aziridines nor the methylamphetamine dimer are route specific compounds [2]. Ko *et al*. [3] identified the presence of napthalenes and isomers of propanone and propenamide as having potential route specificity for the Nagai synthesis [3]. Extensive research conducted by Kunalan [4] which involved the repetitive synthesis of methylamphetamine using seven methods (Leuckart, Reductive Amination, Nagai, Birch, Moscow, Rosenmund and Emde) has provided clarity in relation to the literature surrounding the Nagai synthesis confirming the impurities suggested by Ko *et al*. as those linked to Nagai synthesised samples, and these results are presented in Table 5.1 [5]. However, Kunalan *et al.* also reported that impurities apparent within repetitive Moscow synthesised samples, were the same as those though to be Nagai specific together with an additional two impurities of unknown identity and these results are presented in Table 5.2. This is hardly surprising given the

closeness of the synthetic methods, however it does suggest that there are no impurities specific to the Nagai synthesis alone.

Table 5.1. Nagai Impurities identified by Windahl *et al*. [1], Tanaka *et al. [*2] and Kunalan *et al. [*5]. Samples in red were suggested as route specific.

Impurities	**Windahl.[1]**	**Tanaka[2]**	**Kunalan [5]**
cis-1,2-dimethyl-3-phenylazridine	√		√
trans-1,2-dimethyl-3-phenylazridine	√		√
Methylamphetamine dimer		√	√
1,3-dimethyl-2-phenylnaphthalene	√		√
1-benzyl -3-methylnaphthalene	√		√
Isomers of N-methyl-N-(α-methylphenylethyl)amino-1-phenyl-2-propanone	√		√
Isomers of N-methyl-N-(α-methylphenylethyl)-3-phenylpropenamide	√		√

.

Table 5.2. Moscow Impurities identified by Kunalan [1].

Compound	**Peak m/z(base peak in bold)**
Unknown 5	**43**, 125, 89, 168, 105, 91, 63
Unknown 6	**91**, 145, 262
1,3-dimethyl-2-phenylnaphthalene	**232**, 217, 202, 77
1-benzyl -3-methylnaphthalene	**232**, 217, 202, 58
Isomers of *N*-methyl-N-(α-methylphenethyl) amino-1-phenyl-2-propanone	**238**, 91, 105, 190, 120
Isomers of (*Z*)-*N*-methyl-*N*-(α-methylphenethyl)-3-phenylpropenamide	**131**, 91, 58, 103, 188, 77

5.2 Experimental methods

Sub samples of each batch of synthesised methylamphetamine were extracted and analysed by GCMS according to the methods outlined in Chapter 4. The impurity profiles were scrutinized using the data analysis software associated with the GCMS instrument (HPChemstation Hewlett Packard HP3365) and the compounds were identified using the available literature, previous studies and the NIST library (version 2.0). In each chromatogram presented the main peak at 9 mins is methylamphetamine and the peak at 18.4 mins is the internal standard, eicosane.

5.2.1 Within batch variation

Within batch variation is observed when a single batch of methylamphetamine sample is homogenized, divided into subsamples, analysed and the results compared. Within batch results can be used to assess the extraction method and the variation of selected target impurities within a synthetic batch. The relative standard deviation (RSD) of the normalized peak area of each impurity was calculated using the six sub batches.

5.2.2 Between batch variation

The between batch variation relates to the variation in the impurity profile observed when six batches of sample produced from six repetitive synthesis using the exact same conditions and apparatus. The relative standard deviation (RSD) of the normalized peak area of each impurity was calculated using the six separate batches.

5.3 Results and Discussion

A phosphate buffer (pH 10.5) extract was used to expose the organic impurity profiles obtained from the samples produced using the Moscow and Hypo routes. The organic impurity profiles are presented for samples produced using laboratory grade chemicals initially and these are compared with previous the literature and existing research data. Secondly, the impurity profiles obtained from samples derived from precursors and essential chemicals extracted from Sudafed, iodine tinctures and matchbooks were

compared to each other and to the samples produced using the laboratory precursor chemicals.

There are differences in the impurity profiles of methylamphetamine synthesized from both routes which is evident and expected. Some significant between batch variation were also very evident despite careful repetitive synthesis using the same conditions , equipment and chemicals.

Sections 5.3.1 and 5.3.11 present the within and between batch variations and the route specific impurities obtained for both the Moscow and Hypophosphorous synthetic routes using laboratory grade chemicals as precursors. Sections 5.3.3-5.3.11 and 5.3.13-5.3.20 present the chromatograms derived from samples of methylamphetamine synthesized using the Moscow and Hypophosphorous routes respectively using *pseudo*ephedrine extracted from cold medication using ethanol, ethanol/methanol (90:10 % vol:vol) and commercial methylated spirits respectively. In each case iodine and red phosphorous were used in the synthetic phase. Regions of variability in the impurity profiles are highlighted.

5.3.1 Within batch variation of methylamphetamine synthesized via Moscow route using laboratory grade *pseudo*ephedrine

The chromatographic impurity profiles for each of 6 sub samples of one batches of methylamphetamine (ML 1 (i-iv)) synthesized via the Moscow route using laboratory grade *pseudo*ephedrine hydrochloride is presented in Figure 5.1 and the within batch RSD (%) values of the derived impurities are presented in Table 5.3. Good reproducibility was demonstrated for the extraction method with relatively low relative standard deviations obtained across the range of impurities obtained (11 out of 16 peaks having %RSD of 10 or less) which was in keeping with other reported RSD values in similar studies [6]. Four of the six suggested Nagai/Moscow specific impurities identified by Kunalan [4], were apparent in the samples and these included the unidentified impurity MLUK 1 (unknown 6 in Kunalan's work) [4]. These are highlighted red in the table.

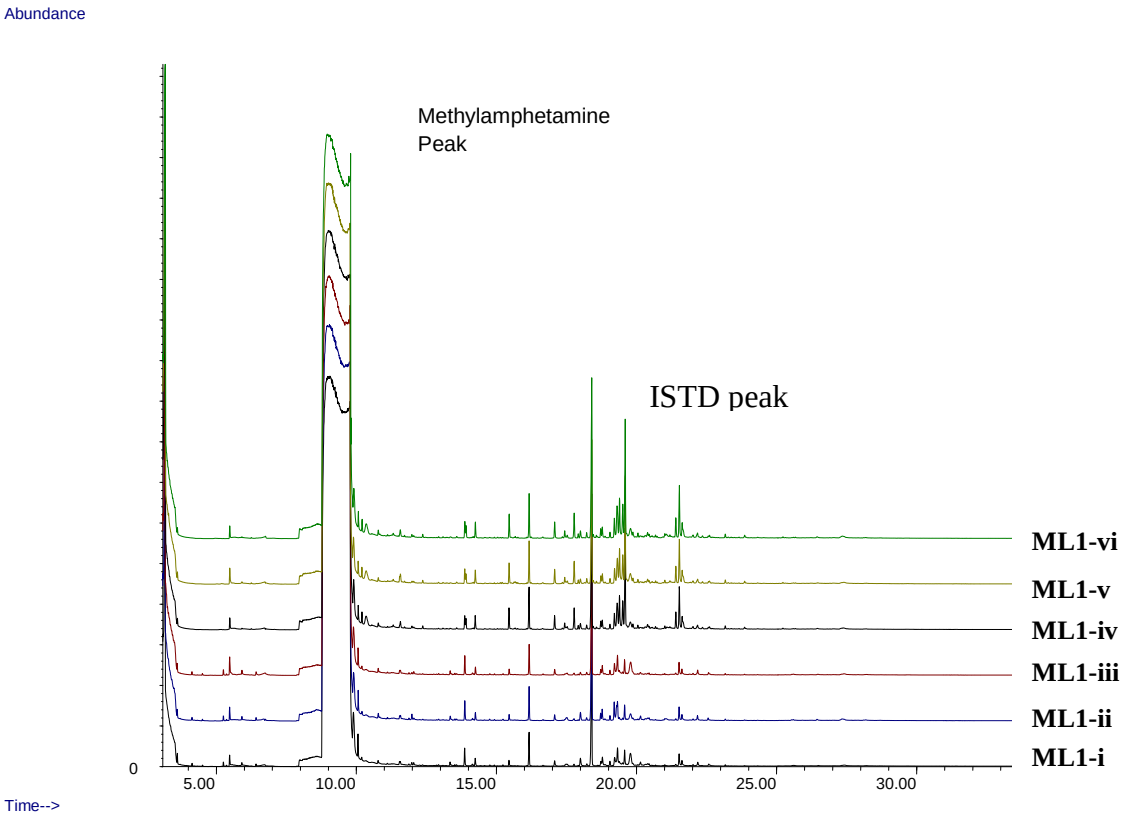

Figure 5.1. Impurity profiles illustrating within batch variation of 6 sub batches of methylamphetamine synthesized via the Moscow route (ML1).

Table 5.3. Table of impurity peaks identified in methylamphetamine synthesized via the Moscow route using laboratory grade *pseudo*ephedrine hydrochloride.

Retention Time (RT)	Impurity Peak	RSD(%) of normalized to IS values
5.484	Benzaldehyde	10
7.984	Benzyl-methyl-ketone(BMK)	27
10.067	Dimethylamphetamine(DMA)	5
10.785	Z(1-phenylpropan-2-one oxime)	35
11.576	N-Methylbenzamide	7
13.876	N-formylmethamphetamine	19
14.254	Methamphetamine acetylated	2
16.172	Amphetaminil	1
17.084	3,4-Diphenyl-3-buten-2-one	30
17.775	N-β-(phenylisopropyl)benzylmethyl ketimine	2
18.727	MLUK 1	24
19.057	Benzylmethnaphtalene	10
19.389	N-methyl-N-(α-methylphenyl)amino-1-phenyl-2-propanone	5
19.577	benzoylmethylamphetamine	8
19.796	N,N-di-(β-phenylisopropyl) formamide	9
21.623	(z)-N-methyl-N-(α-methyphenethyl)-3-phenylpropenamide	4

5.3.2 Between batch variation of methylamphetamine synthesized via Moscow route using laboratory grade *pseudo*ephedrine

The chromatographic impurity profiles for each of six repetitively synthesized batches of methylamphetamine via the Moscow route using laboratory grade *pseudo*ephedrine hydrochloride are presented in Figure 5.2. Visual comparison of the impurity profiles illustrates clear differences between sample profiles particularly within the 15-25 minute range. Methylamphetamine synthesized using the Moscow route exhibits a large benzyl-methyl-ketone (BMK) peak, shown in Figure 5.2. These differences are apparent in the high %RSD values presented in Table 5.4. The previous identified Nagai and Moscow route specific impurities (in red in the table) are still apparent within the samples.

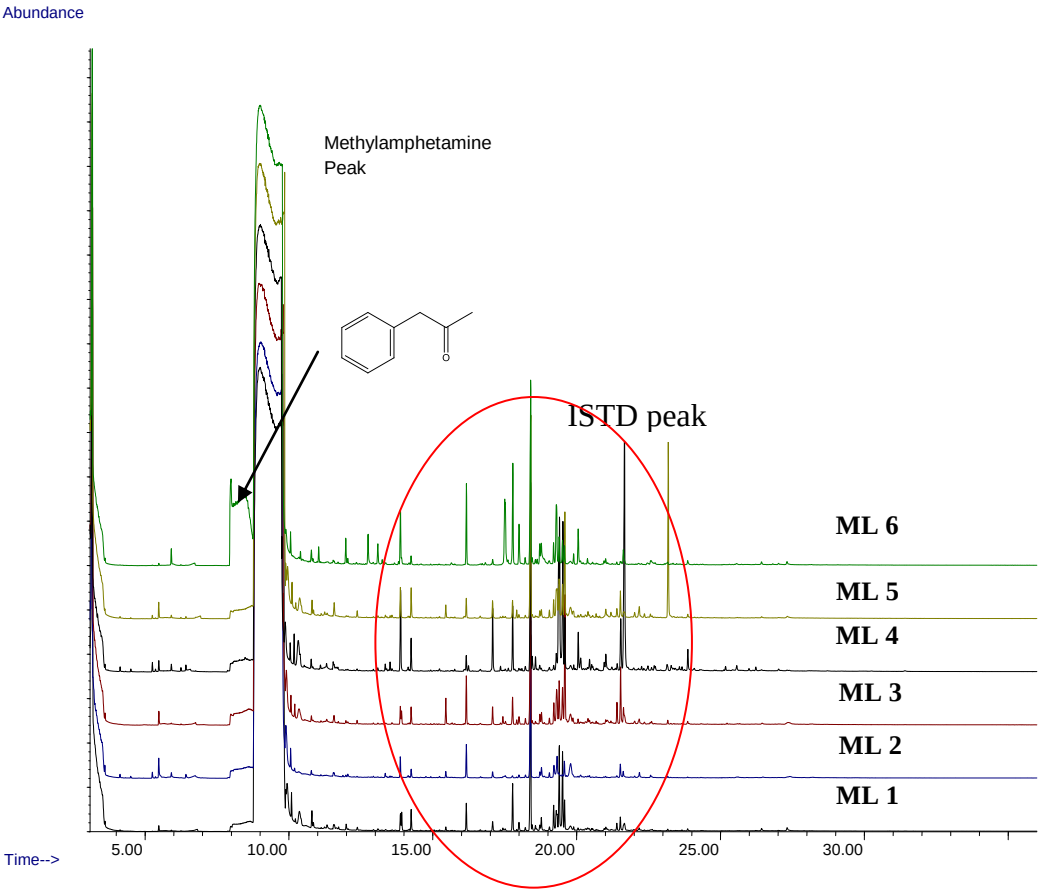

Figure 5.2. Impurity profiles of between batch variation of ML 1 to 6 of methylamphetamine synthesized via the Moscow route using laboratory grade *pseudo*ephedrine.

Table 5.4. Impurity peaks identified methylamphetamine synthesized via the Moscow route using laboratory grade *pseudo*ephedrine hydrochloride.

Retention Time (RT)	Impurity Peak	RSD(%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant values mg/mL	m/z (base peak in bold)
5.484	Benzaldehyde	70	81	76	0.002	**105**,77,51,63
7.984	Benzyl-methyl-ketone(BMK)	192	192	187	0.012	**91**,134,65,77,51
10.067	Dimethylamphetamine(DMA)	24	16	17	0.008	**72**,58,65,77,91
10.785	Z(1-phenylpropan-2-one oxime)	51	46	45	0.002	**91**,149,116,131
11.576	N-Methylbenzamide	85	75	84	0.001	**105**,77,58,134,91
13.876	N-formylmethamphetamine	76	76	76	0.009	**86**,58,118
14.254	Methamphetamine acetylated	49	44	49	0.004	**58**,100
16.172	Amphetaminil	63	64	59	0.008	**132**,105,91,77,65
17.084	3,4-Diphenyl-3-buten-2-one	105	88	107	0.005	**179**,178,222,221
17.775	N-β-(phenylisopropyl)benzylmethyl ketimine	85	87	82	0.011	**91**,160,119,65,77,207
18.727	MLUK 1	71	72	66	0.002	**91,**142,262
19.057	Benzylmethnaphtalene	28	25	25	0.001	**232**,217,202,58
19.389	N-methyl-N-(α-methylphenyl)amino-1-phenyl-2-propanone	86	88	89	0.016	**238**,91,105,190,120
19.577	benzoylmethylamphetamine	83	84	86	0.015	**105**,162,77,91
19.796	N,N-di-(β-phenylisopropyl) formamide	48	58	55	0.005	**190**,91,58,119,77,105
21.623	(z)-N-methyl-N-(α-methyphenethyl)-3-phenylpropenamide	188	188	190	0.017	**131**,91,58,103,188

RSD values are presented for raw data and peak areas normalized to the peak area of the internal standard (IS) and to the total peak area (TPA). Impurities were semi-quantified to the internal standard using a single point estimate.

Three of the four route specific impurities observed witin the Moscow prepared samples have also been previously reported as impurities in Nagai route samples. (N-methyl-N-(α-methyphenethyl)-3-phenylpropenamide, N-methyl-N-(α-methylphenyl)amino-1-phenyl-2-propanone and benzylmethnaphtalene (1-benzyl-3-methylnaphthalene). The unknown compound (MLUK 1) may be route specific for the Moscow synthesis, corroborating the previous work by Kunalan in this regard.

5.3.3 Overview of variation of methylamphetamine impurity profiles synthesized via the Moscow route using *pseudo*ephedrine extracted from proprietary cold medication (Sudafed- UK) using the three different solvent systems

The preparation of methylamphetamine using non laboratory precursor materials represents a more realistic scenario of some clandestine synthesis, particularly small scale synthesis. The mechanisms of extraction of the precursor chemical from available over the counter cold medication (Sudafed) has been previously described and is relatively straight forward. The effect of using different precursor extracting solvents on the final methylamphetamine product is currently unknown with no reports addressing this in the literature. Figure 5.3 represents on overlay comparing of batches of methylamphetamine synthesised using laboratory precursor and precursors extracted from proprietary cold medication using the three different solvent systems and exposes significant differences within the resultant impurity profiles which appear to be solvent dependent. The rest of the work discussed in this section explores these differences in greater depth.

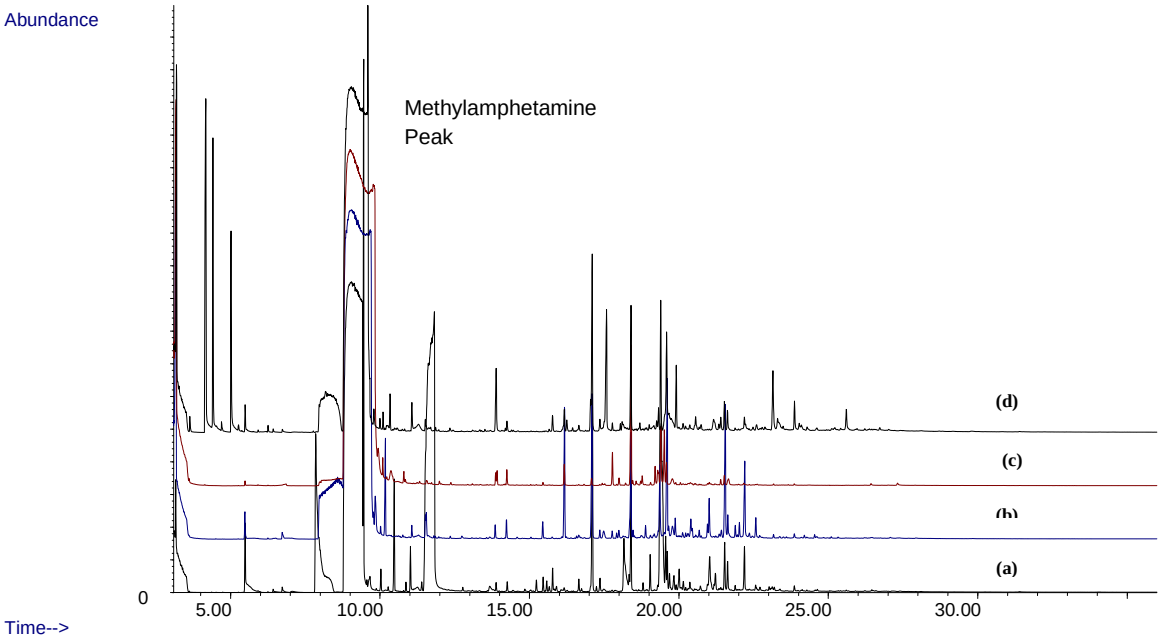

Figure 5.3. Chromatograms of methylamphetamine synthesized via the Moscow route (a) laboratory grade precursors and precursors extracted from 'Sudafed' using (b) ethanol, (c) ethanol/methanol (90:10) % vol/vol and (d)methylated spirits.

5.3.4 Within batch variation of methylamphetamine synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed-UK) using ethanol.

The chromatographic impurity profiles and related data for six subsamples of methylamphetamine produced via the Moscow synthesis from precursor extracted from Sudafed using ethanol are presented in Figure 5.4 and Table 5.5. Ten of the 28 peaks presenting within the impurity profile display an RSD value of 10% of less while no peak has a variation greater than 28% and again this is in accordance with previous literature values for similar studies [6].

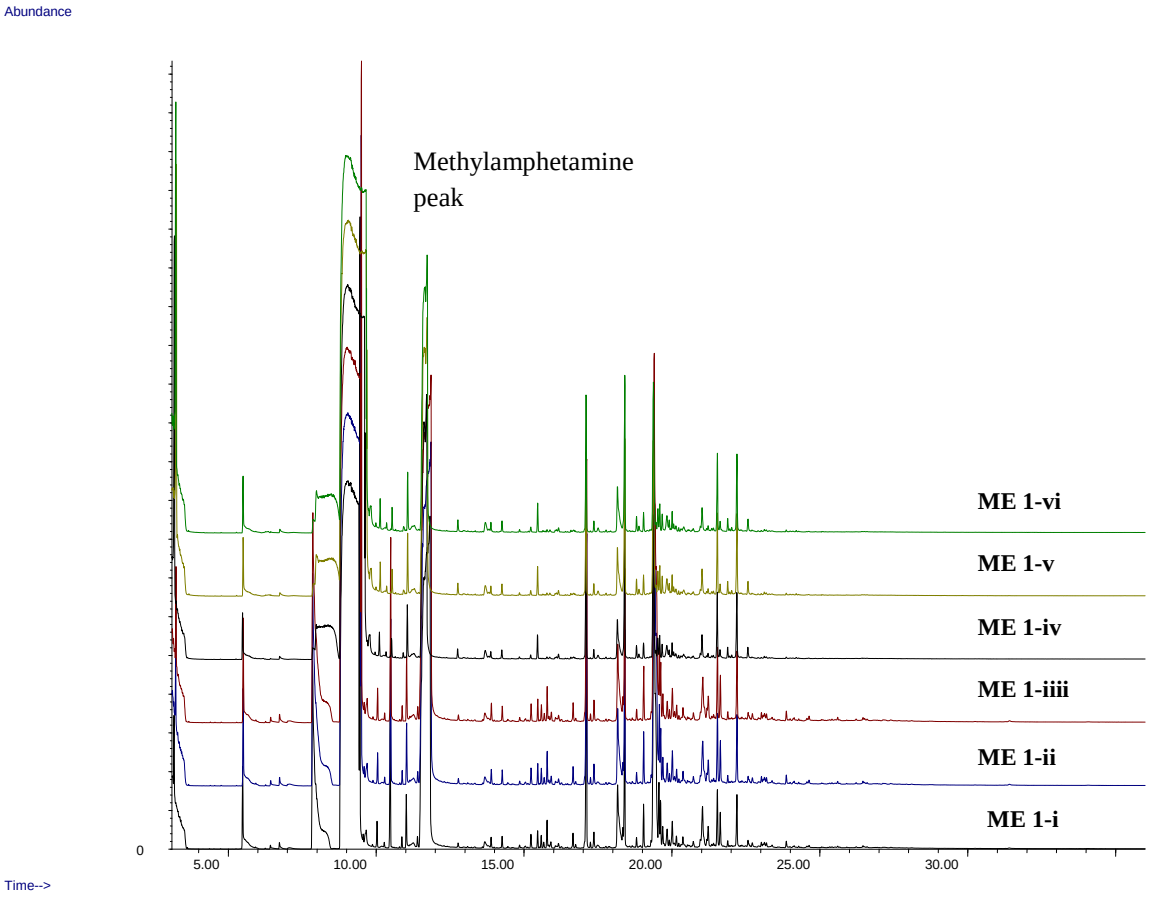

Figure 5.4. Impurity profiles illustrating within batch variation of 6 sub batches of methylamphetamine synthesized via the Moscow route (ME 1) using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) with ethanol.

Table 5.5. Impurity peaks identified in methylamphetamine synthesized via the Moscow route using using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) with ethanol.

Retention Time (RT)	Impurity Peak	RSD(%) of normalized to IS values
6.436	2-propenyl benzene	28
6.737	N-(phenylmethylene methanamine	26
8.356	Benzyl Methyl Ketone(BMK)	15
10.881	N-Methyl-1-phenylethanamine	18
13.333	Bibenzyl	12
13.677	**MEUK 1**	16
14.265	Acetylated methylamphetamine	7
14.855	**MEUK 2**	17
15.240	**MEUK 3**	8
15.465	**MEUK 4**	9
15.783	1,2-diphenyl-ethanone,	17
16.065	N-Acetylpseudoephedrine	23
16.168	2 phenyl-morpholine-3-methyl,	24
17.116	3,4-diphenyl trans 3-buten-2-one	15
17.480	n-β-phenylisopropylbenzyl methyl ketimine	20
18.170	1,2-dimethyl 1,3-phenyl azridine,	14
18.893	**MEUK 5**	10
19.047	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	27
19.184	N-benzoylamphetamine	15
19.300	**MEUK 6**	5
19.694	**MEUK 7**	35
20.089	**MEUK 8**	19
20.236	**MEUK 9**	1
20.568	**MEUK 10**	2
20.726	**MEUK 11**	11
20.964	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	24
21.044	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	10
22.021	**MEUK 12**	3

It is immediately evident that the impurity profile is more complex than that observed when laboratory grade materials were used. This is not unexpected as the purity of the chemicals will be different. Furthermore, a greater number (12) of unidentified compounds are present within the profiles. Interestingly an impurity previously identified as a route specific impurity for the Emde route was found present in the batches of methylamphetamine, the impurity was N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine.

5.3.5 Between batch variation of methylamphetamine synthesized via Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol

The chromatographic impurity profiles for each of the 6 batches synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from Sudafed tablets using ethanol and associated data are presented in Figure 5.5 and Table 5.6. Visual comparison of the impurity profiles illustrates considerable differences between sample profiles particularly within the 14-24 minute range. The large benzyl-methyl-ketone (BMK) peak, present in the previous samples is also evident.

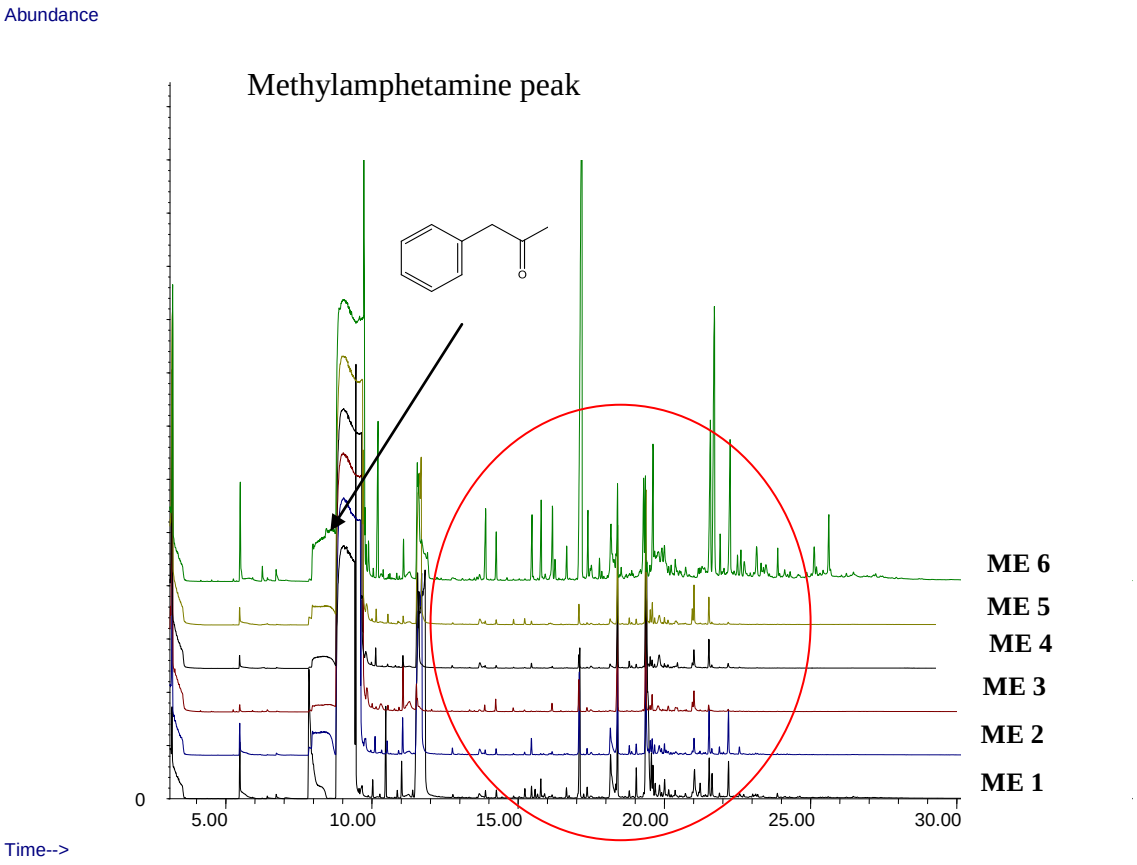

Figure 5.5. Impurity profiles of between batch variation of ME 1 to 6 of methylamphetamine synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol.

Table 5.6. Impurity peaks identified methylamphetamine synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol as the extraction solvent.

Retention Time (RT)	Impurity Peak	RSD (%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant values mg/mL	m/z(base peak in bold)
6.436	2-propenyl benzene	64	63	57	0.001	**117**,91,103,77,60,51
6.737	N-(phenylmethylene methanamine	86	78	69	0.002	**118**,77,91,63,51
8.356	Benzyl Methyl Ketone(BMK)	59	52	42	0.015	**91**,134,65,77,51
10.881	N-Methyl-1-phenylethanamine	52	57	51	0.002	**120**,58,77,91,105,134
13.333	Bibenzyl	80	67	55	0.001	**91**,58,65,77,182
13.677	**MEUK 1**	33	36	35	0.005	**148**,176,133,91,113,58,77
14.265	Acetylated methylamphetamine	123	106	83	0.007	**58**,100
14.855	**MEUK 2**	57	64	65	0.001	**118**,147,58,77,91,105,191,176
15.240	**MEUK 3**	82	88	79	0.002	**118**,147,58,77,91,105,191,176
15.465	**MEUK 4**	153	136	118	0.009	**105**,190,77,86,176,51
15.783	1,2-diphenyl-ethanone,	188	176	166	0.009	**105**,77,91,51,65,115,128
16.065	N-Acetylpseudoephedrine	76	82	73	0.000	**58**,77,100,86,117
16.168	2 phenyl-morpholine-3-methyl,	187	166	155	0.008	**132**,105,91,77,65,51
17.116	3,4-diphenyl trans 3-buten-2-one	179	166	150	0.115	**179**,221,207,152,105,77
17.480	n-β-phenylisopropylbenzyl methyl ketimine	72	54	39	0.002	**91**,160,119,58,77
18.170	1,2-dimethyl 1,3-phenyl azridine	101	95	88	0.029	**146**,132,105,117,91,77,65
18.893	**MEUK 5**	93	76	60	0.002	**131**,148,190,103,91,77,58
19.047	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	98	103	96	0.005	**58**,118,141,193,238
19.184	N-benzoylamphetamine	170	167	134	0.003	**105**,77,148,91,118
19.300	**MEUK 6**	214	205	194	0.012	**148**,190,91,117,105,77,58
19.694	**MEUK 7**	104	88	72	0.007	**148**,190,91,117,105,77,58
20.089	**MEUK 8**	134	116	96	0.004	**203**,91,148,188,58
20.236	**MEUK 9**	156	140	117	0.003	**58**,77,91,105,118,148,176,188,247
20.568	**MEUK 10**	164	149	126	0.002	**259**,115,58,91,176,105,77
20.726	**MEUK 11**	156	140	121	0.003	**176**,148,91,117,133,58
20.964	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	42	48	51	0.004	**58**,208,239,195
21.044	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	57	62	54	0.015	**58**,208,239,195
22.021	**MEUK 12**	146	129	107	0.002	**105**,91,77,58,118,262

RSD values are presented for raw data and peak areas normalized to the peak area of the internal standard (IS) and to the total peak area (TPA). Impurities were semi-quantified to the internal standard using a single point estimate.

5.3.6 Within batch variation of methylamphetamine synthesized via Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol:methanol (90:10% vol/vol) as the extraction solvent.

The chromatographic impurity profiles and related data for six subsamples of methylamphetamine produced via the Moscow synthesis from *pseudo*ephedrine hydrochloride extracted from Sudafed tablets using ethanol:methanol (90:10% vol/vol) as the extraction solvent is Figure 5.6 and Table 5.7. Ten of the 28 peaks presenting within the impurity profile display an RSD value of 10% of less while no peak has a variation greater than 28% and again this is in accordance with previous literature values for similar studies [6].

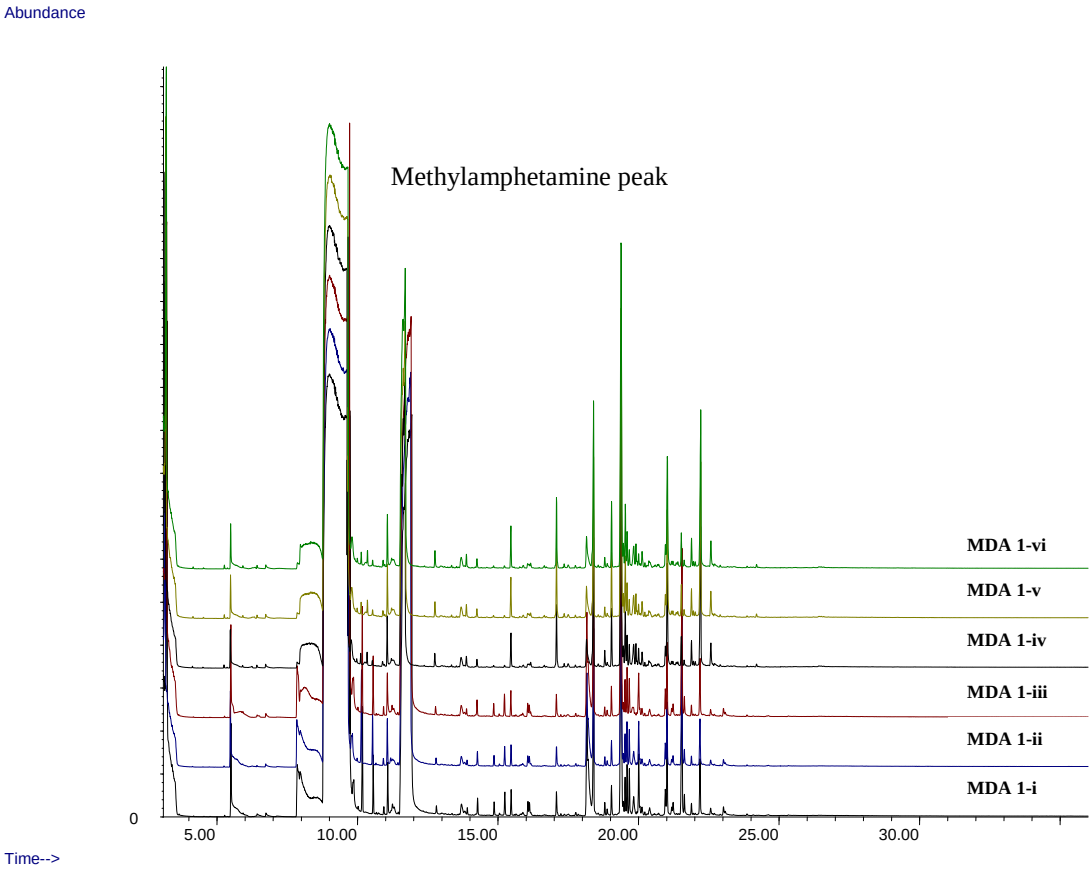

Figure 5.6. Impurity profiles illustrating within batch variation of 6 sub batches of methylamphetamine synthesized via the Moscow route (MDA1) *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol:methanol (90:10 % vol/vol) as the extraction solvent.

Table 5.7. Table of impurity peaks identified in methylamphetamine synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed-UK) using ethanol:methanol (90:10)% vol/vols as the extraction solvent.

Retention Time (RT)	Impurity Peak	RSD(%) normalized to IS
5.481	Benzaldehyde	30
6.432	**MDUK 1**	20
6.734	N(phenylmethylene) methanamine ,	22
7.872	1,2-dimethyl-3-phenyl aziridine,	12
7.977	Benzylmethylketone (BMK)	28
10.563	N,α-dimethyl -benzenemethanamine,	5
12.374	**MDUK 2**	6
12.758	*Pseudo*ephedrine	5
13.095	**MDUK 3**	10
13.247	**MDUK 4**	15
13.354	Bibenzyl	5
13.584	N,N-dimethyl -benzenepropanamine,	12
13.692	**MDUK 5**	7
14.248	Methamphetamine acetylated	20
15.209	2,6-Diisopropylnaphthalene	4
15.457	3-Benzoyl-2-t-butyl-oxazolidin-5-one	16
15.771	N-benzoylamphetamine	3
15.906	**MDUK 6**	15
16.042	N-Acetylephedrine	12
16.654	Bibenzoyl	8
17.361	4,4-diphenyl-3-buten-2-one,	10
17.923	1,2-diphenyl-2-propen-1-one,	5
18.152	Aziridine, 1,2-dimethyl-3-phenyl-, trans	19
19.042	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	10
19.378	**MDUK 7**	23
21.236	benzoylmethamphetamine	10
21.556	Acetylephedrine	23
22.706	E-N-methyl-N-(α-methylpheneethyl)-3-phenylpropenamide	13
23.169	**MDUK 8**	21

As with the ethanol extracted precursor samples, the impurity profiles are more complex than those observed when laboratory grade materials were used. In this case eight unidentified compounds are present within the profiles, and only the E-N-methyl-N-(α-methylpheneethyl)-3-phenylpropenamide impurity from the Nagai specific group is present.

5.3.7 Between batch variation of methylamphetamine synthesized via Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol:methanol (90:10% vol/vol) as the extraction solvent.

The chromatographic impurity profiles for each of the 6 batches synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from Sudafed tablets using ethanol:methanol (90:10 % vol/vol) and associated data are presented in Figure 5.7 and Table 5.8. As before visual comparison of the impurity profiles illustrate considerable differences between sample profiles particularly within the 14-22 minute range and the large benzyl-methyl-ketone (BMK) peak, present in the previous samples is also evident.

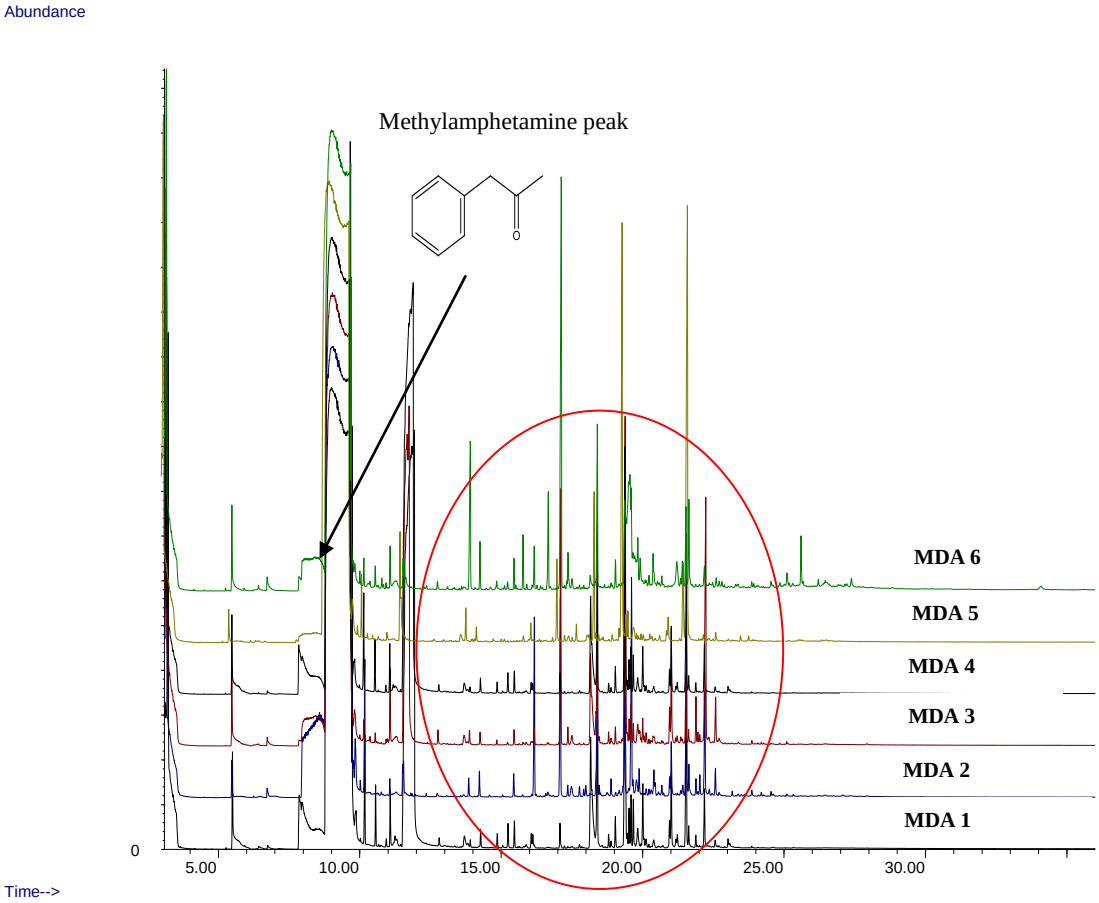

Figure 5.7. Impurity profiles of between batch variation of MDA 1 to 6 of methylamphetamine synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using ethanol:methanol (90:10 % vol/vol) as the extraction solvent.

Table 5.8. Impurity peaks identified methylamphetamine synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol:methanol (90:10% vol/vol) as the extracting solvent.

Retention Time (RT)	Impurity Peak	RSD(%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant values mg/mL	m/z (base peak in bold)
5.481	Benzaldehyde	50	46	46	0.021	**106**,77,51,63,74
6.432	**MDUK 1**	68	78	71	0.001	**60**,117,91,103,51
6.734	N(phenylmethylene) methanamine ,	7	77	71	0.004	**118**,77,91,103,63,51
7.872	1,2-dimethyl-3-phenyl aziridine,	17	170	170	0.008	**146**,105,132,117,91,77
7.977	Benzylmethylketone(BMK)	118	107	108	0.022	**91**,134,65,77,105,115
10.563	N,α-dimethyl benzenemethanamine,	81	73	72	0.006	**120**,58,77,91,105,63,71
12.374	**MDUK 2**	75	67	67	0.001	**105**,77,58,51,163
12.758	*Pseudo*ephedrine	44	35	40	0.003	**58**,77,62,70,105,117
13.095	**MDUK 3**	47	47	43	0.001	**58**,118,72,91,77,65,105
13.247	**MDUK 4**	52	41	44	0.000	**58**,71,91,117,176,105,158
13.354	benzyl	49	38	40	0.000	**91**,58,65,77,182
13.584	N,N-dimethyl benzenepropanamine	49	55	49	0.000	**58**,91,163,118
13.692	**MDUK 5**	61	57	63	0.004	**148**,176,58,91,113,133
14.248	Methamphetamine acetylated	77	83	77	0.005	**58**,100,91,77,65
15.209	2,6-Diisopropylnaphthalene	107	98	98	0.002	**105**,77,86,190,51,65,212
15.457	3-Benzoyl-2-t-butyl-oxazolidin-5-one	5	60	63	0.005	**105**,77,86,190,51,65
15.771	N-benzoylamphetamine	179	187	182	0.002	**105**,77,148,91,118
15.906	**MDUK 6**	43	45	44	0.001	**230**,58,86,77,105,177,188,245
16.042	N-Acetylephedrine	104	96	94	0.001	**58**,100,77,86,70
16.654	Bibenzoyl	191	195	192	0.005	**105**, 77,51, 61, 86
17.361	4,4-diphenyl-3-buten-2-one,	84	90	84	0.003	**179**,222,152,207,186
17.923	1,2-diphenyl-2-propen-1-one,	82	88	80	0.000	**105**,77,51,91
18.152	Trans 1,2-dimethyl-3-phenyl- aziridine	9	86	86	0.026	**146**,105,132,91,77,65
19.042	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	67	98	75	0.007	**58**,208,239
19.378	**MDUK 7**	46	45	50	0.107	**58**,118,149,133,91,77
21.236	benzoylmethamphetamine	139	150	142	0.005	**105**,162,77,91
21.556	Acetylephedrine	60	61	56	0.032	**58**,100,77,86,70

Retention Time (RT)	Impurity Peak	RSD(%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant values mg/mL	m/z (base peak in bold)
22.706	E-N-methyl-N-(α-methylpheneethyl)-3-phenylpropenamide	52	89	50	0.003	**131**,91,58,103,188
23.169	**MDUK 8**	48	56	50	0.001	**105**,204,91,77,58,118,149,178

RSD values are presented for raw data and peak areas normalized to the peak area of the internal standard (IS) and to the total peak area (TPA). Impurities were semi-quantified to the internal standard using a single point estimate.

One of the four specific impurities observed for the Nagai route (N-methyl-N-(α-methyphenethyl)-3-phenylpropenamide) was also observed in all batches of methylamphetamine synthesised via the moscow route using *pseudo*ephedrine hydrochloride extracted using ethanol/methanol (90:10 % vol/vol)

5.3.8 Within batch variation of methylamphetamine synthesized via Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using commercial methylated spirit as the extraction solvent.

The chromatographic impurity profiles for each of within 6 sub batches of MMS 1 synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using commercial methylated spirit as the extraction solvent is presented in Figure 5.8 shown below. The RSD (%) values of list of impurities identified in within sub batch of MMS 1 is presented in Table 5.9 shown below.

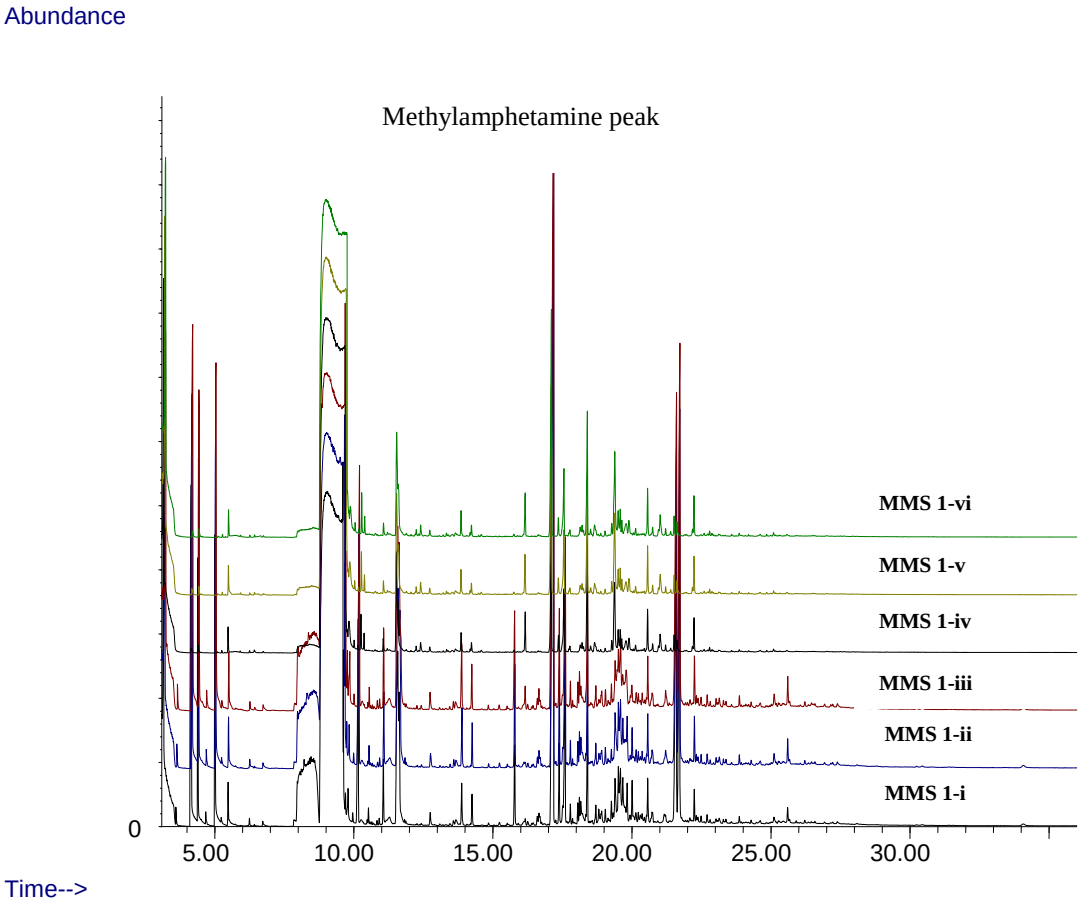

Figure 5.8. Impurity profiles illustrating within batch variation of 6 sub batches of methylamphetamine synthesized via the Moscow route (MMS1) using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using commercial methylated spirit as the extraction solvent.

Table 5.9. Impurity peaks identified in methylamphetamine synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using commercial methylated spirit as the extraction solvent.

Retention Time (RT)	Impurity Peak	RSD(%) normalized to IS
5.261	2,4 dimethyl pyridine	10
6.443	2-propenyl benzene,	10
6.744	N-(phenylmethylene) methanamine	11
8.089	Benzyl methyl ketone(BMK)	18
10.373	2-Chloro-acetophenone	6
10.848	1-phenyl- oxime-2-propanone	20
11.285	1-2-methylaziridine	28
12.744	*Pseudo*ephedrine	40
13.086	**MMUK 1**	28
13.201	1-methyl-2-phenylhexahydropyrimidine	18
13.661	**MMUK 2**	20
15.229	**MMUK 3**	15
15.785	1,2-diphenylethanone	10
16.163	Amphetaminil	2
16.398	**MMUK 4**	2
16.659	Bibenzoyl	2
17.000	4,4-diphenyl-3-buten-2-one	10
17.392	4,4,diphenyl-3-buten-2-one	8
17.792	**MMUK 5**	20
18.166	Trans 1,2 dimethyl-3-phenyl azridine	15
19.182	**MMUK 6**	23
19.266	**MMUK 7**	17
19.596	Benzoylmethamphetamine	12
19.672	**MMUK 8**	13
20.157	**MMUK 9**	8
20.235	**MMUK 10**	5
20.374	**MMUK 11**	7
20.467	**MMUK 12**	19
21.061	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	20
21.217	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	24
22.491	**MMUK 13**	6
23.030	**MMUK 14**	21
23.388	**MMUK 15**	10
23.868	**MMUK16**	11

As with the previous two Sudafed prepared sets of methylamphetamine samples, the impurity profile is more complex than that observed when laboratory grade materials were used. The largest number of unidentified compounds (16) in comparison to all other Moscow prepared samples are present within the profiles, and both isomers of the impurity compound associated with the Emde route was found present in this batches of methylamphetamine similar to the batches of methylamphetamine synthesised using *pseudo*ephedrine HCl extracted using ethanol, the mentioned

impurity N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine were present.

5.3.9 Between batch variation of methylamphetamine synthesized via Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using commercial methylated spirit as the extraction solvent.

The chromatographic impurity profiles for each of the 6 batches synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from Sudafed tablets using commercial methylated spirits and associated data are presented in Figure 5.9 and Table 5.10. As before visual comparison of the impurity profiles illustrate considerable differences between sample profiles particularly within the 14-22 minute range and the large benzyl-methyl-ketone (BMK) peak, present in the previous samples is also evident.

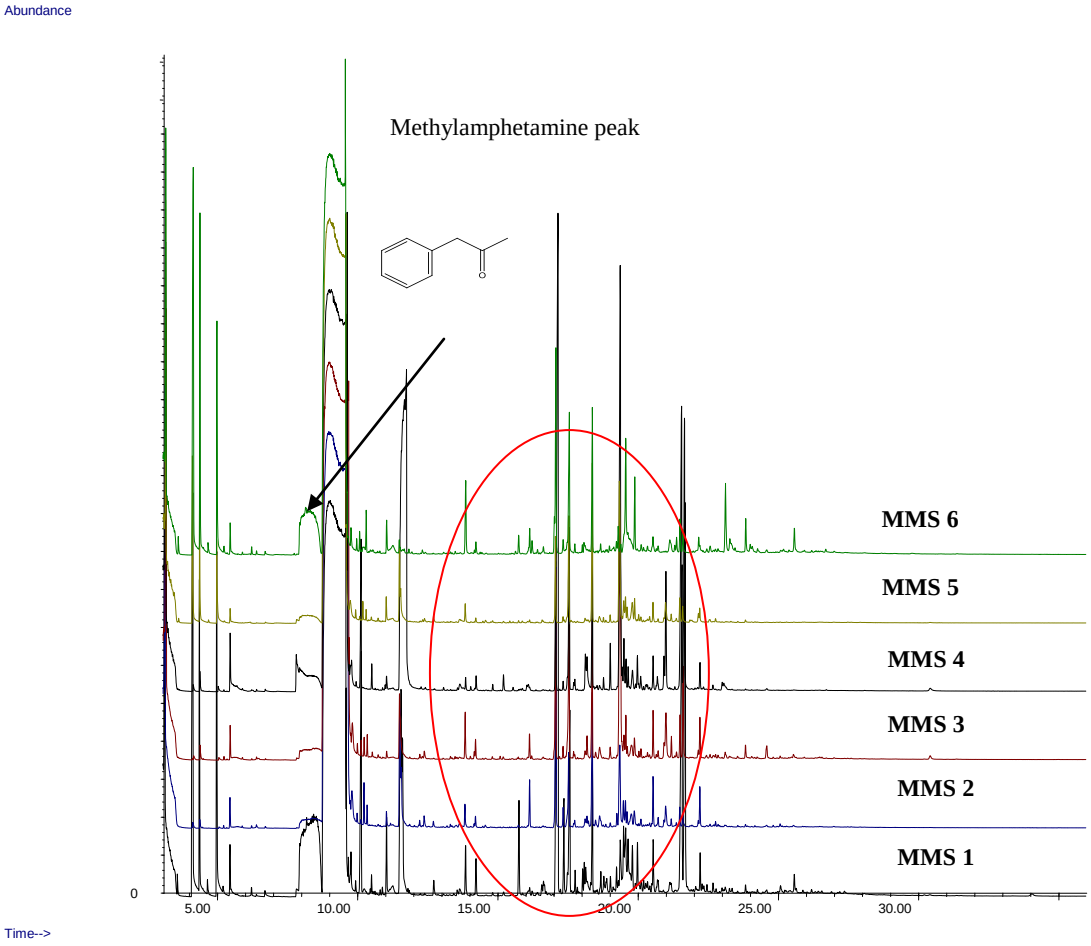

Figure 5.9. Impurity profiles of between batch variation of MMS 1 to 6 of methylamphetamine synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using commercial methylated spirit as the extraction solvent.

Table 5.10. Impurity peaks identified methylamphetamine synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using commercial methylated spirits as the extracting solvent.

Retention Time (RT)	Impurity Peak	RSD (%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant mg/mL	m/z (base peak in bold)
5.261	2,4 dimethyl pyridine,	94	87	77	0.042	**107**,79,92,65,51,115
6.443	2-propenyl benzene,	75	69	65	0.001	**117**,91,103,77,60,51
6.744	N-(phenylmethylene) methanamine	86	79	68	0.001	**118**,77,91,103,63,51
8.089	Benzyl methyl ketone(BMK)	99	91	79	0.013	**91**,134,65,77,105,115
10.373	2-Chloro-acetophenone	63	58	61	0.006	**105**,77,51,58,91,154
10.848	1-phenyl-oxime-2-Propanone	58	49	35	0.002	**91**,58,77,105,119,130
11.285	1-2-methylaziridine	78	70	60	0.009	**56**,63,77,54,91,105,115
12.744	*Pseudo*ephedrine	107	99	83	0.003	**58**,77,62,70,105,117
13.086	**MMUK 1**	34	31	25	0.001	**118**,58,72,91,171,105
13.201	1-methyl-2-phenylhexahydropyrimidine	44	45	46	0.000	**58**,118,146,175,189
13.661	**MMUK 2**	64	62	46	0.001	**148**,176,58,91,113,133,77
15.229	**MMUK 3**	115	123	113	0.001	**111**,91,58,77,176,129
15.785	1,2-diphenylethanone	181	177	160	0.008	**105,**77,91,58,196
16.163	Amphetaminil	81	80	86	0.007	**132**,105,117,91,77,65,51
16.398	**MMUK 4**	69	61	49	0.000	**180**,77,58,91,51
16.659	Bibenzoyl	117	110	93	0.002	**105**,77,51,210
17.000	4,4-diphenyl-3-buten-2-one,	91	89	95	0.039	**179**,222,152,207,186
17.392	4,4-diphenyl-3-buten-2-one	131	123	100	0.009	**179**,222,152,207,186
17.792	**MMUK 5**	91	83	64	0.004	**91**,119,222,104
18.166	Trans 1,2 dimethyl-3-phenyl azridine	60	63	50	0.008	**146**,105,132,117,91,77
19.182	**MMUK 6**	76	69	66	0.001	**105**,186,58,77,91,170,144,118, 160
19.266	**MMUK 7**	87	81	72	0.004	**179**,207,256,58,114,152
19.596	Benzoylmethamphetamine	96	90	90	0.026	**105**,162,77,91
19.672	**MMUK 8**	75	66	46	0.010	**193**,165,250,89,148
20.157	**MMUK 9**	50	43	30	0.005	**58**,160,91,179,251
20.235	**MMUK 10**	91	83	69	0.002	**188**,247,91,105,58,77,148,176

Retention Time (RT)	Impurity Peak	RSD (%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant mg/mL	m/z (base peak in bold)
20.374	**MMUK 11**	82	73	56	0.003	**118**,56,162,279,239,186,105,91
20.467	**MMUK 12**	46	39	34	0.002	**71**,58,91,115,236,251,179
21.061	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	97	100	91	0.017	**58**,208,239,195
21.217	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	82	76	71	0.007	**58**,208,239,195
22.491	**MMUK 13**	80	72	60	0.002	**91**,131,176,202,264,58,77,105, 118
23.030	**MMUK 14**	89	88	77	0.001	**58**,190,105,91,77
23.388	**MMUK 15**	108	103	105	0.002	**105**,120,77,198,275,289,91,51
23.868	**MMUK16**	126	122	125	0.005	**247**,162,91,119,70,56

RSD values are presented for raw data and peak areas normalized to the peak area of the internal standard (IS) and to the total peak area (TPA). Impurities were semi-quantified to the internal standard using a single point estimate.

5.3.10 Unknown impurity peaks of methylamphetamine synthesized via the Moscow route using the various precursor materials.

Besides known impurities listed in the above sections, a number of unknown impurities for methylamphetamine synthesized via the Moscow route for each of the different precursor samples prepared from Sudafed have been exposed and are summarised in Table 5.12. Some of these peaks have been mentioned previously in the literature relating to Moscow synthesised samples.

Table 5.11. Summary of the unknown peaks for the Moscow route

Sample	Mass ions for identification	Retention Time of GC-MS
MLUK 1	**105**,77,58,134,91	11.576
MLUK 2	**91**,119,104,222,71	17.775 Kunalan [4]
MLUK 3	**58**,91,165,178,77	18.727
MLUK 4	**174**,91,56,115,148,265	19.796 (**FSI 160 (2006) 1-10)[7]**
MEUK 1	**148**,176,133,91,113,58,77	13.677
MEUK 2	**118**,147,58,77,91,105,191,176	14.855 (**FSI 164(2006)210-210)[8]**
MEUK 3	**118**,147,58,77,91,105,191,176	15.240 (**FSI 164(2006)210-210)[8]**
MEUK 4	**105**,190,77,86,176,51	15.465
MEUK 5	**131**,148,190,103,91,77,58	18.893
MEUK 6	**148**,190,91,117,105,77,58	19.300
MEUK 7	**148**,190,91,117,105,77,58	19.694
MEUK 8	**203**,91,148,188,58	20.089
MEUK 9	**58**,77,91,105,118,148,176,188,247	20.236
MEUK 10	**259**,115,58,91,176,105,77	20.568
MEUK 11	**176**,148,91,117,133,58	20.726
MEUK 12	**105**,91,77,58,118,262	22.021 (**FSI 160(2006) 1-10)[7]**
MDUK 1	**60**,117,91,103,51	6.432
MDUK 2	**105**,77,58,51,163	12.374 (**FSI164(2006) 201-210)[8]**
MDUK 3	**58**,118,72,91,77,65,105	13.095
MDUK 4	**58**,71,91,117,176,105,158	13.247
MDUK 5	**148**,176,58,91,113,133	13.692
MDUK 6	**230**,58,86,77,105,177,188,245	15.906
MDUK 7	**105**,204,91,77,58,118,149,178	23.169
MMUK 1	**118**,58,72,91,171,105	13.086
MMUK 2	**148**,176,58,91,113,133,77	13.661
MMUK 3	**111**,91,58,77,176,129	15.229 (**FSI 164(2006) 201-210)[8]**
MMUK 4	**180**,77,58,91,51	16.398
MMUK 5	**91**,119,222,104	17.792 Kunalan [4]
MMUK 6	**105**,186,58,77,91,170,144,118,160	19.182
MMUK 7	**179**,207,256,58,114,152	19.266
MMUK 8	**193**,165,250,89,148	19.672
MMUK 9	**58**,160,91,179,251	20.157
MMUK 10	**188**,247,91,105,58,77,148,176	20.235
MMUK 11	**118**,56,162,279,239,186,105,91	20.374
MMUK 12	**71**,58,91,115,236,251,179	20.467 (**FSI 161(2006) 209)[9]**

Sample	Mass ions for identification	Retention Time of GC-MS
MMUK 13	**91,**131,176,202,264,58,77,105,118	22.491
MMUK 14	**58**,190,105,91,77	23.030
MMUK 15	**105**,120,77,198,275,289,91,51	23.388
MMUK 16	**247**,162,91,119,70,56	23.868

One unknown impurity appears across all of the synthetic routes where the precursor was extracted from Sudafed. This peak has been labeled MEUK1, MDUK5 and MMUK2 shown in Figure 5.10 below. The suggested route specific impurity previously identified by Kunalan [4] has also been identified in both the laboratory grade preparation and in the samples prepared from *pseudo*ephedrine extracted using commercial methylated spirits, however it was absent from both of the other sets of samples and as such cannot be described as route specific.

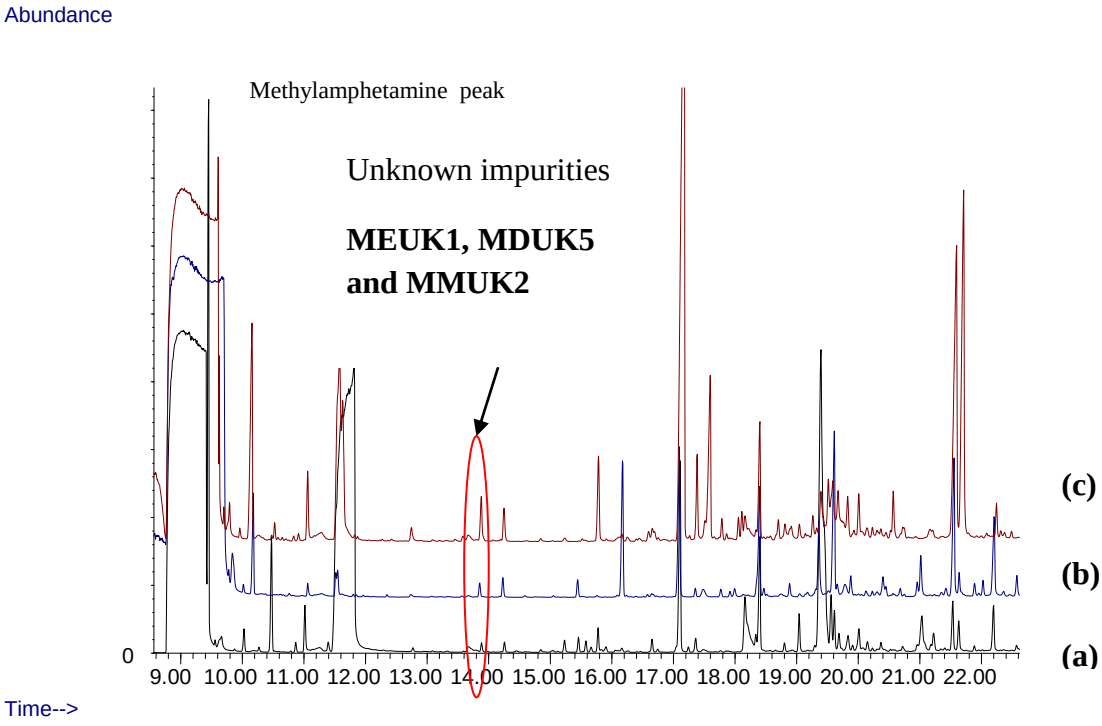

Figure 5.10. Chromatograms of methylamphetamine synthesized via the Moscow route precursors extracted from 'Sudafed' using (a) ethanol, (b) ethanol/methanol (90:10) % vol/vol and (c) methylated spirits

5.3.11 Within batch variation of methylamphetamine synthesized via Hypo route using laboratory grade *pseudo*ephedrine

The chromatographic impurity profiles for each of 6 sub samples of one batches of methylamphetamine (HL 1 i-vi) synthesized via the Hypo route using laboratory grade *pseudo*ephedrine hydrochloride is presented in Figure 5.11 and the within batch RSD (%) values of the derived impurities are presented in Table 5.13. Impurity peaks identified methylamphetamine synthesized via the Hypo route using laboratory grade *pseudo*ephedrine hydrochloride. Good reproducibility was demonstrated for the extraction method with relatively low relative standard deviations obtained across the range of impurities obtained (7 out of 16 peaks having %RSD of 10 or less) which was in keeping with other reported RSD values in similar studies [6]. One of the six suggested Nagai specific impurities identified by Kunalan were apparent in the samples[4, 5].

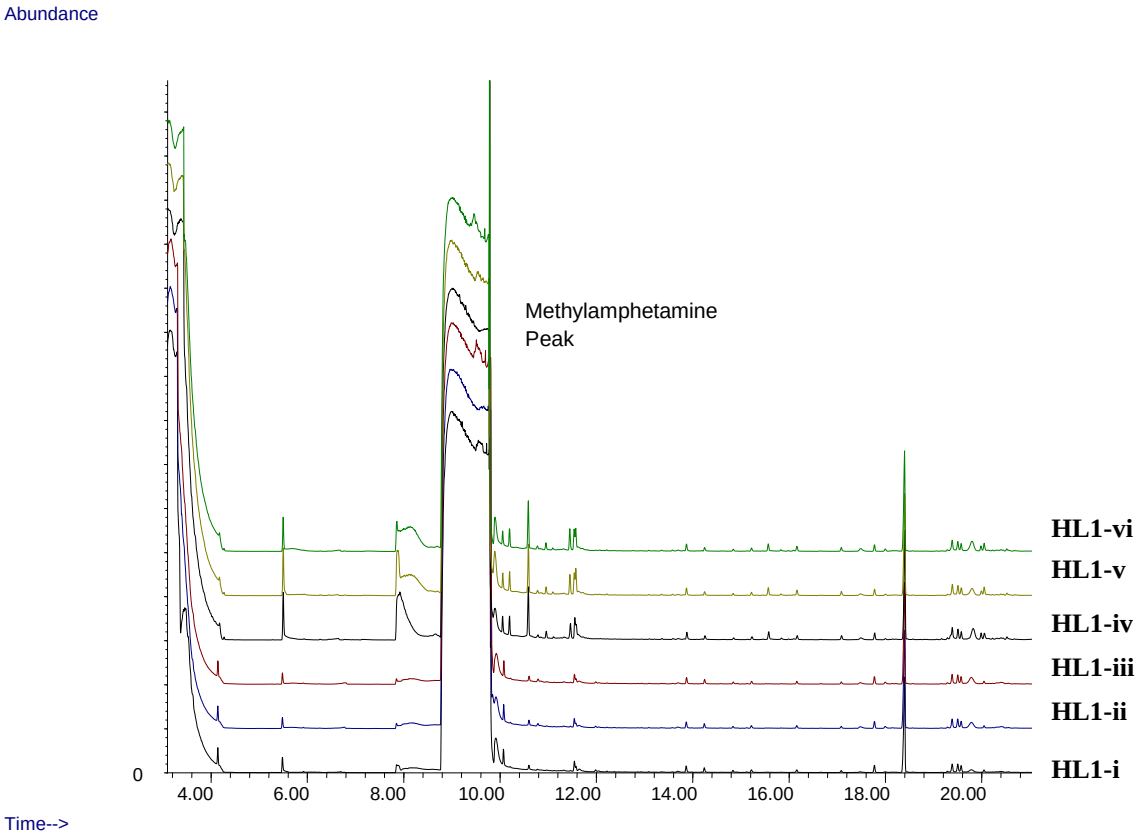

Figure 5.11. Impurity profiles of within batch variation of 6 sub batches of [HL 1] methylamphetamine synthesized via the Hypo route using laboratory grade *pseudo*ephedrine.

Table 5.12. Table of impurity peaks identified in methylamphetamine synthesized via the Hypo route using laboratory grade *pseudo*ephedrine hydrochloride.

Retention Time(RT)	Impurity Peak	RSD(%) of normalized to IS values
6.687	Acetic Acid	2%
7.787	Trans 1,2-dimethyl-3-phenyl azridine,	10%
10.062	Dimethylamphetamine(DMA)	8%
11.556	3-methyl-2-phenyl morpholine	20%
11.635	**HLUK 1**	10%
13.881	N-formylmethamphetamine	15%
14.257	Methylamphetamine acetylated	6%
16.093	Benzeneethanamine, α-methyl-N-phenylmethylene)-	20%
17.088	Benzylamphetamine	10%
17.484	N-β-(phenylisopropyl)benzylmethylketimine	14%
18.003	**HLUK 2**	4%
18.159	1,2-dimethyl-3-phenyl-aziridine,	20%
19.391	N-methyl-N-(α-methylphenethyl)amino-1-phenyl-2-propanone	18%
19.577	Benzoylmethylamphetamine	20%
19.831	**HLUK 3**	17%
20.405	**HLUK 4**	4%

5.3.12 Between batch variation of methylamphetamine synthesized via Hypo route using laboratory grade *pseudo*ephedrine

The chromatographic impurity profiles for each of six repetitively synthesized batches of methylamphetamine via the Hypo route using laboratory grade *pseudo*ephedrine hydrochloride are presented in Figure 5.12. Visual comparison of the impurity profiles illustrate clear differences between sample profiles particularly within the 15-25 minute range. These differences are apparent in the high %RSD values presented in Table 5.13. The previous identified Nagai route specific impurities (in red in the table) are still apparent within the samples.

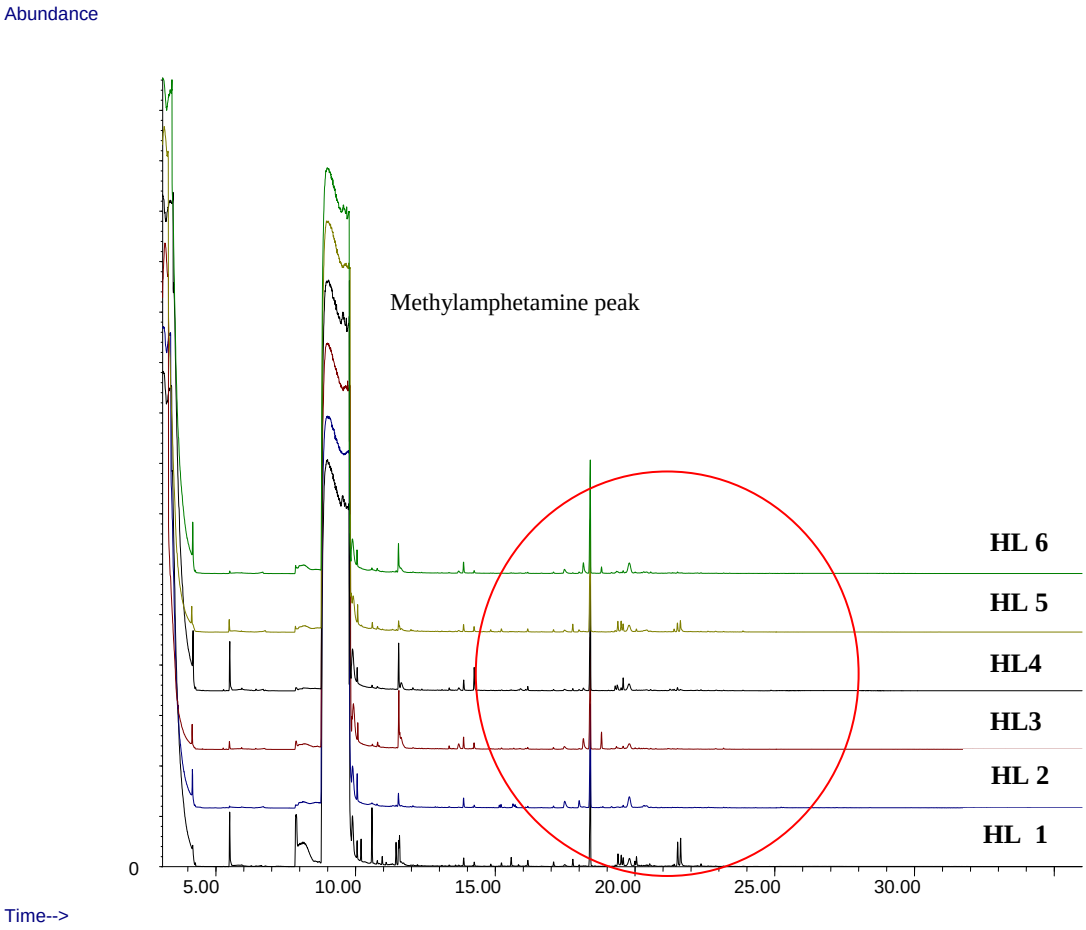

Figure 5.12. Impurity profiles of between batch variation of HL 1 to 6 of methylamphetamine synthesized via the Moscow route using laboratory grade *pseudo*ephedrine.

Table 5.13. Impurity peaks identified methylamphetamine synthesized via the Hypo route using laboratory grade *pseudo*ephedrine hydrochloride.

Retention Time (RT)	Impurity Peak	RSD(%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant values mg/mL	m/z (base peak in bold)
6.687	Acetic Acid	16	14	24	0.002	**60**,76,91,117,133
7.787	Trans 1,2-dimethyl-3-phenyl azridine,	78	80	77	0.007	**146**,105,132,117,91,77
10.062	Dimethylamphetamine(DMA)	27	27	36	0.021	**72**,58,91,65,115,134
11.556	3-methyl-2-phenyl morpholine	55	56	64	0.009	**71**,56,77,91,105
11.635	**HLUK 1**	61	52	68	0.008	**85**,148,70,58,117,133
13.881	N-formylmethamphetamine	71	70	73	0.004	**86**,58,65,77,91,118,132
14.257	Methylamphetamine acetylated	97	100	111	0.002	**58**,100
16.093	α-methyl-N-phenylmethylene- benzeneethanamine	55	53	46	0.002	**132**,71,91,105,117,165
17.088	Benzylamphetamine	50	51	61	0.001	**91**,148,179,222,58,77
17.484	N-β-(phenylisopropyl)benzylmethylketimine	49	51	60	0.002	**91**,160,119,58,77
18.003	**HLUK 2**	41	39	50	0.000	**172**,91,131,58,77,103, 65
18.159	1,2-dimethyl-3-phenyl-aziridine,	116	115	107	0.003	**146**,105,132,117,91,77
19.577	Benzoylmethylamphetamine	57	59	68	0.003	**105**,162,77,91
19.831	**HLUK 3**	46	44	56	0.020	**174**,91,56,115,148,65, 77,265
20.405	**HLUK 4**	26	23	24	0.001	**186**,91,105,128,58,77, 115,162

RSD values are presented for raw data and peak areas normalized to the peak area of the internal standard (IS) and to the total peak area (TPA). Impurities were semi-quantified to the internal standard using a single point estimate.

One of the four route specific impurities observed for the Nagai route were also observed for the batches of methylamphetamine synthesised via the Hypo route using laboratory grade *pseudo*ephedrine hydrochloride. The mentioned route specific impuritiesobserved were, N-methyl-N-(α-methylphenyl)amino-1-phenyl-2-propanone [1].

5.3.13 Overview of variation of methylamphetamine synthesized via Hypo route using *pseudo*ephedrine extracted from proprietary cold medication(Sudafed) using the three different solvent systems

Figure 5.13 represents on overlay of batches of methylamphetamine synthesised using laboratory precursor and precursors extracted from proprietary cold medication using the three different solvent systems and exposes significant differences within the resultant impurity profiles which appear to be solvent dependent. The rest of the work discussed in this section explores these differences in greater depth.

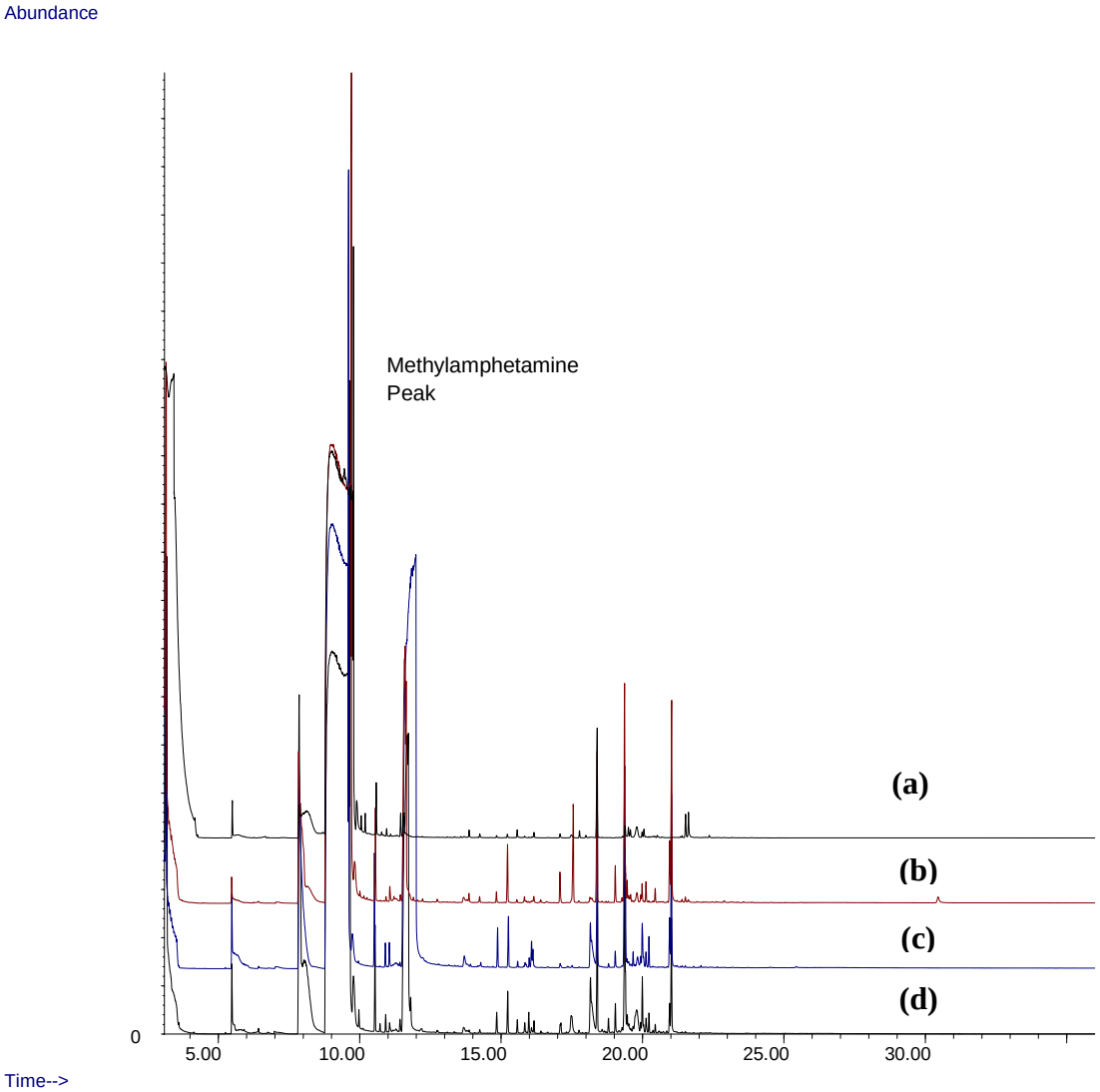

Figure 5.13. Chromatograms of methylamphetamine synthesized via the Hypo route (a) laboratory grade precursors and precursors extracted from 'sudafed' using (b)ethanol, (c) mthanol/methanol(10:90) and (d)methylated spirits.

5.3.14 Within batch variation of methylamphetamine synthesized via Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using ethanol as the extraction solvent

The chromatographic impurity profiles and related data for six subsamples of methylamphetamine produced via the Hypo synthesis from precursor extracted from Sudafed using ethanol are presented in Figure 5.14 and Table 5.14. Four of the 29 peaks presenting within the impurity profile display an RSD value of 10% of less while again this is in accordance with previous literature values for similar studies [6].

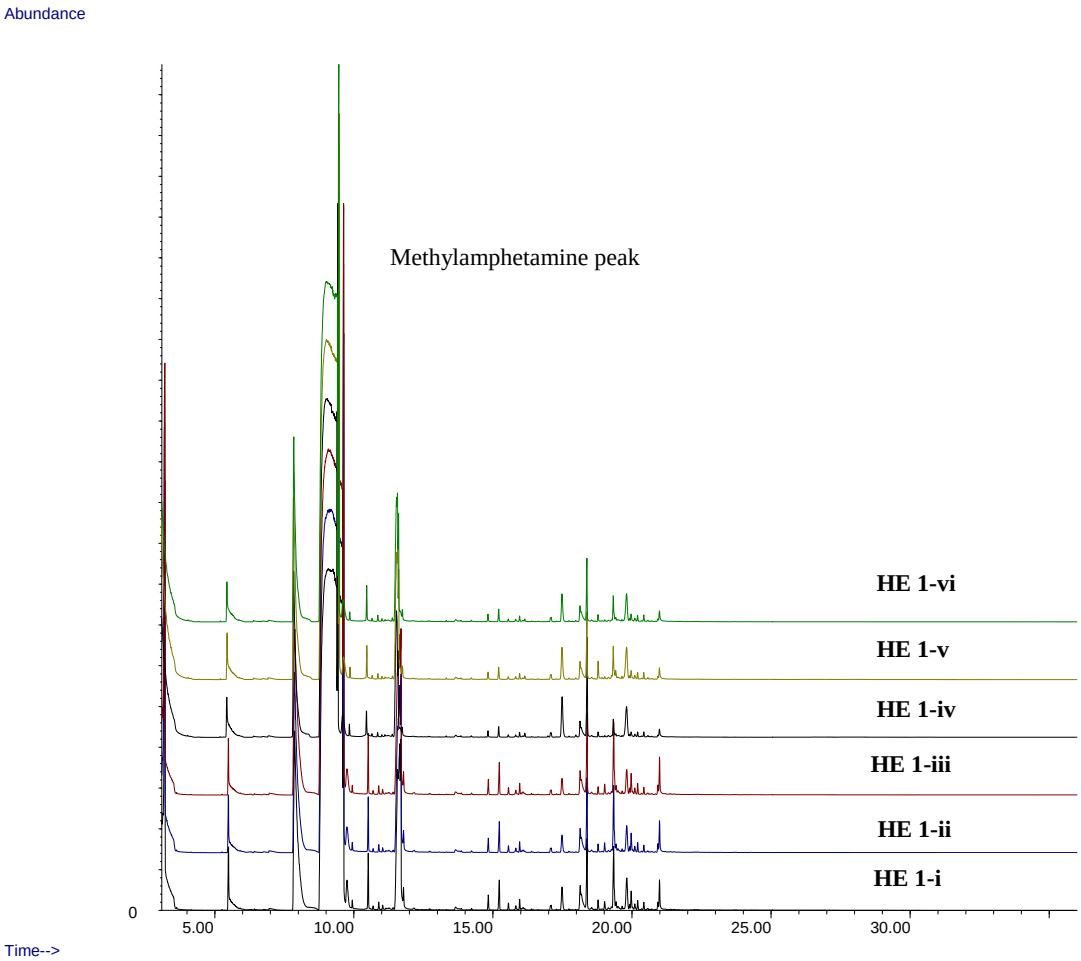

Figure 5.14. Impurity profiles illustrating within batch variation of 6 sub batches of methylamphetamine synthesized via the Hypo route (HE 1) using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) with ethanol.

Table 5.14. Table of impurity peaks identified in methylamphetamine synthesized via the Hypo route using using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) with ethanol.

Retention Time (RT)	Impurity Peak	RSD(%) of normalized to IS values
5.649	Benzaldehyde	26
5.918	2-methyl- benzeneethanol,	10
6.421	2-propenyl benzene,	27
7.864	Trans 1,2-dimethyl-3-phenyl- azridine,	23
11.244	1-(2-phenylethyl) azridine	20
13.357	Bibenzyl	20
13.688	**HEUK 1**	9
13.885	Ethyl-methamphetamine	21
14.258	N-Acetylmethamphetamine	12
14.859	**HEUK 2**	28
15.247	**HEUK 3**	34
15.846	N-formylephedrine	30
16.081	**HEUK 4**	14
16.170	Amphetaminil	20
16.412	*Pseudo*ephedrine	22
17.508	N-β-(phenylisopropyl)benzylmethylketimine	7
17.757	**HEUK 5**	10
18.161	1,2dimethyl-3-phenyl azridine	11
19.287	**HEUK 6**	31
19.464	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	16
19.519	**HEUK 7**	13
19.590	Benzoylmethylamphetamine	20
19.940	**HEUK 8**	18
20.128	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	16
20.234	**HEUK 9**	19
20.370	**HEUK 10**	13
20.567	**HEUK 11**	25
21.031	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropan-2-amine	24
21.519	**HEUK 12**	33

5.3.15 Between batch variation of methylamphetamine synthesized via Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using ethanol as the extraction solvent

The chromatographic impurity profiles for each of the 6 batches synthesized via the Hypo route using *pseudo*ephedrine hydrochloride extracted from Sudafed tablets using ethanol and associated data are presented in Figure 5.15 and Table 5.15. Visual comparison of the impurity profiles illustrate considerable differences between sample profiles particularly within the 14-24 minute range. These differences are apparent in the high %RSD values presented in Table 5.15.

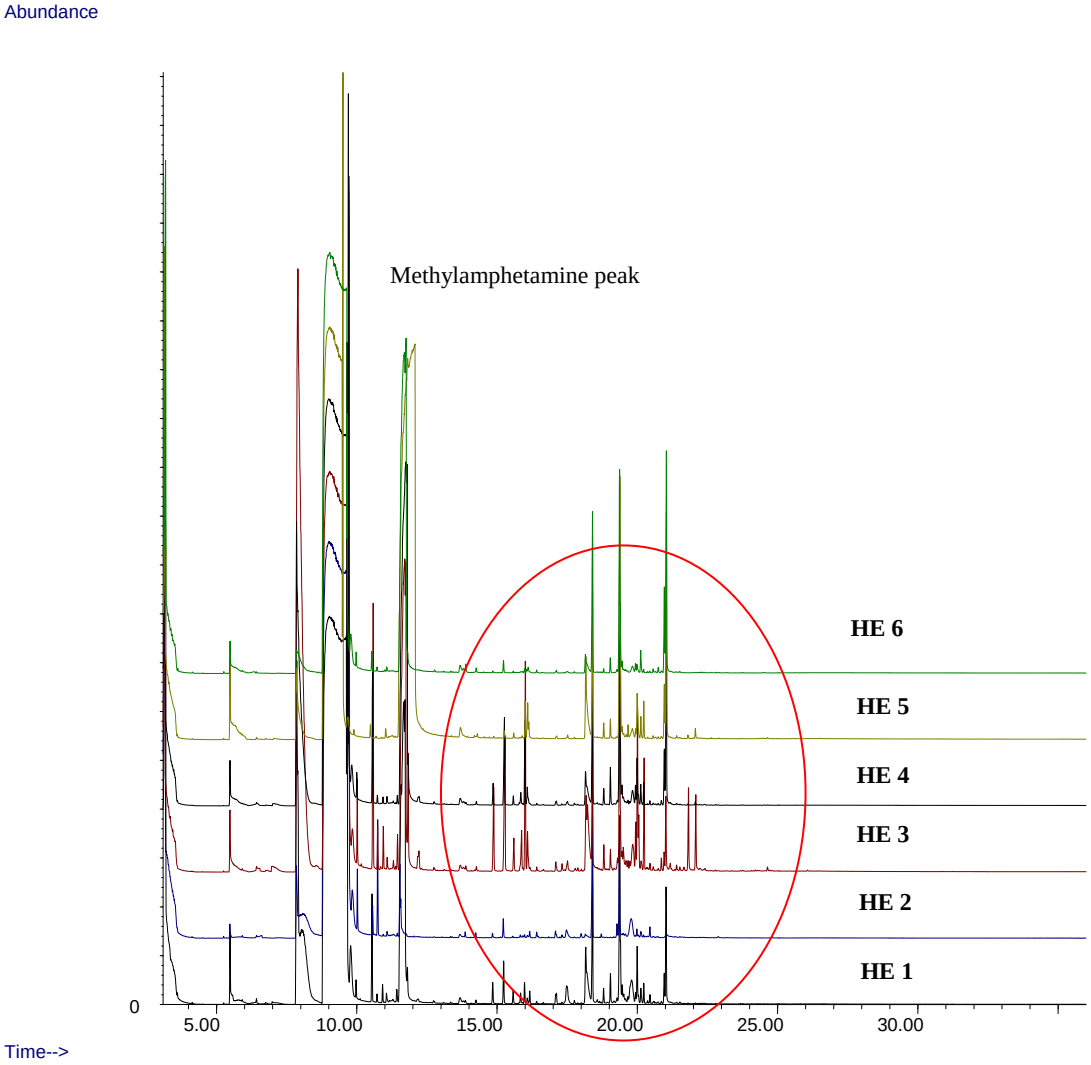

Figure 5.15. Impurity profiles of between batch variation of HE 1 to 6 of methylamphetamine synthesized via the Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol.

Table 5.15. Impurity peaks identified methylamphetamine synthesized via the Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol as the extracting solvent.

Retention Time (RT)	Impurity Peak	RSD(%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant values mg/mL	m/z (base peak in bold)
5.649	Benzaldehyde	115	116	113	0.007	**106**,77,51
5.918	2-methyl- benzeneethanol,	60	59	53	0.004	**105**,77,91,51,63
6.421	2-propenyl benzene,	88	86	84	0.002	**117**,60,91,103,77,51
7.864	Trans 1,2-dimethyl-3-phenyl- azridine,	91	91	87	0.234	**146**,105,132,117,91,77,65
11.244	1-(2-phenylethyl) azridine	70	69	67	0.001	**56**,65,77,91,104,117,146
13.357	Bibenzyl	92	91	89	0.000	**91**,58,77,105,146,182
13.688	**HEUK 1**	53	52	51	0.005	**148**,176,113,91,58
13.885	Ethyl-methamphetamine	44	43	52	0.001	**86**,58,65,77,91,97,118
14.258	N-Acetylmethamphetamine	30	32	36	0.001	**58**,100,65,77,91,117
14.859	**HEUK 2**	146	148	134	0.008	**118**,147,191,77,91,105, 191(2 isomers)
15.247	**HEUK 3**	114	114	100	0.020	**118**,147,191,77,91,105, 191
15.846	N-formylephedrine	125	126	110	0.004	**57**,65,77,91,105,117,132
16.081	**HEUK 4**	168	169	156	0.014	**176**,58,91,115,77
16.170	Amphetaminil	63	62	62	0.002	**132**,58,71,77,91,105,117
16.412	*Pseudo*ephedrine	61	69	75	0.000	**58**,77,91,105
17.508	N-β-(phenylisopropyl)benzylmethylketimine	69	73	75	0.003	**91**,160,119,146,168,206,65,77
17.757	**HEUK 5**	53	52	40	0.000	**85**,58,70,91,105,146,178
18.161	1,2dimethyl-3-phenyl azridine	89	89	85	0.024	**146**,132,105,91,77,65
19.287	**HEUK 6**	64	67	67	0.002	**58**,148,190,91,77,105,118

Retention Time (RT)	Impurity Peak	RSD(%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant values mg/mL	m/z (base peak in bold)
19.464	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	66	56	64	0.005	**58**,118,141,193,238
19.519	**HEUK 7**	81	81	63	0.004	**58**,118,203,49,77,91
19.590	Benzoylmethylamphetamine	58	59	42	0.002	**105**,162,77,91
19.940	**HEUK 8**	87	88	71	0.005	**58**,118,179,91,105
20.128	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	26	22	29	0.006	**58**,118,141,193,238
20.234	**HEUK 9**	118	119	104	0.010	**118**,237,91,58,77,146
20.370	**HEUK 10**	46	47	36	0.000	**118**,56,148,162,91,77,105, 132,239
20.567	**HEUK 11**	52	51	47	0.001	**259**,115,58,77,91,105
21.031	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropan-2-amine	60	59	65	0.049	**58**,239,208,193,165
21.519	**HEUK 12**	87	87	70	0.000	**58**,190,91,105,118,132, 146,77,65

The impurity compound associated with the Emde route was found present in this batches of methylamphetamine similar to the batches of methylamphetamine synthesised using *pseudo*ephedrine HCl extracted using ethanol, the mentioned impurity N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine were present.

5.3.16 Within batch variation of methylamphetamine synthesized via Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using ethanol:methanol (90:10)% vol/vols as the extraction solvent

The chromatographic impurity profiles and related data for six subsamples of methylamphetamine produced via the Hypo synthesis from precursor extracted from Sudafed using ethanol:methanol (90:10)% vol/vols are presented in Figure 5.16 and Table 5.16. Thirteen of the 27 peaks presenting within the impurity profile display and RSD value of 10% of less and again this is in accordance with previous literature values for similar studies [6].

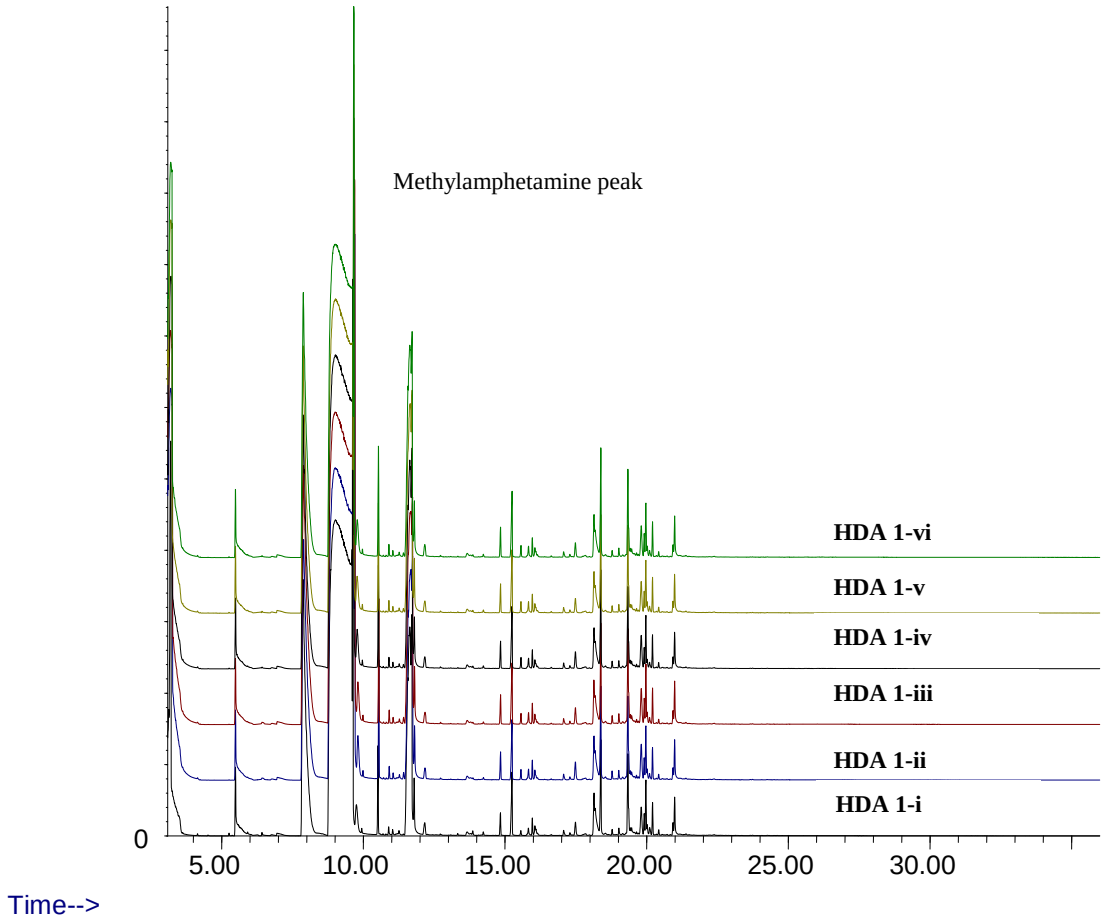

Figure 5.16. Impurity profiles illustrating within batch variation of 6 sub batches of methylamphetamine synthesized via the Hypo route (HDA1) *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol:methanol (90:10 % vol/vol) as the extraction solvent.

Table 5.16. Impurity peaks identified in methylamphetamine synthesized via the Hypo route using using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) with ethanol:methanol (90:10)% vol/vols.

Retention Time (RT)	Impurity Peak	RSD(%) of normalized to IS values
5.584	Benzaldehyde	24%
6.441	2-propenyl benzene	20%
11.805	*Pseudo*ephedrine	2%
13.365	Bibenzyl	2%
13.683	**HDUK 1**	30%
14.248	Methamphetamine acetylated	22%
15.238	**HDUK 2**	12%
16.074	Acetylephedrine	28%
16.652	**HDUK 3**	5%
17.501	N-β-(phenylisopropyl)benzylmethyl ketimine	21%
17.758	**HDUK 4**	6%
18.154	1,2-dimethyl-3-phenyl azridine	5%
19.042	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	10%
19.386	**HDUK 5**	5%
19.466	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	18%
19.593	Benzoylmethylamphetamine	7%
19.662	**HDUK 6**	7%
19.824	**HDUK 7**	4%
19.942	**HDUK 8**	16%
19.996	N-methyl-N-(α-methylphenethyl)amino-1-phenyl-2-propanone	3%
20.368	**HDUK 9**	12%
20.563	**HDUK 10**	20%
20.881	**HDUK 11**	8%
20.961	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1phenylpropa-2-amine	4%
21.028	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	10%
21.517	**HDUK 12**	5%
21.807	**HDUK 13**	19%

5.3.17 Between batch variation of methylamphetamine synthesized via Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed-UK) using ethanol:methanol (90:10)% vol/vols as the extraction solvent

The chromatographic impurity profiles for each of the 6 batches synthesized via the Moscow route using *pseudo*ephedrine hydrochloride extracted from Sudafed tablets using ethanol :methanol (90:10)% vol/vols and associated data are presented in Figure 5.17 and Table 5.17. Visual comparison of the impurity profiles illustrate considerable differences between sample profiles particularly within the 14-22 minute range.

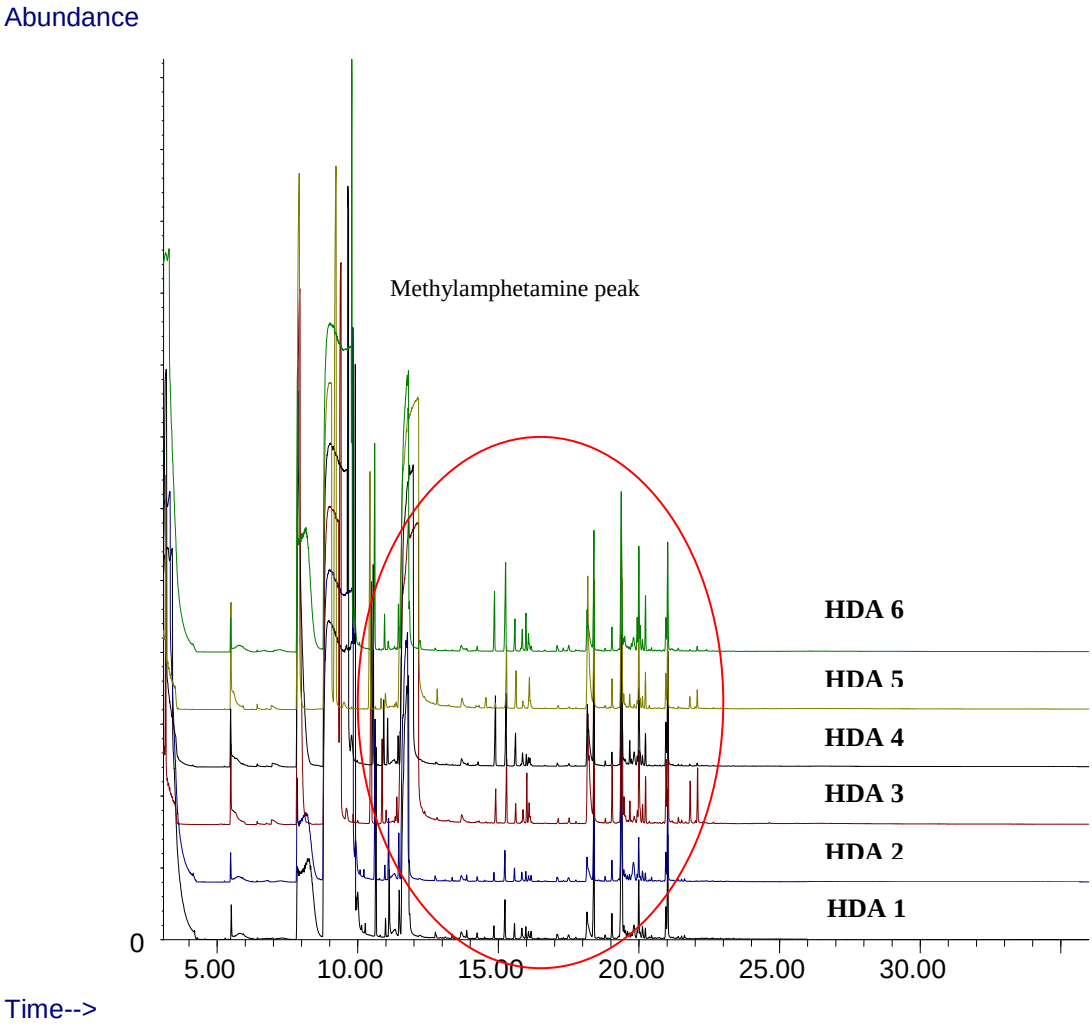

Figure 5.17. Impurity profiles of between batch variation of HDA 1 to 6 of methylamphetamine synthesized via the Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed-UK) using ethanol:methanol (90:10)% vol/vols as the extraction solvent

Table 5.17. Impurity peaks identified methylamphetamine synthesized via the Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using ethanol:methanol (90:10)% vol/vols as the extracting solvent.

Retention Time (RT)	Impurity Peak	RSD (%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant mg/mL	m/z (base peak in bold)
5.584	Benzaldehyde	93	92	98	0.014	**106**,77,51
6.441	2-propenyl benzene	69	66	71	0.002	**117,**60,91,103,77,51
11.805	*Pseudo*ephedrine	62	60	71	0.480	**58**,77,91,105
13.365	Bibenzyl	73	73	68	0.001	**91**,58,77,105,146,182
13.683	**HDUK 1**	32	31	39	0.008	**148**,176,58,91,113,133, 84,77,98
14.248	Methamphetamine acetylated	90	90	74	0.001	**58**,100,65,77,91,117
15.238	**HDUK 2**	38	40	34	0.025	**118**,147,191,77,91,105
16.074	Acetylephedrine	81	80	84	0.006	**58**,77,86,100,117,207
16.652	**HDUK 3**	35	36	18	0.000	**91**,134,58,77,105
17.501	N-β-(phenylisopropyl)benzylmethyl ketimine	54	56	40	0.002	**91**,160,119,146,168,206, 65,77
17.758	**HDUK 4**	54	56	39	0.000	**85**,58,70,77,91,117,250
18.154	1,2-dimethyl-3-phenyl azridine	79	77	97	0.046	**146**,132,105,91,77,65
19.042	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	57	56	59	0.009	**58**,118,141,193,238
19.386	**HDUK 5**	20	18	21	0.107	**118**,58,149,91,133,71
19.466	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	52	52	55	0.108	**58**,118,141,193,238
19.593	Benzoylmethylamphetamine	37	38	20	0.002	**105**,162,77,91
19.662	**HDUK 6**	50	48	52	0.005	**148**,190,203,118,105,91, 77,58,265
19.824	**HDUK 7**	49	49	40	0.008	**174**,105,252,91,77,56,14 8,265
19.942	**HDUK 8**	67	71	52	0.005	**58**,118,237,91,179
19.996	N-methyl-N-(α-methylphenethyl)amino-1-phenyl-2-propanone	34	33	36	0.031	**238**,190,148,120,105,91
20.368	**HDUK 9**	28	27	38	0.000	**118**,148,162,58,105,91, 77,132
20.563	**HDUK 10**	38	39	29	0.000	**259**,146,115,58,77,91, 105
20.881	**HDUK 11**	37	38	26	0.000	**58**,146,241,91,77,105, 117

Retention Time (RT)	Impurity Peak	RSD (%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant mg/mL	m/z (base peak in bold)
20.961	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1phenylpropa-2-amine	32	31	35	0.015	**58**,239,208,193,165
21.028	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	23	23	26	0.047	**58**,239,208,193,165
21.517	**HDUK 12**	52	52	46	0.000	**58**,190,91,105,118,132,146,160
21.807	**HDUK 13**	160	160	155	0.003	**254**,148,120,58,91,105,132

One of the four route specific impurities observed for the Nagai route were also observed for the batches of methylamphetamine synthesised via the Hypo route using laboratory grade *pseudo*ephedrine hydrochloride. The mentioned route specific impurities observed were, N-methyl-N-(α-methylphenyl)amino-1-phenyl-2-propanone [1]. the impurity compound associated with the Emde route was found present in this batches of methylamphetamine the mentioned impurity N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine were present.

5.3.18 Within batch variation of methylamphetamine synthesized via Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using commercial methylated spirit as the extraction solvent

The chromatographic impurity profiles and related data for six subsamples of methylamphetamine produced via the Hypo synthesis from precursor extracted from Sudafed using commercial methylated spirit are presented in Figure 5.18 and Table 5.18

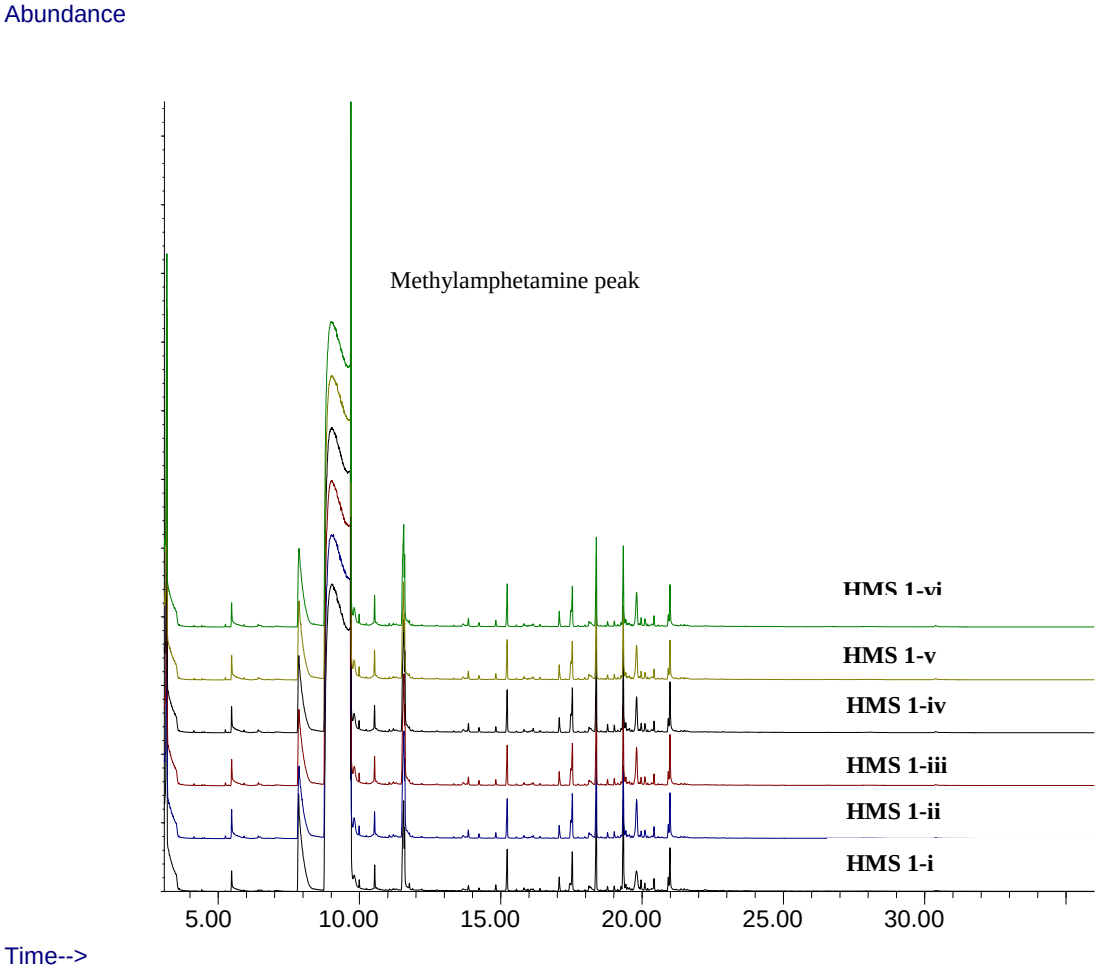

Figure 5.18. Impurity profiles illustrating within batch variation of 6 sub batches of methylamphetamine synthesized via the Hypo route (HMS1) using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) using commercial methylated spirit as the extraction solvent

Table 5.18. Impurity peaks identified in methylamphetamine synthesized via the Hypo route using using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed) with commercial methylated spirit.

Retention Time (RT)	Impurity Peak	RSD(%) of normalized to IS values
5.467	Benzaldehyde	27%
6.420	2-propenyl benzene,	25%
10.163	p-Chloro-N-methylamphetamine	18%
10.270	**HMUK 1**	20%
10.945	**HMUK 2**	25%
12.063	**HMUK 3**	20%
13.696	**HMUK 4**	7%
13.818	**HMUK 5**	17%
13.874	Ethyl methamphetamine	16%
14.250	N-acetyl methamphetamine	16%
15.837	Dimethylphenyloxazolidone	4%
16.094	**HMUK 6**	19%
16.410	**HMUK 7**	18%
17.093	Benzylamphetamine	6%
18.001	**HMUK 8**	21%
18.148	1,2 dimethyl-3-phenyl azridine,	9%
19.355	**HMUK 9**	7%
19.452	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	12%
19.507	**HMUK 10**	5%
19.657	**HMUK 11**	6%
19.987	N-methyl-N-(α-methylphenethyl)amino-1-phenyl-2-propanone	8%
20.223	**HMUK 12**	10%
20.448	**HMUK 13**	11%
21.013	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	15%
21.224	N-benzoylmethylamphetamine	20%
21.517	**HMUK 14**	28%
22.146	**HMUK 15**	17%
30.46	Leucocrystal Violet	18%

5.3.19 Between batch variation of methylamphetamine synthesized via Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed-UK) using commercial methylated spirit as the extraction solvent

The chromatographic impurity profiles for each of the 6 batches synthesized via the Hypo route using *pseudo*ephedrine hydrochloride extracted from Sudafed tablets using commercial methylated spirit and associated data are presented in Figure 5.19 and Table 5.19. Visual comparison of the impurity profiles illustrate considerable differences between sample profiles particularly within the 14-20 minute range.

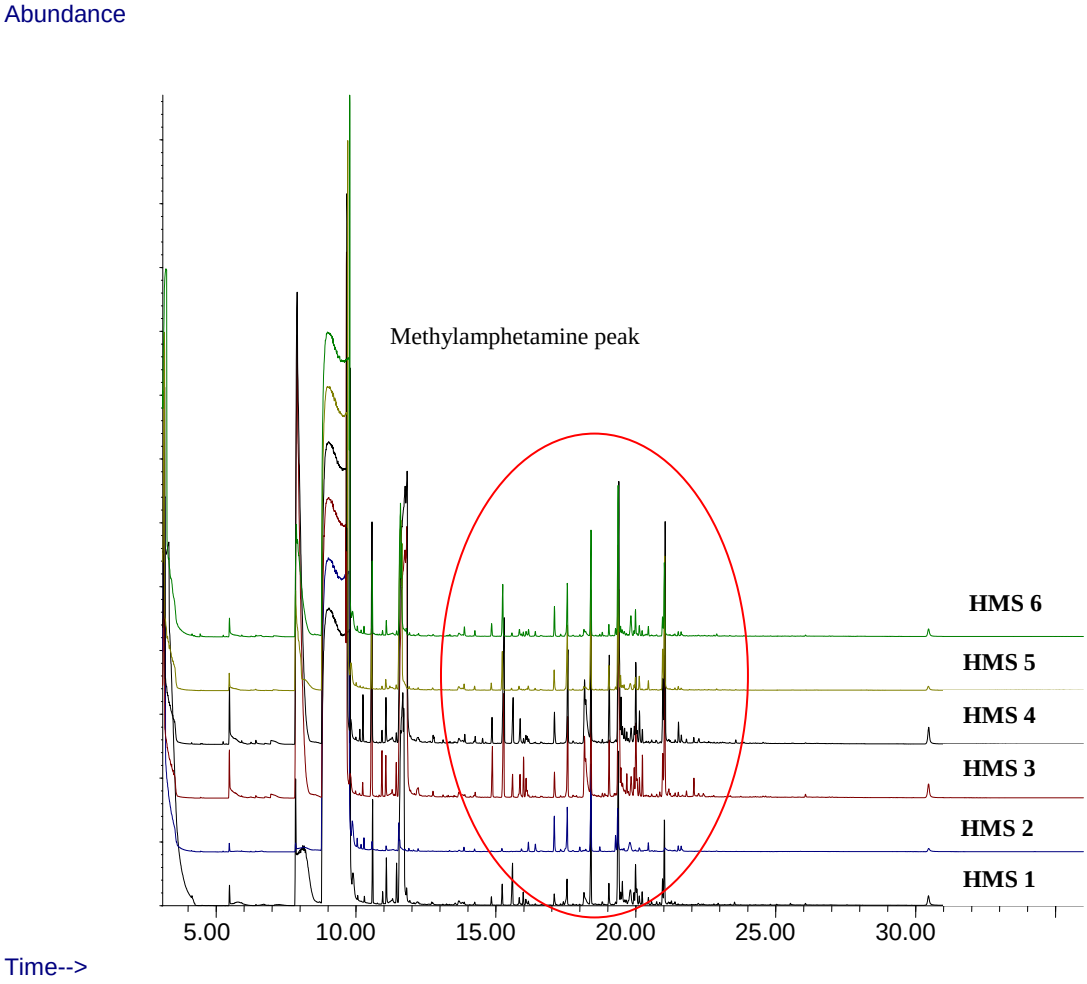

Figure 5.19. Impurity profiles of between batch variation of HMS 1 to 6 of methylamphetamine synthesized via the Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Sudafed-UK) using commercial methylated spirit as the extraction solvent

Table 5.19. Impurity peaks identified methylamphetamine synthesized via the Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication using commercial methylated spirit as the extracting solvent.

Retention Time (RT)	Impurity Peak	RSD (%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant mg/mL	m/z (base peak in bold)
5.467	Benzaldehyde	85	80	63	0.016	**106**,77,51
6.420	2-propenyl benzene,	91	84	70	0.001	**117**,60,91,103,77,51
10.163	p-Chloro-N-methylamphetamine	35	31	30	0.005	**58**,91,125,63,77,105
10.270	**HMUK 1**	68	61	53	0.008	**58**,117,90,144,104,130,77,65
10.945	**HMUK 2**	105	105	91	0.005	**120**,58,77,91
12.063	**HMUK 3**	45	43	37	0.001	**138**,58,181,91,82,100,110,152
13.696	**HMUK 4**	33	31	33	0.004	**148**,176,58,91,113,133,84,98, 77
13.818	**HMUK 5**	57	55	51	0.002	**148**,176,113,91,84,58,77,105, 133
13.874	Ethyl methamphetamine	41	37	42	0.002	**86**,58,65,77,91,97,118
14.250	N-acetyl methamphetamine	42	44	53	0.001	**58**,100,65,77,91,117
15.837	Dimethylphenyloxazolidone	90	86	72	0.006	**57**,77,91,105,117,191
16.094	**HMUK 6**	87	85	75	0.003	**58**,100,77,91,86,176
16.410	**HMUK 7**	86	97	99	0.001	**58**,91,105,115,146
17.093	Benzylamphetamine	31	35	37	0.011	**91**,148,65,77,102,116,130
18.001	**HMUK 8**	60	72	76	0.000	**58**,91,219,176,119,77,105,146, 234
18.148	1,2 dimethyl-3-phenyl azridine	105	101	90	0.017	**146**,132,105,91,77,65
19.355	**HMUK 9**	55	50	37	0.106	**58**,118,149,91,77,133
19.452	N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine	80	74	60	0.009	**58**,118,141,193,238

Retention Time (RT)	Impurity Peak	RSD (%)	RSD(%) normalized to IS	RSD(%) normalized to TPA	Semi quant mg/mL	m/z (base peak in bold)
19.507	**HMUK 10**	62	62	65	0.007	**58**,91,118,238,190,149,133,105 72
19.657	**HMUK 11**	99	97	82	0.004	**148**,190,58,77,91,105,133
19.987	N-methyl-N-(α-methylphenethyl)amino-1-phenyl-2-propanone	70	73	80	0.009	**238**,190,148,120,105,91
20.223	**HMUK 12**	72	66	54	0.007	**188**,118,105,91,58,77,247
20.448	**HMUK 13**	46	52	57	0.002	**71**,56,91,115,78,236,251
21.013	N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine	67	61	52	0.057	**58**,239,208,193,165
21.224	N-benzoylmethylamphetamine	95	93	75	0.002	**105**,162,77,91,58
21.517	**HMUK 14**	96	87	73	0.003	**58**,190,91,105,118
22.146	**HMUK 15**	59	56	39	0.000	**58**,146,105,91,77,132,176,202
30.46	Leucocrystal Violet	52	45	31	0.011	**253**,373,359,329,126,165,208

One of the four route specific impurities observed for the Nagai route were also observed for the batches of methylamphetamine synthesised via the Hypo route using laboratory grade *pseudo*ephedrine hydrochloride. The mentioned route specific impurities observed were, N-methyl-N-(α-methylphenyl)amino-1-phenyl-2-propanone [1]. The impurity compound associated with the Emde route was found present in this batches of methylamphetamine the mentioned impurity N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine were present.

5.3.20 Unknown impurity peaks of the methylamphetamine synthesized from the Hypo route

Besides known impurities listed in the above sections, this research also has identified a number of unknown impurities for methylamphetamine synthesized via the Hypo route. The unknown impurities are listed in Table 5.20. Some of the peaks presented in the table below were listed as unknowns in literature.

Table 5.20. Summary of the unknown peaks for the Hypophosphorous route.

HUK (Hypo unknown impurity peaks)	Mass ions for identification(base peak in bold)	Retention Time of GC-MS(unknowns reported in other citations)
HLUK 1	**85**,148,70,58,117,133,105	11.635(**FSI 164(2006)201-210)[8]**
HLUK 2	**172**,91,131,58,77,103,65	18.003
HLUK 3	**174**,91,56,115,148,65,77,265	19.831 **(FSI 160(2006))[7]**
HLUK 4	**186**,91,105,128,58,77,115,162	20.405
HEUK 1	**148**,176,113,91,58	13.688
HEUK 2	**118**,147,191,77,91,105,191(2 isomers)	14.859 (**FSI 164(2006) 201-210)[8]**
HEUK 3	**118**,147,191,77,91,105,191	15.247 (**FSI 164(2006) 201-210)[8]**
HEUK 4	**176**,58,91,115,77	16.081
HEUK 5	**85**,58,70,91,105,146,178	17.757
HEUK 6	**58**,148,190,91,77,105,118	19.287
HEUK 7	**58**,118,203,49,77,91	19.519
HEUK 8	**58**,118,179,91,105	19.940
HEUK 9	**118**,237,91,58,77,146	20.234 **(FSI 160 (2006) 1-103)[7]**
HEUK 10	**118**,56,148,162,91,77,105,132,239	20.370 (**FSI 160 (2006) 1-10)[7]**
HEUK 11	**259**,115,58,77,91,105	20.567
HEUK 12	**58**,190,91,105,118,132,146,77,65	21.519
HDUK 2	**148**,176,58,91,113,133,84,77,98	13.683
HDUK 3	**118**,147,191,77,91,105	15.238 (**FSI 164 (2006) 201-210)[8]**
HDUK 4	**91**,134,58,77,105	16.652
HDUK 5	**85**,58,70,77,91,117,250	17.758
HDUK 6	**118**,58,149,91,133,71	19.386
HDUK 7	**148**,190,203,118,105,91,77,58,265	19.662
HDUK 8	**174**,105,252,91,77,56,148,265	19.824 (**FSI 160(2006) 1-10)[7]**
HDUK 9	**58**,118,237,91,179	19.942 (**FSI 160(2006) 1-10)[7]**
HDUK 10	**118**,148,162,58,105,91,77,132	20.368
HDUK 11	**259**,146,115,58,77,91,105	20.563
HDUK 12	**58**,146,241,91,77,105,117	20.881
HDUK 13	**58**,190,91,105,118,132,146,160	21.517
HDUK 14	**254**,148,120,58,91,105,132	21.807(**FSI 160(2006) 1-10)[7]**
HMUK 1	**58**,117,90,144,104,130,77,65	10.270

HUK (Hypo unknown impurity peaks)	Mass ions for identification(base peak in bold)	Retention Time of GC-MS(unknowns reported in other citations)
HMUK 2	**120**,58,77,91	10.945 (**Analytica Chimica acta 619 (2008) 20-25)[10]**
HMUK 3	**138**,58,181,91,82,100,110,152	12.063
HMUK 4	**148**,176,58,91,113,133,84,98,77	13.696
HMUK 5	**148**,176,113,91,84,58,77,105,133	13.818
HMUK 6	**58**,100,77,91,86,176	16.094
HMUK 7	**58**,91,105,115,146	16.410
HMUK 8	**58**,91,219,176,119,77,105,146,234	18.001
HMUK 9	**58**,118,149,91,77,133	19.355
HMUK 10	**58**,91,118,238,190,149,133,105,72	19.507
HMUK 11	**148**,190,58,77,91,105,133	19.657
HMUK 12	**188**,118,105,91,58,77,247	20.223
HMUK 13	**71**,56,91,115,78,236,251	20.448 (**FSI 161 (2006) 209-215)[6]**
HMUK 14	**58,**190,91,105,118	21.517
HMUK 15	**58**,146,105,91,77,132,176,202	22.146

A number of common impurities were observed across the unknown impurities within the Hypo samples. In each of the clandestine mimicked sample set, the impurity previously associated with the clandestine mimicked Moscow samples was present at approximately 13.6 minutes. This is identified as HEUK1, HDUK2 and HMUK4. Again these did not appear in the samples produced using laboratory grade chemicals.

Each of the clandestine mimicked Hypo samples produced one common impurity at approximately 21.5 minutes (HEUK12, HDUK13, HMUK14) which was not present in the samples prepared from laboratory grade materials. Finally the impurity at approximately 20.5 minutes (HEUK11, HDUK13) was common across the ethanol and ethanol:methanol extracted precursor samples but did not appear in the methylated spirit extracted precursor samples. This also appeared to be the same impurity as MEUK10 in the Moscow prepared samples.

5.4 Methylamphetamine synthesized from precursors sourced from Malaysia

5.4.1 Methylamphetamine synthesized via Hypo route using *pseudo*ephedrine extracted from proprietary cold medication (Allerpid-Malaysia) using acid base extractions

Two batches of methylamphetamine hydrochloride were synthesized via Hypo route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Allerpid-Malaysia) shown below in Figure 5.20 and Table 5.21 using acid base extraction as detailed in Chapter 3. The impurity profiles of these samples are presented in figure 5.20 and the table of extracted peaks presented in table 5.21.

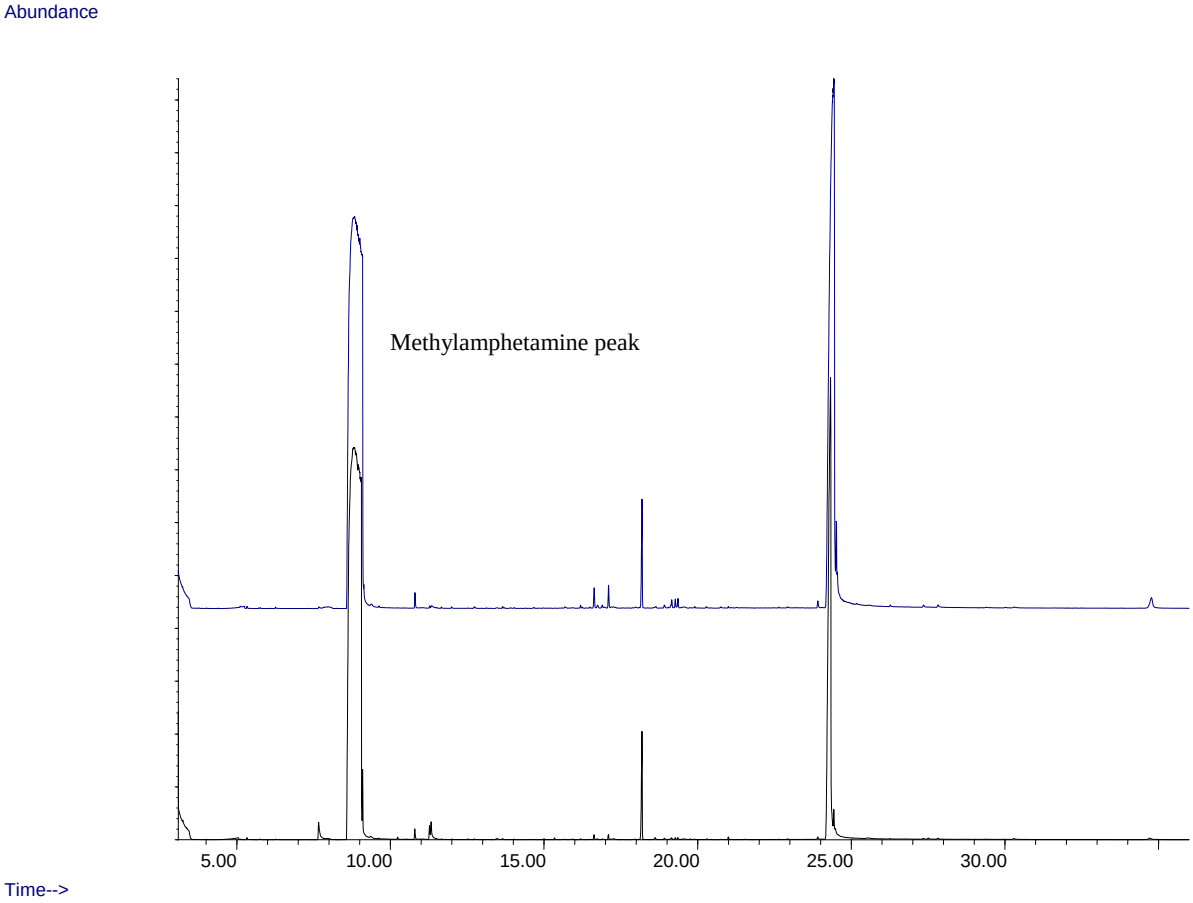

Figure 5.20. Impurity profiles of different batches (AllepH 1 to Allep H2) of methylamphetamine synthesized via the Hypophosphorous route using *pseudo*ephedrine hydrochloride (Allerpid-Malaysia) extracted using acid base extraction

Table 5.21. List of some of the impurities of methylamphetamine synthesized via Hypophosphorous route using *pseudo*ephedrine hydrochloride extracted from (Allerpid-Malaysia) using acid base extraction.

No	RT	Peak	m/z fragments(base peak in bold)
1.	5.329	Benzaldehyde	**106**,77,51,60,85
2.	7.674	1,2 dimethyl-3-phenylazridine	**146**,105,132,117,91,77,65
3.	10.803	Methcathinone	**58**,73,77,86,92,105,115
4.	11.282	3-methyl-2-phenylmorpholine	**71**,56,77,91,117,148,176
5.	16.632	**Unknown**	**162**,91,119,154,56,71
6.	17.102	**Unknown**	**156**,99,91,58,70,78
7.	18.641	**Unknown**	**114**,171,203,58,91,99,81
8.	18.918	**Unknown**	**229**,214,194,170,179,58
9.	19.157	N-methyl-N(-α-methylpheneethyl)amino-1-phenyl-2-propanone	**238**,190,91,120,105,133,146
10.	19.359	**Unknown**	**162**,190,91,119,154,56,71
11.	21.001	Triprolidine	**208,**58,193,181,167,117
12.	23.916	Desloratadine	**280**,324,216,245,70,96,115

One of the four route specific impurities (N-methyl-N-(α-methylphenyl)amino-1-phenyl-2-propanone [1]), observed for the Nagai route was also observed within these samples

5.4.2 Methylamphetamine synthesized via Hypo route using *pseudo*ephedrine extracted from proprietary cold medication (Panadol-Malaysia) using acid base extractions

A single batch of methylamphetamine hydrochloride were synthesized via Moscow route using *pseudo*ephedrine hydrochloride extracted from Panadol sourced from Malaysia using acid base extraction as detailed in Chapter 3 shown in Figure 5.21 and Table 5.22. The impurity profile and associated data is presented in figure 5.21 and table 5.22.

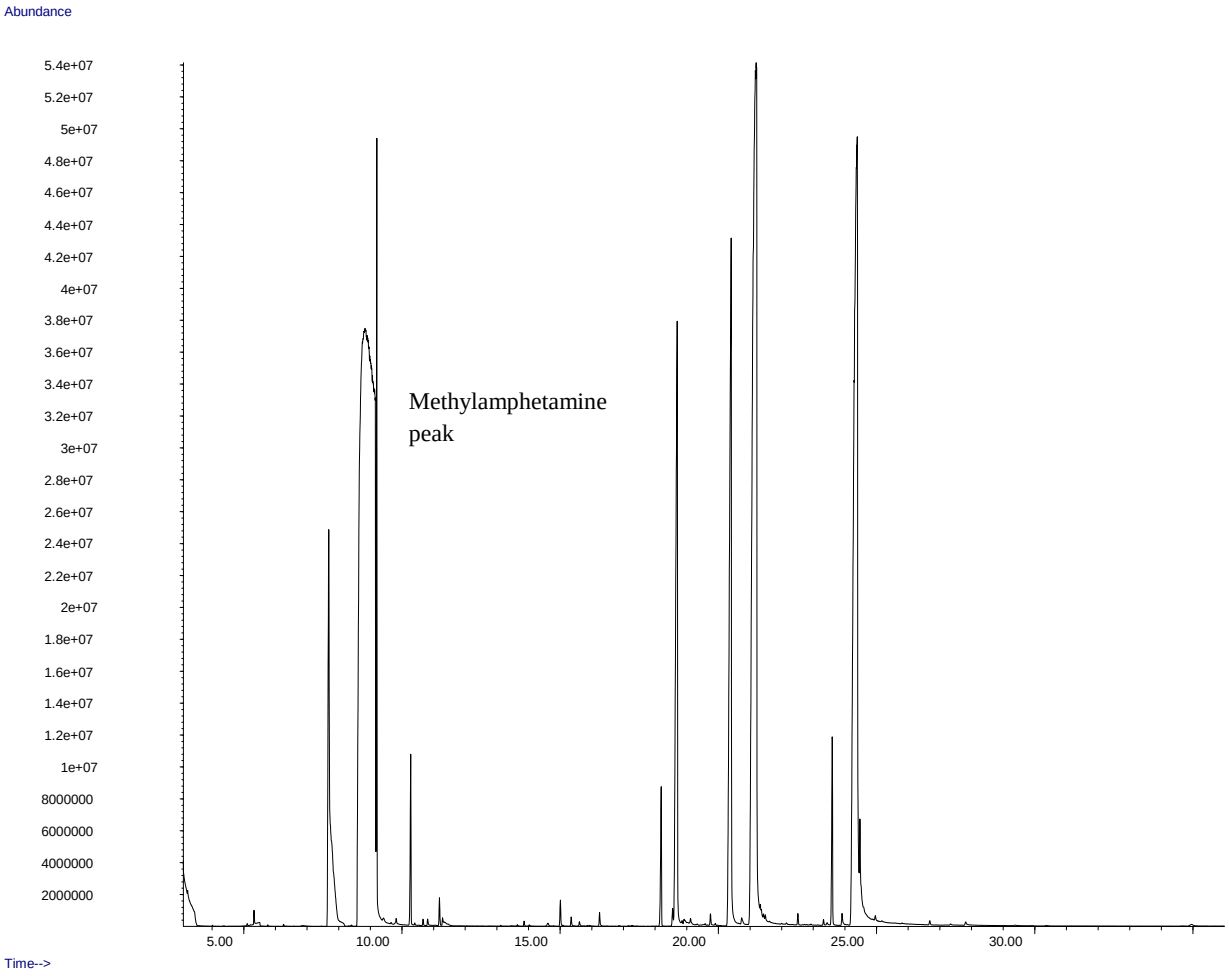

Figure 5.21. Impurity profile of methylamphetamine synthesized via the Hypophosphorous route using *pseudo*ephedrine hydrochloride (Panadol-Malaysia) extracted using acid base extraction.

Table 5.22. List of some of the impurities of methylamphetamine synthesized via Hypophosphorous route using *pseudo*ephedrine hydrochloride extracted from (Panadol-Malaysia) using acid base extraction.

No	RT	Peak	m/z fragments(base peak in bold)
1.	7.69	1,2 –Dimethyl-3-phenylazridine	**146**,105,132,117,91,77
2.	10.672	N-methyl-1-phenylethanamine	**120**,146,77,91,105,63,51
3.	10.819	Methcathinone	**58**,77,105,50,91,160
4.	11.188	1,2-dimethyl-3-phenylazridine	**58**,146,132,117,105,91
5.	11.287	3-methyl-2-phenylmorpholine	**71**,58,91,105,117,132,146
6.	13.867	3,5-Dimethyl-2-phenylpyridine	**82**,167,58,77,90,105,115
7.	15.013	2H-Indol-2-one, 1,3-dihydro-1-methyl	**118**,147,191,77,91,105
8.	15.617	2H-Quinoline-1-carboxylic acid,3,4-dihydro-methylester	**57**,191,176,163,77,91,105
9.	16.25	2-methylphenyl-2-pyridinyl methanone	**119**,169,91,196,182,65,51
10.	18.559	5-Methyl-2-phenylindolizine	**207**,191,102,110,97
11.	18.702	Chlorphenamine	**203**,167,58,72,139
12.	19.123	**Unknown**	**118**,58,203,134,149,91,77
13.	19.757	Methylamphetamine dimer	**238**,91,120,148
14.	20.406	**Unknown**	**183**,167,98,84,196
15.	21.206	Triprolidine	**208**,58,193,181,167,117
16.	22.52	**Unknown**	**272**,256,244,128,136
17.	23.33	**Unknown**	**286**,271,207,143,258
18.	23.436	**Unknown**	**224**,207,183,276,294,117
19.	23.612	**Unknown**	**276**,247,219,205,117,102
20.	23.921	Desloratadine	**280**,266,310,254,245,230

One of the Nagai route impurities (methylamphetamine dimer [1].) was in this sample. The samples prepared from the Malaysian pharmaceutical samples produced, in general, much cleaner impurity profiles than those prepared using Sudafed tablets. This may be in part because of the extraction methodology used for prepare the precursor chemical

5.4.3 Methylamphetamine synthesized via Moscow route using *pseudo*ephedrine extracted from proprietary cold medication (Allerpid-Malaysia) using acid base extractions

A total of 2 batches of independent samples of methylamphetamine hydrochloride were synthesized via Moscow route using *pseudo*ephedrine hydrochloride extracted from proprietary cold medication (Allerpid-Malaysia) using acid base extraction as detailed in Chapter 3 shown in Figure 5.22 and Table 5.23 below.

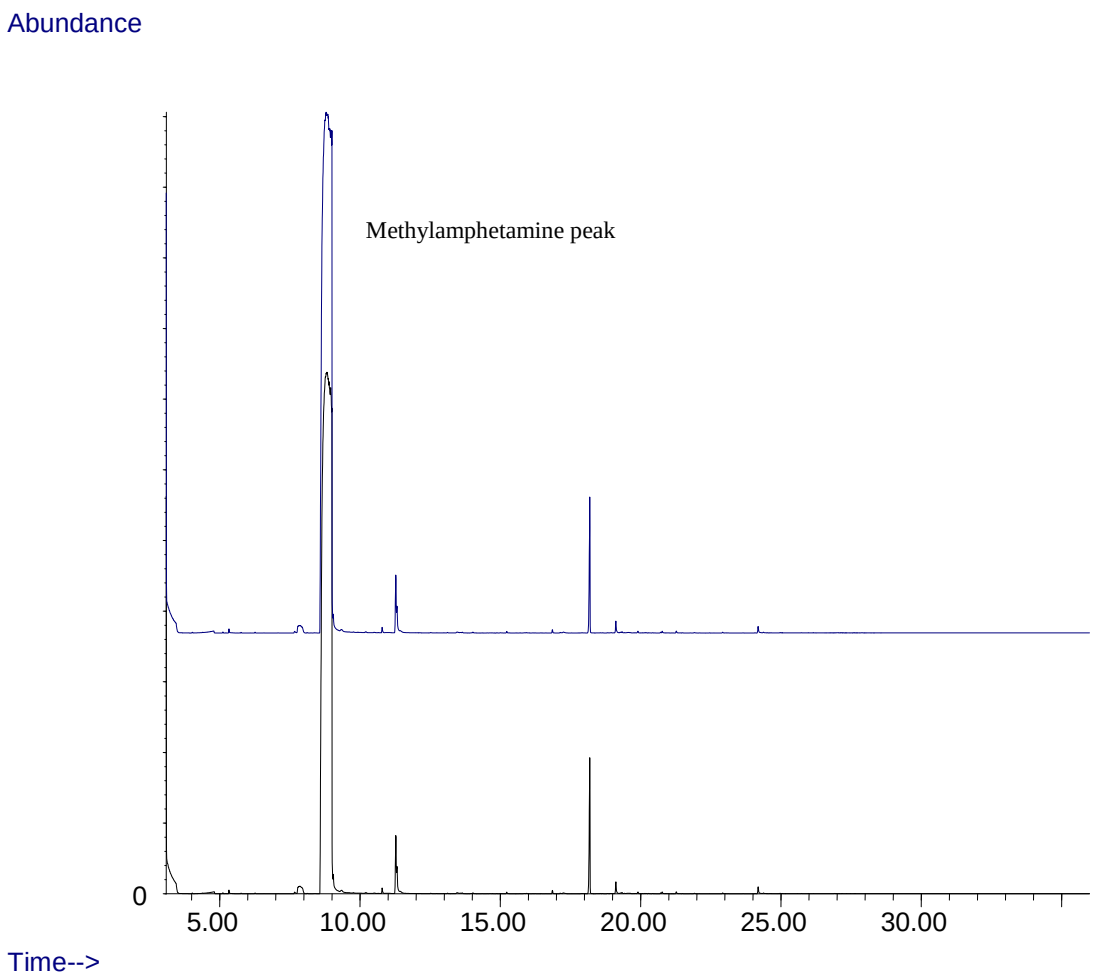

Figure 5.22. Impurity profiles of interbatch variation of 2 batches(AllepM 1 to Allep M2) of methylamphetamine synthesized via the hypoHypophosphorous route using *pseudo*ephedrine hydrochloride (Allerpid-Malaysia) extracted using acid base extraction

Table 5.23. List of some of the impurities of methylamphetamine synthesized via Moscow route using *pseudo*ephedrine hydrochloride extracted from (Allerpid-Malaysia) using acid base extraction.

No	RT	Peak	m/z fragments(base peak in bold)
1.	5.332	Benzaldehyde	**105**,77,51,61,85
2.	7.672	1,2-Dimethyl-3-phenylazridine	**146**,105,117,132,91,77
3.	7.812	Benzyl methyl ketone	**91**,134,65,77,84,51,57
4.	9.05	1,2-dimethyl-3-phenylazridine	**146**,105,117,132,91,77
5.	10.791	Methcathinone	**58**,73,77,86,92,105,115
6.	11.278	*Pseudo*ephedrine	**58**,71,79,91,105,117,132
7.	15.23	**Unknown**	**105**,190,77,51,64,86,131
8.	16.858	4,4-diphenyl-3-buten-2-one,	**179**,222,207,152,115,77,51
9.	19.119	**Unknown**	**118**,58,149,91,133,77,105
10.	19.908	**Unknown**	**160**,91,144,131,117,103,77
11.	20.773	**Unknown**	**58,**208,239,193,165,178
12.	21.274	**Unknown**	**58**,91,105,190,77,118
13.	24.189	Desloratadine	**280**,266,310,254,245,230

5.5 Comparison of the chromatographic profiles of methylamphetamine synthesized using precursors (*pseudo*ephedrine) extracted from Sudafed tablets using the three different solvent systems using the Pearson Correlation matrix approach

The impurity profiles were compared with each other using a Pearson correlation coefficient matrix where the linkages threshold defined the goodness of fit for samples within their synthetic group. In total, three data pre treatment refinements of the various GCMS data sets were investigated in an effort to gain the most accurate mathematical discrimination of the samples using Pearson correlation matrix. The data pretreatment methods were selected based on those suggested by the CHAMP authors [11](i.e. normalization, square root and fourth root). In this study a further pretreatment method, the sixteen root was also included. The three refinements were as follows:

1. Target impurities from this study normalized to the sum of targets and pre-treated with square root method.
2. Target impurities from this study normalized to the sum of targets and pre-treated with fourth root method.
3. Target impurities from this study normalized to the sum of targets and pre-treated with sixteenth root method.

The success of the GCMS profiling method was assessed by its ability to produce Pearson correlation coefficients which would facilitate the correct allocation of individual drug batches to their synthetic route, while not permitting the batches between synthetic routes to be deemed similar [11].Before discussing the results, a discussion of the calculation of Pearson correlation coefficients and effect of the data pre-treatment methods is undertaken in the sections below.

5.5.1 Pearson Correlation Coefficient

Pearson correlation coefficient, r, is a measure of the correlation between two variables. The values usually ranges from -1 to +1 (with +1 indicating a positive linear relationship, -1 indicating a negative linear relationship , and 0 indicating no linear relationship between the two variables), although the coefficients may be scaled over a different range for ease of use if required [11, 12, 13]. The coefficient can be calculated using the following equation 5.1:

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[\, n\Sigma x^2 - (\Sigma x)^2 \,][\, n\Sigma y^2 - (\Sigma y)^2 \,]}}$$

Equation 5.1

where x and y represent the two samples under the comparison and n is the number of variables per sample [11,12].

An Excel macro, which allows convenient calculation of Pearson correlation coefficients for data input by the user, has been developed by the European Network of Forensic Institutes (ENFSI) drug working group and was used in this study.[11,12] The coefficients are scaled such that a maximum positive correlation corresponds to a value of +100, maximum negative correlation corresponds to a value of -100, and no correlation corresponds to a value of 0. For the purpose of comparing chromatograms, a value of +100 represents maximum similarity between profiles[11,12,13].

While Pearson correlation coefficients conveniently qualify the relative similarity between impurity profiles in a data set, it is up to the user to evaluate the meaning of the value of r. Application of this statistic to a data set of samples of which the origin (i.e. similarity) is known will allow the threshold value to be set such that all of the known samples within each synthetic route are grouped together [11, 12, 13].

5.5.2 Data Pre-Treatment Methods

In order to reduce the influence of larger peak areas in, for example, a chromatogram, data can be pre-treated, or transformed, before statistical analysis. Two pre-treatment methods investigated by the CHAMP authors were the square root and fourth root methods [11]. When pre-treating the data with the square root method, each data point is replaced by its square root. Similarly, the fourth root and sixteenth roots require replacing each data point by its fourth root and sixteenth roots respectively. These types of pretreatment effectively reduce the range over which data points are spread by reducing the magnitude of the larger data points (or increasing the magnitude of the smaller points for values between 0 and 1) [14,15] This effect is presented in Table 5.24 in which a Hypothetical set of 10 randomly generated data points is pre-treated using both methods.

Table 5.24. The effects of square root, fourth root and sixteen root data pre-treatments on a set of randomly generated data.

Random Raw Data	After Square Root Pre-treatment	After Fourth Root Pre-treatment	After Sixteen Root Pre-treatment
9.78	3.12	1.76	1.15
3.33	1.82	1.35	1.07
7.10	2.66	1.63	1.13
0.96	0.97	0.98	0.99
6.80	2.60	1.61	1.12
8.72	2.95	1.71	1.14
4.67	2.16	1.47	1.10
1.27	1.12	1.06	1.01
5.44	2.33	1.52	1.11
2.45	1.56	1.25	1.05

From data shown the Table 5.24 (above), the apparent spread of raw data originally from 0.96 through to 9.78, is reduced to a range of 0.97 to 3.12 after the square root pre-treatment and further reduced to 0.98 to 1.76 and 0.99 to 1.15 after pre-treating

with fourth and sixteenth root techniques respectively. The graphical representation of the figures in the table above is shown in Figure 5.23 below.

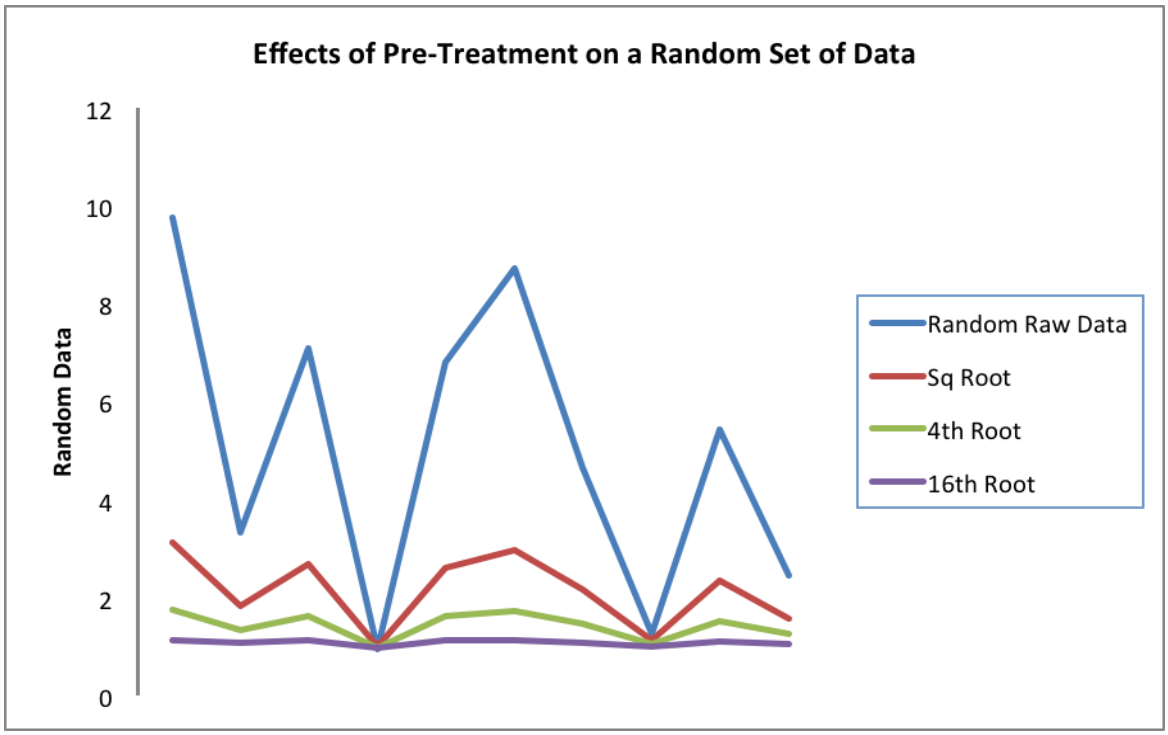

Figure 5.23. Illustration of the effect of square, fourth and sixteen root pre-treatment on a random set of data.

The square root method and, to a lesser extend, the fourth and sixteen root methods are accepted and used relatively widely as data transformation methods [14, 15] similar to the common log transformation [14,15]. The square, fourth and sixteenth roots methods are more suitable than the log transformation when the data set has many zeroes (as in the case with drug profiling data in which some of the target impurities are not present)[14,15].

The Pearson correlation coefficient matrices are presented in tables 5.25 to 5.27

5.5.3 Results of Pearson Correlation Coefficient analysis

ME1	100.00											
2.00	86.05	100.00										
3.00	86.45	88.44	100.00									
4.00	89.43	84.84	88.93	100.00								
5.00	69.73	68.33	84.88	81.63	100.00							
6.00	72.26	74.38	86.90	78.89	77.21	100.00						
MDA7	41.87	40.70	61.05	62.04	49.85	71.29	100.00					
8.00	31.85	38.08	48.40	50.25	30.79	61.11	91.86	100.00				
9.00	32.41	34.21	40.40	47.28	17.53	50.29	86.76	92.57	100.00			
10.00	30.70	30.06	47.63	51.75	44.60	58.04	89.69	91.28	77.87	100.00		
11.00	31.69	38.36	60.50	54.62	56.23	69.03	94.02	85.73	74.00	88.78	100.00	
12.00	36.04	39.32	49.57	53.26	28.87	60.08	92.24	98.11	95.46	88.33	83.88	100.00
MMS 13	26.44	7.64	24.93	37.69	36.80	27.26	39.78	33.92	24.94	49.53	37.55	33.04
14.00	24.02	16.66	29.11	37.57	47.29	36.56	36.87	37.38	20.85	54.54	37.91	31.06
15.00	30.21	15.53	35.17	47.73	54.06	43.09	50.53	44.94	28.81	64.49	48.96	40.14
16.00	34.24	23.55	33.47	46.91	47.87	40.13	43.10	41.65	26.02	58.92	39.70	36.70
17.00	28.24	14.43	34.28	45.06	52.06	41.26	47.46	43.98	26.30	62.74	46.49	38.63
18.00	35.50	23.00	39.11	44.79	45.47	42.53	51.79	48.34	35.22	63.35	50.35	44.98
ML19	47.38	48.81	59.27	57.93	58.39	62.26	68.85	54.81	57.99	56.55	64.78	54.46
20.00	48.14	48.14	59.59	57.87	56.05	63.75	71.17	57.43	62.46	56.55	65.04	58.15
21.00	53.92	49.48	59.00	51.66	55.39	57.31	50.30	34.84	39.27	38.09	45.75	35.69
22.00	54.40	60.64	58.27	68.32	56.09	57.46	58.79	49.23	56.96	46.14	52.13	50.67
23.00	77.44	81.61	83.57	84.49	66.84	70.16	56.23	44.28	41.14	44.76	49.48	46.59
24.00	58.97	59.87	65.74	61.57	56.26	63.56	61.47	49.15	55.26	46.95	55.16	50.85
HE25	-5.55	-8.44	-15.48	-12.94	-5.41	-7.56	-6.98	-6.94	4.80	-6.43	-2.00	-7.18
26.00	59.98	60.14	42.53	55.36	38.37	29.36	-1.59	-6.96	-0.04	-6.93	-1.11	-4.45
27.00	32.45	31.62	17.42	24.90	21.64	16.33	-7.98	-8.48	1.90	-11.45	-7.20	-8.93
28.00	43.87	44.17	26.41	36.78	27.09	15.70	-6.20	-8.15	0.65	-7.78	-3.40	-6.79
29.00	24.28	26.16	8.66	19.21	8.94	-0.22	-10.87	-6.97	-1.98	-3.82	-6.10	-6.75
30.00	48.52	49.17	31.77	42.48	28.57	15.86	-5.93	-6.79	-1.94	-3.80	-2.07	-4.44
HDA31	82.86	84.84	77.72	80.83	58.17	61.98	41.40	38.29	36.38	37.30	34.15	39.15
32.00	67.56	62.75	56.73	59.84	34.74	37.70	22.00	17.16	24.13	13.14	9.39	20.91
33.00	73.15	63.88	63.75	66.51	44.04	48.52	35.29	28.24	31.09	28.45	22.49	30.20
34.00	62.76	52.88	53.75	50.49	25.92	37.40	27.28	20.30	29.90	12.77	12.05	25.37
35.00	86.00	79.37	75.59	77.88	47.86	60.31	43.46	41.20	42.55	37.20	30.73	44.52
36.00	76.22	66.81	66.95	66.71	42.28	50.57	38.79	33.38	36.05	32.47	25.93	35.71
HMS 37	34.93	48.14	30.19	43.48	25.48	24.77	11.78	15.43	11.67	11.74	9.75	15.95
38.00	32.96	35.93	29.99	26.93	20.89	23.08	12.87	10.29	13.21	7.53	12.66	14.03
39.00	12.02	16.47	7.98	8.38	6.29	0.89	-8.68	-8.71	-6.52	-7.63	-4.15	-4.78
40.00	31.58	33.13	28.81	23.84	19.79	19.26	7.27	6.63	9.03	7.44	11.84	11.50
41.00	42.39	40.91	34.96	32.46	23.28	27.93	12.50	10.90	13.40	8.93	11.84	15.27
42.00	52.19	52.78	45.83	41.59	32.01	37.43	15.43	15.20	16.77	11.48	14.21	18.81
HL43	54.46	57.62	54.86	58.99	49.93	53.46	44.76	35.45	36.02	35.12	39.93	35.78
44.00	43.58	41.42	44.41	51.87	51.22	47.40	42.19	28.56	32.67	31.17	38.33	29.64
45.00	50.26	51.45	53.99	60.90	53.02	55.75	54.22	44.67	40.49	48.94	49.72	43.94
46.00	48.87	59.56	50.47	51.59	30.30	48.57	42.82	40.44	46.30	26.30	33.59	43.27
47.00	47.90	49.60	53.53	59.46	52.44	55.36	54.18	43.99	40.91	46.10	49.33	43.89
48.00	59.08	55.40	58.67	67.78	57.76	58.22	50.27	36.85	39.17	36.68	43.09	39.32

MMS 13	100.00											
14.00	57.17	100.00										
15.00	67.10	93.83	100.00									
16.00	63.68	90.15	94.54	100.00								
17.00	80.34	87.91	94.64	91.05	100.00							
18.00	89.23	72.85	79.62	78.62	89.08	100.00						
ML19	37.02	29.23	36.52	35.69	36.63	46.01	100.00					
20.00	33.48	24.40	32.15	30.37	30.97	39.97	98.24	100.00				
21.00	30.92	18.40	24.15	27.17	23.78	36.88	90.78	90.19	100.00			
22.00	30.79	19.56	25.92	28.12	25.81	32.27	91.08	90.59	79.30	100.00		
23.00	20.15	16.78	24.99	26.52	23.51	30.58	62.80	63.13	60.06	67.86	100.00	
24.00	28.92	19.26	24.88	27.27	24.45	37.70	96.18	95.50	95.11	89.77	69.02	100.00
HE25	14.39	-5.17	-9.94	-8.36	-6.68	4.93	38.75	35.63	40.57	36.00	-18.15	37.08
26.00	11.58	-5.31	-4.55	-0.84	-4.77	2.58	27.96	25.99	31.36	50.28	49.74	35.76
27.00	12.26	-5.44	-8.41	-1.40	-3.62	2.68	37.80	34.41	41.26	50.93	11.86	41.45
28.00	13.28	-9.57	-11.37	-5.27	-8.05	2.17	32.34	28.35	34.78	50.43	26.69	37.43
29.00	13.60	-9.84	-13.28	-9.18	-9.45	1.99	19.16	15.54	18.53	33.66	20.36	21.17
30.00	10.02	-10.50	-11.02	-7.63	-9.51	0.93	15.36	12.43	16.71	35.50	36.68	20.92
HDA31	13.56	28.00	25.34	28.68	21.95	25.93	45.78	44.73	43.11	55.35	82.76	53.57
32.00	1.15	-0.22	-2.35	-0.85	-1.83	3.18	31.90	33.98	31.56	42.57	69.99	40.64
33.00	14.56	16.62	16.60	17.36	16.82	21.39	40.32	41.55	39.69	44.19	73.63	46.55
34.00	1.08	-8.01	-8.20	-8.96	-7.19	2.19	35.04	39.75	38.76	38.10	65.16	45.20
35.00	18.71	22.23	22.85	26.45	21.76	30.11	43.22	43.34	44.81	48.65	79.06	54.22
36.00	16.40	17.64	17.70	19.87	18.11	26.42	41.23	42.17	42.19	42.22	74.47	49.09
HMS 37	5.40	-4.62	0.53	5.09	1.67	9.77	5.68	3.61	-1.04	20.47	38.12	9.01
38.00	0.40	-13.70	-9.46	-5.10	-8.90	7.04	22.95	20.48	30.24	18.57	25.19	32.43
39.00	-8.70	-18.02	-15.93	-12.54	-14.83	-6.22	1.90	-1.93	5.91	2.35	8.03	8.15
40.00	2.82	-11.48	-8.03	-4.26	-6.06	8.31	22.85	18.67	29.28	20.01	21.55	31.81
41.00	5.79	-10.63	-5.16	-1.07	-4.08	13.48	24.84	21.94	32.39	21.25	29.52	34.88
42.00	12.57	-8.16	-4.25	-0.18	-1.45	17.90	27.90	25.87	37.70	28.21	34.34	40.72
HL43	28.94	21.10	28.40	33.71	28.35	40.37	59.02	54.75	58.45	53.54	54.60	62.60
44.00	36.20	19.14	29.39	30.72	30.79	39.02	66.01	62.24	63.78	60.35	41.86	65.72
45.00	39.81	35.07	44.64	48.06	44.55	52.47	57.50	52.84	53.48	49.45	55.13	57.66
46.00	8.25	-1.87	2.93	9.72	3.70	18.66	47.55	47.64	46.48	47.48	53.83	55.78
47.00	38.15	29.14	39.50	41.46	40.72	48.52	55.60	52.25	51.08	48.03	53.01	55.59
48.00	35.27	19.04	31.73	32.26	31.68	38.88	58.65	57.04	56.50	57.25	56.30	60.99

HE25	100.00											
26.00	36.43	100.00										
27.00	75.07	73.35	100.00									
28.00	67.85	86.99	89.53	100.00								
29.00	65.96	71.76	70.32	87.30	100.00							
30.00	44.55	87.17	67.42	90.40	90.88	100.00						
HDA31	-8.20	61.94	27.07	45.24	36.91	55.13	100.00					
32.00	-5.14	54.80	22.81	42.31	40.02	53.92	87.23	100.00				
33.00	-6.65	50.18	20.48	36.48	33.56	46.78	90.42	96.17	100.00			
34.00	-2.37	40.53	14.25	30.28	29.71	39.12	76.51	94.65	92.69	100.00		
35.00	-8.98	53.73	22.52	37.36	30.60	46.76	94.85	86.25	90.93	81.76	100.00	
36.00	-6.07	48.43	19.54	34.92	31.84	45.40	90.84	93.16	98.32	90.82	93.81	100.00
HMS 37	-14.54	35.83	13.98	29.25	23.74	36.41	39.70	29.82	28.58	20.43	34.91	26.61
38.00	15.13	21.75	23.43	27.86	15.52	20.78	23.99	18.57	19.58	22.18	29.00	22.83
39.00	9.89	16.45	18.24	24.57	20.54	22.55	8.44	6.09	2.86	5.00	9.02	2.93
40.00	24.07	26.46	30.84	37.69	31.79	33.93	22.39	16.71	15.49	18.56	26.36	18.60
41.00	18.23	29.06	28.48	35.17	25.19	30.81	31.00	24.12	25.95	27.40	37.52	29.73
42.00	24.25	40.55	34.39	42.66	31.35	40.44	38.32	34.66	34.41	36.91	46.14	38.94
HL43	21.05	37.87	32.41	32.21	15.37	21.83	47.05	28.32	39.12	28.05	49.82	44.08
44.00	38.00	35.20	41.94	37.17	17.61	19.26	29.57	15.90	25.08	17.41	31.63	27.13
45.00	10.14	26.80	18.74	18.42	5.48	11.15	44.22	21.08	35.63	19.94	46.92	40.79
46.00	10.85	31.02	21.65	23.40	9.79	16.51	45.67	35.07	39.82	39.38	51.66	45.04
47.00	9.27	24.28	16.77	16.35	3.23	9.11	39.93	20.54	33.73	21.26	43.40	38.29
48.00	17.03	39.80	31.19	32.51	13.52	23.16	44.09	29.99	39.03	30.93	47.66	41.27

HMS 37	100.00											
38.00	73.24	100.00										
39.00	70.79	86.43	100.00									
40.00	60.90	87.39	91.64	100.00								
41.00	73.44	95.89	87.19	92.96	100.00							
42.00	50.00	71.12	41.03	61.85	72.45	100.00						
HL43	45.23	59.57	22.50	34.05	53.23	66.63	100.00					
44.00	37.81	58.41	25.62	35.79	51.22	60.58	93.49	100.00				
45.00	41.44	50.22	14.91	24.50	43.81	55.37	96.70	89.55	100.00			
46.00	50.65	61.73	23.90	33.07	53.85	67.93	90.88	80.70	84.60	100.00		
47.00	41.73	50.82	13.88	22.93	42.99	58.01	96.45	91.13	98.78	87.59	100.00	
48.00	47.34	57.71	21.85	32.62	51.59	66.58	94.93	95.01	92.27	87.11	94.89	100.00

Table 5.25. Pearson correlation coefficients for every pair of samples in the 48 batches synthesised by two synthetic routes using normalized to the sum of targets and pre-treated with square root method. Red numbers corresponds to the coefficients greater than a threshold of 70.26. The coefficients inside each box should be high as they denote the comparison of samples from within a synthetic route (Hypo is green and Moscow is blue).

ME1	100.00
2.00	79.30	100.00
3.00	76.45	79.81	100.00
4.00	85.82	77.11	91.86	100.00
5.00	79.59	76.05	93.65	94.97	100.00
6.00	76.41	72.41	74.87	73.48	75.30	100.00
MDA7	29.77	34.55	58.53	52.37	59.88	29.51	100.00
8.00	25.18	27.19	50.39	47.18	55.58	26.30	90.17	100.00
9.00	15.95	42.31	35.95	28.89	35.70	13.02	74.45	76.03	100.00
10.00	15.80	25.64	49.29	40.93	52.30	22.68	88.97	93.28	73.20	100.00
11.00	27.32	25.40	54.54	51.42	60.16	28.48	92.48	85.55	58.27	82.22	100.00
12.00	29.93	31.82	54.53	52.32	59.24	27.25	91.80	97.70	77.87	91.45	86.20	100.00
MMS13	13.21	9.62	26.64	26.55	26.17	26.31	29.17	21.27	5.23	23.87	29.22	21.29	100.00
14.00	12.68	6.52	32.87	33.74	39.67	26.86	33.21	31.08	-3.84	30.65	49.50	26.70	55.91	100.00
15.00	3.19	-2.02	26.69	23.32	30.26	15.11	23.21	18.31	-19.18	24.53	39.82	14.81	58.60	89.01	100.00
16.00	1.97	-9.13	22.02	22.90	23.62	8.34	27.86	23.41	-4.50	22.50	37.63	21.58	66.95	80.52	84.31	100.00
17.00	22.11	16.50	43.87	44.78	51.44	32.96	44.13	38.01	6.30	42.02	52.46	37.06	60.07	84.42	80.98	74.63	100.00
18.00	34.68	24.76	51.88	55.48	55.05	38.38	47.80	36.31	7.26	37.46	54.03	36.97	79.40	76.05	73.98	71.61	78.21	100.00
ML19	57.16	50.01	65.27	65.55	63.39	51.57	53.32	42.86	36.71	31.48	43.84	45.11	27.42	11.90	6.64	19.23	23.90	38.59	100.00
20.00	55.93	50.25	62.55	61.23	61.77	53.63	51.45	40.59	36.57	29.70	41.36	42.97	25.18	9.96	3.28	13.80	21.19	33.69	98.41	100.00
21.00	59.46	48.82	58.45	60.82	60.96	55.05	48.38	36.12	29.54	24.19	38.96	38.45	25.19	8.54	2.26	10.38	19.99	33.93	93.86	95.62	100.00
22.00	50.51	41.58	55.22	55.85	55.81	46.99	48.65	37.02	30.06	25.88	40.38	38.17	25.10	10.62	4.47	15.39	20.16	34.86	96.75	97.13	94.04	100.00
23.00	48.04	40.87	49.56	52.52	50.90	42.25	43.06	33.18	28.46	21.26	34.25	34.86	20.04	4.40	-0.88	9.95	17.70	28.87	93.11	93.07	91.84	95.48	100.00
24.00	56.46	46.57	59.23	62.07	61.51	51.66	51.04	40.29	32.30	28.62	42.52	42.14	25.64	10.39	4.19	15.20	23.10	35.87	97.60	98.02	96.31	98.38	95.76	100.00
HE25	15.01	12.49	5.80	8.81	16.06	20.73	-0.36	-3.40	1.62	-9.22	4.61	-5.42	-9.86	-1.18	-6.63	-0.73	-8.51	-7.74	45.84	49.68	45.96	51.12	48.10	50.82	100.00
26.00	19.05	18.97	19.57	18.35	26.49	23.20	3.83	1.16	1.48	-5.16	6.87	-1.62	-19.94	-3.18	-8.98	-5.73	-8.90	-12.93	49.60	53.33	49.56	52.54	50.36	52.59	90.65	100.00
27.00	12.54	16.75	10.61	12.40	21.77	17.32	7.00	2.85	9.44	-1.49	12.01	1.52	-9.39	-2.96	-9.21	-8.29	-8.21	-7.40	40.10	44.42	41.99	44.97	40.22	44.28	90.03	87.03	100.00
28.00	11.40	10.47	5.01	7.89	15.91	18.91	-0.81	-4.53	-1.08	-9.20	6.38	-6.34	-9.39	1.99	-3.71	-0.05	-5.23	-6.57	39.38	42.71	40.06	43.60	39.58	43.60	97.66	90.29	92.44	100.00
29.00	18.75	11.20	9.11	14.44	17.26	22.55	-4.65	-3.18	-4.86	-10.39	4.25	-4.05	-6.23	5.63	-3.04	4.22	-2.53	-1.67	43.49	45.28	41.06	44.09	43.86	44.85	91.49	85.48	80.62	92.12	100.00
30.00	14.06	11.71	9.85	11.60	17.66	24.05	-4.81	-4.38	-7.01	-9.14	5.06	-5.98	-11.38	7.10	1.08	4.66	-1.91	-4.77	37.04	39.71	36.14	37.70	37.94	38.92	91.09	89.41	79.39	92.31	96.16	100.00
HDA31	73.70	74.25	67.79	69.01	66.51	58.76	42.46	36.80	33.71	30.06	33.23	43.54	-3.86	-7.44	-14.83	-16.98	5.10	13.24	47.63	47.64	52.65	39.60	40.67	47.54	12.32	18.83	18.56	10.71	10.90	11.69	100.00
32.00	57.17	58.37	46.24	47.30	42.62	34.94	35.18	32.33	34.70	23.46	28.34	37.77	-18.91	-19.47	-29.35	-28.04	-20.18	-2.64	34.54	34.05	37.96	29.27	31.55	34.35	12.94	13.46	17.73	9.50	11.72	10.35	88.44	100.00
33.00	65.18	56.15	53.37	56.16	51.39	43.44	40.06	38.48	29.99	27.25	35.06	43.76	-8.10	-11.50	-20.21	-16.62	-9.50	9.65	41.22	39.47	44.86	35.78	36.94	41.34	12.51	13.93	17.64	9.51	12.97	10.45	90.65	95.66	100.00
34.00	55.21	54.54	47.33	44.75	40.87	32.44	41.00	37.17	37.87	28.31	32.13	42.27	-13.53	-17.84	-26.22	-20.10	-17.63	2.58	38.79	38.15	40.29	33.91	34.20	37.83	15.82	15.20	18.10	11.04	15.02	12.46	85.43	95.82	95.06	100.00
35.00	73.68	64.70	60.02	63.75	57.09	50.72	37.47	34.36	30.53	23.44	29.64	41.22	-8.96	-11.70	-23.09	-16.98	-7.59	9.76	44.23	43.40	46.94	36.30	36.31	42.80	12.21	16.36	14.40	9.40	14.64	12.77	93.42	91.77	94.57	90.82	100.00
36.00	66.03	59.05	54.39	54.74	49.50	44.56	37.30	33.87	31.62	22.27	31.68	39.90	-7.46	-14.37	-23.71	-18.26	-12.15	7.63	41.93	40.62	44.76	35.54	35.67	40.41	11.44	12.22	17.02	8.68	12.54	8.89	89.45	95.39	98.33	94.60	94.62	100.00
HMS37	6.50	6.58	0.23	7.77	1.06	2.60	-2.66	-6.10	-1.81	-15.94	-2.38	-8.37	1.58	-0.72	-5.38	1.96	-6.87	8.17	16.00	12.67	7.47	20.99	23.76	16.05	9.17	4.77	12.13	8.36	4.65	-1.72	-1.26	-5.38	-0.60	0.06	1.20	-1.12	100.00
38.00	-1.82	0.87	1.16	4.68	1.15	-1.35	12.41	3.22	7.09	0.40	10.01	3.31	0.65	-8.90	-8.02	-0.28	2.03	10.53	9.53	6.47	0.85	11.18	13.75	10.67	1.60	-0.80	3.72	4.26	-2.01	-3.66	0.36	-9.90	-4.37	-1.58	0.93	-5.26	81.55	100.00
39.00	-15.04	-3.89	-1.67	-4.16	-7.61	-9.77	9.17	5.51	10.84	3.72	7.83	6.07	2.42	-10.73	-9.70	2.25	-2.67	6.32	-5.15	-8.97	-14.44	-5.14	-1.64	-6.60	-7.58	-4.02	-3.34	-3.98	-3.90	-3.51	-6.26	-11.42	-6.56	-0.92	-2.83	-5.90	62.81	84.51	100.00
40.00	-9.25	-0.50	-1.36	-1.48	-7.17	-9.32	5.54	0.54	9.09	-2.47	4.31	1.80	-3.65	-11.48	-10.80	1.07	-4.38	5.44	-0.99	-5.05	-12.95	-0.33	0.67	-3.15	-1.36	-1.94	-1.56	-1.18	-1.37	-2.08	-8.57	-10.74	-7.49	-0.28	-0.93	-5.78	63.70	82.08	94.26	100.00
41.00	-8.36	-4.53	-6.07	-0.49	-5.90	-11.05	2.30	-2.43	1.20	-8.57	4.00	-3.43	-0.83	-0.86	-0.61	7.49	3.45	9.74	3.98	-0.05	-6.46	5.78	8.07	3.40	4.15	1.41	4.57	5.27	0.97	-1.64	-11.93	-19.67	-14.80	-11.82	-8.76	-14.68	84.05	90.70	84.68	89.21	100.00
42.00	-8.05	-3.67	-13.64	-8.24	-14.31	-6.44	-10.35	-12.74	-4.08	-18.29	-10.98	-15.45	-9.03	-12.64	-14.32	-4.79	-11.90	-4.54	-0.12	-2.33	-8.99	3.34	6.86	0.73	8.20	5.62	5.77	8.19	4.37	2.99	-13.16	-18.73	-16.81	-11.28	-9.93	-16.68	86.50	91.08	81.29	82.94	93.88	100.00
HL43	51.50	37.62	56.62	60.11	55.68	42.28	47.06	34.90	21.76	23.24	41.62	36.77	24.96	12.51	8.13	20.64	21.56	39.09	90.18	88.18	83.13	93.44	89.60	90.69	39.44	42.98	37.63	32.32	32.45	26.25	37.71	28.71	36.73	33.07	37.73	35.92	35.56	24.95	9.47	15.26	20.90	17.69	100.00
44.00	43.12	34.57	49.46	51.07	49.13	37.38	44.78	31.36	24.92	21.31	37.96	32.50	19.74	5.41	2.55	15.08	13.13	30.74	88.94	87.74	82.48	93.22	89.77	90.46	50.25	51.40	47.93	43.12	39.24	34.30	33.01	26.65	31.90	30.67	32.63	31.30	36.95	27.98	10.55	16.80	22.78	21.29	97.41	100.00
45.00	48.19	31.74	55.79	59.35	55.51	41.09	47.54	36.41	18.62	24.66	42.73	38.02	25.49	15.10	9.88	22.88	24.36	40.47	88.62	86.22	82.38	92.09	88.49	89.28	39.50	45.36	38.33	33.72	34.36	28.81	35.02	25.30	34.47	29.65	35.12	33.21	31.34	22.27	9.89	13.73	18.25	14.72	98.77	95.83	100.00
46.00	45.64	40.59	56.16	55.92	52.04	39.10	50.36	38.37	32.93	26.84	40.12	40.30	19.92	4.94	-3.66	12.60	14.84	31.20	89.31	87.71	82.21	91.85	89.37	88.69	38.91	46.13	39.58	33.04	33.13	27.97	40.66	33.53	38.79	38.19	40.70	39.35	33.74	25.23	16.20	19.34	20.72	19.08	96.64	95.53	96.78	100.00
47.00	46.02	34.05	56.37	56.93	54.32	42.95	48.68	38.41	23.11	26.12	43.27	39.04	26.75	14.38	8.22	21.37	21.73	38.60	89.59	87.47	83.58	93.30	89.15	90.00	40.02	45.98	38.91	33.88	34.15	29.19	34.16	26.90	35.10	30.76	34.47	34.78	27.67	17.83	8.53	12.92	14.79	11.26	97.60	96.03	98.60	97.21	100.00
48.00	51.96	43.11	57.50	59.49	54.39	42.59	45.68	33.87	26.59	21.63	37.81	35.71	22.32	5.83	1.06	15.86	15.89	34.24	91.16	88.91	83.81	93.76	90.38	91.30	39.95	43.67	37.41	32.26	32.42	26.65	41.10	33.43	39.82	37.75	41.25	39.89	34.59	24.60	11.48	18.14	21.09	18.59	98.73	97.49	96.93	97.56	97.55	100.00

Table 5.26. Pearson correlation coefficients for every pair of samples in the 48 batches synthesised by two synthetic routes using normalized to the sum of targets and pre-treated with fourth root method. Red numbers corresponds to the coefficients greater than a threshold of 71.26. The coefficients inside each box should be high as they denote the comparison of samples from within a synthetic route (Hypo is green and Moscow is blue).

ME1	100
2	98.89	100
3	91.27	93.32	100
4	98.5	99.56	93.73	100
5	98.37	99.35	93.68	99.52	100
6	97.98	98.21	91.15	97.3	97.18	100
MDA7	12.66	18.58	28.51	19.39	20.08	14.82	100
8	13.71	20.3	29.63	19.52	22.06	17.46	83.09	100
9	18.16	24.94	32.53	25.49	27.17	19.1	72.81	84.99	100
10	4.045	10.94	19.87	11.26	13.55	8.961	76.4	87.52	72.98	100
11	2.637	8.777	18.67	9.753	10.64	4.526	86.2	74.4	66.36	67.56	100
12	12.11	18.71	28.68	18.33	20.31	14.49	82.2	95.12	86.73	84.52	77.03	100
MMS 13	-6.139	-2.507	6.968	-1.689	-1.907	-7.295	26.34	12.95	13.89	9.285	21.06	21.04	100
14	1.528	5.053	6.068	5.31	2.634	5.037	15.86	-0.814	0.563	13.47	6.536	2.729	38.65	100
15	-5.436	0.599	9.846	3.218	2.293	-2.56	39.34	23.05	21.86	34.01	25.8	27.98	68.81	59.47	100
16	-2.134	1.701	9.577	3.331	2.725	0.42	26.94	13.48	9.605	23.97	19.82	19.44	66.65	48.1	86.68	100
17	4.154	5.816	7.049	6.939	6.902	2.709	12.93	-0.735	2.061	12.27	-0.951	6.281	27.2	21.95	40.37	26.97	100
18	5.351	9.826	20.59	13.01	11.78	3.071	41.43	25.03	31.85	26.84	30.33	33.39	79.64	49.31	84.14	72.04	36.24	100
ML19	54.28	56.33	48.13	56.68	55.97	51.81	21.18	19.44	35.07	9.075	17.36	19.59	-15.2	-6.165	-3.213	-0.386	-6.414	1.288	100
20	54.19	56.35	48.27	56.68	56.01	51.97	19.3	17.8	33.21	7.748	16.13	17.83	-17.48	-7.309	-4.483	-1.93	-5.382	-0.92	99.79	100
21	53.83	55.79	47.69	56.14	55.66	51.46	17.15	15.48	31.39	6.36	14.33	14.98	-19.05	-4.854	-3.536	-1.546	-4.598	-1.469	99.3	99.61	100
22	54.13	56.37	48.02	56.63	56.13	51.65	18.33	17.45	34.16	7.124	16.01	17.35	-16.19	-8.577	-5.153	-2.355	-5.932	-0.78	99.69	99.78	99.42	100
23	52.36	54.26	47.18	54.77	54.59	50.65	13.98	14.32	33.43	11.03	12.43	16.06	-23.8	-8.17	-6.838	-5.825	-5.149	-7.452	92.12	92.59	92.3	92.38	100
24	54.45	56.53	48.21	56.86	56.26	51.99	19.25	17.21	33.46	7.046	16.36	17.15	-16.66	-6.51	-4.418	-2.515	-4.394	-0.545	99.68	99.83	99.65	99.81	92.54	100
HE25	79.96	79.52	72.15	78.62	79.15	82.62	2.719	8.89	16.18	-1.666	0.396	3.506	-19.92	-9.326	-16.02	-10.79	-15.91	-12.85	62.36	62.6	62.51	62.94	60.45	62.53	100
26	77.96	77.65	70.15	76.94	77.72	78.98	0.415	4.885	10.45	0.628	-1.462	0.946	-14.65	-4.467	-11.8	-4.723	-10.74	-8.204	66.87	67.11	67.04	67.24	64.94	67	93.74	100
27	80.25	79.52	72	78.87	79.52	82.36	2.492	9.03	18.26	-1.332	-1.623	3.174	-19.95	-10.35	-15.62	-11.46	-13.5	-10.17	61.52	61.74	61.86	61.98	59.91	61.62	98.65	92.91	100
28	79.44	78.53	71.2	77.8	78.44	81.83	1.755	6.856	14.53	-3.455	-1.366	1.05	-20.98	-9.555	-16.72	-11.41	-16.2	-13.79	60.54	60.75	60.84	60.96	59.17	60.65	99.68	93.5	98.97	100
29	65.75	62.22	54.89	61.25	62.24	68.16	-14	-8.944	-8.931	-14.45	-12.57	-13.81	-34.52	-11.3	-29.91	-21.39	-25.51	-29.22	45.02	45.16	46.18	45.44	44.6	45.11	88.26	81.22	86.72	88.61	100
30	79.23	78.71	72.17	78.08	78.5	81.99	2.525	7.289	14.54	-1.246	-1.447	2.094	-22.03	-5.569	-15.97	-11.61	-16.71	-13.39	59.67	59.92	59.9	59.97	59.46	59.8	99.04	93.92	97.56	99.02	89.09	100
HDA31	83.91	84.42	77.86	84.36	84.87	83.51	15.1	21.77	32.92	13.68	18.07	22.91	-18.22	-4.734	-10.82	-7.93	-0.933	1.326	52.97	52.94	52.92	53.35	53.03	53.26	73.89	72.82	74.2	72.81	60.99	72.67	100
32	78.25	79.04	72.87	79	79.8	78.04	17.61	25.12	37	17.73	23.44	26.37	-18.51	-3.994	-13.84	-10.78	-11	0.181	49.65	49.36	49.44	49.91	50.38	49.93	69.85	69.31	70.31	69.05	56.89	68.97	98.15	100
33	80.88	81.7	76.17	81.87	82.28	80.5	15.24	22.49	34.81	16.46	19.95	23.54	-19.07	-2.032	-12.6	-9.712	-9.014	0.695	50.87	50.63	50.89	51.11	52.62	51.15	72.47	71.82	72.91	71.67	59.45	71.64	98.49	99.32	100
34	78.32	79.43	73.75	79.59	79.97	77.72	18.15	24.52	36.28	16.69	22.93	25.59	-16.8	-2.944	-12.09	-9.166	-11.2	2.328	51.01	50.68	50.68	51.2	51.73	51.27	70.02	70.19	70.57	69.12	55.54	69.14	97.7	99.44	99.31	100
35	82.08	82.26	75.82	82.13	82.61	81.42	11.62	19.04	31.56	11.27	15.6	20.05	-18.23	-5.035	-13.3	-10.31	-5.507	-0.032	49.16	49	49.18	49.47	49.87	49.45	72.65	71.27	73.08	71.8	58.53	71.42	98.99	98.69	99.01	98.59	100
36	81.39	81.72	76.07	81.89	82.2	80.65	14.92	22.03	33.89	15.48	19.49	22.67	-20.39	-3.97	-14.63	-11.48	-10.42	-0.865	50.23	49.97	50.2	50.59	51.65	50.6	72.3	71.43	72.47	71.43	60	71.51	98.4	99.12	99.7	99.03	98.88	100
HMS37	6.572	6.835	0.996	7.579	6.368	6.055	-13.43	1.658	8.534	-3.522	1.11	8.83	1.213	16.4	3.352	4.332	-11.43	4.978	12.34	12.04	13.57	11.06	12.87	12.44	15.45	11.6	14.22	14.42	18.74	16.44	10.92	11.16	12.63	10.51	11.17	10.6	100
38	12.6	12.25	16.93	13.62	12.43	14.22	-6.289	12.69	17.41	8.649	-6.379	16.81	9.575	12.86	14.75	8.86	2.899	20.24	2.295	1.668	2.775	0.555	4.719	1.923	13.63	8.069	14.86	12.16	15.46	16.31	23.22	20.91	22.32	20.96	22.95	20.94	73.4	100
39	2.714	1.011	9.48	1.403	1.187	5.352	-5.758	11.63	8.654	6.607	-8.585	12.52	2.664	13.38	8.2	1.783	-13.37	10.42	-4.939	-5.478	-4.21	-7.13	-1.41	-5.347	10.97	9.681	12.5	10.51	18.93	15.83	13.17	12.83	13.51	13.39	13.7	12.81	57.9	87.87	100
40	-4.396	-6.874	0.985	-6.238	-5.712	-3.551	-12.85	5.538	3.296	-0.075	-10.89	6.998	9.674	14.27	9.908	5.279	-11.49	13.56	-11.95	-12.41	-10.29	-13.41	-9.946	-12.03	4.655	4.507	6.018	4.793	14.61	8.607	8.421	8.588	8.914	8.674	9.153	8.202	60.39	84.14	95.22	100
41	-0.169	-1.618	2.722	-0.048	-0.621	0.038	-13.02	3.528	6.786	-3.008	-2.213	6.649	5.807	12.42	7.578	2.899	-6.562	12.12	-4.769	-4.932	-2.739	-6.069	-2.952	-4.301	6.788	5.721	7.338	6.702	12.46	10.09	14.36	14.47	14.63	14.1	15.55	13.78	79.87	88.74	85.24	88.91	100
42	0.207	-2.145	-2.577	-1.992	-1.98	1.807	-31.72	-11.65	-11.96	-17.3	-24.33	-8.516	-3.751	10.77	-0.925	-0.668	-9.919	-1.703	-6.63	-6.156	-3.598	-7.788	-1.154	-6.043	6.604	1.396	7.691	6.684	19.28	9.568	8.909	8.062	8.7	7.081	9.754	7.126	79.87	86.03	79.83	82.26	90.11	100
HL43	50	51.33	44.69	51.61	51.54	47.25	29.87	22.62	35.55	5.547	27.9	22.59	0.392	-10.46	-0.569	5.593	-4.503	7.274	88.66	87.7	86.51	88.43	80.93	88.81	55.73	59.3	53.5	53.68	36.67	52.78	48.51	45.98	45.14	47.93	44.83	45.48	9.908	1.549	-5.391	-11.49	-1.696	-6.892	100
44	49.34	50.48	43.99	50.72	50.82	46.72	28.31	21.33	34.61	5.207	26.94	20.71	-2.554	-11.16	-3.325	4.17	-8.358	3.568	88.53	87.5	86.51	88.38	81.29	88.69	56.48	59.93	54.01	54.63	39.04	53.76	48.15	46.13	45.35	47.86	44.59	45.76	9.662	0.167	-5.805	-11.49	-2.011	-6.863	99.67	100
45	50.15	51.62	45.02	51.72	51.74	47.79	30.46	23.2	35.25	6.845	27.96	23.05	0.659	-10.15	-0.273	6.372	-3.998	6.55	88.45	87.5	86.35	88.21	80.81	88.59	55.7	59.49	53.26	53.56	36.51	52.78	48.39	45.84	45.03	47.7	44.63	45.36	8.491	0.636	-6.111	-12.56	-3.276	-7.615	99.82	99.6	100
46	50.4	51.49	44.88	51.66	51.55	47.69	29.54	21.6	33.32	5.665	27.31	21.29	-0.434	-10.35	-1.698	5.426	-5.485	5.458	88.48	87.49	86.42	88.24	80.91	88.57	55.94	59.64	53.42	53.89	38.43	53.16	47.98	45.28	44.63	47.05	44.04	45.17	8.85	0.505	-6.044	-12.17	-2.762	-6.96	99.63	99.63	99.82	100
47	49.7	51.15	44.68	51.15	51.33	47.21	29.98	22.84	35.33	6.506	27.63	21.75	0.051	-9.862	-0.604	6.312	-6.772	5.769	88.55	87.56	86.57	88.43	80.66	88.6	56.14	59.93	53.68	54.1	37.49	53.27	48.23	45.81	44.9	47.55	44.58	45.33	6.548	-1.767	-7.908	-13.86	-4.55	-9.152	99.55	99.59	99.72	99.7	100
48	49.9	51.14	44.48	51.43	51.28	47.02	28.68	21.02	34.26	4.515	26.95	20.38	-0.881	-10.22	-1.331	5.385	-6.064	5.619	88.73	87.77	86.69	88.61	80.89	88.86	56.05	59.51	53.58	54.06	37.49	53.07	48.38	45.75	45.03	47.71	44.75	45.47	8.337	-0.523	-7.348	-13.01	-2.84	-8.347	99.8	99.76	99.62	99.62	99.7	100

Table 5.27. Pearson correlation coefficients for every pair of samples in the 48 batches synthesised by two synthetic routes using normalized to the sum of targets and pre-treated with sixteenth root method. Red numbers corresponds to the coefficients greater than a threshold of 69.26. The coefficients inside each box should be high as they denote the comparison of samples from within a synthetic route (Hypo is green and Moscow is blue).

5.5.3.1 Discussion on the Pearson Correlation Coefficient analysis

The Pearson correlation coefficients were calculated for each pair of samples using the three data sets discussed previously shown in Table 5.25, Table 5.26 and Table 5.27. Since the 'true' relationships of the samples were known, a threshold value for the calculated coefficients was sought such that values above the threshold indicated the related samples and values below the threshold indicated unrelated samples.

The most accurate discrimination by synthetic route of the 48 batches of methylamphetamine was achieved using the impurities normalized to the sum of targets and pre-treated with the fourth root shown in table 5.26. The lowest coefficient calculated for a pair of samples from within a synthetic route was 71.26; thus this was the maximum threshold that would allow the six samples within each route to be deemed similar. The red numbers in table 5.26 indicate coefficients greater than 71.26, and it is clear that the set of Moscow route batches are deemed similar to one another, as are the Hypo route batches within their respective sets.

The best linkages were observed for the methylamphetamine synthesised from laboratory grade chemicals for both of the Hypo and Moscow routes. While discrimination between the Hypo and Moscow routes using laboratory grade chemicals is encouraging, it is unfortunate that the precursors extracted using different solvents from proprietary cold medication using the two mentioned routes could not be completely resolved correctly.

Batches of methylamphetamine synthesised from the *pseudo*ephedrine extracted from proprietary cold medication using ethanol/methanol and commercial methylated spirit as extraction solvent via the Moscow route consistently had the lowest Pearson correlation coefficient, indicating that these samples were the most difficult to link together using the data pre-treatment methods and reflects the observed variation in the six chromatographic profiles of batches of methylamphetamine synthesised from these precursors. The same observation was observed in batches of methylamphetamine synthesised via the Hypo route.

5.6 Conclusion

Organic impurity profiling of methylamphetamine hydrochloride synthesized from proprietary cold medication using gas chromatography mass spectrometry (GCMS) is one of the major objectives of this research. From the results presented the variation of the impurity profiles generated from between batches synthesized from the same chemist showed greater differences. Potential route specific impurities identified from the individual batches demonstrated a possible link between the batches of methylamphetamine synthesized.

The aim of the study was to synthesize methylamphetamine in an analogous manner to that employed by clandestine laboratories [16]. The impurity profiles related to batches of methylamphetamine synthesized from laboratory grade materials have been contrasted with those associated with material extracted from cold medication. To our knowledge this is the first time a comparison of this nature has been reported.

Existing impurities suggested to be route specific impurities in the Emde preparative route were identified within methylamphetamine synthesized using the Moscow and Hypo routes following clandestine methods, (N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine (2 isomers) and N,N'-dimethyl-3,4-diphenylhexane-2,5-diamine) [17].

The new impurities identified in the methylamphetamine synthesized from proprietary cold medication sourced from United Kingdom was not present in methylamphetamine synthesized from proprietary cold medication sourced from Malaysia. The different tablet formulation of the brands from sourced from Malaysia compared to the cold medication sourced from United Kingdom, coupled with different precursor extraction methods may account for these differences. A number of common impurities were associated with the Moscow and Hypophosphorous synthesis were identified some of which were also associated with the Nagai route [1-4]. A number of unknown impurities were also identified some of which appeared only in the clandestine mimicked samples and some which may be specific to each route.

5.7 References

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Chapter 6 Inorganic impurity profiling of precursors, essential chemicals and methylamphetamine synthesized via the Moscow and Hypophosphorous routes using inductively coupled plasma mass spectrometry (ICP-MS).

6.1 Introduction

Inorganic profiling or elemental analysis was expected to reveal information relating to the catalyst (red phosphorous extracted from matchboxes), essential chemicals (iodine extracted from iodine tinctures), precursor chemical and extracting solvents used in the synthesis of methylamphetamine. In total 48 batches of methylamphetamine were analysed using inductively coupled plasma mass spectrometry (ICPMS) and included all of the samples prepared from Sudafed, iodine tinctures and red phosphorous from match books together with six repetitive samples of each method prepared from laboratory grade chemicals as a control set. The batches of methylamphetamine synthesised from the cold medication sourced from Malaysia (Panadol and Allerpid tablets) were not analysed using ICPMS due to limited amount of sample.

Previous ICPMS work has reported that elemental profiles could contribute information supporting linkage of methylenedioxymethylamphetamine (MDMA) and other illcit drug samples [1-11]. ICPMS has also been reported to provide information relating to diluents added into MDMA powder during the tableting process. C.Koper *et al.* [12] reported that elemental profiles suggested information about the synthetic phase and production stage of MDMA [12].

Suh *et al.* [13] analysed 51 seized methylamphetamine samples using ICPMS and identified iodine (I) in the majority of samples known to have been prepared via the Nagai route, and palladium (Pd) and barium (Ba) in samples known to have been prepared via the Emde route. Bromine (Br) was also detected in samples identified as Nagai, Emde and one other undetermined route [13]. Marumo *et al*. [5] analysed seized samples of methylamphetamine using ICPMS and atomic absorption spectrometry (AAS) and identified seven elements (barium (Ba), antimony (Sb), palladium (Pd),

strontium (Sr), bromine (Br), zinc (Zn) and copper (Cu)) and suggested that samples could be classified into five groups, however no route identification was possible [5].

Other studies examining natural or semi-synthetic drugs such as cannabis, cocaine and heroin have also suggested the potential for elemental analysis to provide useful information in relation to sample linkages [6-11].

6.2 Experimental Methods

6.2.1 Reagents and Standards

Trace metal grade nitric acid (65%, w/w) and laboratory grade tetramethylammonium hydroxide (TMAH) (25%, v/v) were both obtained from Sigma Aldrich (UK). Potassium iodide was obtained from Fluka. Ultrapure water was used throughout. Regenerated cellulose syringe filters (brown code) were purchased from Spec and Burke Analytical (Scotland). Multi element standards were obtained from Merck (Germany) and CPI international (USA). Calibration solutions were prepared from a Spex "CertiPrep" certified standard diluted as required with 2% Fischer Trace Metal grade nitric acid.

6.2.2 Sample Preparation

Multi element analysis

100 mg of sample was weighed into a 10 mL polypropylene tube and 1% HNO_3 (4 mL) was added. The tubes were placed on an Edmund Buhler Swip KS-10 rotative shaker overnight. The solution was filtered with a regenerated cellulose syringe filter (25 mm and diameter 0.45 µm pore size). The following elements were analysed in multi element analysis with ICP-MS (isotopic abundances): Li (7), Be (9), B (11) , Na (23), Al (27) , Si (28), P (31), S (34), K (39), Ca (42), Sc (45), Ti (47), V (51), Cr (52), Mn (55), Fe (56), Co (59), Ni (60), Cu (63), Zn (66), Ga (69), Ge (72), As (75), Se (78), Br (79), Rb (85), Ru (101), Rh (103), Pd (105), Ag (107), Cd (111), Sn (118), Sb (121), I (127), Ba (137), Pt (195), Au (197), Hg (202), Pb (208), Bi (209), U (238).

Iodine Analysis

Batches of synthesized methylamphetamine were subjected to separate iodine analysis. This is because at low pH, iodide is easily oxidized to volatile molecular iodine via dissolved oxygen. Iodine is not a stable element in dilute nitric acid and carry over can result even with nitric acid washes between samples. Due to iodine's volatility and complex redox chemistry, the sample was prepared in an alkaline media to avoid the oxidation of iodine. The presence of strong alkali conditions leads to the conservation of iodine as iodide or iodate which can then be determined by ICPMS analysis.

Approximately 100 mg of a sample was weighed into a 10 mL polypropylene tube; 1% tetramethylammonium hydroxide (4 mL) was added and the procedure outlined previously was followed. A series of iodine calibration standards were prepared from potassium iodide [14].

6.2.3 ICPMS Instrument Parameters

An Agilent 7700 quadrupole instrument was used with a Cetac ASX-520 autosampler. The instrument was operated with a Peltier cooled conical single-pass spray chamber with impact bead and has an integral peristaltic pump for sample uptake from the autosampler. A hexapole for CCT ED (Collision Cell Technology with Energy Discrimination) mode was used to remove polyatomic interferences.

Instrumental operating conditions used were 1400 W RF forward power; 13 L/min plasma flow; 1.0 L/min nebulizer flow and 0.8 L/min auxiliary flow, respectively. For the ICPMS a sample flush time of 60 s, a wash time of 90 s and peak hopping scan mode was used with a dwell time per isotope of 10 ms.

A solution of 1% HNO_3 was used as the wash solution for Li, Pd and Hg analysis and 1% TMAH was used as the wash solution for iodine as discussed previously.

6.3 Results and Discussion

The analysis of the precursor and essential chemicals used in the preparation of methylamphetamine by both synthetic routes are presented. The effect of the different solvent extractions on the extraction of *pseudo*ephedrine is discussed and the influences of the synthetic processes are exposed.

6.3.1 Analysis of precursor chemicals

6.3.1.1 Solvent analysis:

Six separate samples of each of the extracting solvents (ethanol, ethanol:methanol (90:10)% vol/vol and commercial methylated spirits) were analysed using ICPMS as described previously and the results presented in Figure 6.1 to Figure 6.3 and Table 6.1 to Table 6.3. Visual comparison of the elemental profiles indicate high concentrations of silicone in all samples and additional high concentrations of potassium in the ethanol:methanol (90:10)% vol/vol samples and sulfur, potassium, zinc and sodium in the commercial methylated spirit samples. The relative standard deviations obtained for the concentrations of these elements were also quite high demonstrating some variability across aliquots of the samples.

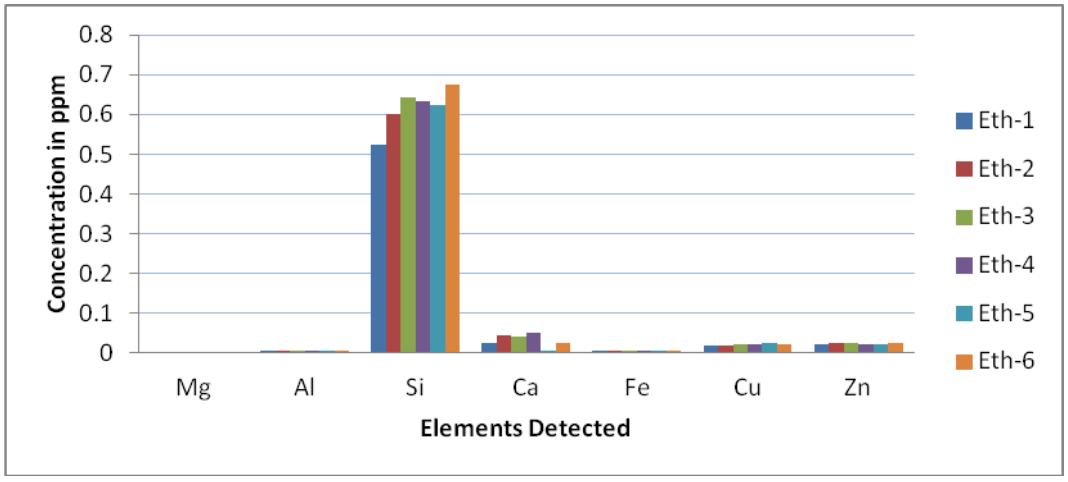

Figure 6.1 Graph of ICPMS analysis of 6 samples of ethanol.

Table 6.1. ICPMS analysis of elements detected in ppm for 6 samples of ethanol.

Element/ ppm	ETH-1	ETH-2	ETH-3	ETH-4	ETH-5	ETH-6	Mean	Std Dev	RSD (%)
Mg	0.001	0.002	0.002	0.002	0.001	0.002	0.002	0.000	18.88
Al	0.006	0.005	0.006	0.006	0.006	0.007	0.006	0.000	8.82
Si	0.522	0.601	0.643	0.633	0.624	0.674	0.616	0.051	8.41
Ca	0.025	0.043	0.039	0.050	0.004	0.025	0.031	0.016	52.58
Fe	0.005	0.004	0.004	0.005	0.005	0.006	0.005	0.000	13.94
Cu	0.020	0.019	0.020	0.020	0.023	0.021	0.021	0.001	6.81
Zn	0.022	0.023	0.023	0.021	0.022	0.026	0.023	0.001	7.41

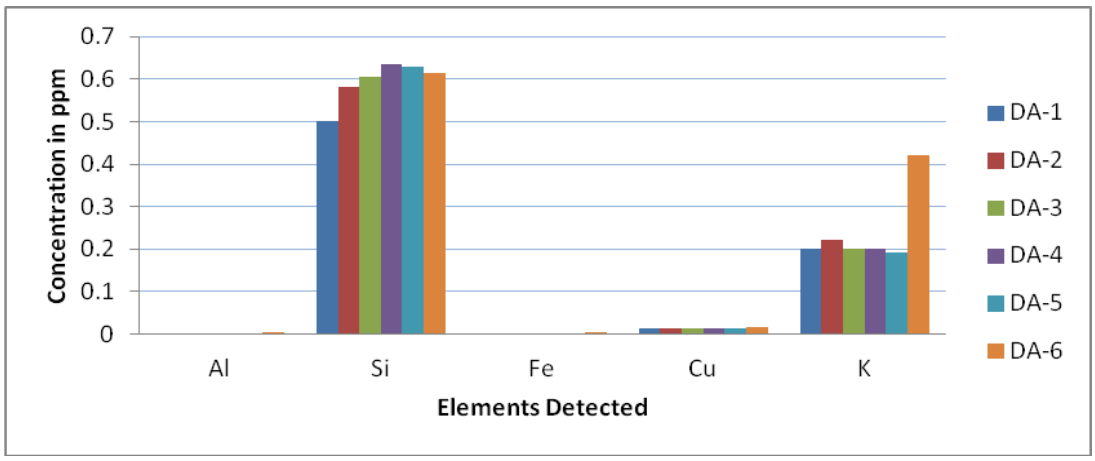

Figure 6.2. Graph of ICPMS analysis of 6 samples of ethanol :methanol (90:10)% vol/vol.

Table 6.2. ICPMS analysis of elements detected in ppm for 6 samples of ethanol: methanol (90:10) % vol/vol.

Element / ppm	DA-1	DA-2	DA-3	DA-4	DA-5	DA-6	Mean	Std Dev	RSD (%)
Al	0.002	0.002	0.001	0.002	0.002	0.003	0.002	0.000	29.54
Si	0.501	0.582	0.606	0.634	0.628	0.613	0.594	0.048	8.241
Fe	0.002	0.002	0.002	0.002	0.002	0.005	0.003	0.001	34.548
Cu	0.013	0.014	0.013	0.013	0.013	0.017	0.014	0.001	9.488
K	0.200	0.220	0.200	0.200	0.190	0.420	0.238	0.089	37.425

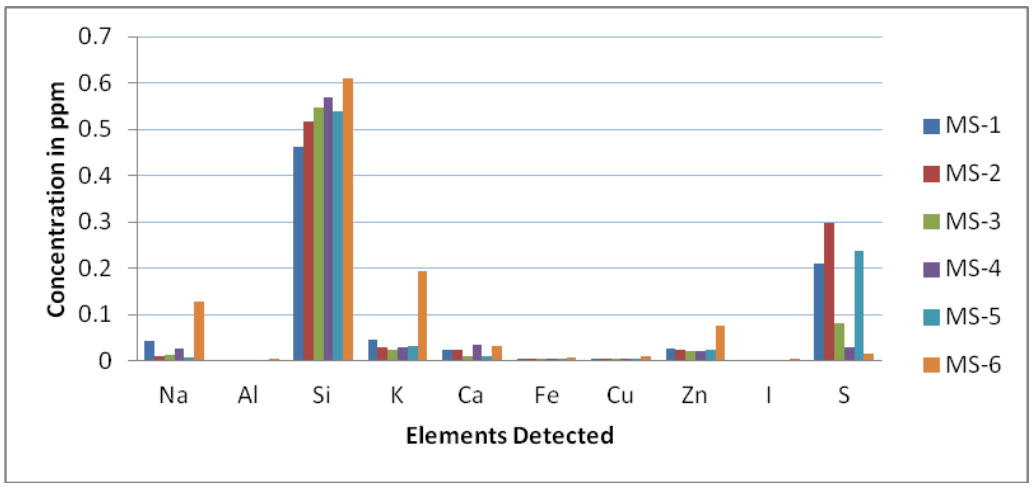

Figure 6.3. Graph of ICPMS analysis of 6 samples of commercial methylated spirits.

Table 6.3. ICPMS analysis of elements detected in ppm for 6 samples of commercial methylated spirit.

Element/ ppm	MS-1	MS-2	MS-3	MS-4	MS-5	MS-6	Mean	Std Dev	RSD (%)
Na	0.043	0.010	0.012	0.025	0.007	0.128	0.037	0.046	122.09
Al	0.002	0.001	0.001	0.001	0.001	0.004	0.002	0.001	58.80
Si	0.463	0.515	0.547	0.569	0.537	0.610	0.540	0.049	9.20
K	0.046	0.028	0.022	0.028	0.030	0.192	0.058	0.066	114.08
Ca	0.022	0.024	0.009	0.033	0.009	0.032	0.022	0.010	47.97
Fe	0.004	0.004	0.003	0.003	0.003	0.007	0.004	0.001	30.45
Cu	0.004	0.004	0.004	0.004	0.004	0.009	0.005	0.001	34.94
Zn	0.025	0.024	0.020	0.021	0.022	0.074	0.031	0.021	68.70
I	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.000	16.78
S	0.208	0.297	0.081	0.029	0.238	0.014	0.145	0.118	81.63

6.3.2 Laboratory grade chemicals

Six samples of laboratory grade *pseudo*ephedrine were analysed using ICPMS as described previously and the results presented in Figure 6.4 and Table 6.4. Visual comparison of the elemental profiles demonstrate consistency in the concentration of potassium and silicone with very good relative standard deviations expected because of the nature of the material (commercially synthesised).

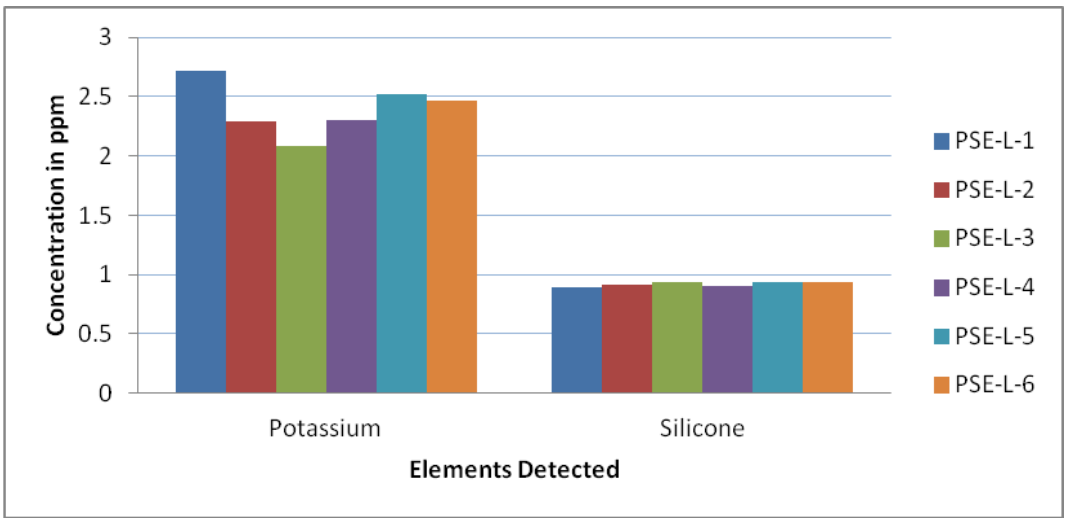

Figure 6.4. Graph of ICPMS analysis of 6 batches of laboratory grade *pseudo*ephedrine.

Table 6.4. ICP-MS analysis of elements detected in ppm for 6 batches of laboratory grade *pseudo*ephedrine hydrochloride.

Element/ppm	L-1	L-2	L-3	L-4	L-5	L-6	Mean	Std Dev	RSD(%)
K	2.71	2.29	2.08	2.30	2.53	2.46	2.40	0.22	9.1
Si	0.89	0.92	0.94	0.90	0.94	0.93	0.92	0.02	2.0

A possible explanation for the potassium concentration in the laboratory grade *pseudo*ephedrine may be due to acid-base extractions [15], when the *pseudo*ephedrine is extracted from the plant Ephedra. Potassium carbonate is a common base used in the acid base extractions. The presence of silicone may be due to artefacts from the manufacturing process and/or contaminants from glassware and was also observed in some of the extracted samples.

6.3.3 The analysis of proprietary medication and the effect of solvent extraction

*Pseudo*ephedrine was extracted from Sudafed tablets using the three different solvent systems. Initially six Sudafed tablets were analysed using ICPMS to gain an understanding of the background inorganic profiles which may be present within the pharmaceutical preparations and these are presented in Figure 6.5 and Table 6.5.

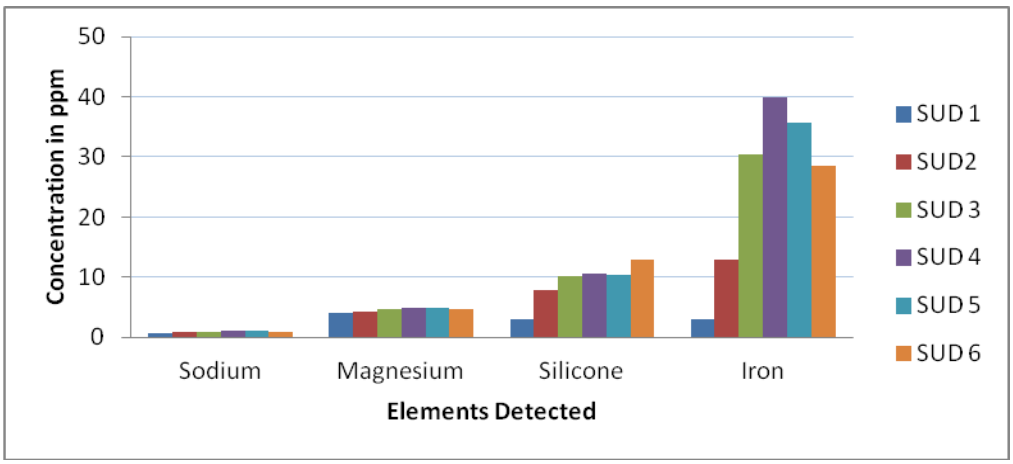

Figure 6.5. Graph of ICPMS analysis of 6 batches of Sudafed tablets (UK).

Table 6.5. ICPMS analysis of elements detected in ppm for 6 batches of Sudafed tablets purchased from pharmacies.

Element ppm	SUD 1	SUD2	SUD 3	SUD 4	SUD 5	SUD 6	Mean	Std Dev	RSD (%)
Na	0.62	0.79	0.97	1.14	1.06	0.94	0.92	0.18	20.22
Mg	4.05	4.36	4.72	4.82	4.86	4.68	4.58	0.31	6.85
Si	3.00	7.79	10.07	10.57	10.46	12.86	9.12	3.40	37.29
Fe	2.95	12.81	30.43	39.89	35.61	28.50	25.03	14.21	56.79

The Sudafed tablets consistently contained four main elements, sodium, magnesium, silicon and iron. The tablets were red in colour and were coated with red iron oxide (E172). Magnesium stearate was also added as a binder and both of these elements are reflected in the ICPMS profiles. Visual comparison of the elemental profiles shown in Figure 6.6 reveals some variation within the concentration of the dominant four elements present in the tablets. The presence of silicone may be due to coatings present on the tablets and/or contaminants from glassware. Figure 6.6 illustrates the degree of variation present across these elements for the six samples analysed. In general more iron is present in the Sudafed tablets than the other three elements and this is reflected in the elemental variation presented in the boxplot below.

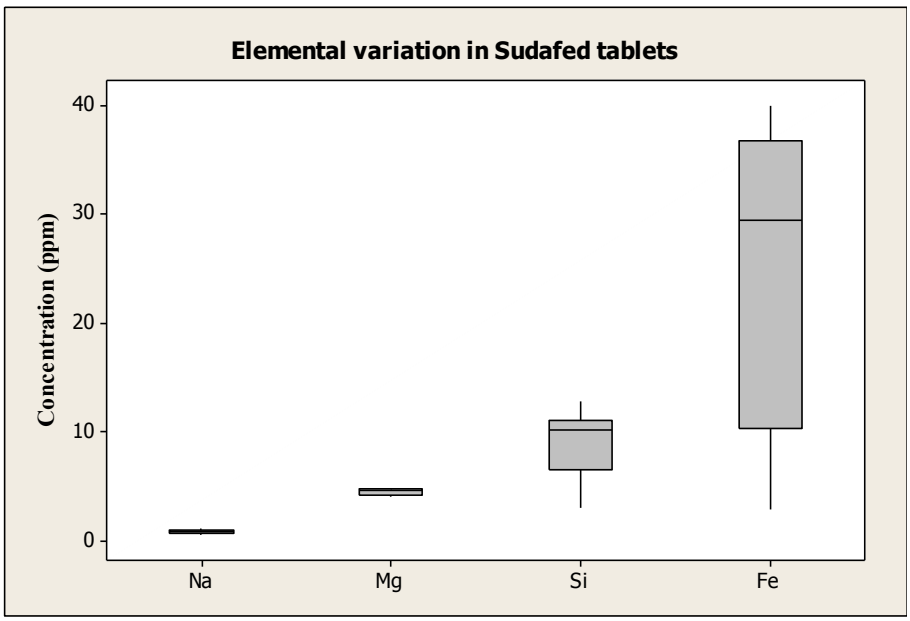

Figure 6.6. Boxplot analysis of the elemental variation present in the 6 batches of Sudafed tablets (UK).

6.3.4 The analysis of proprietary medication extracted using different solvents.

Six samples of *pseudo*ephedrine extracted using each of the solvent systems were analysed by ICPMS and are presented in Figure 6.7 to Figure 6.9.

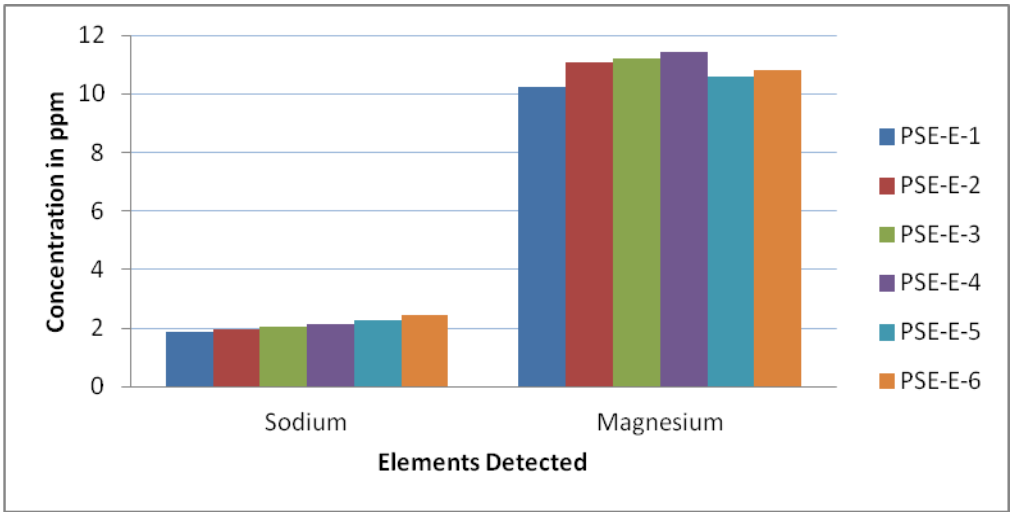

Figure 6.7. Graph of ICPMS analysis of 6 batches of *pseudo*ephedrine extracted from Sudafed tablets using ethanol as the extraction solvent.

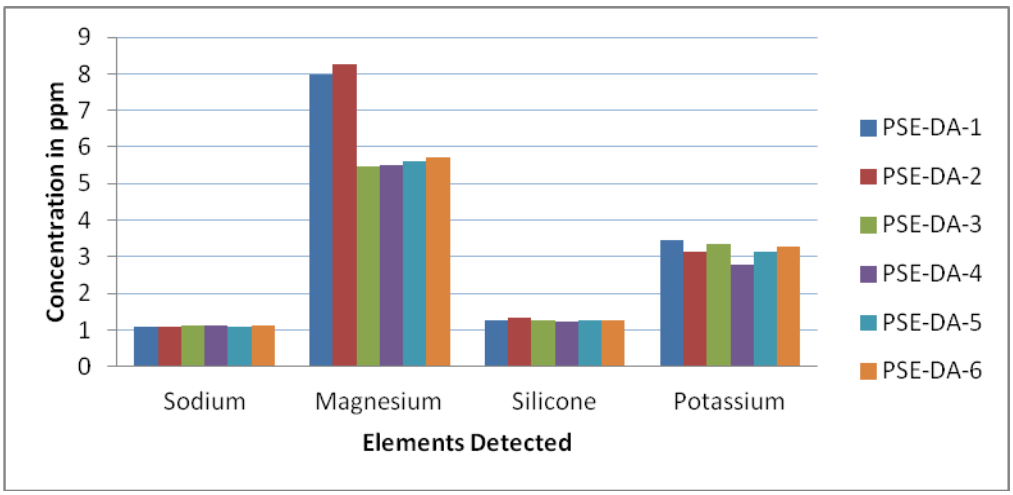

Figure 6.8. Graph of ICPMS analysis of 6 batches of *pseudo*ephedrine extracted from Sudafed tablets using ethanol:methanol (90:10) % vol/vol as the extraction solvent.

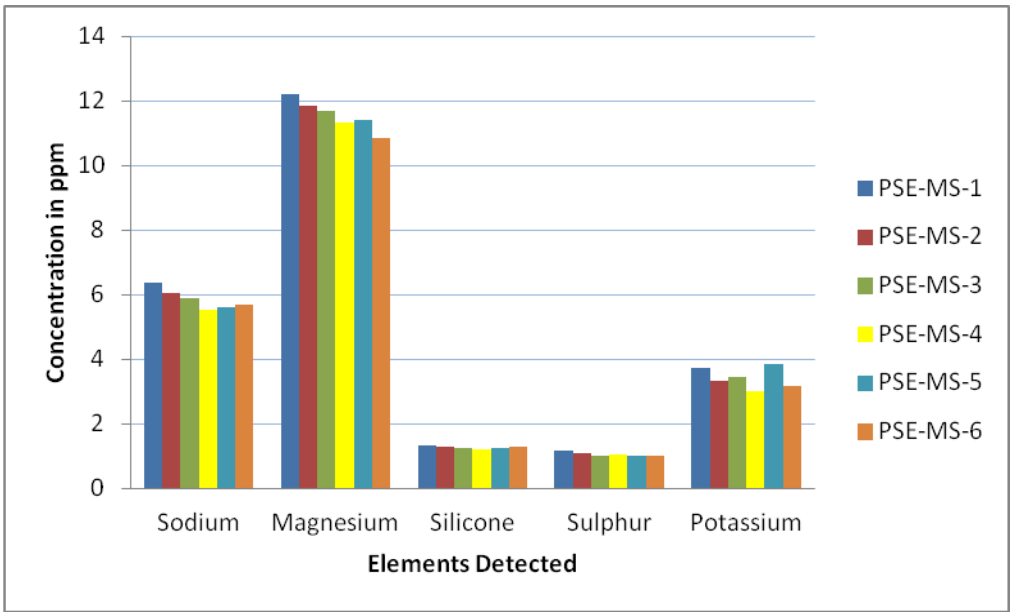

Figure 6.9. Graph of ICPMS analysis of 6 batches of *pseudo*ephedrine extracted from Sudafed tablets using commercial methylated spirit as the extraction solvent.

Table 6.6. ICP-MS analysis of elements detected in ppm for 6 batches of;

(a) *pseudo*ephedrine hydrochloride extracted from ethanol.

Element/ ppm	PSE-E-1	PSE-E-2	PSE-E-3	PSE-E-4	PSE-E-5	PSE-E-6	Mean	Std Dev	RSD (%)
Na	1.86	1.97	2.06	2.14	2.27	2.43	2.12	0.20	9.78
Mg	10.22	11.09	11.24	11.41	10.60	10.80	10.89	0.43	4.00

(b) *pseudo*ephedrine hydrochloride extracted from ethanol:methanol (90:10 % vol/vol).

Element/ ppm	PSE-DA-1	PSE-DA-2	PSE-DA-3	PSE-DA-4	PSE-DA-5	PSE-DA-6	Mean	Std Dev	RSD (%)
Na	1.08	1.08	1.10	1.10	1.09	1.10	1.09	0.00	0.82
Mg	7.97	8.24	5.48	5.48	5.59	5.71	6.41	1.31	20.50
Si	1.26	1.31	1.25	1.23	1.26	1.24	1.26	0.02	2.20
K	3.46	3.14	3.34	2.76	3.15	3.29	3.19	0.24	7.59

(c) *pseudo*ephedrine hydrochloride extracted from commercial methylated spirits.

Element/ ppm	PSE-MS-1	PSE-MS-2	PSE-MS-3	PSE-MS-4	PSE-MS-5	PSE-MS-6	Mean	Std Dev	RSD (%)
Na	6.37	6.03	5.88	5.51	5.59	5.67	5.84	0.32	5.51
Mg	12.21	11.83	11.69	11.32	11.39	10.83	11.54	0.47	4.12
Si	1.33	1.29	1.26	1.18	1.22	1.26	1.26	0.05	4.22
S	1.15	1.09	1.01	1.04	1.01	0.99	1.05	0.05	5.58
K	3.74	3.34	3.45	3.01	3.85	3.17	3.43	0.32	9.43

Distinct elemental variations were observed for the precursors extracted from the different solvent systems. Iron was absent in the extracted precursor samples reflecting the removal of the red iron oxide colouration as part of the extraction process. In general, a greater concentration of magnesium and sodium was present in the extracted samples when compared with the Sudafed samples. The introduction of methanol to the extraction solvent appears to have facilitated the extraction of both potassium and silicon and/or the transference of these elements from the solvents themselves to the extracted product. When commercial methylated spirits was used to extract the samples, sulfur was also present in the resultant extracts reflecting its presence within the solvent and its transference to the final extracted product. As a consequence of the elemental analysis it was possible to distinguish between the extraction solvents used to prepare *pseudo*ephedrine from the cold medication.

6.3.5 Iodine extraction

Six batches of iodine extracted from different iodine tinctures (as previously described in section 3.2.3) was analysed by ICPMS and the results presented in Figure 6.10 and Table 6.7. In total eight elements were consistently present in the samples and a visual comparison of the elemental profiles reveal the variation, particularly in iodine content where two samples contained significantly greater amounts of iodine than the remaining four samples. These variations are reflected in the high relative standard deviation values for iodine.

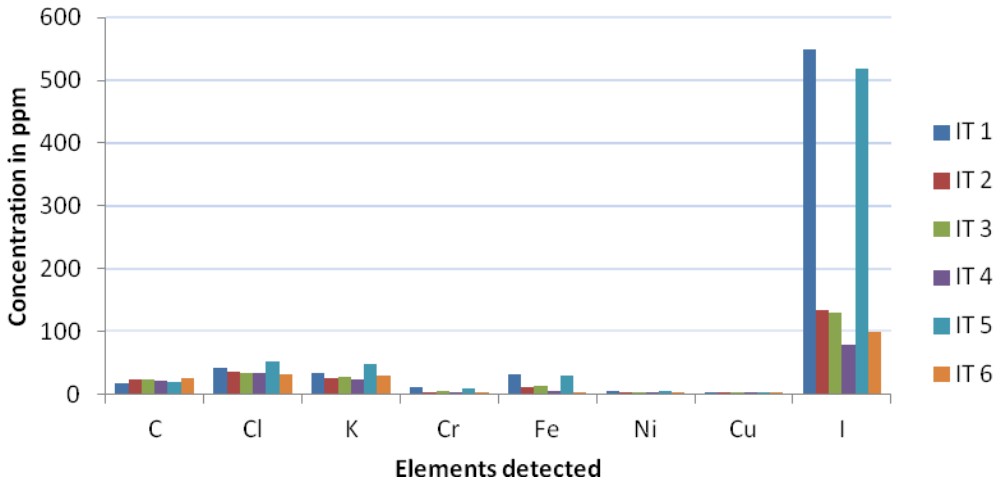

Figure 6.10. ICPMS analysis of iodine extracted from iodine tinctures.

Table 6.7. ICP-MS analysis of elements detected in ppm for 6 batches of iodine extracted from iodine tinctures.

Element/ ppm	IT 1	IT 2	IT 3	IT 4	IT 5	IT 6	Mean	Std Dev	RSD (%)
C	17.53	22.57	23.32	21.72	18.68	25.61	21.57	3.00	13.92
Cl	41.84	35.20	34.44	33.35	51.72	32.41	38.16	7.43	19.47
K	33.05	26.31	28.38	23.34	48.37	28.91	31.39	8.91	28.38
Cr	10.28	3.52	4.18	1.18	8.91	0.45	4.75	4.02	84.57
Fe	32.23	11.71	13.39	4.13	30.36	1.85	15.61	12.92	82.74
Ni	4.60	1.62	1.82	0.54	4.18	0.22	2.16	1.836	84.64
Cu	1.29	0.47	0.56	0.14	1.26	0.06	0.63	0.53	84.00
I	549.69	134.80	128.99	78.94	517.90	99.02	251.56	219.79	87.37

6.3.6 Red phosphorous extraction from matchboxes

Six samples of red phosphorous extracted from the striking pad of six different matchboxes (as described previously in section 3.2.36.2) were analysed using ICPMS and the results are presented in Figure 6.11 and Table 6.8. In total, fourteen elements were present across all six samples with phosphorous and sulfur dominating. The striking pad typically contains, 50% red phosphorous, 5% neutralizer, 4% carbon black and 16% binder together with a coat of adhesive that glues the above mentioned compounds to the striker pads of the matchboxes [16].

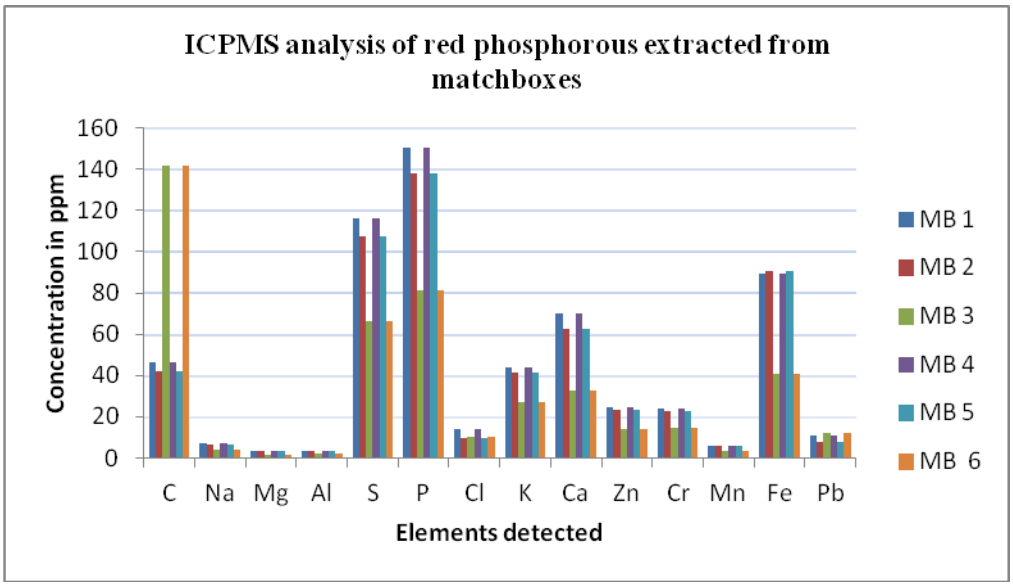

Figure 6.11. Graph of ICPMS analysis of red phosphorous extracted from matchboxes.

Table 6.8. ICP-MS analysis of elements detected in ppm for six batches of red phosphorous extracted from matchboxes.

Element/ ppm	MB 1	MB 2	MB 3	MB 4	MB 5	MB 6	Mean	Std Dev	RSD (%)
C	46.42	41.99	141.90	46.42	41.99	141.90	76.77	50.48	65.75
Na	7.06	6.70	4.20	7.06	6.70	4.20	5.99	1.39	23.22
Mg	3.32	3.14	1.61	3.32	3.14	1.61	2.69	0.84	31.23
Al	3.28	3.09	1.89	3.28	3.09	1.89	2.75	0.67	24.32
S	115.89	107.71	66.39	115.89	107.71	66.39	96.67	23.73	24.55
P	150.52	137.82	81.19	150.52	137.82	81.19	123.18	33.01	26.80
Cl	13.78	9.82	10.06	13.78	9.82	10.06	11.22	1.98	17.69
K	43.63	41.28	26.80	43.63	41.28	26.80	37.28	8.15	21.89
Ca	70.30	62.68	32.44	70.30	62.68	32.44	55.14	17.90	32.47
Zn	24.64	23.17	14.14	24.64	23.17	14.14	20.65	5.08	24.62
Cr	23.77	22.88	14.61	23.77	22.88	14.61	20.42	4.52	22.13
Mn	5.99	5.65	3.25	5.99	5.65	3.25	4.96	1.33	26.81
Fe	89.29	90.76	40.99	89.29	90.76	40.99	73.68	25.33	34.37
Pb	10.82	7.56	12.32	10.82	7.56	12.32	10.23	2.179	21.28

6.3.7 Analysis of methylamphetamine synthesised via the Moscow and Hypophosphorous routes.

Six batches of methylamphetamine synthesized using (a) laboratory grade precursor, iodine and red phosphorous and (b) extracted precursor and extracted iodine and red phosphorous were analysed using ICPMS and the results are presented in Figure 6.12 to Figure 6.16 and Table 6.9.

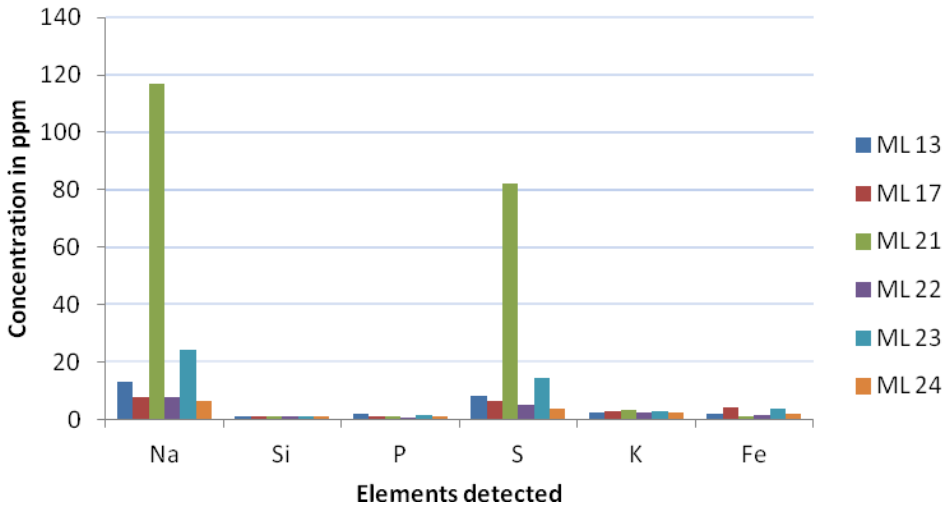

Figure 6.12. ICPMS analysis of methylamphetamine via Moscow Route using laboratory grade *pseudo*ephedrine HCl.

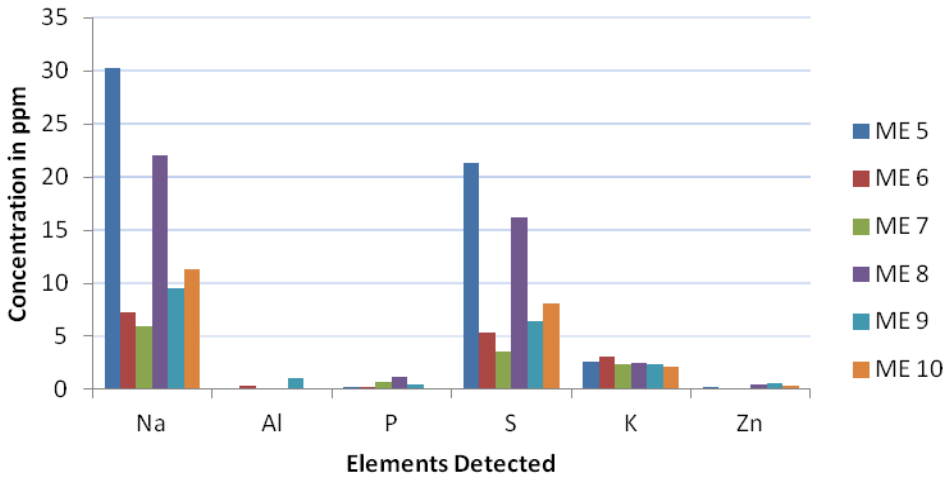

Figure 6.13. ICPMS analysis of methylamphetamine via Moscow Route using *pseudo*ephedrine extracted from Sudafed using ethanol.

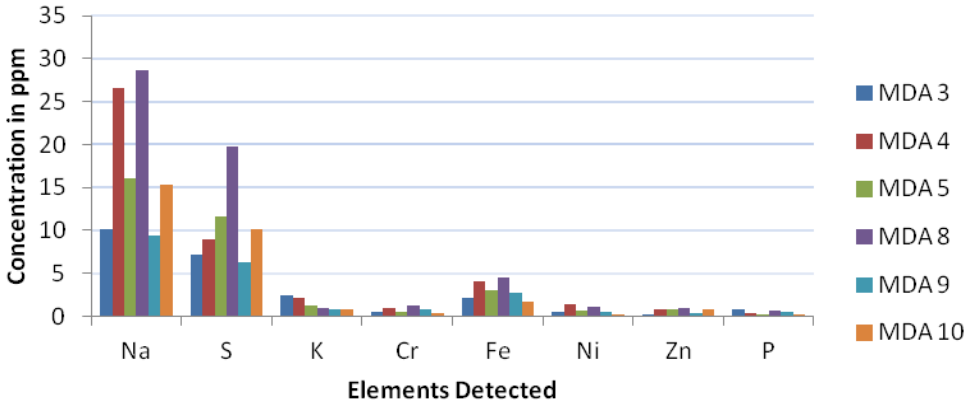

Figure 6.14. ICPMS analysis of methylamphetamine via Moscow Route using *pseudo*ephedrine extracted from Sudafed using ethanol:methanol (90:10)% vol/vol.

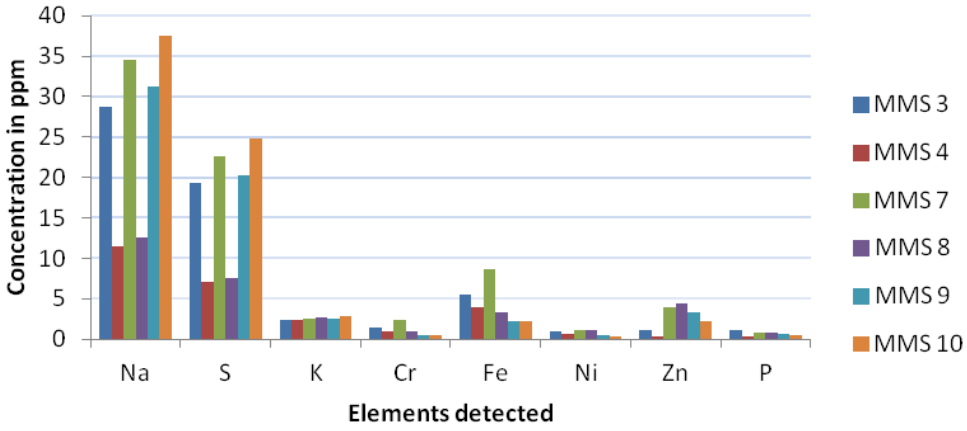

Figure 6.15. ICPMS analysis of methylamphetamine via Moscow Route using *pseudo*ephedrine extracted from Sudafed using commercial methylated spirits.

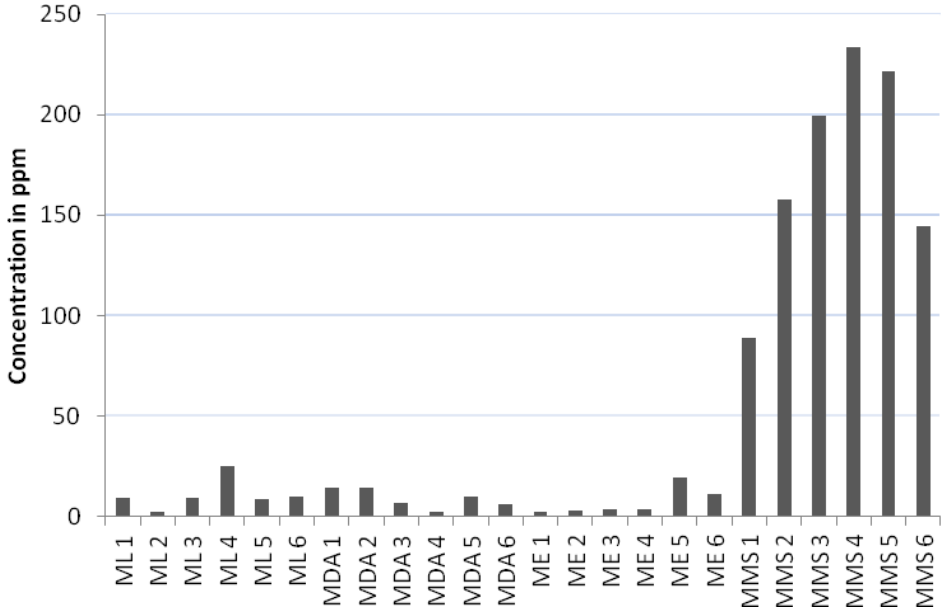

Figure 6.16. Iodine analysis of methylamphetamine batches synthesized using the Moscow route using *pseudo*ephedrine extracted from Sudafed using the three solvents.

Table 6.9. ICP-MS analysis of elements detected in ppm for six batches of methylamphetamine synthesized via Moscow route from:

(a) laboratory grade *pseudo*ephedrine.

Element	ML 13	ML 17	ML 21	ML 22	ML 23	ML 24	Mean	Std Dev	RSD (%)
Na	13.21	7.87	117.10	7.74	24.25	6.44	29.43	43.44	147.58
Si	1.02	0.99	1.02	1.04	0.99	0.96	1.00	0.027	2.72
P	2.07	0.98	1.07	0.63	1.58	1.19	1.25	0.50	39.97
S	8.02	6.59	81.97	5.22	14.21	3.79	19.97	30.58	153.14
K	2.55	2.90	3.44	2.59	2.72	2.58	2.80	0.34	12.15
Fe	2.02	4.14	1.26	1.40	3.58	1.88	2.38	1.19	50.11

(b) *pseudo*ephedrine extracted ethanol.

Element	ME 5	ME 6	ME 7	ME 8	ME 9	ME 10	Mean	Std Dev	RSD (%)
Na	30.26	7.25	5.92	22.07	9.50	11.26	14.38	9.66	67.21
Al	0.04	0.29	0.12	0.055	1.10	0.05	0.27	0.41	148.14
P	0.20	0.17	0.76	1.19	0.49	0.12	0.49	0.41	85.02
S	21.35	5.38	3.56	16.22	6.43	8.08	10.17	7.02	68.99
K	2.58	3.08	2.41	2.49	2.36	2.12	2.51	0.32	12.81
Zn	0.19	0.10	0.10	0.43	0.55	0.37	0.29	0.18	63.92

(c) *pseudo*ephedrine extracted ethanol : methanol (90:10) vol/vol.

Element	MDA 3	MDA 4	MDA 5	MDA 8	MDA 9	MDA 10	Mean	Std Dev	RSD (%)
Na	10.19	26.53	16.13	28.70	9.38	15.32	17.71	8.15	46.05
S	7.21	8.97	11.57	19.75	6.29	10.13	10.66	4.84	45.47
K	2.45	2.20	1.25	0.97	0.87	0.81	1.42	0.71	50.31
Cr	0.44	0.88	0.44	1.18	0.77	0.40	0.68	0.31	45.49
Fe	2.14	4.13	2.98	4.52	2.67	1.71	3.03	1.10	36.43
Ni	0.46	1.36	0.61	1.04	0.48	0.25	0.70	0.41	59.29
Zn	0.25	0.81	0.80	1.02	0.37	0.87	0.69	0.30	43.75
P	0.82	0.39	0.22	0.64	0.58	0.22	0.48	0.24	50.42

(d) *pseudo*ephedrine extracted commercial methylated spirit

Element	MMS 3	MMS 4	MMS 7	MMS 8	MMS 9	MMS 10	Mean	Std Dev	RSD (%)
Na	28.70	11.48	34.51	12.64	31.29	37.48	26.02	11.21	43.09
S	19.37	7.14	22.65	7.50	20.31	24.84	16.97	7.71	45.42
K	2.41	2.39	2.51	2.64	2.50	2.87	2.55	0.18	7.07
Cr	1.47	1.04	2.37	0.90	0.57	0.53	1.15	0.69	60.07
Fe	5.55	3.92	8.58	3.35	2.22	2.20	4.30	2.43	56.54
Ni	0.95	0.62	1.19	1.10	0.56	0.28	0.78	0.35	44.91
Zn	1.18	0.39	3.90	4.50	3.38	2.25	2.60	1.60	61.67
P	1.08	0.35	0.87	0.77	0.60	0.46	0.69	0.27	39.39

Six batches of methylamphetamine synthesized via the Hypo route using (a) laboratory grade precursor, iodine and red phosphorous and (b) extracted precursor and extracted iodine and red phosphorous were analysed using ICPMS and the results are presented in Figure 6.17 to Figure 6.21 and Table 6.10.

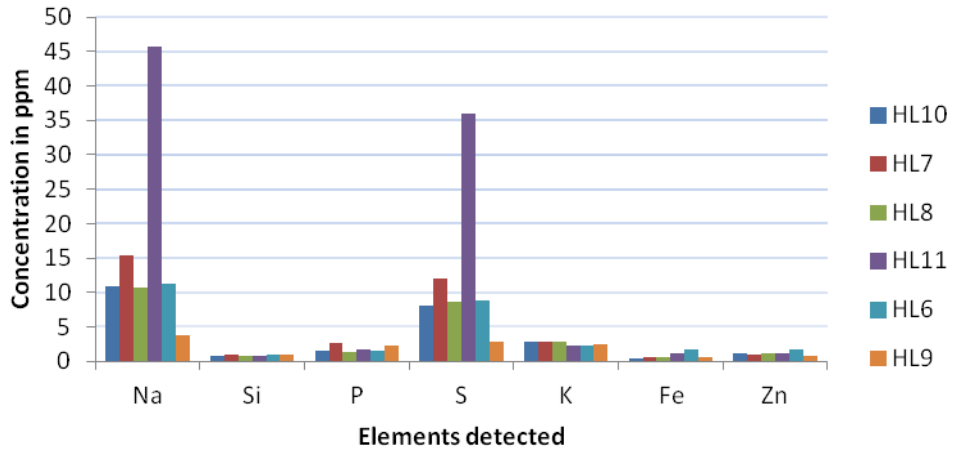

Figure 6.17. ICPMS analysis of methylamphetamine via Hypo Route using lab grade *pseudo*ephedrine HCl.

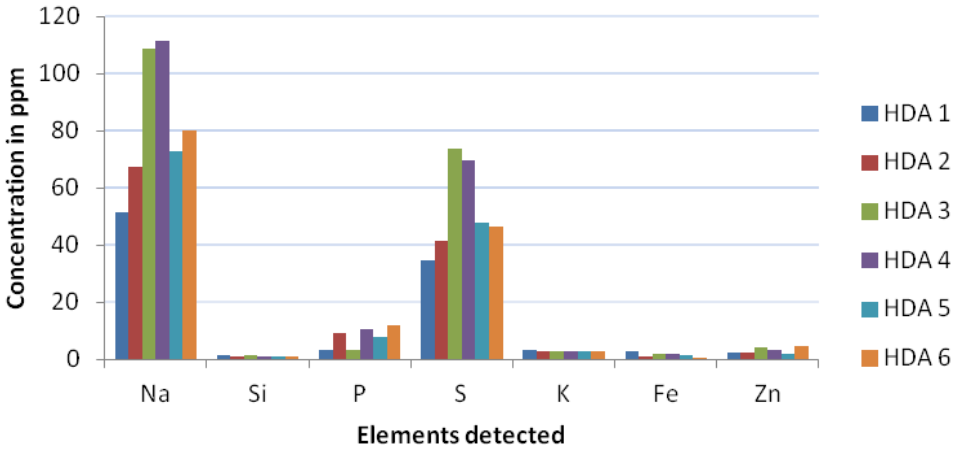

Figure 6.18. ICPMS analysis of methylamphetamine via Hypo Route using *pseudo*ephedrine extracted from Sudafed using ethanol:methanol (90:10)% vol/vol.

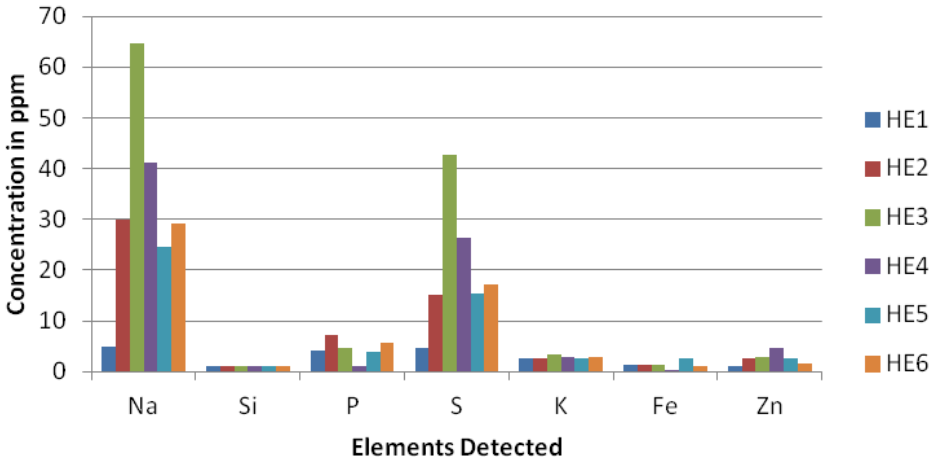

Figure 6.19. ICPMS analysis of methylamphetamine via Hypo Route using *pseudo*ephedrine extracted from Sudafed using ethanol.

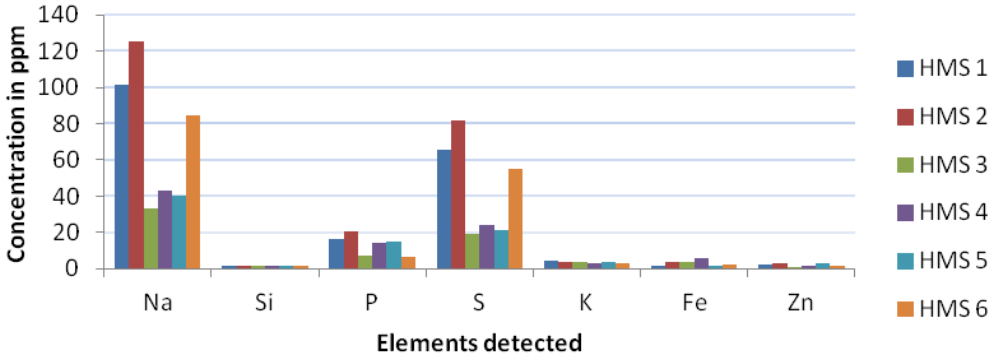

Figure 6.20. ICPMS analysis of methylamphetamine via Hypo Route using *pseudo*ephedrine extracted from Sudafed using commercial methylated spirits.

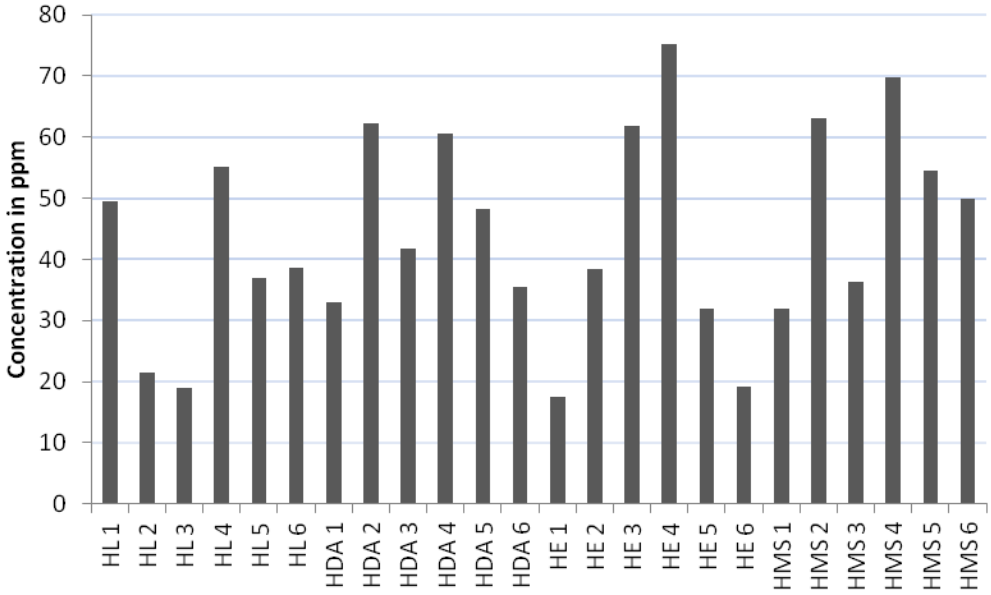

Figure 6.21. Iodine analysis of methylamphetamine batches synthesized via the Hypo route using *pseudo*ephedrine extracted from Sudafed using the three solvents.

Table 6.10. ICP-MS analysis of elements detected in ppm for six batches methylamphetamine synthesized via Hypo route from:

(a) laboratory grade *pseudo*ephedrine.

Elements	HL10	HL7	HL8	HL11	HL6	HL9	Mean	Std Dev	RSD (%)
Na	10.82	15.31	10.70	45.76	11.32	3.71	16.27	14.92	91.69
Si	0.82	1.05	0.86	0.86	1.03	0.98	0.93	0.09	10.51
P	1.48	2.60	1.29	1.64	1.44	2.32	1.79	0.53	29.79
S	8.16	11.93	8.58	35.93	8.84	2.85	12.72	11.74	92.31
K	2.79	2.93	2.82	2.287	2.28	2.54	2.61	0.28	10.90
Fe	0.40	0.68	0.63	1.071	1.63	0.54	0.82	0.45	54.85
Zn	1.08	1.03	1.19	1.253	1.62	0.87	1.180	0.25	21.69

(b) *pseudo*ephedrine extracted ethanol : methanol (90:10) vol/vol.

Elements	HDA 1	HDA 2	HDA 3	HDA 4	HDA 5	HDA 6	Mean	Std Dev	RSD (%)
Na	51.36	67.18	108.80	111.58	72.75	79.98	81.94	23.83	29.09
Si	1.31	1.25	1.27	1.24	1.18	1.13	1.23	0.06	5.24
P	3.22	9.24	3.25	10.41	7.70	11.84	7.61	3.65	47.98
S	34.63	41.44	73.82	69.81	48.01	46.49	52.37	15.82	30.21
K	3.38	2.63	2.80	3.03	2.70	3.05	2.93	0.27	9.52
Fe	2.74	1.12	1.91	1.92	1.65	0.56	1.65	0.74	45.31
Zn	2.49	2.40	4.05	3.28	1.97	4.65	3.14	1.04	33.23

c) ***pseudo*ephedrine extracted from ethanol.**

Elements	HE1	HE2	HE3	HE4	HE5	HE6	Mean	Std Dev	RSD (%)
Na	4.98	29.89	64.72	41.27	24.60	29.25	32.45	19.75	60.87
Si	1.12	1.08	1.08	1.07	1.05	1.01	1.071	0.03	3.47
P	4.20	7.13	4.65	1.19	3.85	5.71	4.46	1.99	44.67
S	4.58	15.10	42.64	26.35	15.48	17.26	20.23	12.97	64.13
K	2.67	2.69	3.29	2.90	2.64	2.80	2.83	0.24	8.53
Fe	1.38	1.38	1.42	0.45	2.53	1.10	1.38	0.67	48.85
Zn	1.01	2.53	2.97	4.67	2.73	1.51	2.57	1.27	49.60

(d) *pseudo*ephedrine extracted from commercial methylated spirit.

Elements	HMS 1	HMS 2	HMS 3	HMS 4	HMS 5	HMS 6	Mean	Std Dev	RSD (%)
Na	101.72	125.53	33.17	43.22	40.26	84.67	71.43	38.08	53.31
Si	1.50	1.51	1.54	1.49	1.45	1.30	1.47	0.08	5.76
P	16.62	20.83	7.26	14.59	15.12	6.51	13.49	5.56	41.27
S	65.85	81.50	19.48	23.91	20.93	55.26	44.49	26.63	59.85
K	4.53	3.76	3.89	3.24	3.78	3.07	3.71	0.51	13.91
Fe	1.74	3.36	3.71	5.77	1.92	2.17	3.11	1.52	49.11
Zn	2.37	2.97	1.23	1.50	2.71	1.92	2.12	0.68	32.34

The various elements detected within each sample and route are summarised in Table 6.11.

Table 6.11. Various elements detected in batches of methylamphetamine, precursors and essential chemicals.

Precursor source	*Pseudo*-ephedrine	Moscow route	Hypo route
Laboratory chemicals	P Si	Na S K I P Fe	Na S K I P Zn
Ethanol extraction	Na Mg	Na S K I P Zn Al	Na S K I P Zn Fe
Ethanol/Methanol (90:10% vol/vol)	Na Mg K Si	Na S K I P Zn Fe Cr Ni	Na S K I P Zn Fe
Commercial methylated spirits	Na S K Si Mg	Na S K I P Zn Fe Cr Ni	Na S K I P Zn Fe
Iodine tincture	C, Cl, K, Cr, Fe, I		
Matchbook stripe pad	C, Cl, K, Cr, Fe, Na, Mg, S, P, Zn, Mn, Pb		

Various elements were present across the range of samples analysed. Specific combinations of elements were also present in the extracting solvents and iodine and red phosphorous which appeared to carry through to the final products. Five elements, (sodium, sulfur, potassium, iodine and phosphorous), were present in all synthesised samples, all be it, in variable quantities. Zinc was also present in all samples synthesised from the extracted precursor chemicals and may be present due to its presence in red phosphorous prepared from matchbook stripe pads.

Sodium (presented in Figure 6.22) was present in all extracted *pseudo*ephedrine samples and presumably carried through to the final synthetic product although the presence of this element as an environmental contaminant cannot be ruled out. The synthetic process itself may also introduce sodium and sulphur as a consequence of the basification of methylamphetamine from the oil where sodium hydroxide and sulphuric acid are used. The generation of gaseous hydrochloric acid occurs via a reaction between sodium chloride and sulphuric acid introduced to the methylamphetamine base via a cannula providing another potential sodium source [17]. Methylamphetamine samples synthesized from the Hypo route generally had increased concentrations and a wider spread of sodium compared to methylamphetamine synthesized from the Moscow route. This may be as a consequence of further sodium being introduced with hypophosphorous acid used in the synthesis which itself is prepared industrially via a two step process involving lithium, sodium or potassium hypophosphite salts [18,19].

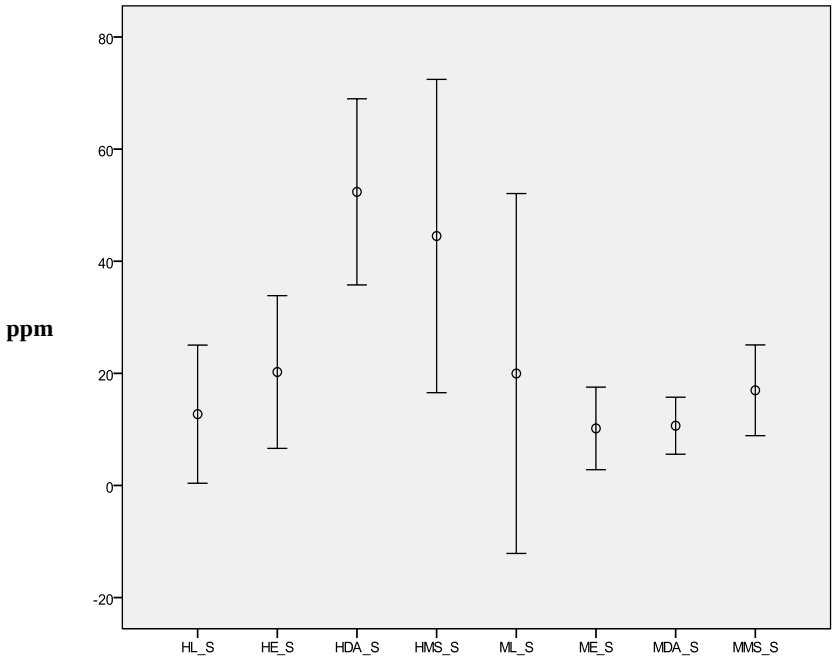

Figure 6.22. Sodium concentrations of methylamphetamine synthesized from both routes using laboratory grade and extracted precursors; HL/ML=hypo/moscow route using laboratory grade chemicals, HE/ME=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol, HDA/MDA= Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol:methanol (90:10% vo/vol), HMS/MMS=Hypo route/Moscow route using *pseudo*ephedrine extracted using commercial methylated sprits.

The values for sulphur within each group of samples is presented in Figure 6.23.

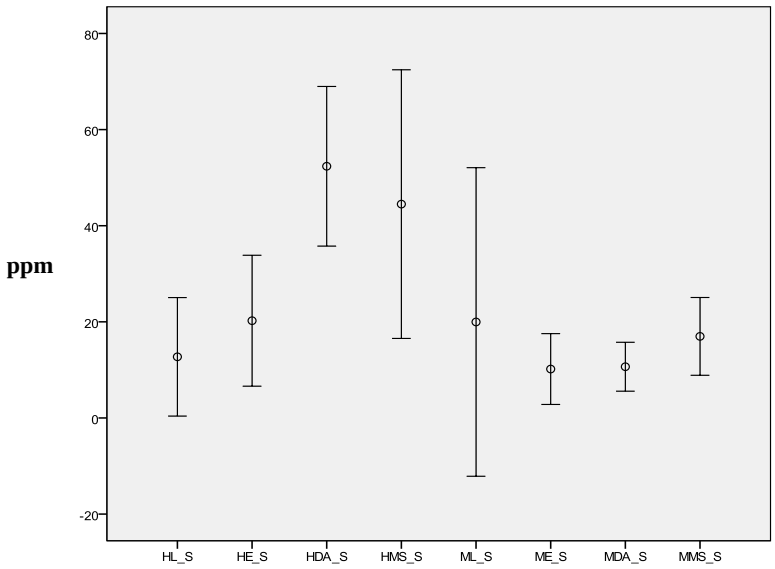

Figure 6.23. Sulfur concentrations of methylamphetamine synthesized from both routes using laboratory grade and extracted precursors HL/ML=hypo/moscow route using laboratory grade chemicals, HE/ME=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol, HDA/MDA=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol:methanol (90:10% vo/vol), HMS/MMS=Hypo route/Moscow route using *pseudo*ephedrine extracted using commercial methylated sprits.

Potassium was most probably due to the presence of the element in the extracted *pseudo*ephedrine, iodine and red phosphorous samples. The distribution of potassium is presented in Figure 6.24 and is reasonably evenly distributed across the samples.

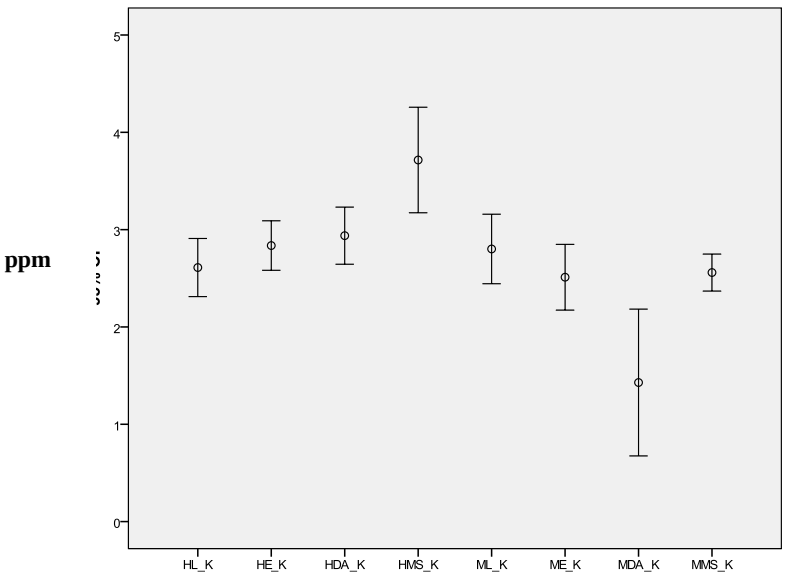

Figure 6.24. Potassium concentrations of methylamphetamine synthesized from both routes using laboratory grade and extracted precursors HL/ML=hypo/moscow route using laboratory grade chemicals, HE/ME=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol, HDA/MDA=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol:methanol (90:10% vo/vol), HMS/MMS=Hypo route/Moscow route using *pseudo*ephedrine extracted using commercial methylated.

Methylamphetamine synthesized from the Hypo route have a higher concentration of phosphorous compared to methylamphetamine synthesized from the Moscow route when compared across each of the specific precursor sources. The highest concentration of phosphorous was detected within batches of methylamphetamine synthesized using precursors extracted from proprietary cold medication using commercial methylated spirit (HMS).

The distribution of phosphorous within the samples is presented in Figure 6.25

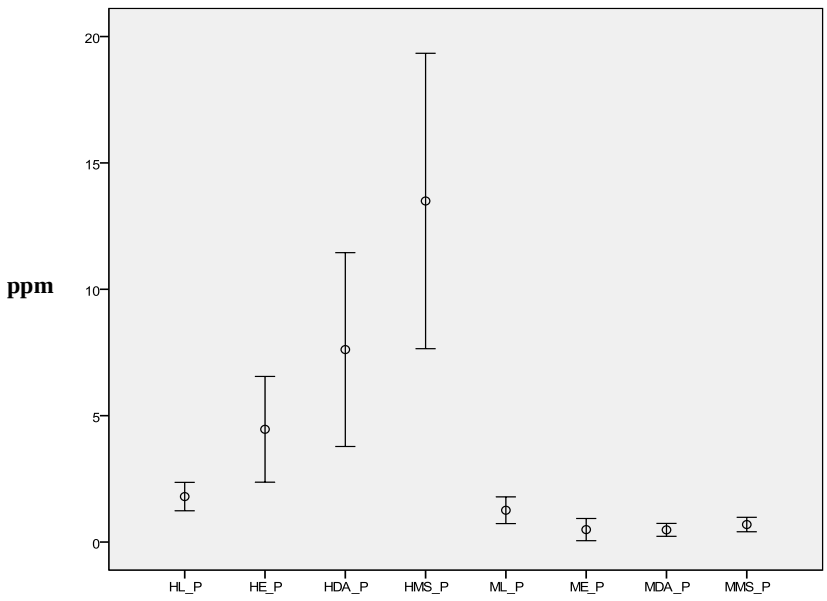

Figure 6.25. Phosphorous concentrations of methylamphetamine synthesized from both routes using laboratory grade and extracted precursors HL/ML=hypo/moscow route using laboratory grade chemicals, HE/ME=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol, HDA/MDA=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol:methanol (90:10% vo/vol), HMS/MMS=Hypo route/Moscow route using *pseudo*ephedrine extracted using commercial methylated.

The source of phosphorous was different for each synthetic route. In the Hypo route *in situ* synthesised hypophosphorous acid was used while red phosphorous was directly used in the Moscow route and this no doubt also contributed to the elemental distribution between routes.

The iodine source is obviously the tinctures used in the synthesis for both routes. Apart from the Moscow route samples where commercial methylated spirit was used as the extracting solvent for Sudafed, the concentrations of iodine did not vary significantly between the various batches of samples, with the Hypo samples having slightly higher concentrations and a wider spread of values than the Moscow samples as illustrated in Figure 6.26.

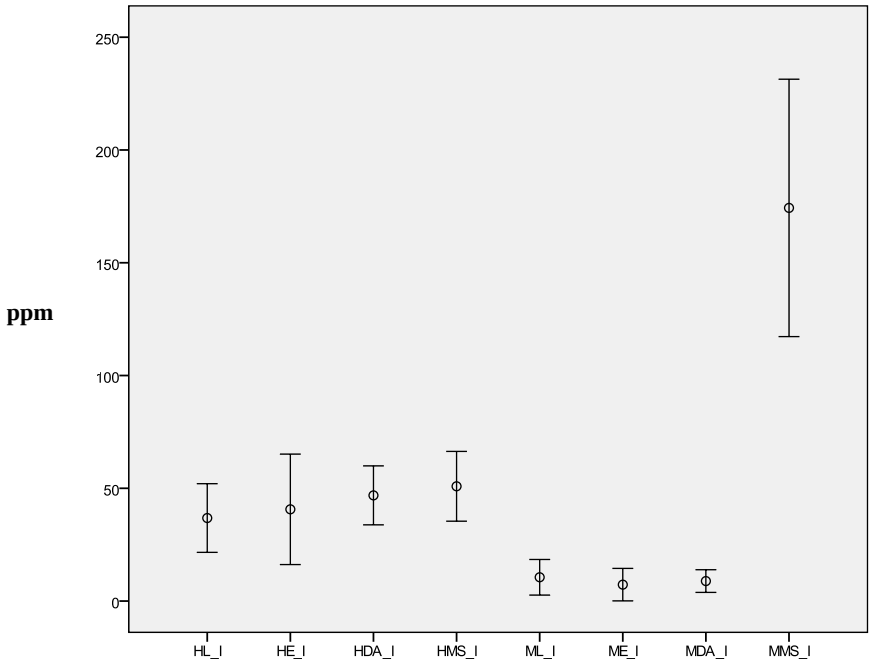

Figure 6.26. Iodine concentrations of methylamphetamine synthesized from both routes using laboratory grade and extracted precursors HL/ML=hypo/moscow route using laboratory grade chemicals, HE/ME=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol, HDA/MDA=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol:methanol (90:10% vo/vol), HMS/MMS=Hypo route/Moscow route using *pseudo*ephedrine extracted using commercial methylated.

Methylamphetamine synthesized from the Moscow route have a higher concentration of iron compared to methylamphetamine synthesized from the Moscow route when compared across each of the specific precursor sources. The only exception to this was Hypo samples synthesised from the precursor extracted using commercial methylated spirit. The highest concentration of iron was detected within batches of methylamphetamine synthesized using precursors extracted using commercial methylated spirit (MMS) which also provided the widest range of values in both the Moscow and Hypo routes. Iron was also detected in the iodine tinctures, phosphorous extracted from the matchboxes and from the Sudafed tablets themselves. The distribution of iron within the samples is presented in Figure 6.27.

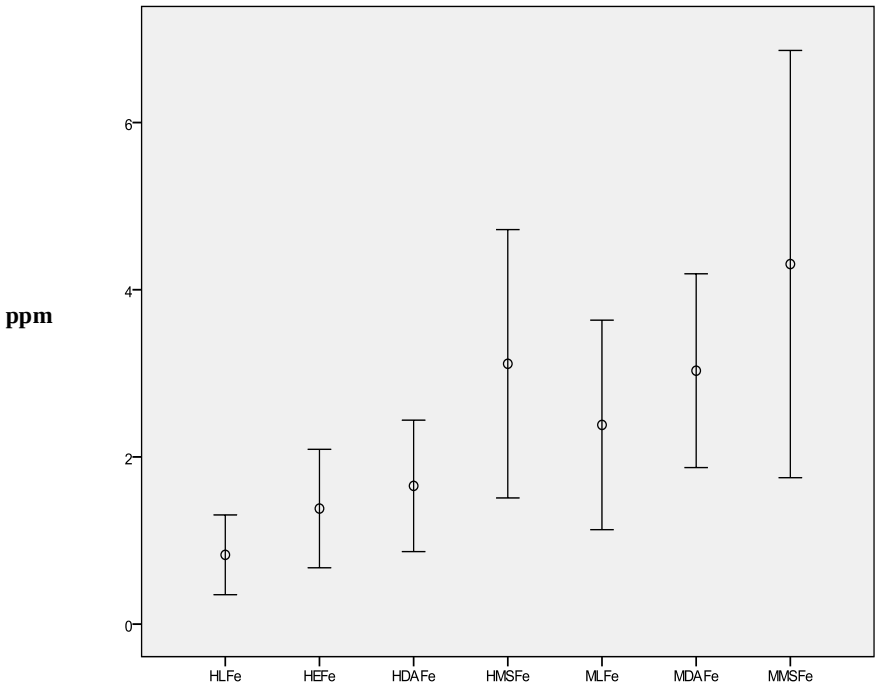

Figure 6.27. Iron concentrations of methylamphetamine synthesized from both routes using laboratory grade and extracted precursors HL/ML=hypo/moscow route using laboratory grade chemicals, HE/ME=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol, HDA/MDA=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol:methanol (90:10% vo/vol), HMS/MMS=Hypo route/Moscow route using *pseudo*ephedrine extracted using commercial methylated.

The zinc detected may be as a result of zinc present within the phosphorous extracted from matchboxes. The distribution of zinc within the samples is presented in Figure 6.28. Methylamphetamine synthesized from the Hypo route had, generally speaking, higher concentrations of zinc compared to samples prepared using the Moscow route with the exception of the methylated spirit extracted Moscow synthesised sample.

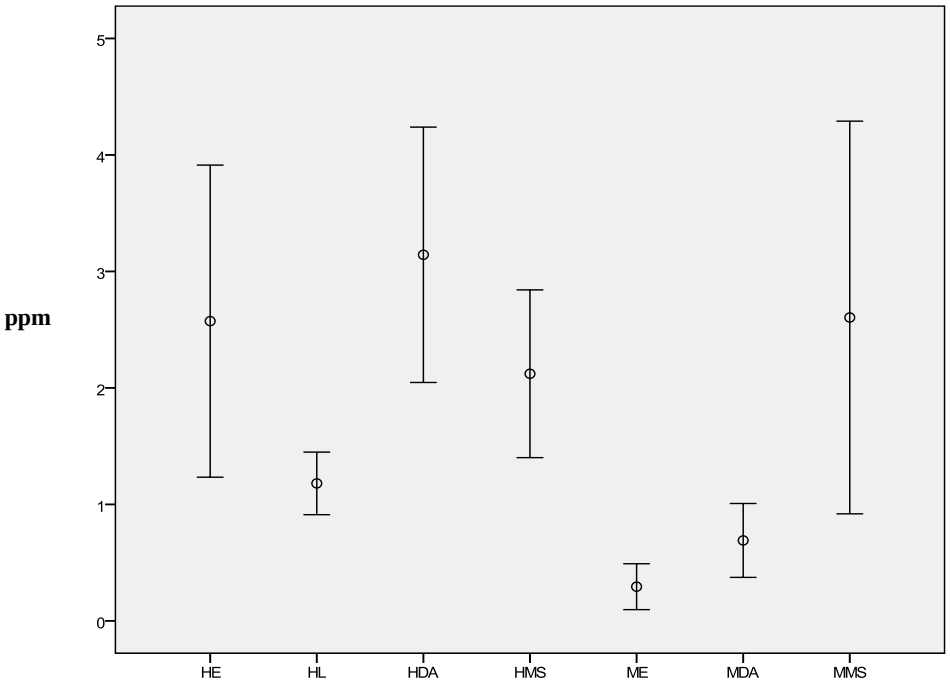

Figure 6.28. Zinc concentrations of methylamphetamine synthesized from both routes using laboratory grade and extracted precursors HL/ML=hypo/moscow route using laboratory grade chemicals, HE/ME=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol, HDA/MDA=Hypo/Moscow route using *pseudo*ephedrine extracted using ethanol:methanol (90:10% vo/vol), HMS/MMS=Hypo route/Moscow route using *pseudo*ephedrine extracted using commercial methylated.

6.4 Conclusions

Inductively coupled plasma mass spectrometry (ICP-MS) analysis is potentially useful in the comparison and discrimination between batches of precursor produced via different extracting solvents and subsequent methylamphetamine produced for each route. Generally speaking, the elemental concentrations within the Hypo samples were higher than those within the Moscow samples.

The elemental analysis of the extracted *pseudo*ephedrine distinguished between the extraction solvents used to prepare the precursor from the cold medication. To our knowledge this is the first time that this discrimination had been exposed.

Methylamphetamine synthesized from precursors sourced from cold medication and essential chemicals extracted from tinctures and matchboxes also demonstrated different elemental profiles from the laboratory grade precursors by constantly exhibiting the presence of zinc in all samples. Of the lab grade synthesised samples, zinc was present only in the Hypo samples. Both zinc and iron were present in the elemental profiles of red phosphorous from matchbook strips, with iron also present consistently in the elemental profiles of iodine from iodine tinctures and the Sudafed tablets themselves. The elemental variation of phosphorous observed within methylamphetamine synthesised via the Hypo and Moscow routes were also different presumably due to the different phosphorous sources used in the synthetic methods.

A trend was also observed in the concentrations of sodium and sulphur within the various samples which may be directly linked to the salting out process used.

6.5 References

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Chapter 7 Investigation of precursor source, synthetic routes and regiospecificity of methylamphetamine samples using stable isotope ratio mass spectrometry (IRMS)

7.1 Introduction

The investigation of carbon, nitrogen and hydrogen stable isotopic ratios are presented for each batch of methylamphetamine prepared via the Hypophosphorous and Moscow routes using the various precursor samples. Six samples were prepared using each synthetic method using laboratory precursors and the various extracted precursors, giving 48 samples in total. This facilitated the investigation of the influence of the extracting solvent on any resultant data. Secondly, 5 batches of methylamphetamine synthesized from precursors extracted from Allerpid and Panadol tablets sourced from Malaysia were also analysed. All IRMS analysis was conducted at the James Hutton Institute, Dundee.

7.2 Experimental Methods

7.2.1 ^{13}C and ^{15}N Isotope analysis by EA-IRMS

Carbon and nitrogen isotope abundances analyses were carried out using an automated nitrogen-carbon analyzer (ANCA) coupled to an automated breath analyzer (ABCA) isotope ratio mass spectrometer (SerCon Ltd, Crewe, United Kingdom). Typically 0.4 mg of sample material was weighed into tin capsules (Elemental microanalysis, Devon, United Kingdom) and introduced via a solid Costech Zero-Blank autosampler (Pelican Scientific Limited, Alford, United Kingdom). The elemental analyzer (EA) reactor tubes were comprised of two quartz glass tubes filled with chromium (III) oxide/copper oxide and reduced copper, held at 1020^{o}C and 620^{o}C for combustion and reduction respectively. A water trap filled with magnesium perchlorate was used to remove water from combustion gases thus generated, and post reactor gas chromatograpy (GC) column was kept at 65^{o}C for separation of evolved N_2 and CO_2. Data were processed using proprietary software (SerCon Limited, Crewe, United

Kingdom). Measured isotope ratios are expressed in the δ notation [$^{o}/_{oo}$] relative to the appropriate international standard material anchoring the isotope scale (e.g., VPDB for ^{13}C or VSMOW for ^{2}H).

Each batch of samples was bracketed by two blanks (empty tin capsules) and two sets of laboratory certified standards of known isotopic composition (Iso-Analytical, Crewe, United Kingdom). This standard was leucine ($\delta^{13}C_{VPDB}$= -30.52 $^{o}/_{oo}$, $\delta^{15}N_{AIR}$= +10.77 $^{o}/_{oo}$).

7.2.2 ^{2}H Isotope analysis by TC/EA-IRMS

A Delta^{Plus}-XP isotope ratio mass spectrometer (IRMS) coupled to a high-temperature conversion/elemental analyzer (TC/EA; both Thermo-Fischer Corporation, Bremen, Germany) was used for $^{2}H/^{1}H$ isotope ratio measurement of synthesized methylamphetamine samples. Typically, 0.2 mg of solid sample was weighed into a silver capsule and placed in a desiccator for one week before the samples were introduced into the TC/EA by means of a solid Costech Zero-Blank solid autosampler (Pelican Scientific Ltd, Alford, United Kingdom). The reactor tube was self packed and comprised of an Alsint ceramic tube, containing a glassy carbon tube filled with glassy carbon granulate, silver and quartz wool (SerCon, Crewe, Cheshire). The reactor temperature was set at 1425^{o}C while the postreactor gas chromatograph column was maintained at 85^{o}C. Helium (99.99% purity, Air Products plc, Crewe, Cheshire) pressure was set at 1.45 bar. The run time per analysis was 350s. Measured $^{2}H/^{1}H$ isotope ratios are expressed as δ values in $^{o}/_{oo}$ relative to VSMOW.

The working reference gas, H_2 (BOC, Guilford, Surrey, United Kingdom), was calibrated against VSMOW ($\delta^{2}H$=0.00 $^{o}/_{oo}$) and checked against international reference material (IRM), IAEA-CH-7 polyethylene ($\delta^{2}H_{VSMOW}$= -100.3 $^{o}/_{oo}$; IAEA, Vienna, Austria). Cross checking the $\delta^{2}H_{VSMOW}$-value obtained for the working reference gas H_2 against a further international reference material (IRM) for H^2 isotope analysis, GISP, yielded a measured $\delta^{2}H_{VSMOW}$-value for GISP of -194.6 $^{o}/_{oo}$ (accepted $\delta^{2}H_{VSMOW=}$ -189.73 $^{o}/_{oo}$; IAEA, Vienna Austria). A typical batch analysis compised of 10 samples

run in triplicate, preceded and followed by a set of standards as reported previously [1]. This consisted of in-house standards (coumarin, δ^2H_{VSMOW}= +62.56 $^{o}/_{oo}$) and one IRM (IAEA-CH-7) as calibration controls at the beginning and end of the set. Each batch was preceded and followed by a blank silver capsule. Precision of ^2H isotope analysis as monitored by the IRMs and lab standards was $\pm$1.15 $^{o}/_{oo}$ or better.

7.2.3 IRMS Sample preparation

Aliquots sufficient for stable isotope analysis were weighed out and dried in a desiccator to remove any traces of moisture (*in vacuo* over P_4O_{10}). To prepare samples for isotope analysis, drug aliquots were removed from the desiccator and approximately 0.2 and 0.4 mg samples were weighed out in triplicate into silver and tin capsules (SerCon Limited, Crewe, United Kingdom) for analysis by TC/EA-IRMS and EA-IRMS respectively. Capsules were subsequently crimped and placed into 96 well-plates already prepared with blanks and appropriate reference materials. Batch run ready well-plates were placed into another desiccator, where they were kept *in vacuo* over P_4O_{10} until analysis.

7.2.4 Solvent and drying studies of *pseudo*ephedrine hydrochloride extracted from Sudafed tablets.

A precursor drying and extraction solvent study was undertaken to determine the effects on isotopic variation that may have occurred when *pseudo*ephedrine hydrochloride was extracted from the Sudafed tablets.

Two sets of the precursor were prepared by extraction from Sudafed samples using solvents sourced from different suppliers. The tablets were extracted according to the method detailed in section 3.2.2. Ethanol and methanol were supplied by Sigma Aldrich and Fluka, commercial methylated Spirits were sourced from two different batches of Home brand purchased locally in Glasgow at B&Q.

Secondly, a set of extracted precursors were split into two batches to explore the effect of drying. In each case, one half of the sample was dried at room temperature in a fume hood and the second half dried *in vacuo* over P_4O_{10} for one week prior to analysis.

7.3 Results and discussion

7.3.1 *Pseudo*ephedrine HCl extracted from Sudafed tablets

Methylamphetamine hydrochloride was prepared via both synthetic routes using laboratory grade *pseudo*ephedrine hydrochloride purchased from Sigma Aldrich. One set of precursors obtained were split and dried either at room temperature in a fume hood or in a desiccator. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values for the precursor samples are presented in the table below. Sudafed tablets extracted using ethanol and methanol purchased from Fluka was initially used in the extraction process of *pseudo*ephedrine. These are highlighted in italics table shown below. Single batches of the samples was analysed initially to determine the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ isotopic values. The results obtained for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ represents the average of triplicate analysis $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values for the precursor samples are presented in Table 7.1.

Table 7.1. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values for the precursor samples.

Precursor Source	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{2}H$**
Pseudoephedrine from Sigma Aldrich	**-24.99**	**3.72**	**-82.50**
Samples dried at room temperature			
Sudafed tablets extracted using ethanol (Fluka)	**-24.97**	**-3.00**	**-20.10**
Sudafed tablets extracted using ethanol:methanol(90:10)%vol/vol (both Fluka)	**-26.78**	**-2.91**	**55.30**
Sudafed tablets extracted using commercial methylated spirit (home brand B&Q 1)	**-25.28**	**-2.88**	**-2.00**
Sudafed tablets extracted using ethanol (Sigma Aldrich)	**-26.57**	**-2.48**	**-34.88**
PSE-E-1	-26.51	-2.43	-35.1
PSE-E-2	-26.60	-2.45	-33.0
PSE-E-3	-26.61	-2.46	-34.3
PSE-E-4	-26.65	-2.52	-35.4
PSE-E-5	-26.55	-2.56	-35.9
PSE-E-6	-26.50	-2.46	-35.6

Sudafed tablets extracted using ethanol:methanol(90:10)%vol/vol (both Sigma Aldrich)	**-26.71**	**-2.16**	**-50.73**
PSE-DA-1	-26.69	-2.09	-50.7
PSE-DA-2	-26.83	-2.19	-51.3
PSE-DA-3	-26.70	-2.14	-50.9
PSE-DA-4	-26.67	-2.21	-49.1
PSE-DA-5	-26.73	-2.13	-52.8
PSE-DA-6	-26.65	-2.20	-49.6
Sudafed tablets extracted using commercial methylated spirit (home brand B&Q 2)	**-27.12**	**-2.09**	**-58.87**
PSE-MMS-1	-27.09	-2.06	-60.7
PSE-MMS-2	-27.15	-2.06	-58.0
PSE-MMS-3	-27.11	-2.12	-60.4
PSE-MMS-4	-27.15	-2.10	-58.0
PSE-MMS-5	-27.12	-2.14	-58.3
PSE-MMS-6	-27.10	-2.08	-57.8
Samples dried in vacuo over P_4O_{10}			
Sudafed tablets extracted using ethanol (Sigma Aldrich)	**-27.12**	**-2.09**	**-30.75**
D-PSE-E-1	-27.09	-2.06	-32.1
D-PSE-E-2	-27.15	-2.06	-30.9
D-PSE-E-3	-27.11	-2.12	-31.0
D-PSE-E-4	-27.15	-2.10	-30.3
D-PSE-E-5	-27.12	-2.14	-30.5
D-PSE-E-6	-27.11	-2.08	-29.7
Sudafed tablets extracted using ethanol:methanol(90:10)%vol/vol (both Sigma Aldrich)	**-27.12**	**-2.09**	**-45.40**
D-PSE-DA-1	-27.09	-2.06	-45.4
D-PSE-DA-2	-27.15	-2.06	-45.7
D-PSE-DA-3	-27.11	-2.12	-44.2
D-PSE-DA-4	-27.15	-2.10	-43.8
D-PSE-DA-5	-27.12	-2.14	-44.3
D-PSE-DA-6	-27.11	-2.08	-49.0
Sudafed tablets extracted using commercial methylated spirit (home brand B&Q batch 2)	**-27.12**	**-2.09**	**-52.28**
D-PSE-MMS-1	-27.09	-2.06	-53.2
D-PSE-MMS-2	-27.15	-2.06	-52.2
D-PSE-MMS-3	-27.11	-2.12	-51.9
D-PSE-MMS-4	-27.15	-2.10	-51.9
D-PSE-MMS-5	-27.12	-2.14	-52.0
D-PSE-MMS-6	-27.11	-2.08	-52.5

Solvent effects

Significant variations are observed within the data for $\delta^{15}N$ values between laboratory grade *pseudo*ephedrine and extracted *pseudo*ephedrine hydrochloride. The precursor extracted from Sudafed tablets demonstrate between them a spread of $\pm 0.91^{o}/_{oo}$ compared with a difference of over $5.81^{o}/_{oo}$ between the nearest sample and the laboratory grade sample. Positive $\delta^{15}N$ values can be used as an indicator of the manufacturing method for the laboratory grade chemical and is indicative of a semi-synthetic route using molasses as the pre precursor [1, 2]. Negative $\delta^{15}N$ values indicate that *pseudo*ephedrine extracted from the Sudafed tablets could have been manufactured from pyruvic acid as the pre precursor and are in agreement of work published by Kurashima *et al*. [2].

Variation in $\delta^{2}H$ values were observed of *pseudo*ephedrine extracted from Sudafed tablets using solvents sourced from different manufactures. This variation was also observed in precursor samples extracted with the two batches of commercial methylated spirit and is in agreement with similar work done by Benson *et al.* relating to explosive analysis [6].

The effect of drying

Variations in $\delta^{2}H$ values can be due to a variety of external factors such as, the drying process, the kind of solvent used and ambient humidity [2]. The variations observed for $\delta^{2}H$ values for the *pseudo*ephedrine hydrochloride extracted from Sudafed tablets using the different drying conditions appeared to also be influenced by the extraction solvent with larger variations observed for the ethanol:methanol extracted samples commercial methylated spirit when compared to ethanol extracted samples. The influence of methanol may be because it is more hygroscopic compared to ethanol, and as such has a greater tendency to absorb moisture from the atmosphere [3]. The hydrogen atoms contained in the hydroxyl group are classed as exchangeable hydrogen, available for exchange with atmospheric hydrogen and as such differences in the moisture content

within the environment of the sample can affect the δ^2H isotopic value. This was further confirmed by studies conducted by Carter *et al*. [5].

Thirty six batches of *pseudo*ephedrine hydrochloride extracted from the Sudafed tablets using the three solvent systems were dried at room temperature and in a desiccator. The δ^{13}C, δ^2H and δ^{15}N data are presented in Figure 7.1 to Figure 7.4.

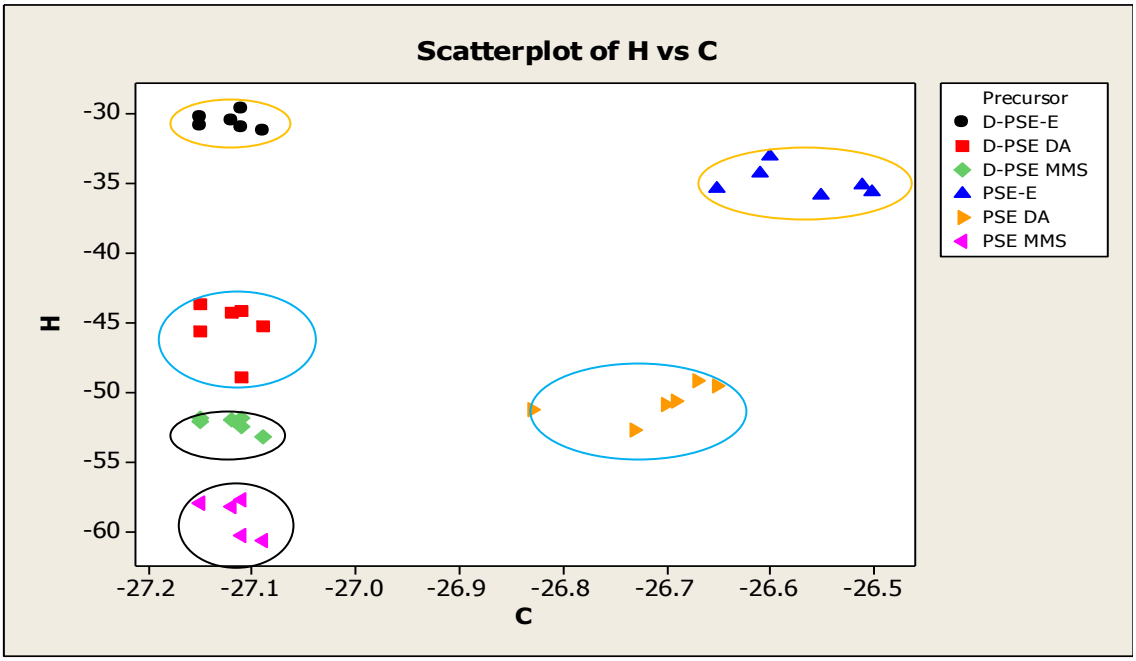

Figure 7.1. Scatter plot of δ^{13}C and δ^{15}H values of batches of *pseudo*ephedrine dried in a desiccator and at room temperature.

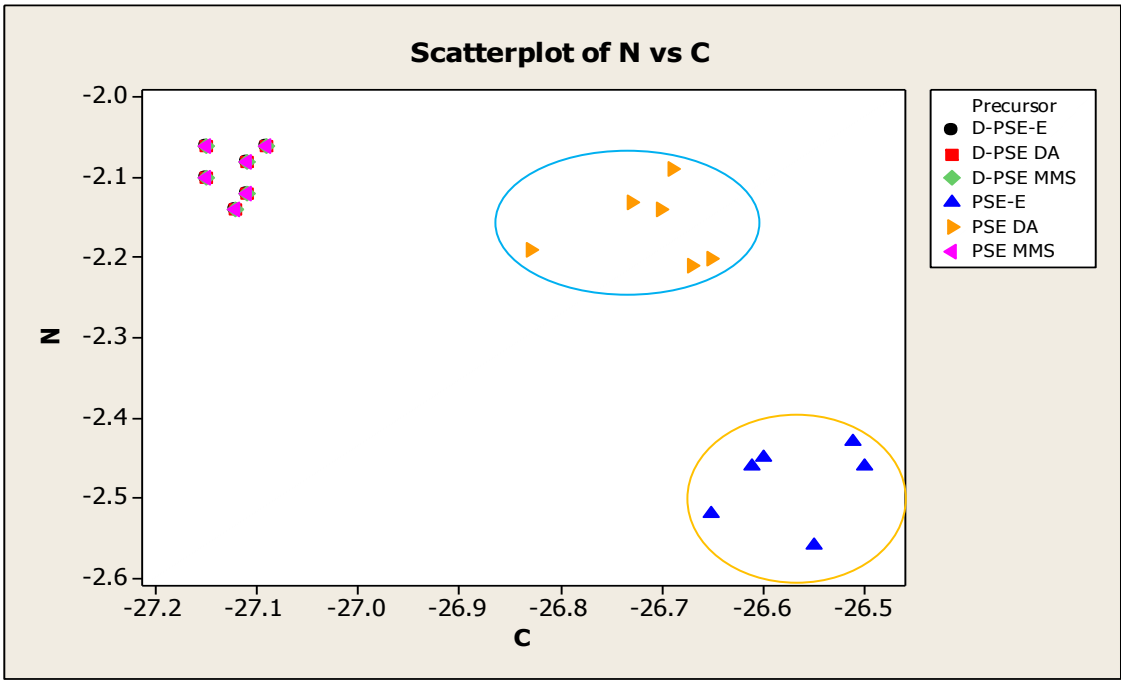

Figure 7.2. Scatter plot of $\delta^{15}N$ and $\delta^{13}C$ values of batches of *pseudo*ephedrine dried in a desiccator and at room temperature.

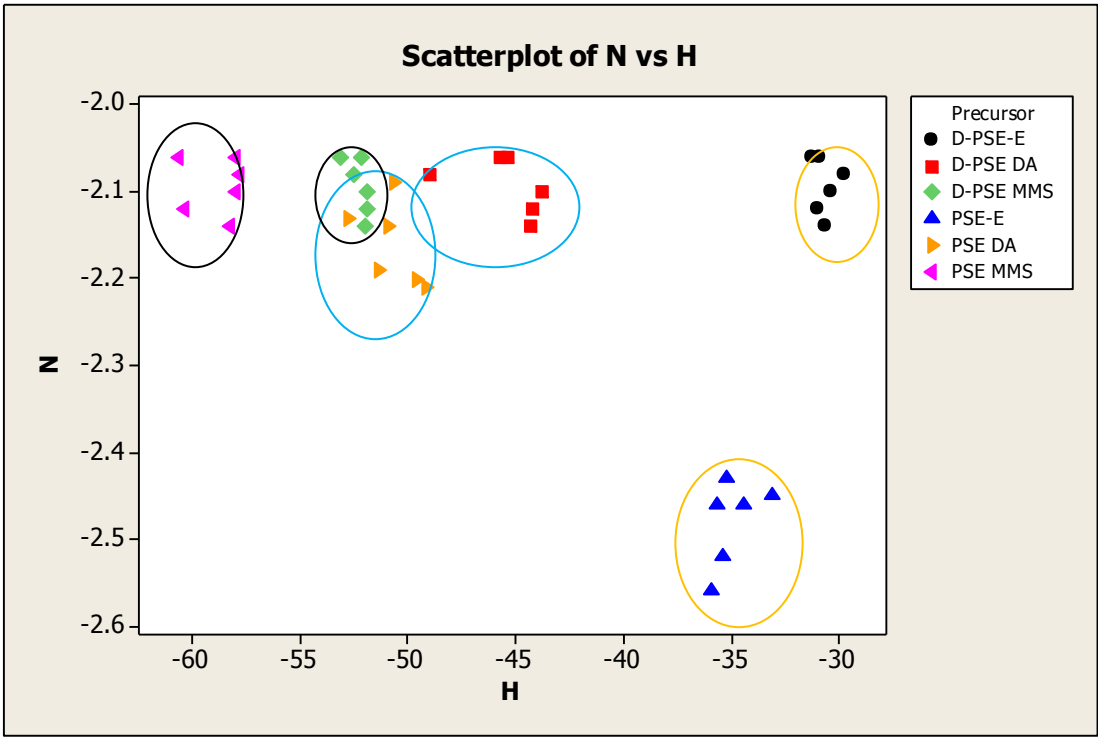

Figure 7.3. Scatter plot of $\delta^{15}N$ and $\delta^{2}H$ values of batches of *pseudo*ephedrine dried in a desiccator and at room temperature.

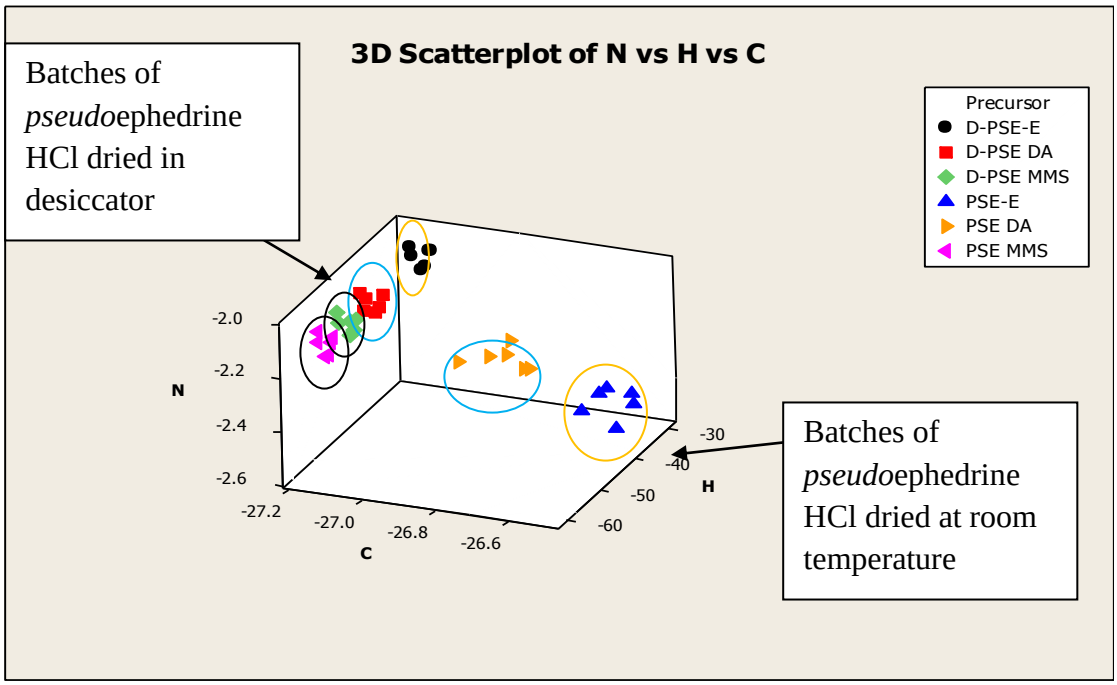

Figure 7.4. 3D scatter plot of $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ analysis of desiccated and non-desiccated *pseudo*ephedrine samples extracted from Sudafed tablets.

*Pseudo*ephedrine hydrochloride extracted using the three different solvents and dried under two different conditions formed some distinct clusters across the different isotopic combinations, however only the carbon and hydrogen isotopic values used in tandem provided sufficient differences to facilitate full discrimination of all the samples. The precursor extracted using ethanol and the ethanol:methanol mixture could be easily discriminated from each other and as a function of drying, specifically on the carbon isotopic values and this may be indicative of the evolution of solvent as the sample dried. Samples extracted using commercial methylated spirits were discriminated from the other precursor samples but the large differences observed in the ethanol and ethanol:methanol extracted samples in relation to the drying of the sample were not apparent in this case. Plotting all three isotopic ratio values against each other reflected the differences observed within the hydrogen and carbon isotopic values. Thus IRMS has been demonstrated as a potential tool for precursor specificity both by extraction

solvent but also as an indicator of some of the synthetic processes which may be involved such as solvent drying.

7.3.2 *Pseudo*ephedrine HCl extracted from Allerpid and Panadol tablets

The combination of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ data in a 3D plot of all stable isotopes was also used to investigate the potential of the technique to discriminate batches of *pseudo*ephedrine extracted from the Malaysian sourced Allerpid and Panadol tablets from the precursor prepared from Sudafed. Figure 7.5 illustrates that this geographical discrimination is possible based largely on the nitrogen isotopic values.

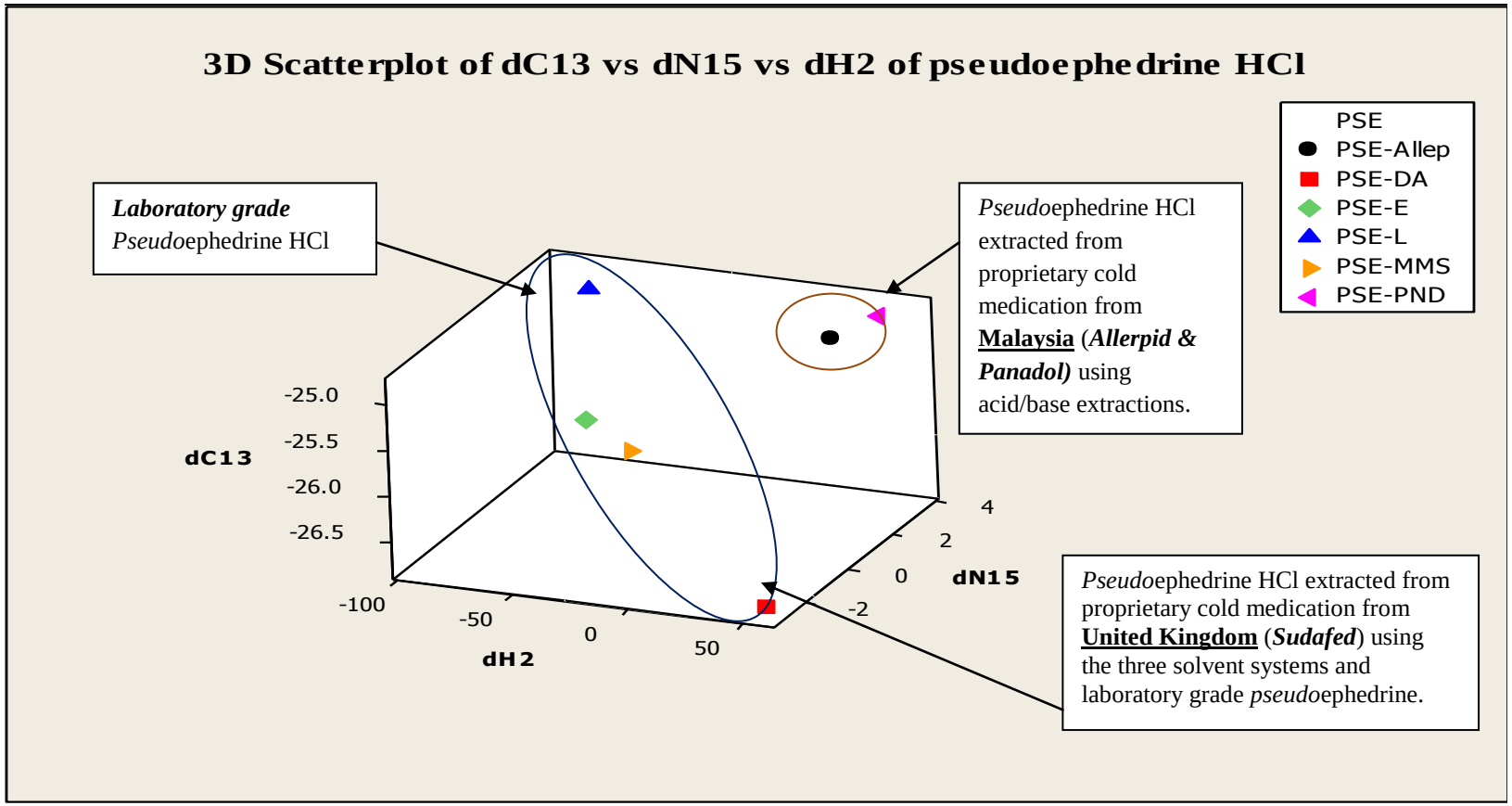

Figure 7.5. 3D scatter plot of mean values $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ analysis of *pseudo*ephedrine samples extracted from laboratory and pharmaceutical grades (Sudafed, Allerpid and Panadol).

7.3.3 IRMS analysis of methylamphetamine

A total of 53 batches of methylamphetamine were prepared from the various *pseudo*ephedrine precursor samples (6 batches from each precursor) and are presented in Table 7.2.

Table 7.2. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of methylamphetamine synthesised from the Hypo and Moscow routes.

Precursor	Route	$\delta^{13}C_{VPDB}(^{0}/_{00})$	$\delta^{15}N_{Air}(^{0}/_{00})$	$\delta^{2}H_{VSMOW}$ $(^{0}/_{00})$
*Pseudo*ephedrine HCl (99%) (Sigma Aldrich)	***Moscow(M)***			
	ML 24	-27.13	5.50	63.8
	ML 22	-27.12	5.59	62.4
	ML 17	-27.35	5.61	61.8
	ML 21	-27.22	6.59	63.0
	ML 23	-26.78	6.10	66.2
	ML 13	-27.29	5.77	60.7
	Hypo(H)			
	HL 6	-25.16	4.75	-150.8
	HL 7	-25.10	8.20	-142.9
	HL 8	-25.07	5.28	-150.8
	HL 9	-25.10	4.57	-152.5
	HL 10	-25.12	5.33	-152.8
	HL 11	-25.16	4.11	-154.4
*Pseudo*ephedrine HCl extracted from Sudafed tablets using ethanol as the solvent (Fluka)	***Moscow(M)***			
	ME 5	-27.27	-1.96	48.3
	ME 6	-26.82	-1.65	46.0
	ME 7	-27.15	-2.35	43.5
	ME 8	-26.85	-3.12	43.3
	ME 9	-26.64	-2.57	60.3
	ME 10	-26.77	-2.27	35.0
	Hypo(H)			
	HE 1	-26.48	-0.48	19.0
	HE 2	-26.26	-1.39	-3.9
	HE 3	-27.09	-3.09	25.7
	HE 4	-26.52	-1.84	21.1
	HE 5	-26.18	-2.58	-0.2
	HE 6	-24.85	-1.96	-37.6
*Pseudo*ephedrine HCl extracted from Sudafed tablets using ethanol:methanol (90:10)% vol/vol as the solvent (Fluka)	***Moscow(M)***			
	MDA 3	-27.06	-1.56	41.4
	MDA 4	-27.15	-3.24	44.0
	MDA 5	-27.31	-0.61	32.9
	MDA 8	-27.03	-2.35	47.7
	MDA 9	-26.73	-2.41	35.1
	MDA 10	-26.75	0.11	37.2
	Hypo(H)			
	HDA 1	-26.10	-1.00	35.4
	HDA 2	-25.99	-1.84	-4.3
	HDA 3	-24.21	-1.31	18.3

	HDA 4	-25.23	0.19	-22.5
	HDA 5	-25.53	-2.42	-13.9
	HDA 6	-25.16	-2.04	-25.8
*Pseudo*ephedrine HCl extracted from Sudafed tablets using commercial methylated spirit as the solvent	***Moscow(M)***			
	MMS 3	-27.31	-1.93	49.7
	MMS 4	-27.13	-2.52	39.6
	MMS 6	-26.79	0.88	38.6
	MMS 7	-26.92	-1.51	49.0
	MMS 8	-26.88	-1.22	37.4
	MMS 10	-26.61	0.23	38.0
	Hypo(H)			
	HMS 1	-25.22	-1.79	-27.6
	HMS 2	-24.91	-2.83	-29.6
	HMS 3	-24.97	-2.95	-36.6
	HMS 4	-25.22	-2.04	-27.0
	HMS 5	-25.31	-2.70	-19.8
	HMS 6	-24.98	-2.36	-39.7
Pseudoephedrine HCl extracted from Allerpid tablets using acid base extractions	***Moscow(M)***			
	Allep-Moscow 1	-24.10	6.87	56.08
	Allep-Moscow 2	-23.73	6.92	55.74
	Hypo(H)			
	Allep-Hypo 1	-23.89	5.26	34.26
	Allep-Hypo *2*	-24.10	6.86	37.90
Pseudoephedrine HCl extracted from Panadol tablets using acid base extractions	***Hypo(H)***			
	Panadol-Hypo	-24.66	3.32	-23.65

In order to discuss the origins of the atoms on a methylamphetamine hydrochloride molecule prepared by each of the two synthetic routes investigated, it is useful to refer to the numbered molecule shown in Figure 7.6. The nitrogen in the molecule is also contributed by the starting material *pseudo*ephedrine HCl.

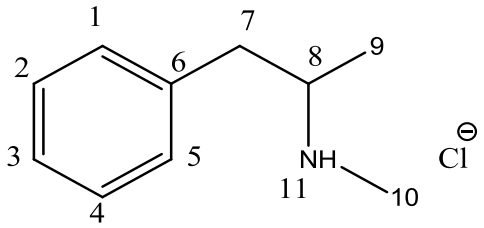

Figure 7.6. The methylamphetamine HCl molecule with numbered atoms.

All of the carbon atoms (1-10) on the methylamphetamine hydrochloride are from the *pseudo*ephedrine HCl used as the starting material or precursor [1, 2], and as such one would have an expectation of a close carbon isotopic link between precursor and final product. One of the hydrogen atoms on the methylamphetamine molecule originate from the formation of hydroiodic acid generated during the reaction process which protonates the hydroxyl group of ephedrine or *pseudo*ephedrine [7, 8]. The origin of hydrogen atom on the nitrogen is from hydrogen gas, formed from the HCl solution used to convert methylamphetamine base to its hydrochloride salt. Proton exchange will also occur during the reaction.

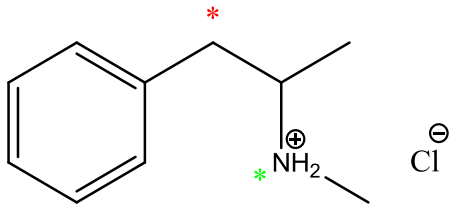

Figure 7.7. The origin of hydrogen atoms on an Methylamphetamine molecule synthesised by the Hypo and Moscow routes. *elimination of hydroxyl group from *pseudo*ephedrine HCl replaced by a hydrogen atom. * Proton source from hydrogen gas.

7.3.4 IRMS analysis of methylamphetamine synthesized from the Hypo and Moscow routes using laboratory grade *pseudo*ephedrine

Twelve batches of methylamphetamine hydrochloride were synthesized using laboratory grade *pseudo*ephedrine hydrochloride, iodine and red phosphorous. The $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ data are presented for these samples in Figure 7.8 to Figure 7.11. Visual discrimination of the methylamphetamine synthesized via the two different routes using the same batch of *pseudo*ephedrine hydrochloride was evident. Methylamphetamine synthesized using the Hypo route produced more negative values for $\delta^{2}H$ compared to the products from the Moscow synthesis possibly due to influence of the hydrogen donor in the benzylic position which, in the case of Moscow synthetic route, is influenced by the addition of water to aid in the formation of hydroiodic acid *in-situ* [9]. Differentiation on the basis of carbon isotopic ratios was also clearly evident. This is

more likely to be connected to the reaction conditions for each synthetic route given that all carbon atoms of the product derive from the precursor.

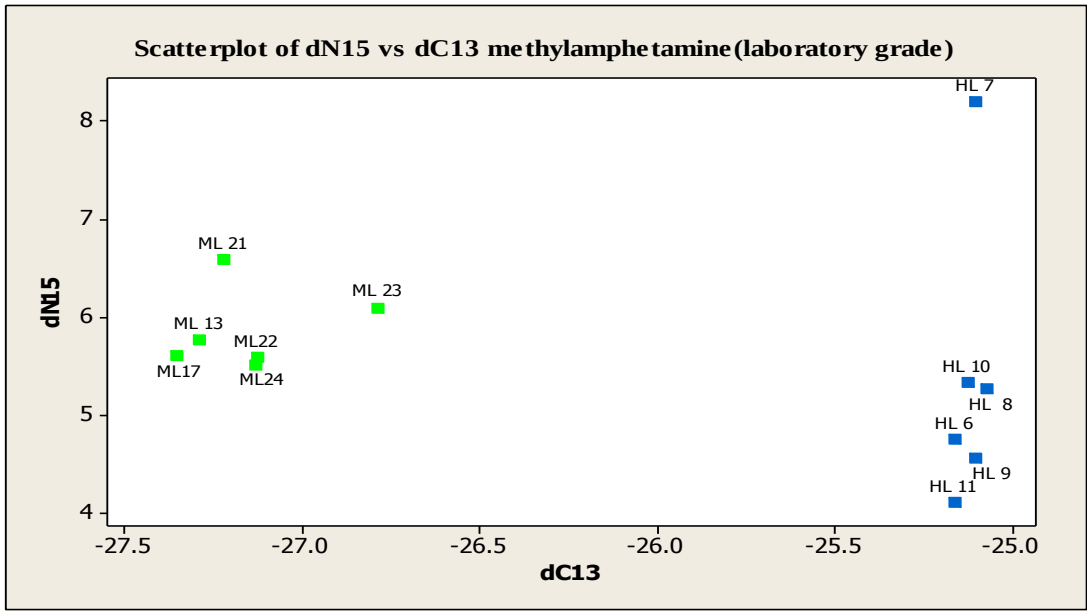

Figure 7.8. Scatter plot of $\delta^{13}C$ and $\delta^{15}H$ values of batches of methylamphetamine synthesized from the hypo (HL) and moscow (ML) routes using laboratory grade *pseudo*ephedrine.

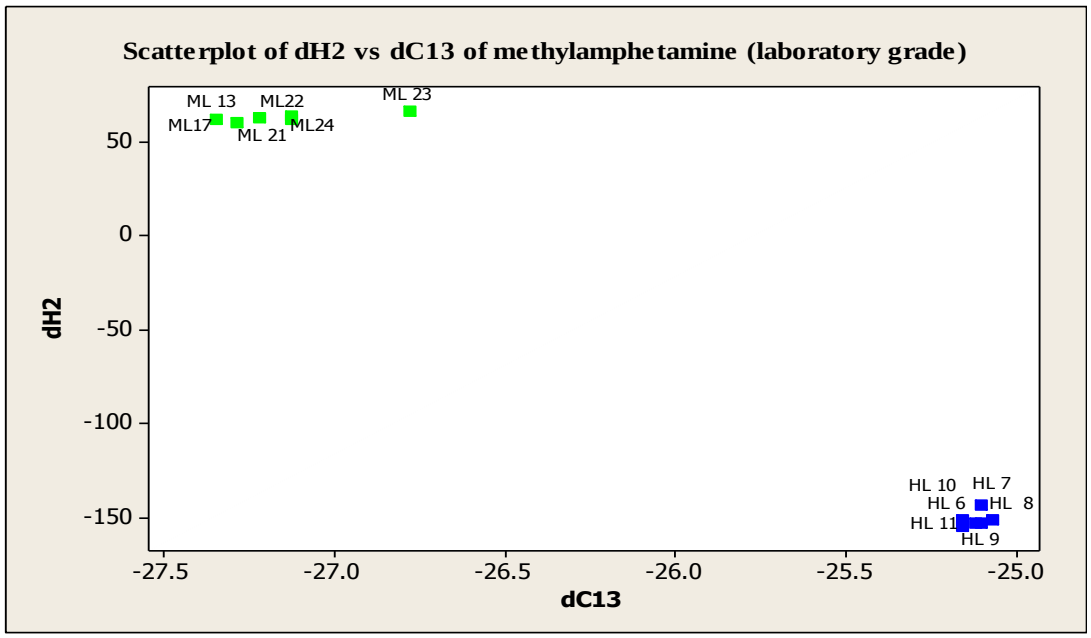

Figure 7.9. Scatter plot of $\delta^{13}C$ and $\delta^{2}H$ values of batches of methylamphetamine synthesized from the hypo (HL) and moscow (ML) routes using laboratory grade *pseudo*ephedrine.

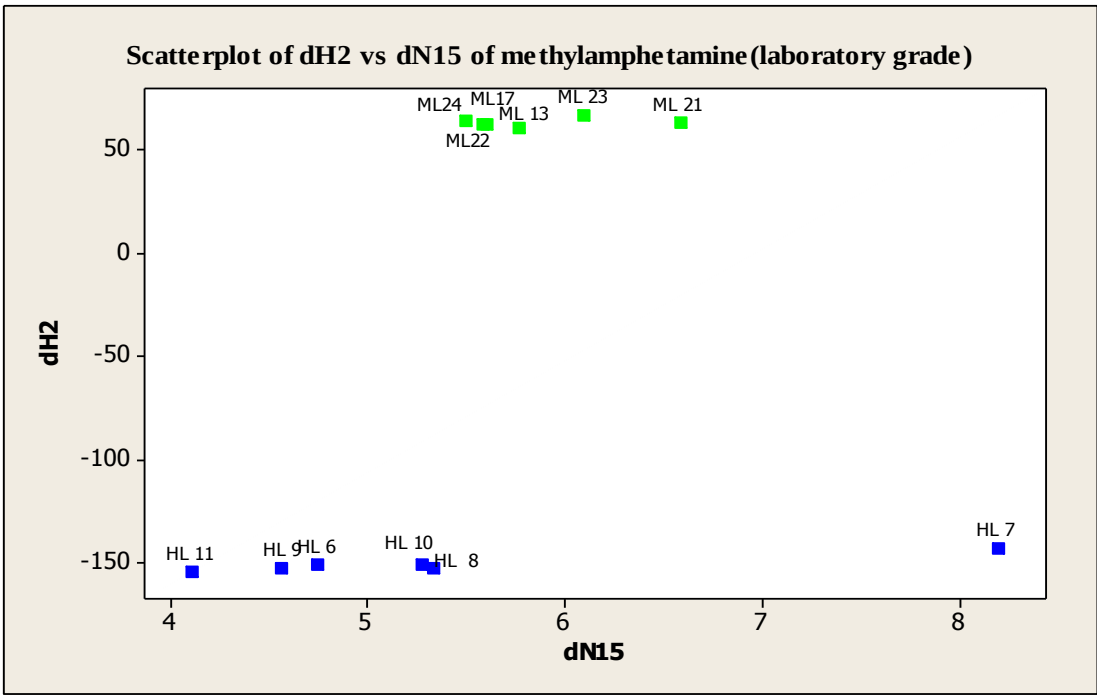

Figure 7.10. Scatter plot of $\delta^{15}N$ and $\delta^{2}H$ values of batches of methylamphetamine synthesized from the hypo (HL) and moscow (ML) routes using laboratory grade *pseudo*ephedrine.

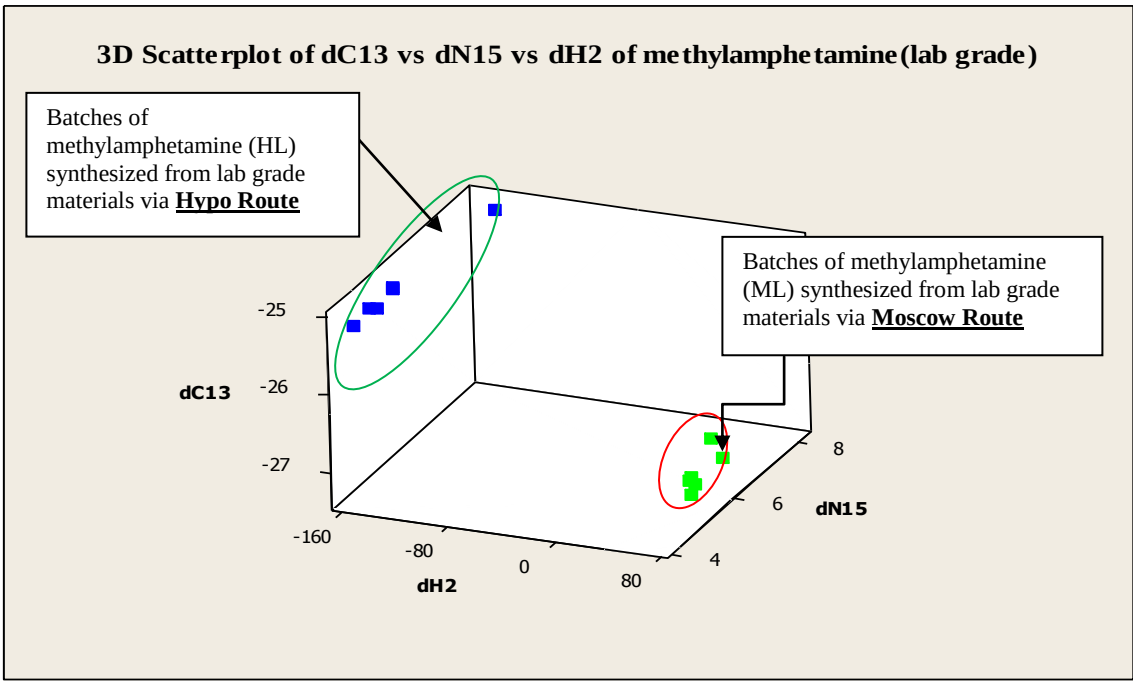

Figure 7.11. 3D scatter plot of $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ for methylamphetamine synthesized from the from the hypo (HL) and moscow (ML) routes using laboratory grade *pseudo*ephedrine.

7.3.5 IRMS analysis of methylamphetamine synthesized *pseudo*ephedrine extracted from pharmaceutical tablets

A total of 41 batches of methylamphetamine hydrochloride were synthesized using *pseudo*ephedrine hydrochloride extracted from pharmaceutical tablets and iodine and red phosphorous extracted from tinctures and matchboxes respectively. The $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ data are presented for these samples in Figure 7.12 to Figure 7.15.

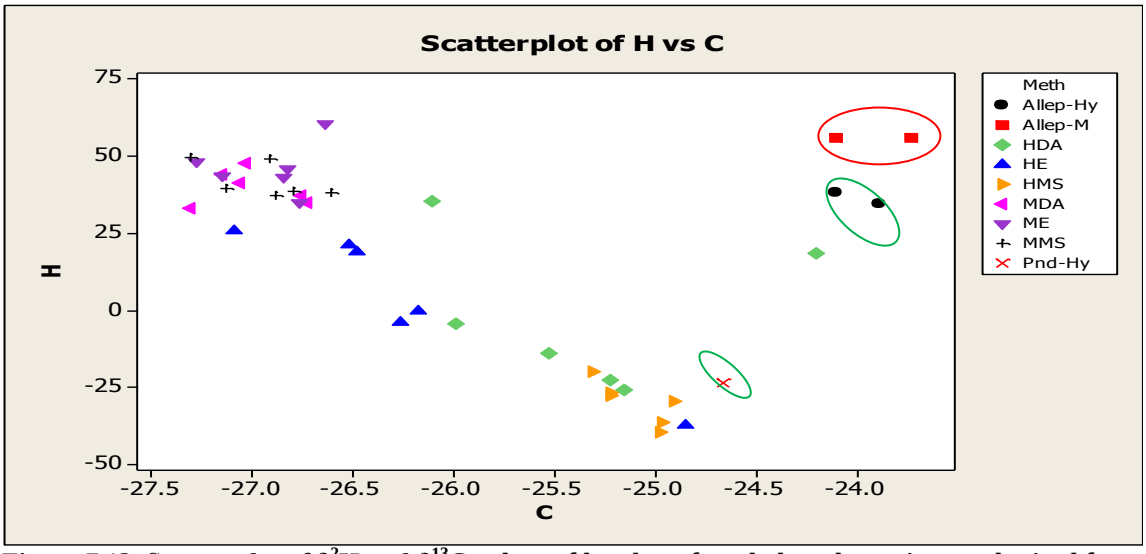

Figure 7.12. Scatter plot of $\delta^{2}H$ and $\delta^{13}C$ values of batches of methylamphetamine synthesized from the Hypo and Moscow routes using *pseudo*ephedrine extracted from proprietary cold medication.

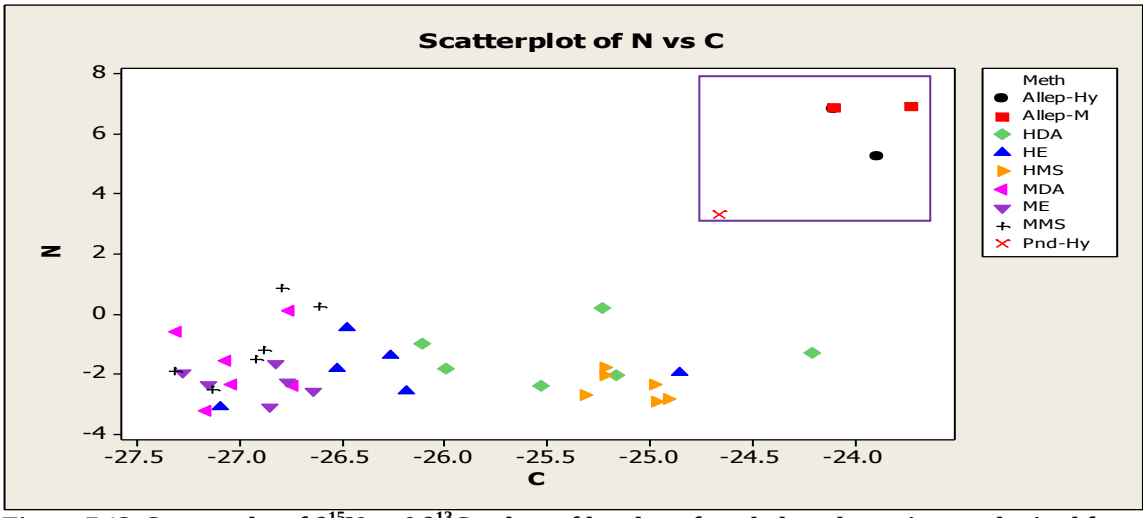

Figure 7.13. Scatter plot of $\delta^{15}N$ and $\delta^{13}C$ values of batches of methylamphetamine synthesized from the hypo and moscow routes using *pseudo*ephedrine extracted from proprietary cold medication.

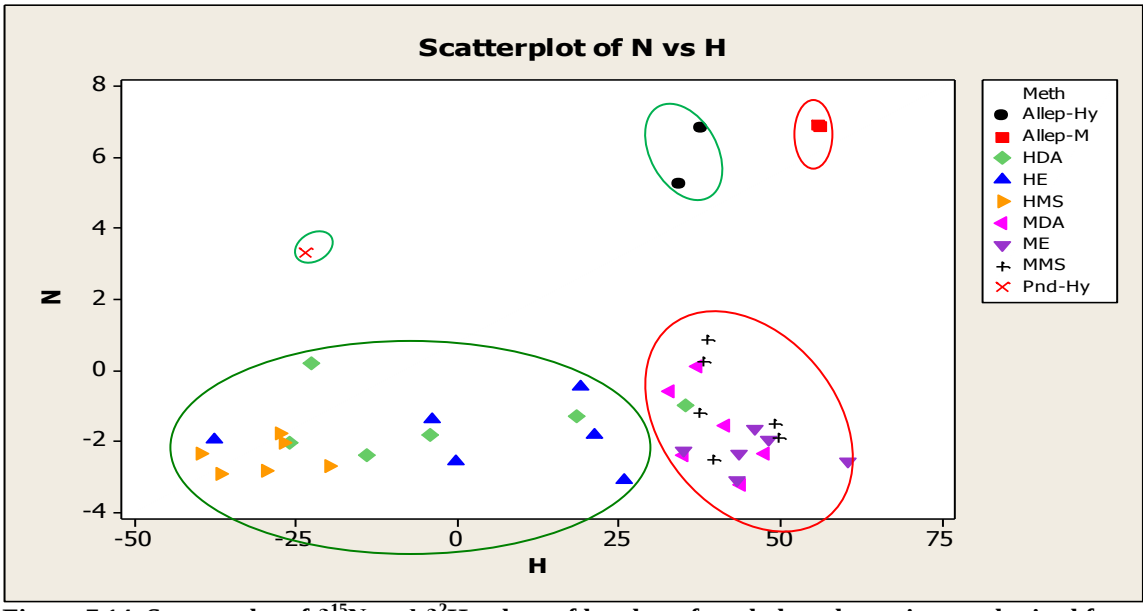

Figure 7.14. Scatter plot of $\delta^{15}N$ and $\delta^{2}H$ values of batches of methylamphetamine synthesized from the hypo and Moscow routes using *pseudo*ephedrine extracted from proprietary cold medication.

Convolution of the samples occurs except when hydrogen or carbon and nitrogen isotopes are plotted against each other (with the exception of one or two samples). In this case the samples synthesised from the Malaysian sourced precursor are clearly separated both from each other by tablet source and also by synthetic route. Separation

occurs both on the nitrogen isotopic values as well as the hydrogen isotopic values. Furthermore, the Sudafed precursor samples are separated from the Malaysian precursor samples specifically by the nitrogen isotopic values with the latter having a more negative value. The samples are also separated according to synthetic route where the Moscow samples have generally more positive hydrogen and negative carbon isotopic values. This suggests that the nitrogen isotopes are presenting a geographical specificity, (given the nitrogen atom in the product comes directly from the precursor) and route specificity presented by the carbon or hydrogen isotope variations. The source specificity for the laboratory precursor samples appears to be more related to differences in the carbon isotope values rather than those of the nitrogen isotopes.

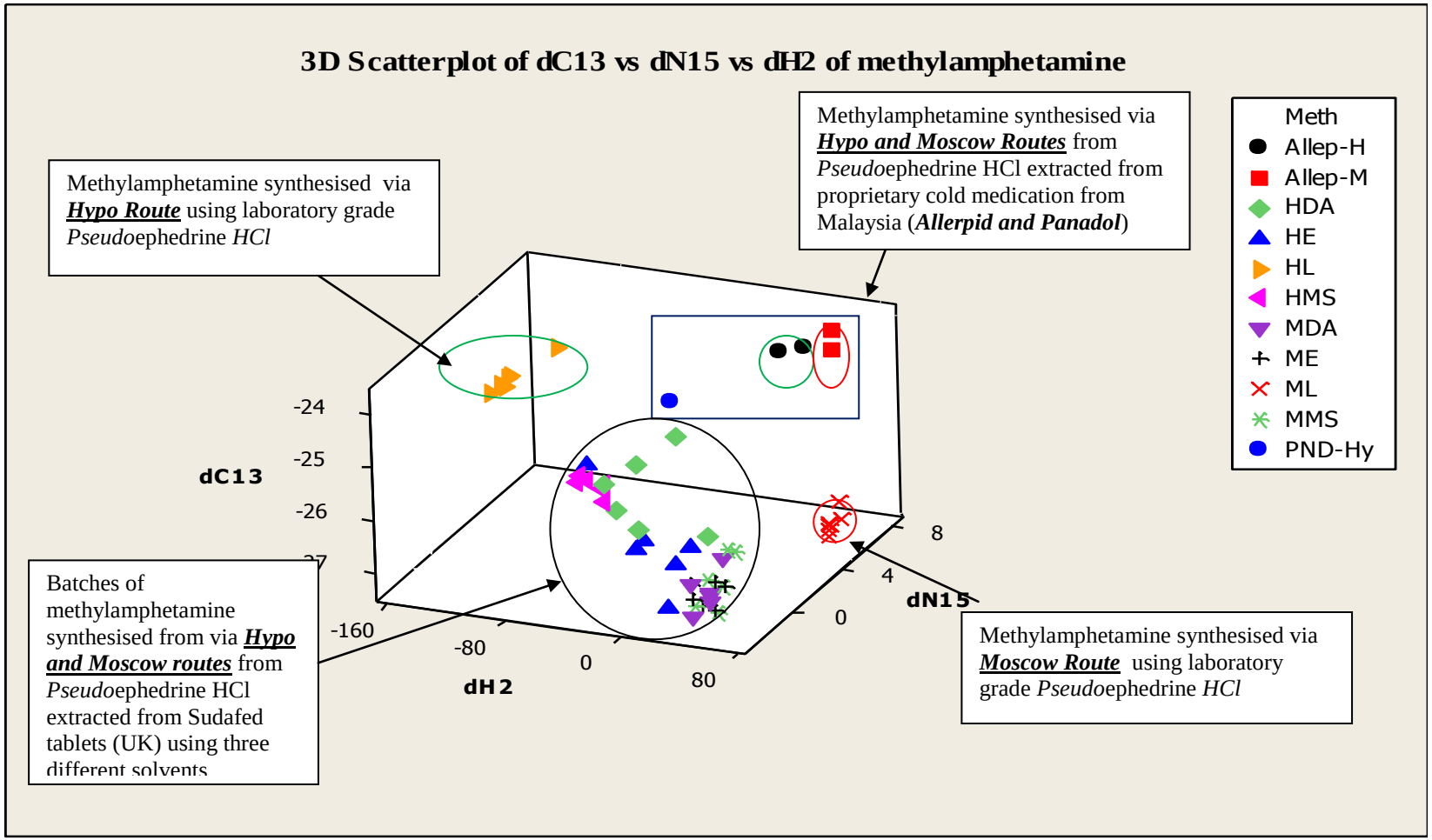

Figure 7.15. 3D scatter plot of $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ for methylamphetamine synthesized from the from the hypo and Moscow routes using laboratory grade *pseudo*ephedrine.

Carbon results

10 of the 11 carbon atoms on the final methylamphetamine molecule are contributed by the *pseudo*ephedrine starting material [1, 2]. Small variations were observed within the carbon values and presented in Figure 7.16 indicate that while fractionation occurs within the synthetic process the reproducibility by the same chemist using the same method and materials is relatively good **[**10]. Within route variation was more obvious for the Hypo synthesised samples and this route was hardest to replicate as the temperature of the exothermic reaction was difficult to control during the preparation of hydroiodic acid when iodine was added into the hypophoshorus acid. The Moscow prepared samples involved a more controlled temperature reaction where the precursor, essential chemicals and water are added together in flask and refluxed. Such differences in preparative method have been confirmed as potential causes of carbon isotopic variation by Carter *et a*l. [10, 11] and Buchanan *et al*. [12]. It can be observed that the Moscow samples have more negative carbon isotope values in all cases.

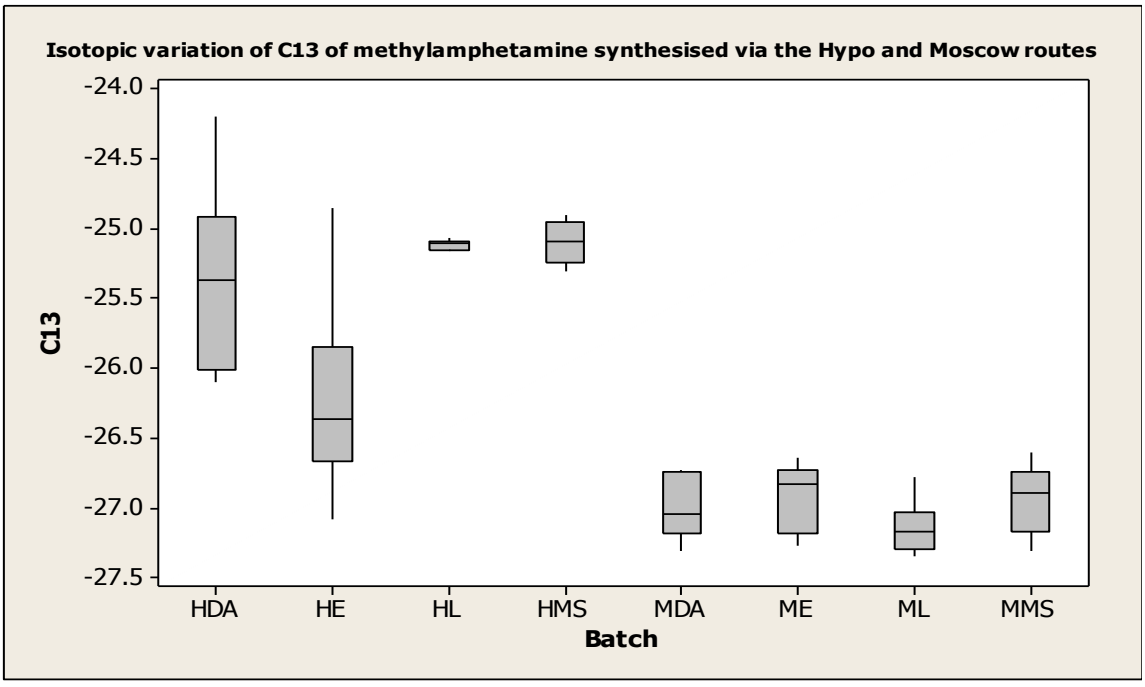

Figure 7.16. Isotopic variation of δ13C of batches of methylamphetamine synthesised via the Hypo and Moscow routes.

Nitrogen results

Variations were observed in $\delta^{15}N$ values across all of the samples and when compared with the nitrogen values of the individual precursor chemicals some general trends are observed and presented in Figure 7.17. The nitrogen atom on the methylamphetamine hydrochloride molecule is contributed exclusively by the precursor molecule [1, 2]. For the laboratory grade precursors, positive values for the nitrogen isotopic values in the precursor chemicals are reflected in the methylamine products for both synthetic routes. Negative isotopic values between -2.88 and -3.00 were obtained . All other Sudafed derived samples produced negative nitrogen isotopic values while the methylamphetamine prepared from medication sourced from Malaysia produced positive nitrogen isotope values.

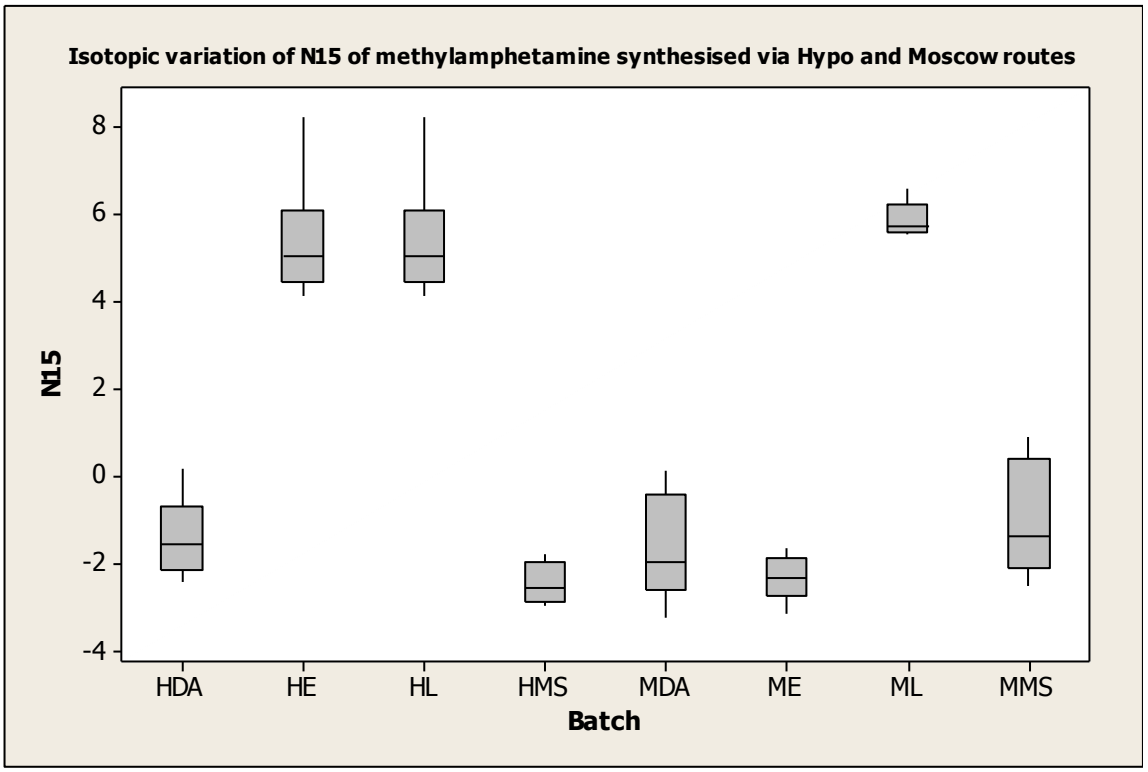

Figure 7.17. Isotopic variation of δ15N of batches of methylamphetamine synthesised via the Hypo and Moscow routes.

This variation observed is most likely due to isotopic fractionation and kinetic isotope effects during the reaction phase of methylamphetamine [9, 10, 11]. The isotopic fractionation that occurs during the synthetic process was also reported in the studies conducted by Buchanan *et al.* [12] and by Carter *et al*. [10] who suggested that during the synthesis of methylamphetamine, seemingly innocuous differences in the synthetic method could result in different $\delta^{15}N$ values of the target compound. The large variability of the $\delta^{15}N$ between the precursors and product was greater than observed by studies conducted by Collins *et al*. [13] and Kurashima *et al*. [2]. This variability may be due to fractionation that occurs during the precipitation of the product as the HCl salt [9, 13, 14].

Hydrogen results

The $\delta^{2}H$ data values of methylamphetamine synthesized for the Hypo and Moscow routes using the *pseudo*ephedrine extracted from Sudafed batches followed the same trend observed with the samples prepared using the laboratory precursor. This re-emphasises that the variations observed between routes are potentially due to the synthetic phase of the reaction and the nature of different essential chemicals used in the individual routes. This is best explained by the catalytic cycle shown in Figure 7.18 and Figure 7.19 [15].

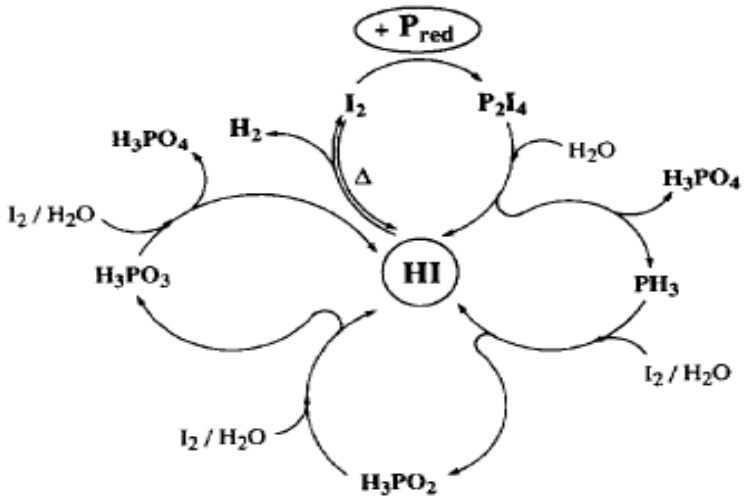

Figure 7.18. Red phosphorous involvement in a catalytic cycle for generation of hydroiodic acid in anhydrous media for the Moscow route [15].

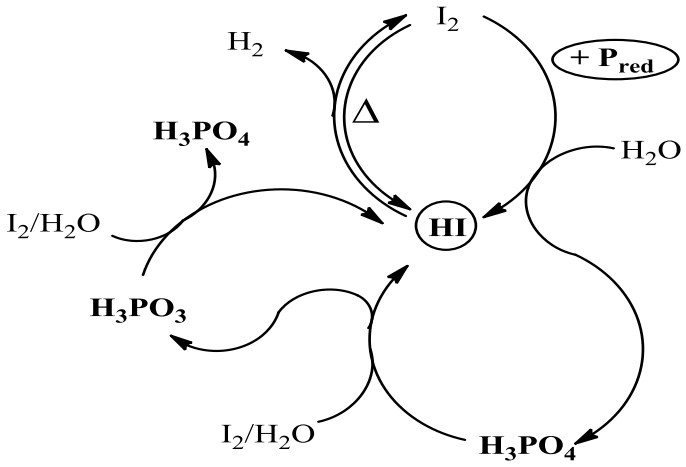

Figure 7.19. Hypophosphorous acid involvement in a catalytic cycle for generation of hydroiodic acid in aqueous media for the Hypophoshorous route [15].

The generation of hydroiodic acid in the Moscow route occurs *in-situ* in the reaction process where higher temperatures are needed when compared to the Hypo route [15, 16] where involvement of hypophosphorous acid in the catalytic cycle for the generation of hydroiodic acid in aqueous media is involved.

A contributing factor for hydrogen isotopic variation is the anhydrous nature of the catalytic cycle in the generation hydroiodic acid *in situ* during the reaction of the Moscow route and aqueous nature of hydroiodic acid generation of the Hypo route. Mass differences arising from the different nature of the respective catalytic routes can give rise to fractionation during the synthetic phase whereby a bond containing the atom or isotope is broken or formed in the rate determining step of the reaction process [17]. Molecules consisting of lighter isotopes moving from the liquid to the vapour phase faster than molecules consisting of heavier isotopes [17]. As more energy is needed to break bonds in anhydrous media compared to aqueous media a difference in the isotopic values between routes is expected.

The formation of the final hydrochloride salt may also have some influence on the final isotopic values for hydrogen [9, 13, 14, 18-21]. During salt formation the nitrogen gains a proton to become positively charged. The deuterium species forms a stronger bond to the nitrogen than hydrogen [9, 13, 14, 18-21]. This proton gain is in rapid equilibrium and the formation of deuterium-nitrogen species will be present for a longer time in a solution than the hydrogen-nitrogen species [9, 13, 14, 18, 19]. The deuterium-nitrogen species thus, have a greater chance of associating with the chloride ions to form the methylamphetamine salt and precipitate from the solution [9, 13, 14, 18, 19].

Figure 7.20 illustrates the isotopic variation observed for hydrogen between the batches of methylamphetamine synthesised via the Hypo and Moscow routes. It can be observed that the Moscow samples have more positive hydrogen isotope values in all cases.

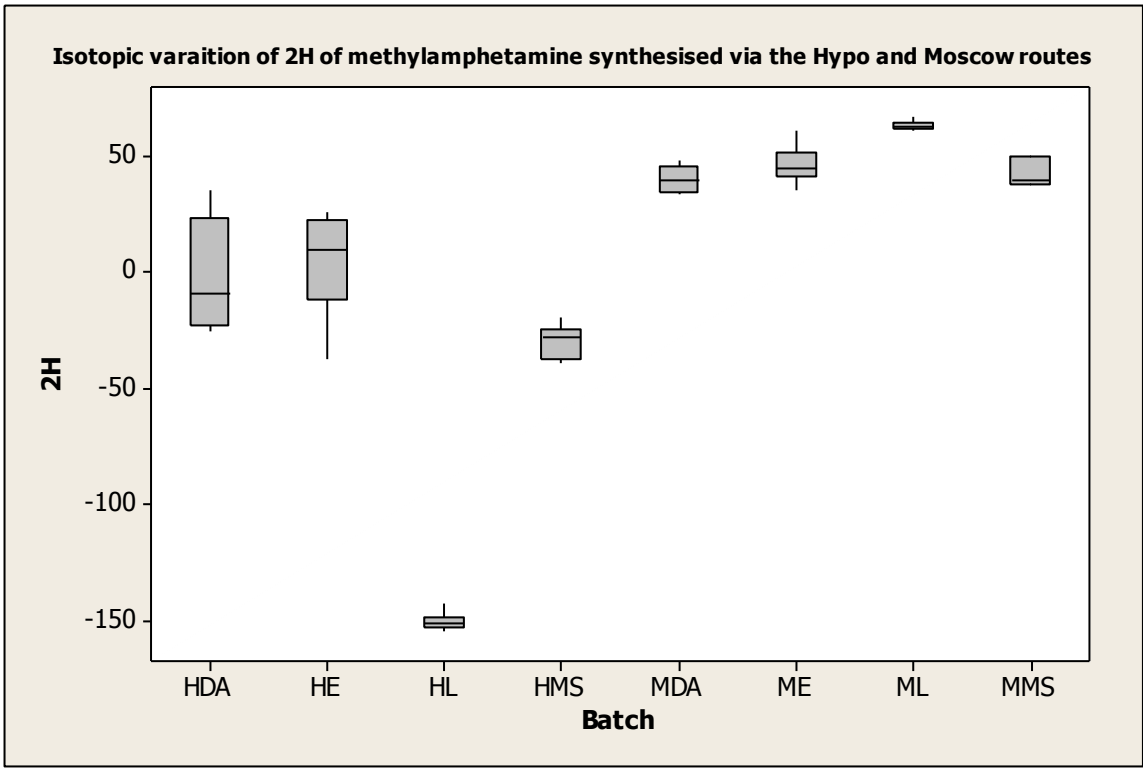

Figure 7.20. Isotopic variation δ2H of batches of methylamphetamine synthesised via the Hypo and Moscow routes.

7.4 Conclusion

53 batches of methylamphetamine synthesized from the two routes using the *pseudo*ephedrine extracted from laboratory grade and cold medication sourced from different regions were analysed to present a comprehensive data set of clandestine synthesised samples. The combination of $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ data facilitated the discrimination of the samples into four major groups according to routes and precursor origin. This is in agreement with studies conducted by Collins *et al*.[13] where $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ values provided a viable means of distinguishing batches of methylamphetamine synthesised from different routes to precursors from different origins. Samples synthesized from laboratory grade *pseudo*ephedrine via the Moscow and Hypo routes could be clearly resolved from each other and all other samples specifically by hydrogen isotopic values. The methylamphetamine synthesized from precursor extracted from Sudafed tablets using the three solvent systems forms one elongated cluster with two further groups consisting of methylamphetamine synthesized from precursor extracted from Panadol and Allerpid tablets.

Sample discrimination according to geographical region was best illustrated using nitrogen isotopic values particularly when plotted against hydrogen values. Carbon and hydrogen isotopic values were more successful at discriminating samples by synthetic route and clear distinctions could be made. IRMS was not capable of discriminating methylamphetamine samples according to the extraction solvent used to prepare the precursor chemical eventhough this was possible for the precursors themselves.

7.5 References

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Chapter 8 Chemometric analysis

8.1 Introduction

PCA and HCA are the two most conventional pattern recognition techniques applied in the analysis of data of forensic science interest whilst SOFM, which is based on an artificial neural network (ANN) learning scheme is relatively new in its application to forensic science problems [1, 2, 3]. SOFM has, however been successfully applied in the data derived from the analysis of wax based products, ink dyes and ignitable liquids for articulating and displaying elusive clusters within datasets where PCA and HCA had failed to do so [1, 2, 3].

This part of the research provides insight to the application of unsupervised pattern recognition techniques for data derived from the analysis of the various methylamphetamine samples prepared. Given the way in which the synthesized samples were produced, discrimination could occur on a number of levels, (a) by starting material (laboratory grade materials, Sudafed decongestant tablets, Allerpid or Panadol tablets) (b) by extracting solvent/method (c) by synthetic route or (d) by a combination of starting material, extraction solvent and synthetic route (referred to as 'lab output'). The data generated from each of the analytical techniques, were subjected to unsupervised pattern recognition analysis using hierarchical cluster analysis (HCA), principal component analysis (PCA) and self organising feature maps (SOFM). This facilitated the identification of clusters that could be used to objectively interpret the respective data sets. The data derived from the three analytical techniques were either analysed on their own or combined with one another. The GCMS data was also pre-processing where the peak areas of the target impurities were normalised to either the internal standard or the sum of the total peak area [4]. The square, fourth and sixteenth root data pre-treatment methods were also investigated using HCA, PCA and SOFM analysis. The summary of the datasets used in the multivariate and SOFM analysis are presented in Table 8.1 and Table 8.2.

Table 8.1. List of datasets used in multivariate and chemometric analysis (HCA, PCA and SOFM).

No	Sample class	Data description	Data pre-processing
1	ML GCMS data (methylamphetamine synthesised via Moscow route using laboratory grade materials	Target impurities identified in this study with acceptable RSD values	Raw data
			Normalised to total peak area (TPA), square root
			Normalised to total peak area (TPA), fourth root
			Normalised to total peak area (TPA), sixteenth root
2	ME GCMS data (methylamphetamine synthesised via Moscow route using precursors extracted from proprietary cold med using ethanol as the extraction solvent)	Target impurities identified in this study with acceptable RSD values	Raw data
			Normalised to total peak area (TPA), square root
			Normalised to total peak area (TPA), fourth root
			Normalised to total peak area (TPA), sixteenth root
3	MDA GCMS data (methylamphetamine synthesised via Moscow route using precursors extracted from proprietary cold med using ethanol/methanol (10:90 % vol/vol) as the extraction solvent)	Target impurities identified in this study with acceptable RSD values	Raw data
			Normalised to total peak area (TPA), square root
			Normalised to total peak area (TPA), fourth root
			Normalised to total peak area (TPA), sixteenth root
4	MMS GCMS data (methylamphetamine synthesised via Moscow route using precursors extracted from proprietary cold med using commercial methylated spirit as the extraction solvent)	Target impurities identified in this study with acceptable RSD values	Raw data
			Normalised to total peak area (TPA), square root
			Normalised to total peak area (TPA), fourth root
			Normalised to total peak area (TPA), sixteenth root
5	HL GCMS data (methylamphetamine synthesised via Hypo route using laboratory grade materials	Target impurities identified in this study with acceptable RSD values	Raw data
			Normalised to total peak area (TPA), square root
			Normalised to total peak area (TPA), fourth root
			Normalised to total peak area (TPA), sixteenth root
6	HE GCMS data (methylamphetamine synthesised via Hypo route using precursors extracted from proprietary cold med using ethanol as the extraction solvent)	Target impurities identified in this study with acceptable RSD values	Raw data
			Normalised to total peak area (TPA), square root
			Normalised to total peak area (TPA), fourth root
			Normalised to total peak area (TPA), sixteenth root

Table 8.2. List of datasets used in multivariate and chemometric analysis (HCA, PCA and SOFM) continued.

No	Sample class	Data description	Data pre-processing
7	HDA GCMS data (methylamphetamine synthesised via Hypo route using precursors extracted from proprietary cold med using ethanol/methanol (10:90 % vol/vol) as the extraction solvent)	Target impurities identified in this study with acceptable RSD values	Raw data
			Normalised to total peak area (TPA), square root
			Normalised to total peak area (TPA), fourth root
			Normalised to total peak area (TPA), sixteenth root
8	HMS GCMS data (methylamphetamine synthesised via Hypo route using precursors extracted from proprietary cold med using commercial methylated spirit as the extraction solvent)	Target impurities identified in this study with acceptable RSD values	Raw data
			Normalised to total peak area (TPA), square root
			Normalised to total peak area (TPA), fourth root
			Normalised to total peak area (TPA), sixteenth root
9	Isotopic values of batches of methylamphetamine via Moscow and Hypo routes using laboratory grade precursors and precursors extracted from proprietary cold medication using IRMS analysis	δC^{13}, δN^{15}, δH^{2} isotopic values	Raw data
10	Elemental values of batches of methylamphetamine via Moscow and Hypo routes using laboratory grade precursors and precursors extracted from proprietary cold medication	Elemental concentration in ppm and ppb	Raw data
11	Combination of GCMS, IRMS and ICPMS analysis batches of methylamphetamine via Moscow and Hypo routes using laboratory grade precursors and precursors extracted from proprietary cold medication	Target impurities in this study with acceptable RSD values, isotopic and elemental values	Raw data for the ICPMS and IRMS. Normalised to total peak area (TPA) and treated with fourth root for GCMS data for HCA and PCA analysis Raw data for the ICPMS and IRMS. Normalised to total peak area (TPA) and treated with sixteenth root for GCMS data for SOFM analysis

8.2 Hierarchical Cluster Analysis (HCA)

Hierarchical Cluster Analysis (HCA) is an unsupervised pattern recognition technique in which a set of data is clustered to produce a dendogram, or a graphical representation of the similarities between the elements in the data set [5, 6]. Dendograms are similar to tree diagrams in that individual elements or clusters are linked to other elements and clusters until finally, all the elements are linked together [5, 6]. Dendograms can be either agglomerative or divisive [5, 6, 7]. The agglomerative method is more popular and involves examination of the data points in a sequential way until the entire data set is linked together. The divisive method begins with one large group and breaks this down until only clusters consisting of one element remain [5, 6, 7]. A number of methods also exist for determining how clusters should be combined, such as single, complete, group-average, centroid, median and Ward clustering [5, 6, 7]. Two common linkages are the nearest neighbour (single linkage) and furthest neighbour (complete linkage) methods [5, 6, 7]. For the nearest neighbour linkage (simplest procedure) the distance between the two clusters is defined as the smallest distance between the two elements, one from each cluster, shown in figure 8.1 [5, 6, 7]. The disadvantage of this particular linkage is 'space contracting' or 'chaining' [5, 6, 7], which in turn will result in poorly separated clusters [5, 6, 7].

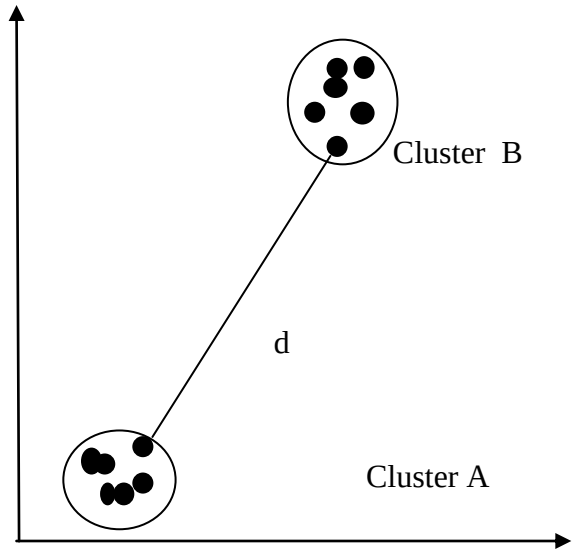

Figure 8.1. Distance, d, between clusters A and B as defined by the nearest neighbour method [5,6].

As implied by the name, the furthest neighbour clustering method is the opposite of nearest neighbour. With this method, the distance between the two clusters is defined by the greatest distance between two objects, one from each cluster [5, 6,]. Figure 8.2 illustrates this concept. The furthest neighbour method can have result in 'space dilating' creating many small clusters and is the opposite of space contracting effect [5, 6].

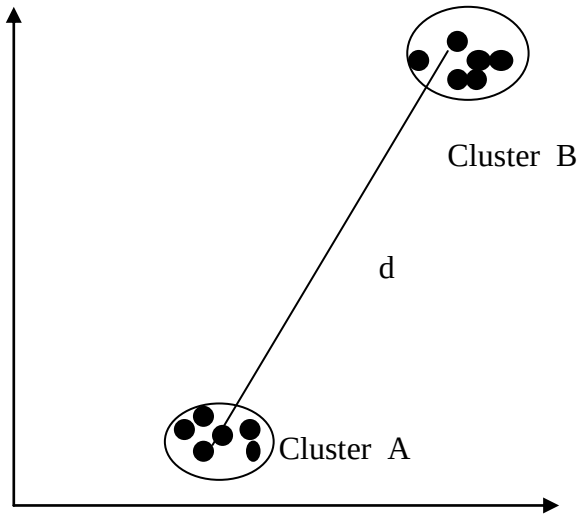

Figure 8.2. Distance, d, between clusters A and B as defined by the furthest neighbour method [5,6].

A method of measuring the distance between clusters must also be selected for hierarchical cluster analysis. Two options to measure the interval are a simple Euclidean distance, in which the straight line distance between the two points is calculated and the squared Euclidean distance, which is the same distance but squared[5,6,7]. Equation 8.1 presents the relationship used for the Euclidean.

$$Euclidean\ distance\ (d) = [\Sigma(X_{ik} - X_{jk})^2]^{1/2} \qquad \textbf{Equation 8.1 [7]}$$

8.2.1 HCA experimental

HCA was performed using Minitab (version 15) on the data sets mentioned previously in table 8.1. Euclidean distance measurements using single, average and complete linkage strategies were used to reveal the best clustering regime which in each case was using complete linkage with Euclidian distance as the measurement.

8.2.2 HCA results and discussion

8.2.2.1 GCMS data

The dendrogram generated from HCA analysis of the raw GCMS data of the target impurities is presented in Figure 8.3 and illustrate some correct linkages for batches of samples (HMS, MMS and HDA) but many misclassifications.

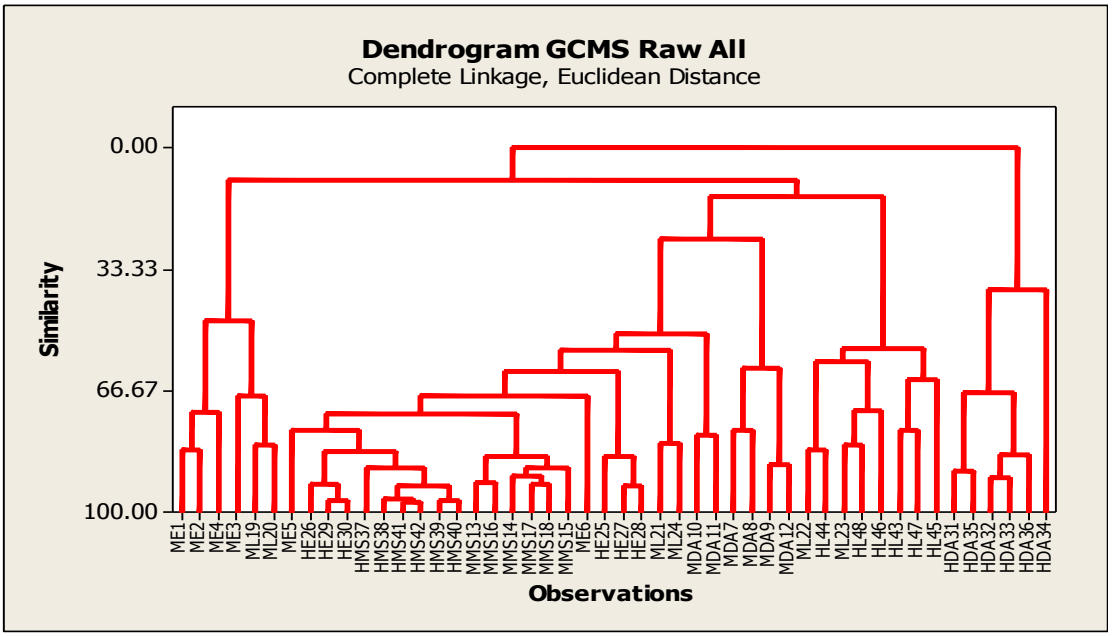

Figure 8.3. Hierarchical clustering of chromatographic profiles of batches of methylamphetamine synthesised via the Hypo and Moscow routes using raw GCMS data. The sample codes provided in Table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

Dendrograms derived from row scaled GCMS data (normalised to total peak area) and pre processed using the square root, fourth root and sixteenth root power transformations of the target impurities identified are presented in Figure 8.4, Figure 8.5 and Figure 8.6 respectively. HCA progressively improved in its ability to classify the batches of samples together depending on extraction solvent and all batches were successfully grouped with fourth and sixteenth root pre processing. However the samples could not be successfully grouped by synthetic route (that is Moscow synthesised samples were grouped more readily with Hypo synthesised samples in some cases) irrespective of the level of pre processing.

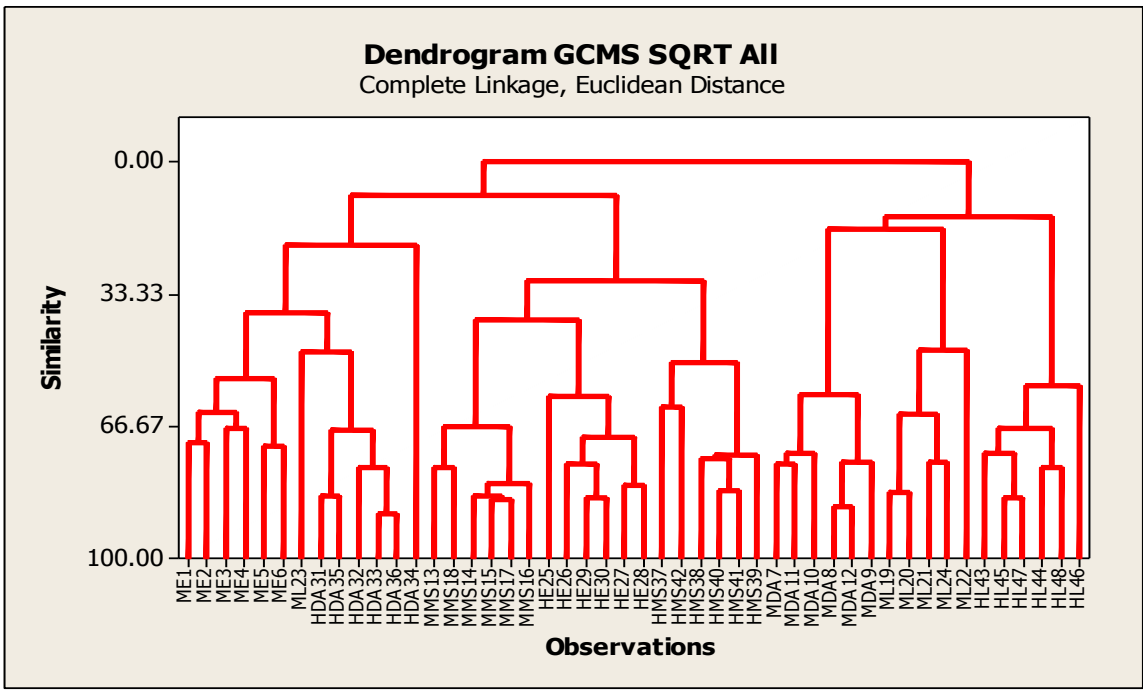

Figure 8.4. Hierarchical clustering of chromatographic profiles of batches of methylamphetamine synthesised via the Hypo and Moscow routes using row scaled GCMS data pre processed using the square root. The sample codes provided in Table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

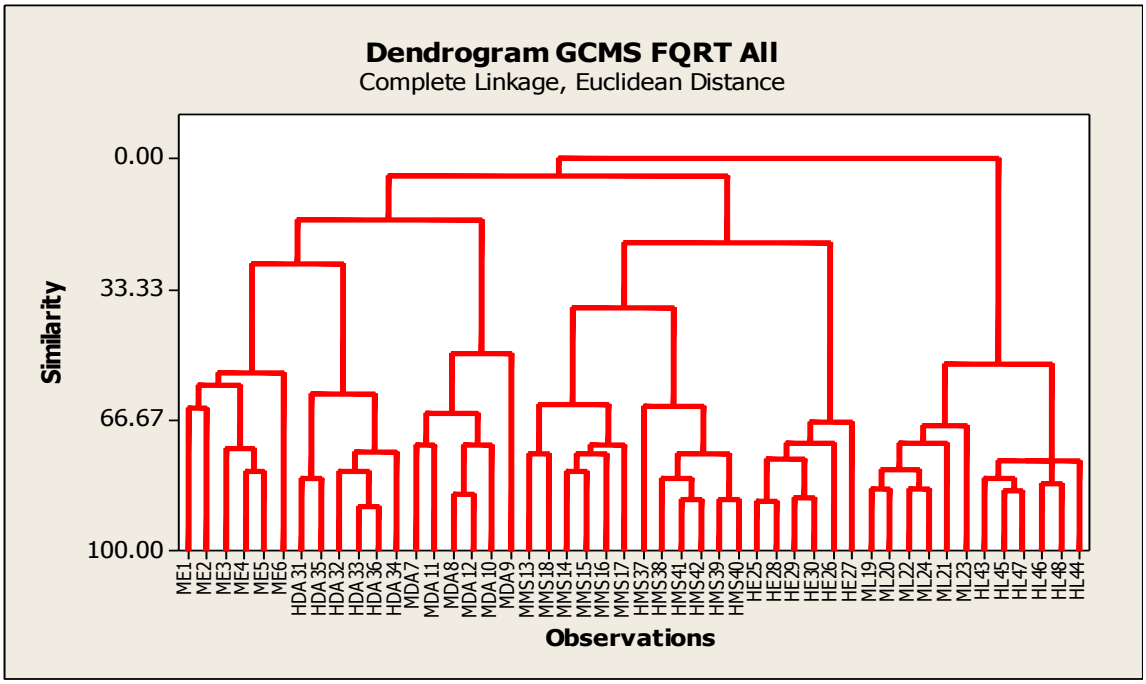

Figure 8.5. Hierarchical clustering of chromatographic profiles of batches of methylamphetamine synthesised via the Hypo and Moscow routes using row scaled GCMS data pre processed using the fourth root. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number. All samples were correctly grouped depending on extracting solvent.

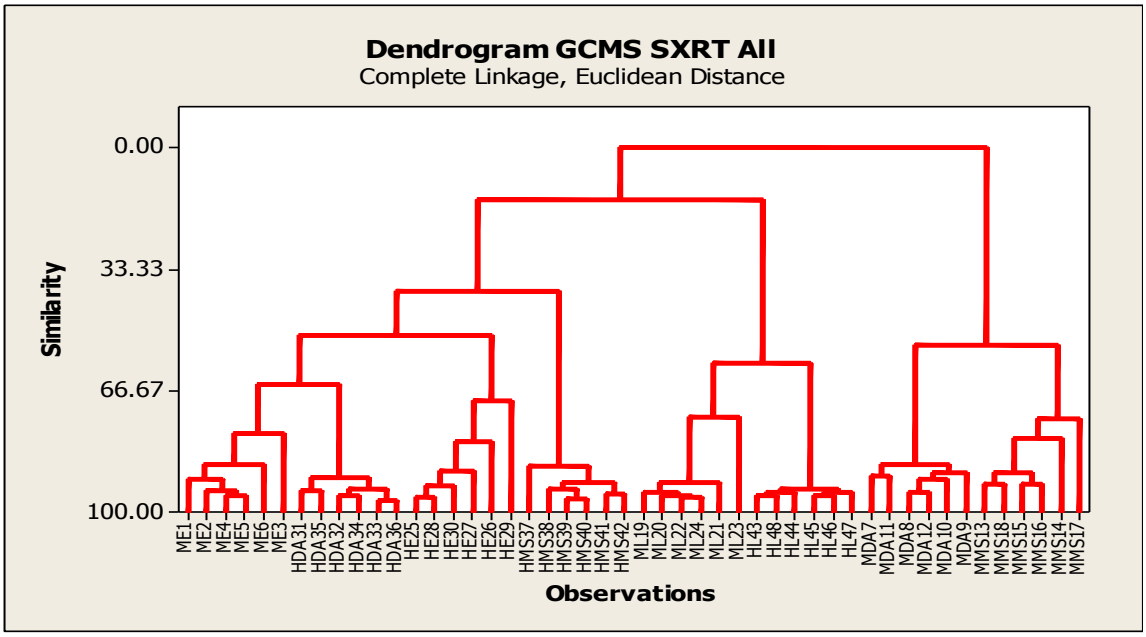

Figure 8.6. Hierarchical clustering of chromatographic profiles of batches of methylamphetamine synthesised via the Hypo and Moscow routes using row scaled GCMS data pre processed using the sixteenth root. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number. All samples were correctly grouped depending on extracting solvent.

The fourth and sixteenth root pre processed data provided discrimination between the methylamphetamine prepared from the laboratory grade chemicals (however both synthetic routes were linked together) and the extracted precursor products.

8.2.2.2 IRMS data

IRMS data for the six repetitive samples of each synthetic batch were averaged and subjected to HCA. The resultant dendrogram is presented in Figure 8.7.

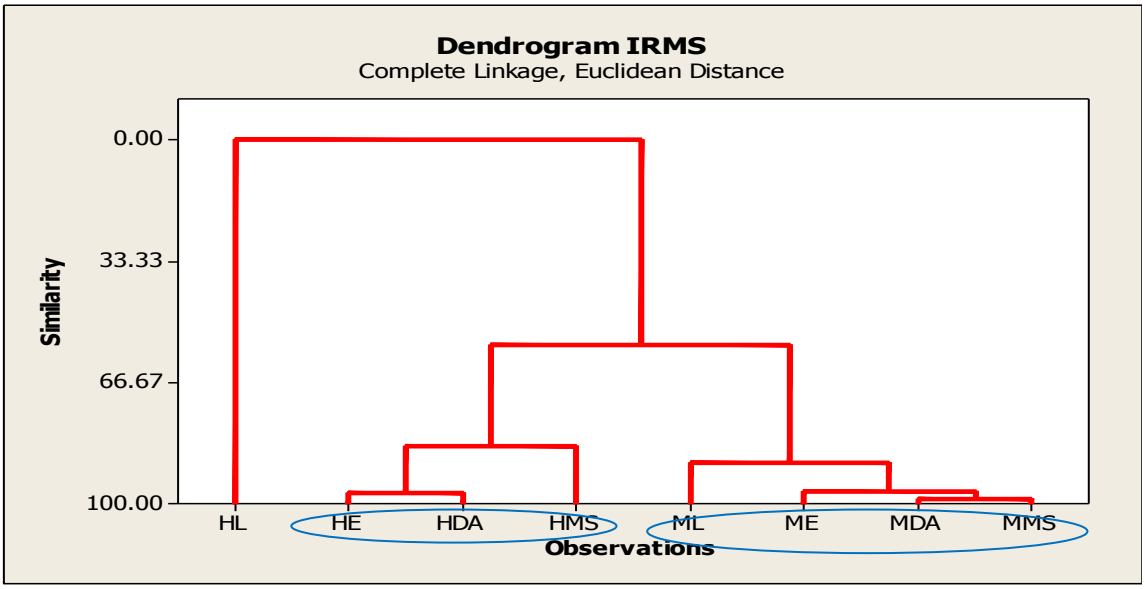

Figure 8.7. Hierarchical clustering of batches of methylamphetamine synthesised via the Hypo and Moscow routes using C, N and H isotopic ratios. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

In each case the samples were readily separated by the solvent used to extract the precursor chemical. All of the Moscow samples were linked together irrespective of precursor, while the Hypo samples prepared from the laboratory precursor were split away from all other samples.

8.2.2.3 ICPMS analysis

The ICPMS data for the six repetitive samples of each synthetic batch were averaged and subjected to HCA and the resultant dendrogram is presented in Figure 8.8.

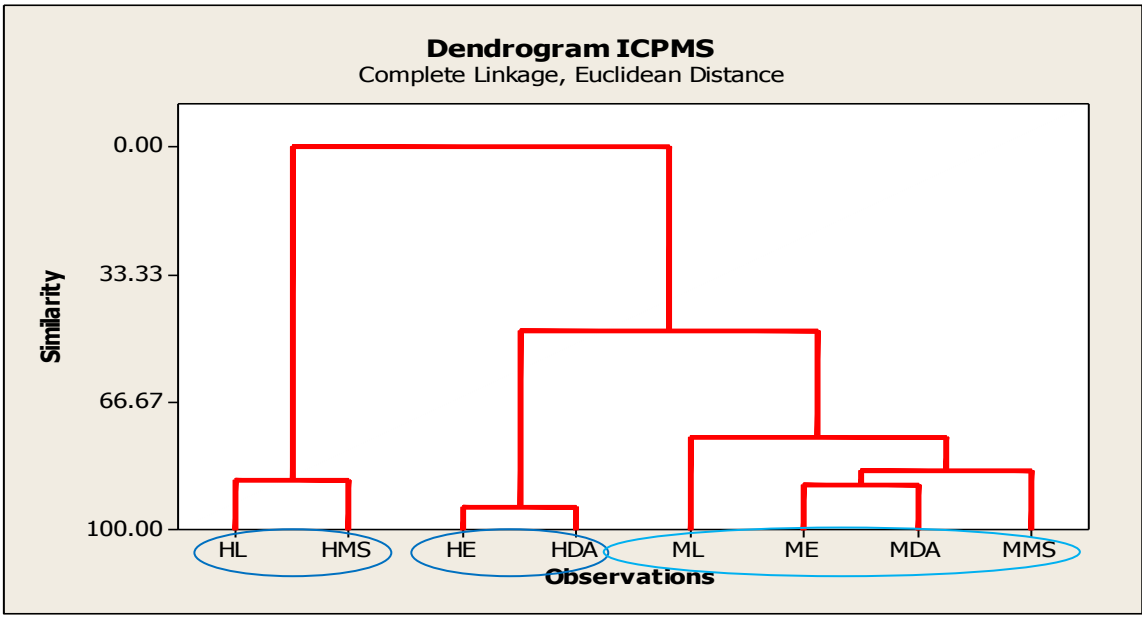

Figure 8.8. Hierarchical clustering of batches of methylamphetamine synthesised via the Hypo and Moscow routes using ICPMS data. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

As with the IRMS data, the samples were readily separated by the solvent used to extract the precursor chemical. The laboratory prepared samples were successfully separated from each other by route. All of the Moscow samples were linked together by route whilst the Hypo samples were split into two separate groups where similarities were suggested between the samples prepared from the laboratory precursor and the precursor extracted by commercial methylated spirits.

8.2.2.4 Combination of IRMS and ICPMS data

A combination ICPMS and IRMS datasets produced a dendrogram shown in Figure 8.9 which was essentially the same as that for ICPMS suggesting that the data derived from this technique dominated and little added value was obtained from combining the IRMS and ICPMS results.

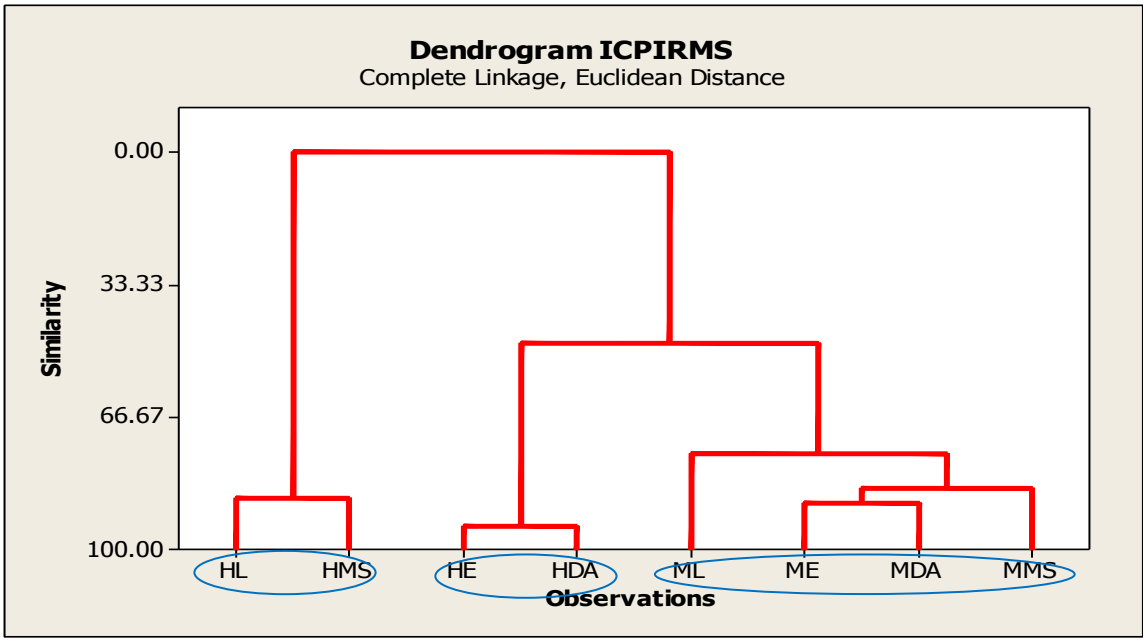

Figure 8.9. Hierarchical clustering of batches of methylamphetamine synthesised via the Hypo and Moscow routes using ICPMS and IRMS data. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.2.2.5 Combination of GCMS, ICPMS and IRMS data

A combination of each of the best performing data sets (GCMS using row scaled fourth root pre processed data, ICPMS and IRMS) produced a dendrogram presented in Figure 8.10 which demonstrated discrimination of all samples by precursor but did not provide any significant route discrimination.

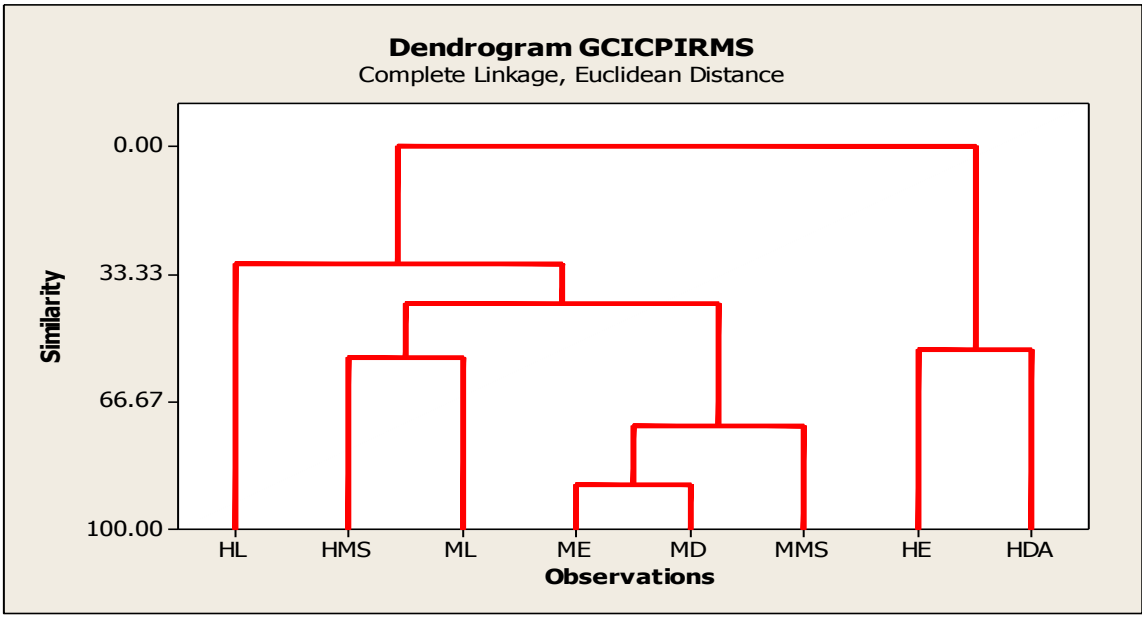

Figure 8.10. Hierarchical clustering of batches of methylamphetamine synthesised via the Hypo and Moscow routes using the combined GCMS (row scaled and fourth root pre processed) ICPMS and IRMS data. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.2.3 HCA conclusions

HCA analysis of the various batches of methylamphetamine samples proved successful in discriminating the samples by precursor when using any of the normalised and pre treated GCMS data (above the square root) or either the IRMS and ICPMS data sets alone or in combination. No complete route discrimination was possible using any of the data sets although the ICPMS and IRMS did provide some linkages between Moscow prepared samples.

8.3 Principal Component Analysis (PCA)

Principal component analysis (PCA), changes the dimensionality of data by the combination, or transformation, of the original variables into new variables, each of which accounts for more variance than the corresponding original variables [8, 9, 10]. PCA seeks to find linear combinations of the original variables, each weighted appropriately, such that the greatest degree of variance is explained by the fewest number of new variables, or principal components (PCs) [8, 9, 10]. Once the first PC is found, a second linear combination is sought to explain the remaining variance. Each principal component is constructed so that it is orthogonal (or uncorrelated) with others. When multivariate data sets contain more than two or three variables, graphical representation of the data becomes complicated, thus rendering the identification of similarities and differences between samples difficult. If two or three PCs can be identified which account for the majority of the variation in the data set, then graphical representation is simplified and it may be possible to visually identify clusters among the samples [11, 12, 13]. The first PC is defined by equation 8.2:

$$PC1 = \alpha_{11}X_1 + \alpha_{12}X_2 + \ldots + \alpha_{1p}X_p \qquad \textbf{Equation 8.2}$$

Where:

α_{1X} represents the weights or loadings for each of the original variables (X_1 to X_p) in PC1.

Large absolute values of these loadings indicate a strong contribution of the corresponding original variable, and loadings near zero indicate a weak contribution [11, 12]. PC's may be created until 100% of the variance in the data is explained however, it is common to select a subset of the PC's such that the dimensionality of the original data set is reduced while very little information is lost overall [11, 12, 13]. Normal practise dictates accepting those PC's which cumulatively account for 80-90% for the overall variance of the data [11, 12, 13]. The PC 'scores' are the elements of the new variables (PC1, PC2, etc) which are derived from the loadings and the original

variables and represent the projection of the original data points onto the new axes [11, 12, 13].

The interpretability of PC's can be improved through rotation. Rotation maximizes the loading of each variable on one of the extracted PC whilst minimizing the loading on all other PC's and works through changing the absolute values of the variables whilst keeping their differential values constant [11, 12, 13]. There are two methods of rotation, orthogonal and oblique rotation. Orthogonal rotation assumes that the factors are at right angles to each other usually described as uncorrelated [12, 13]. For example, if factor loadings are plotted on a two dimensional axes, the variables that load on one factor would lie along one axis, the variables that load on the other factor would lie along the other [12, 13]. Oblique rotations relaxes the assumption that factors must be orthogonal. In this method one set of variables may lie along the axis while the other may lie along a 45 degree angle to the axes [12, 13].

8.3.1 PCA experimental

PCA was performed using Minitab software (version 15) on the data sets described at the beginning of the chapter. The first two principal components (PCs) of each analysis were extracted and plotted to assess which data sets, if any, afforded any discrimination of the samples. The data sets which facilitated clustering of the samples using a plot of the first two PCs were than examined further to identity the variables which loaded most highly on PC1 and PC2. The variable loadings are a measure of the correlation between the variable and the principal component, and strength of this relationship is indicated by the magnitude of the loading (either positive or negative), where -1 indicates maximum negative correlation and + 1 indicates maximum positive correlation.

8.3.2 PCA results and discussion

8.3.2.1 GCMS data

PCA is highly susceptible to variations in the magnitude of variables and the data generally needs to be pre processed in order that the PC's provide meaningful data. As such it was unsurprising that convoluted data clusters were obtained when the raw GCMS data was examined and these results are presented in Figure 8.11. Notwithstanding this, clustering of the Moscow synthesised methylamphetamine from *pseudo*ephedrine extracted from Sudafed using ethanol:methanol (90:10 % vol/vol) and the Hypo and Moscow samples produced from *pseudo*ephedrine extracted from Sudafed using commercial methylated spirits was observed. Furthermore, the data appears to be conditioned on PC1according to the methanol content of the extracting solvent with non methanol containing solvent having negative PC1 values and methanol containing solvents having progressively more positive PC1 values.

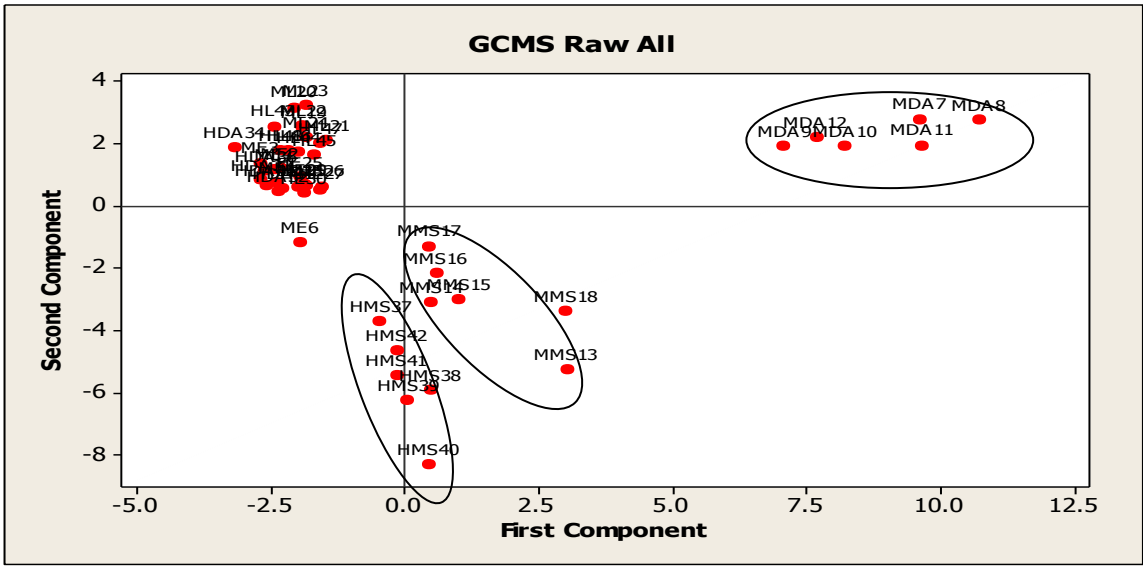

Figure 8.11. Score plots of chromatographic profiles of batches of methylamphetamine synthesised via the Hypo and Moscow routes. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

The score plots for the principal components generated from the dataset for the row scaled pre processed data are presented in Figure 8.12 (square root transformation), Figure 8.13 (fourth root transformation) and Figure 8.14 (sixteenth root transformation). While each dataset provides some deconvolution of the samples, in no case are all of the samples discriminated according to either their precursors (laboratory grade or extracting solvent) or synthetic route. The same general trend was noted with the samples which involved precursor extraction with methanol containing solvents tending to be discriminated better by PC1, this is particularly the case for the fourth and sixteenth root transformed data.

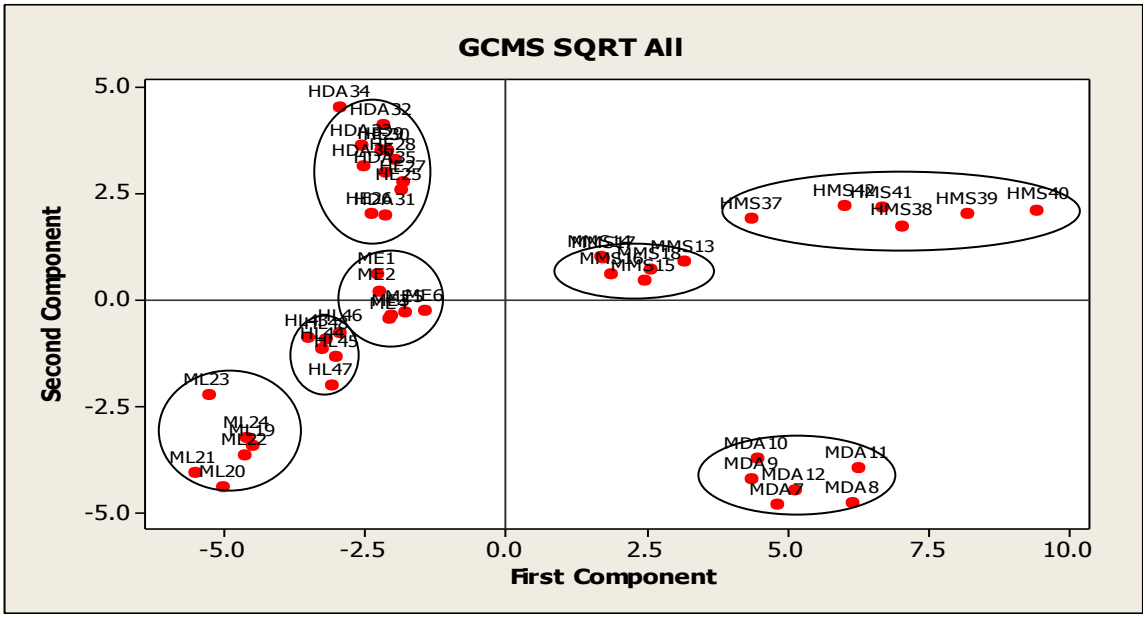

Figure 8.12. Score plots of chromatographic profiles of batches of methylamphetamine synthesised via the Hypo and Moscow routes using row scaled GCMS data pre processed using the square root. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

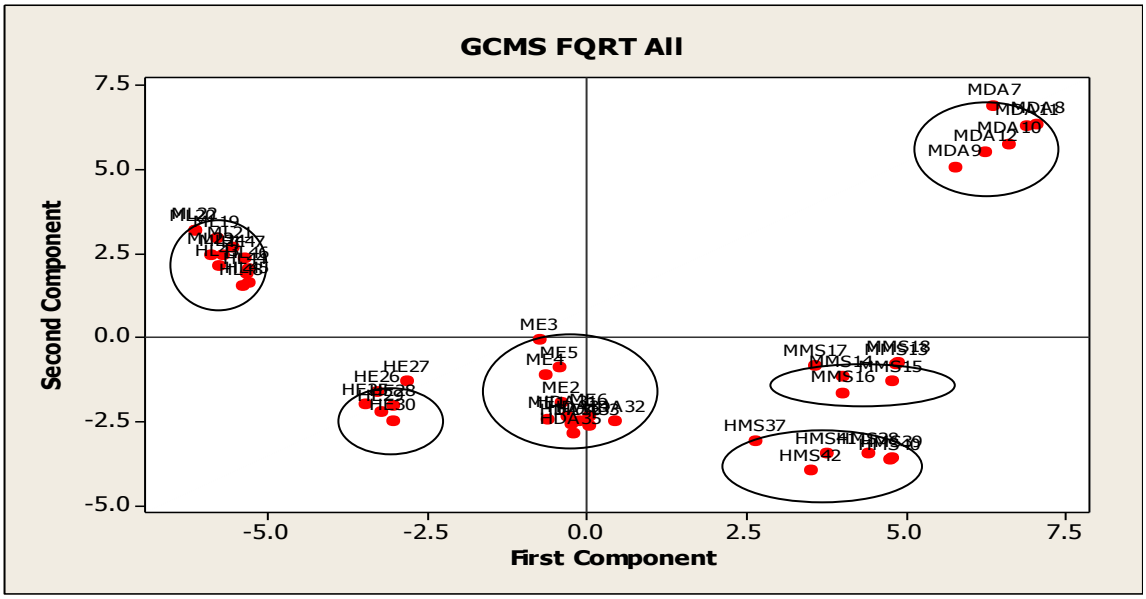

Figure 8.13. Score plots of chromatographic profiles of batches of methylamphetamine synthesised via the Hypo and Moscow routes using row scaled GCMS data pre processed using the fourth root. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

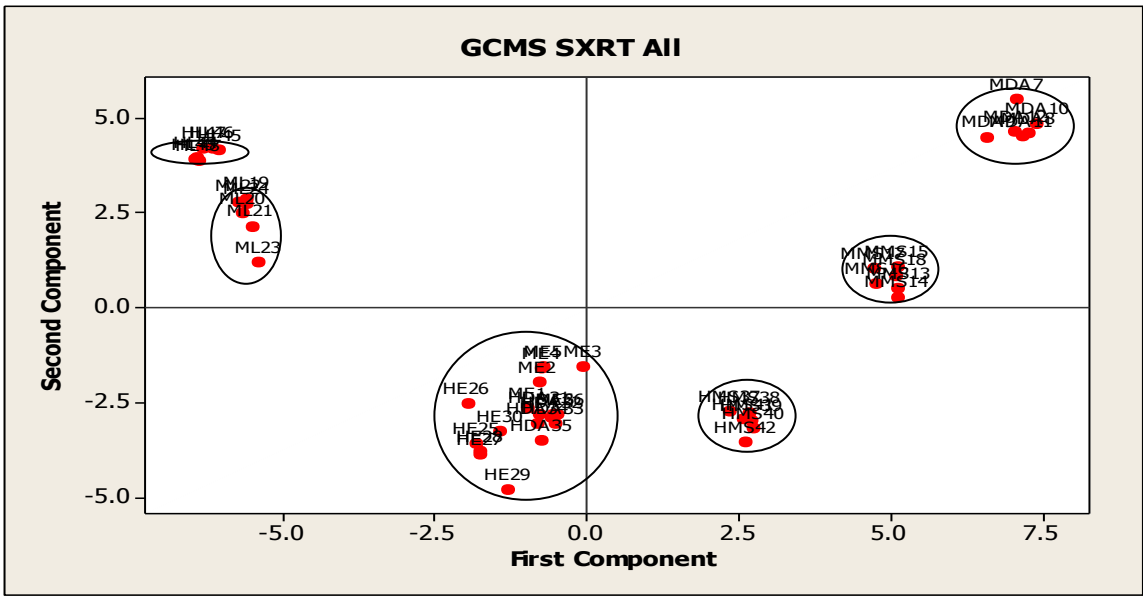

Figure 8.14. Score plots of chromatographic profiles of batches of methylamphetamine synthesised via the Hypo and Moscow routes using row scaled GCMS data pre processed using the sixteenth root. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.3.2.2 IRMS data

The PCA score plot shown in Figure 8.16 clearly illustrates that the batches of samples are discriminated by both precursor and synthetic route.

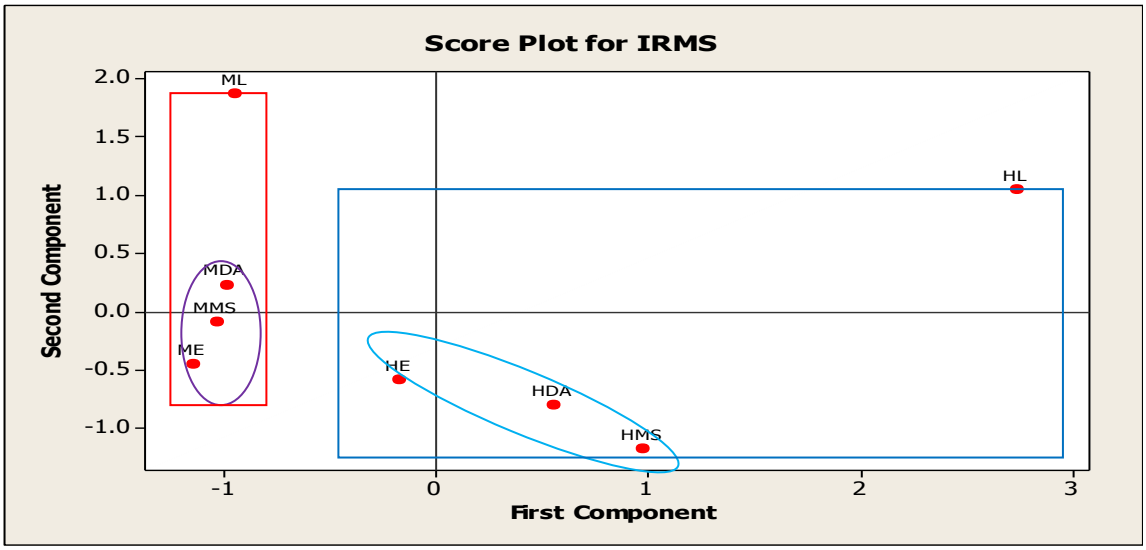

Figure 8.15. Score plot of batches of methylamphetamine synthesised via the Hypo and Moscow routes using average C, N and H isotopic ratios. The sample codes provided in Table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

The products of the laboratory grade precursor have been influenced by both PC 1 and PC2 providing a very clear separation between the two synthetic methods. The extracted precursor samples demonstrate a more negative influence on PC 2 in comparison to the laboratory grade precursors. Furthermore, all of the Hypo synthesised samples are more heavily influenced by PC1 which has spread these samples to towards more positive values of this PC. This provides a distinct delineation between the two synthetic routes and provides a full 'lab output' discrimination of the samples.

8.3.2.3 ICPMS data

Similar to the IRMS data analysis, the PCA score plot shown in Figure 8.16 for the elemental analysis by ICPMS presented as average results for each precursor type has separated all of the samples by precursor. However the same trends are not observed in relation to route specificity and while all of the Moscow synthesised samples have more positive values of PC2 this is also the case for the lab precursor Hypo synthesised samples thus affording no overall route specificity using the ICPMS data.

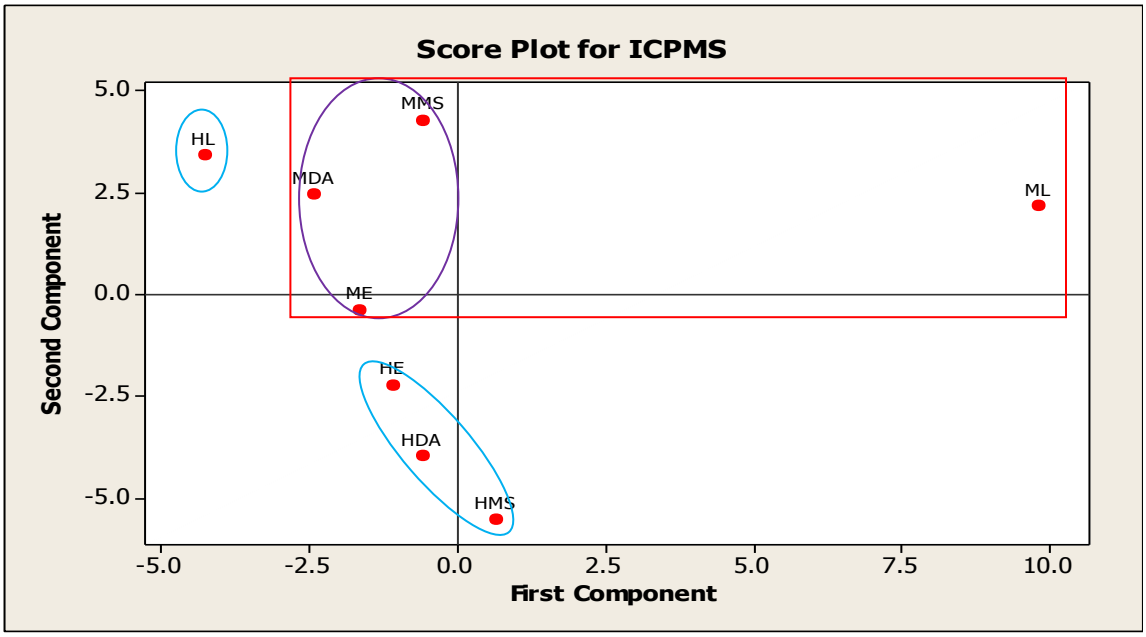

Figure 8.16. Score plot of batches of methylamphetamine synthesised via the Hypo and Moscow routes using average elemental profiles. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.3.2.4 Combination of IRMS and ICPMS data

The PCA score plot of the combined ICPMS and IRMS is shown in Figure 8.17. Little difference is seen from the result presented by ICPMS data alone, again corroborating the dominance of this technique over IRMS that was observed in HCA.

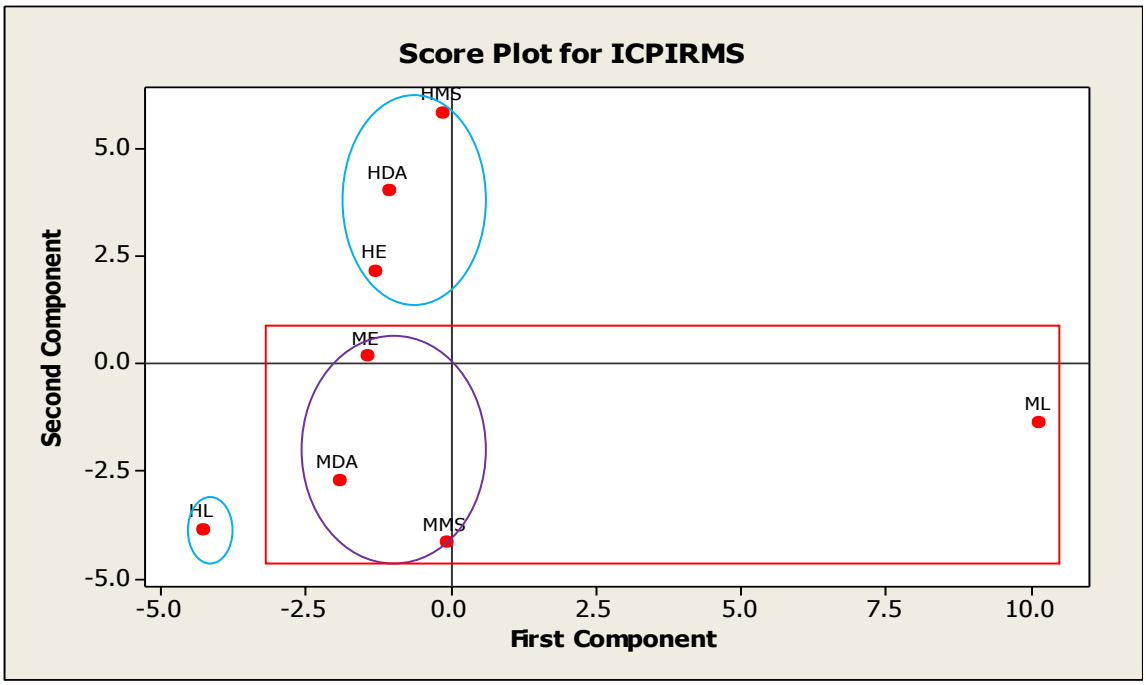

Figure 8.17. Score plot of batches of methylamphetamine synthesised via the Hypo and Moscow routes using average elemental and isotopic profiles. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.3.2.5 Combination of GCMS, IRMS and ICPMS data.

When the results of the row scaled and pre processed with fourth root transformation GCMS data, IRMS and ICPMS data are combined all of the Hypo synthesised samples show strong positive correlations with both PC 1 and 2 providing clear discrimination of this group. The Moscow samples prepared using the precursor extracted from Sudafed are less influenced by PC 1 and group together while the laboratory grade precursor is

clearly more heavily influenced by PC1 and is convincingly separated from the other Moscow synthesised samples.

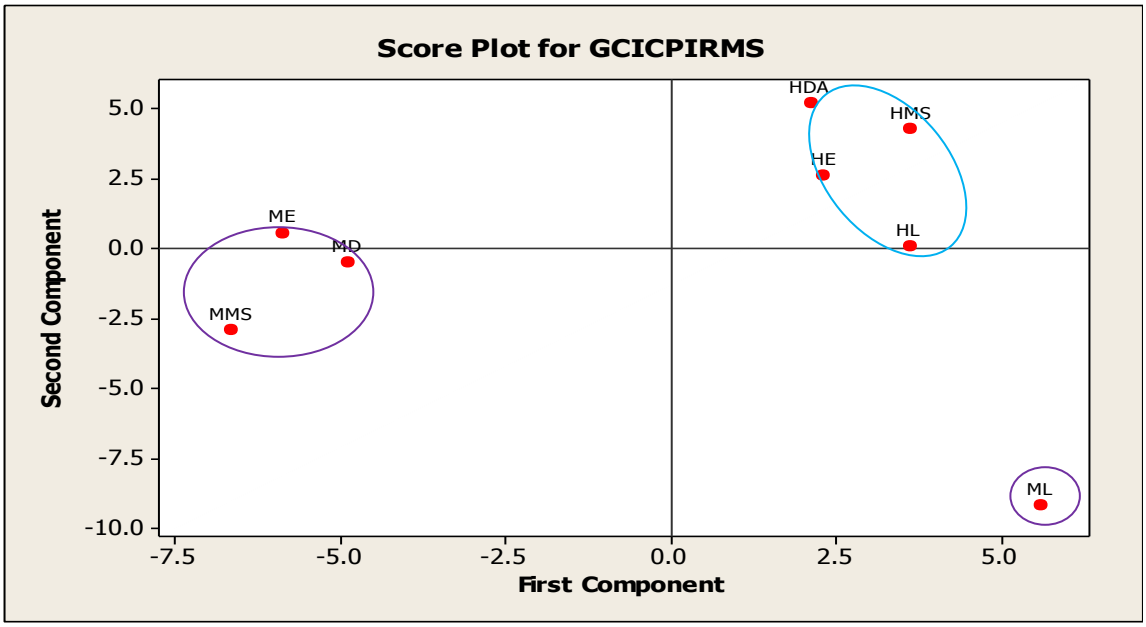

Figure 8.18. Score plots of batches of methylamphetamine synthesised via the Hypo and Moscow routes using the combined GCMS (row scaled and pre processed with fourth root transformation) ICPMS and IRMS data. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.3.3 PCA conclusion

Only PCA analysis performed on IRMS data provided complete discrimination of the samples based on both the precursor (including the extraction method) and synthetic route. All other data produced either convoluted clusters of did not provide clear route discrimination based on the PC's presented.

8.4 Artificial Neural Networks

Artificial Neural Networks (ANN) is a powerful technique that mimics the basic function of the human brain [14, 15, 16, 17, 18]. In their early stages, ANN's were developed thirty years ago using electrical circuits however, with the emergence of computer technology, suitable functions and codes using computer programming software such as Visual Basic, C++ and Pascal were developed [14]. ANN's are used widely in scientific disciplines such as engineering, business, ecology and medicine although their application to forensic science has been much more recent [14].

There are two types of learning schemes are available for ANN's classified as supervised and unsupervised learning mechanisms. In supervised learning, both the input and output signals are required in the learning process and the output signals act as target outcomes. With unsupervised learning schemes only input signals are required in the learning process [14]. An example of an ANN based on a supervised learning scheme is the Multi Layer Perceptron (MLP) or back propagation network, and an example of ANN based on an unsupervised learning scheme is the Self Organising Feature Maps (SOFM) or Kohonen network [14].

8.5 Self Organising Feature Maps (SOFM)

The Self Organising Feature Maps (SOFM) neural network was first introduced by Kohonen in 1982 [14, 18, 19, 20, 21] and represents perhaps the most popular unsupervised pattern recognition ANN [14-20, 22-24] SOFM does not require a prior knowledge of the output patterns and uses only the input patterns (vectors) to devise variable (or class) membership [14]. In doing so, two basic assumptions are made, firstly the membership of each variable is defined by the user and shares common features and secondly, the network will be able to identify these common features across the range of the input vectors [14, 15].

8.5.1 SOFM Algorithm

SOFM is arranged in a two-layer format consisting of an input and an output layer. The input layer represents the 'input neurons' which are the variables from the dataset. The output layer is described as a two-dimensional platform equipped with a number of map units (neurons or pixels) for the input patterns to be mapped onto. The typical architecture of SOFM is shown in the Figure 8.19 [14, 25, 26].

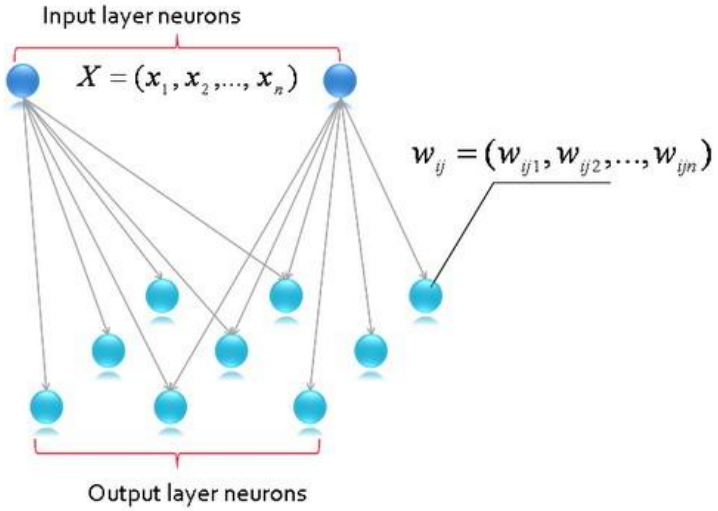

Figure 8.19. Two layer structure of SOFM neural network. Each input variable is connected to all neurons on the platform. Adapted from [26].

The mapping process starts with initialisation of the model where each unit or node on the output layer is given a random weighting. The next stage is the training or the learning process. At this stage, input neurons are introduced to the network iteratively so that all the neurons will be stimulated by the input vectors until the best matching unit (BMU) is identified. The BMU is chosen based on the similarity of the output neurons to the input neurons whereby similarities between the two are measured using Euclidean distances [14, 20, 25, 26].

The weights of the BMU's are corrected so that they become closer to the input neurons in the next iteration of the algorithm. The neighbouring neurons of the BMU undergo weight adjustment in proportion to their distance to the BMU such that the further they

are, the less the adjustment is made. As the iterative process continues, the SOFM organises into a state whereby similar input neurons are mapped onto similar neurons on the output layer [14, 20, 25, 26]. When the process ends, the output neuron is labelled according to the input or object that has been mapped onto it to reveal if clustering has emerged from the training [14, 25, 26]. The SOFM matrix is trained to correctly classify the members of the chosen training set. Once trained, the ability of the specific SOFM algorithm to correctly classify novel samples can be revealed and validated by using a test set of known data [14, 20, 26].

8.5.2 SOFM Visualisation

The output layer or output map is a powerful means of visualising complex multidimensional data using spatial arrangements and colour coding that presents general clustering inherent within the dataset [20, 26]. Apart from the output map, there are other multiple visualisation methods offered depending on the information acquired, for example, various types of distance matrix maps, and two and three dimensional projections of hit histograms [20, 26]. Multiple visualisation techniques allow the analyst to view the results from a variety of perspectives increasing the interpretative value of the data [20, 26]. Individual component maps (associated with each input variable) can be particularly useful as they facilitate the examination of the characteristics of the clusters and explore the association between the variables within the dataset [20, 26].

8.5.3 Limitations of SOFM

SOFM is proposed as a mathematical technique which can complement HCA and PCA. Despite its proven performance, the method is computationally complex and remains to be universally accepted in the interpretation of forensic science data [25, 26]. A major obstacle in the application of SOFM, is that it requires extra computation time as the network learns and becomes optimised. Parameters such as the numbers of nodes and the numbers of iterations in the learning process are carried out largely by trial and error

[26]. In some cases PCA and HCA can be used to condition data sets providing a reduced variable set for subsequent SOFM analysis.

8.5.4 SOFM experimental

SOFM artificial neural network analysis was performed using Viscovery^{R}SOMine (version 5.0.2, Viscovery Software GmbH). Dataset learning was carried out using the optimum specification set by the software whereby the number of iterations (epochs) in each case was 40 with 2000 maps units (neurons).

In order to test both the predictive nature of the SOFM approach as well as provide reassurance that unknown samples would indeed cluster within their source group, cross validation was performed where the overall dataset were split into two; a training and a test set. The training set was used to model the data while the test set was used to test the quality and predictive ability of the model. SOFM analysis was applied to the datasets of the three different analytical techniques presented earlier in the chapter.

8.5.5 SOFM Results and Discussion

8.5.5.1 GCMS Data

The SOFM output maps of raw and row scaled pre processed GCMS data (using the square, fourth and sixteenth root transformations respectively) are presented in Figure 8.20.

Analysis of the raw GCMS data resulted in some misclassification. All samples were correctly classified by precursor for all row scaled and power transformed data however none of the GCMS datasets correctly grouped all samples correctly by synthetic route. The sixteenth root power transformation provided the best classification grouping together all of the Moscow synthesised samples but the Hypo synthesised samples remained split with the laboratory precursor samples being removed from the remaining Hypo samples.

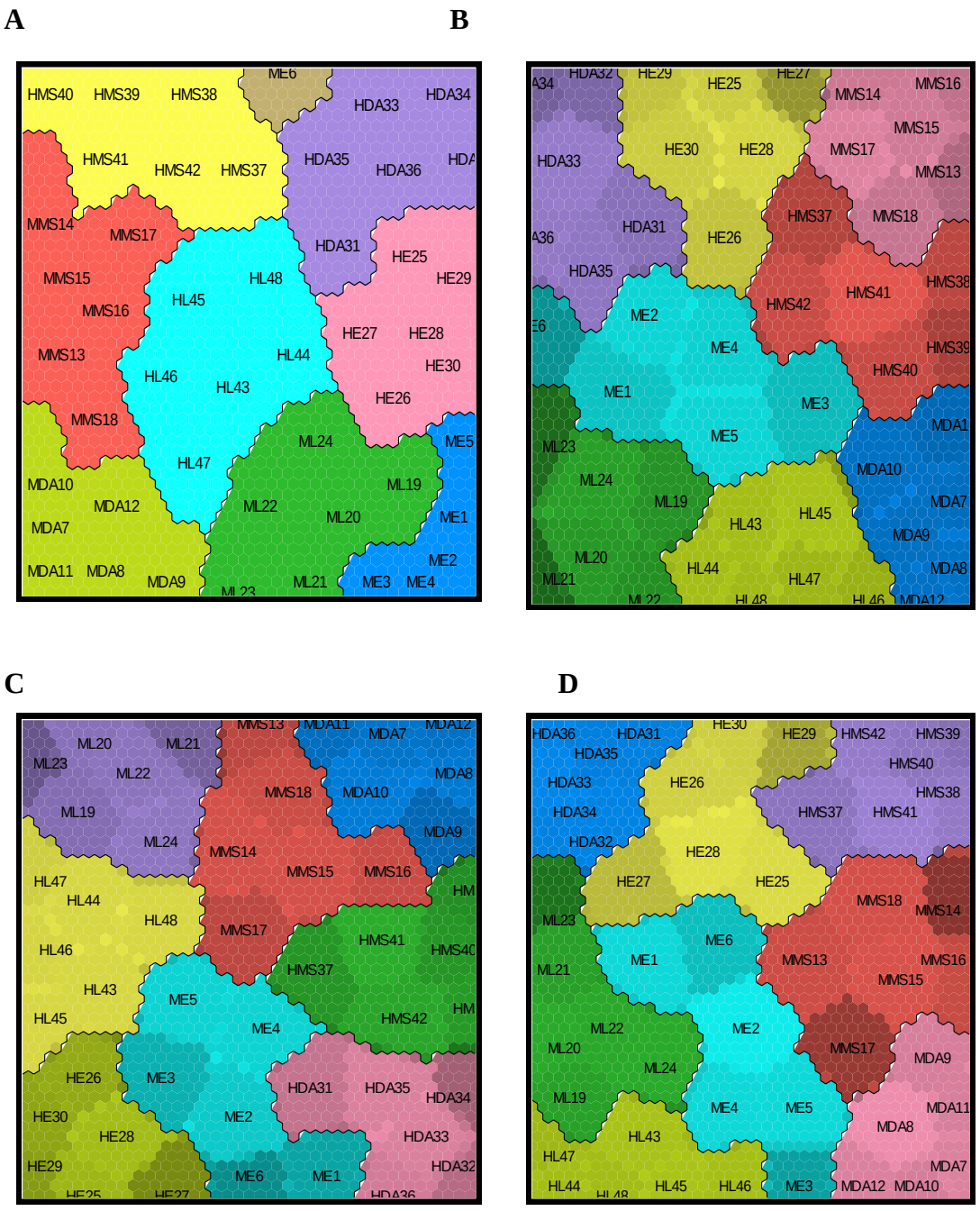

Figure 8.20. SOFM output maps of the GCMS data. A,B,C and D represent the maps of, raw data row scaled and pre processed using the square root transformation, row scaled and pre processed using the fourth root transformation, row scaled and pre processed using the sixteenth root transformation. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.5.5.2 IRMS data

The SOFM output for the average values for the isotopic ratios of all the samples are presented in Figure 8.21 and illustrate a clear and distinctive grouping of the batches of methylamphetamine synthesised from the two routes using the precursors from the various sources. Furthermore, the samples were discriminated by synthetic route with the Hypo samples being placed predominantly to the left of the SOFM output map and the Moscow samples on the right hand side of the map. The samples prepared from laboratory grade precursors were placed side by side indicating a similarity between these products and a distinction from the other samples while at the same time maintaining their synthetic route distinction. The results also suggest that, even though there have been some substantial isotopic changes during the precursor extraction and synthetic process, the samples synthesised from the same route share sufficient features to facilitate being clustered together.

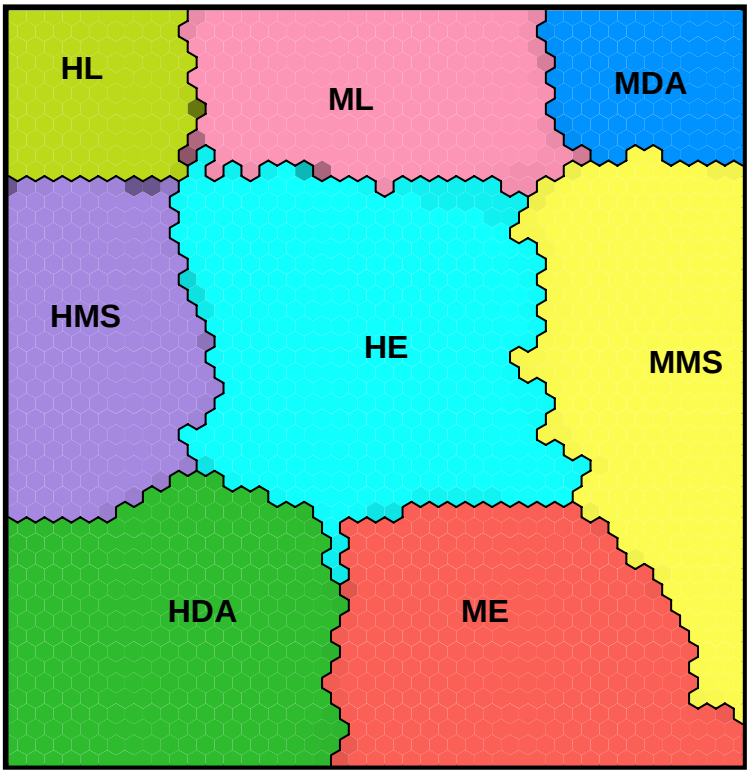

Figure 8.21. SOFM output maps of the IRMS data. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.5.5.3 ICPMS data

The SOFM output for the average values for the elemental profiles of all the samples are presented in Figure 8.22 and these also illustrate a clear and distinctive grouping of the batches of methylamphetamine synthesised from the two routes using the precursors from the various sources. Again, the Hypo and Moscow samples have been separated.

The elemental profiles suggest a linkage both within route but also a connectivity across routes through the nature of the precursors and the extraction solvents used. The output map is much more symmetrical than that obtained for the IRMS data, with the methylated spirit and ethanol:methanol (90:10 % vol/vol) samples placed next to each other. In further contrast to the IRMS results, the methylamphetamine produced from laboratory grade precursors are separated completely by the synthetic method.

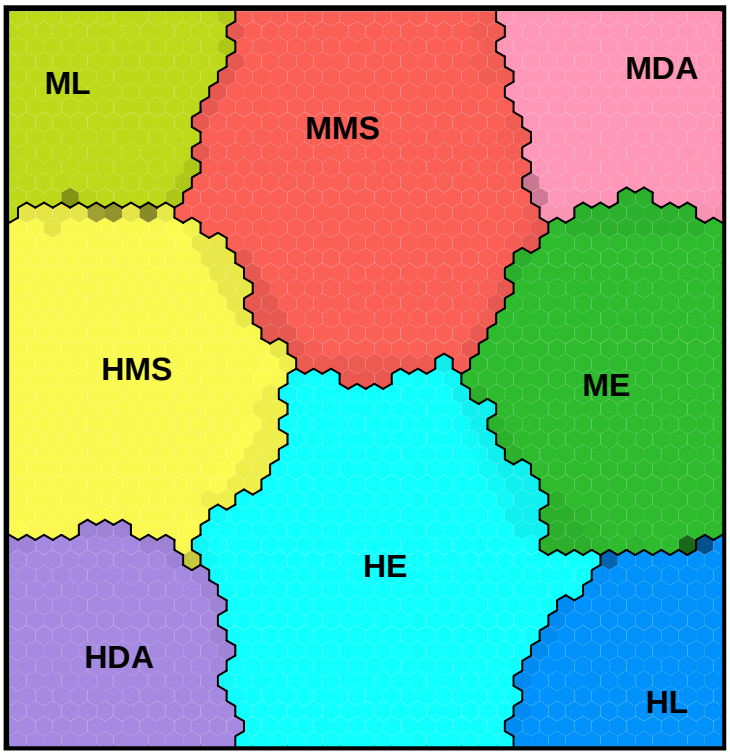

Figure 8.22. SOFM output maps of the ICPMS data. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.5.5.4 Combination of IRMS and ICPMS data

The SOFM obtained from combining the two different datasets together is presented in Figure 8.23. The SOFM map, again illustrate a clear and distinctive grouping of the batches of methylamphetamine synthesised from the two routes using the precursors from the various sources. The dominant trend observed in the ICPMS analysis is again reflected in the combination of IRMS and ICPMS datasets where the methylamphetamine produced from laboratory grade precursors are separated completely by the synthetic method.

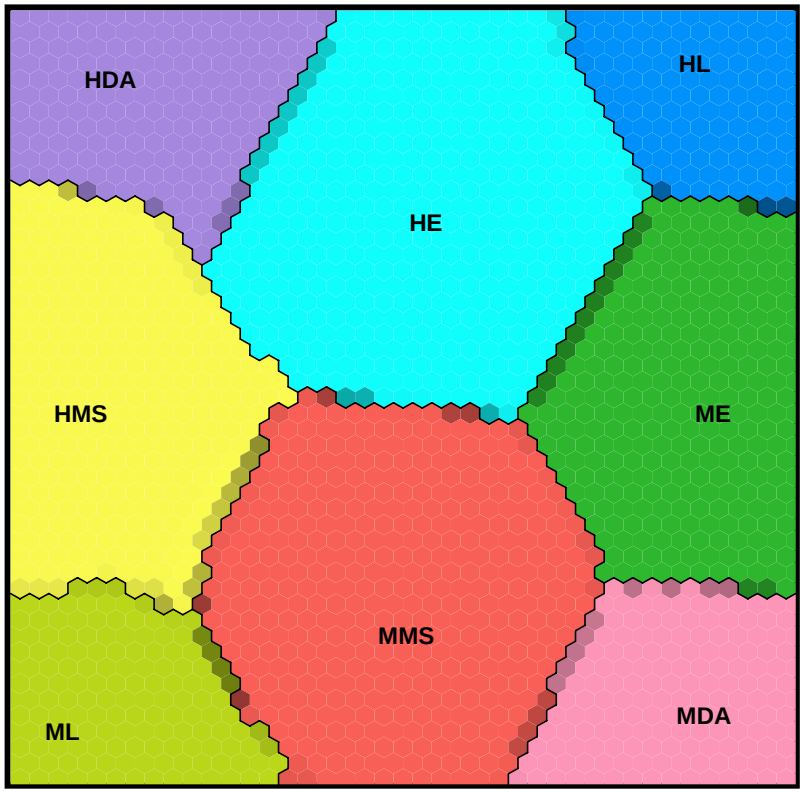

Figure 8.23. SOFM output maps of the ICPMS and IRMS data. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number

8.5.5.5 Combination of GCMS, IRMS and ICPMS data

The SOFM obtained from combining all three data sets together is presented in Figure 8.24. Again complete discrimination of the samples has been achieved by precursor and synthetic method. The influence of the elemental and isotopic profiles appear to have combined to produce a map with the laboratory grade precursors aligning and the samples prepared from the precursor chemicals extracted with greater volumes of methanol present within the extracting solvents being separated further from each other.

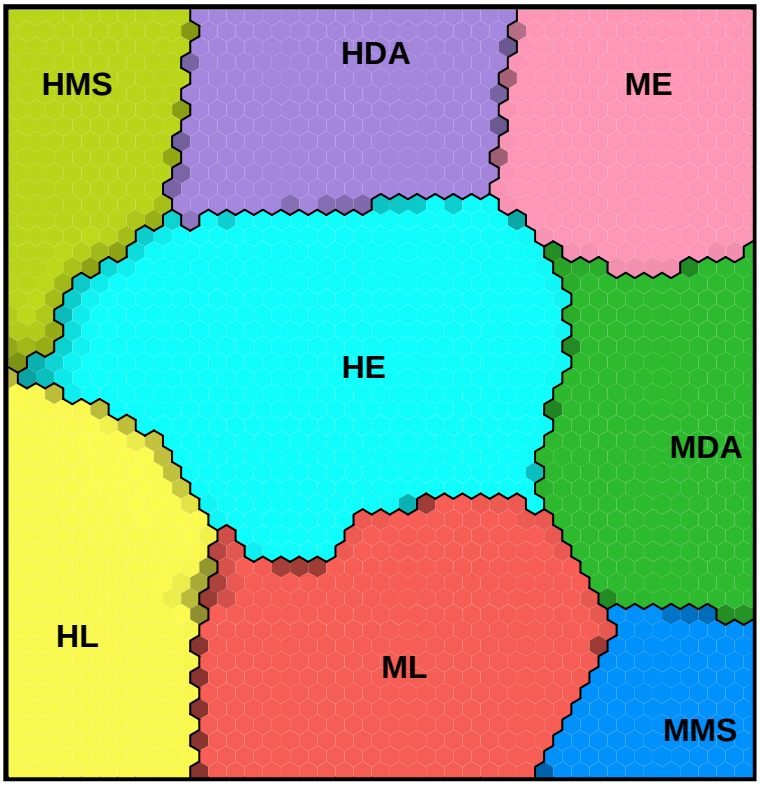

Figure 8.24. SOFM output maps of the GCMS (row scaled and pre processed with sixteenth root transformation)ICPMS and ICPMS data. The sample codes provided in table 8.1 identify each sample type and the numbers refer to a particular synthetic batch number.

8.5.6 SOFM conclusion

SOFM accurately classified batches of methylamphetamine synthesised using two different routes and each of the different source of precursor (laboratory grade and precursors extracted from pharmaceutical products using different solvents). Both the IRMS and ICPMS provided complete discrimination of the samples.

8.6 Conclusion

The HCA had limited success in discriminating samples at 'laboratory output' level that is by both precursor source and synthetic route. The HCA of IRMS and ICPMS data on their own readily discriminated the samples by precursor but not by synthetic method and even the combination of the various data sets did not present route specificity.

PCA analysis performed on samples had similar success as compared to HCA analysis with the exception of PCA analysis performed on the IRMS dataset. In this case complete laboratory output discrimination was achieved. PC loading suggested some influence on the level of methanol present within the extracting solvent may be contributing to the discrimination of samples at the precursor level.

The SOFM successfully discriminated the batches of methylamphetamine by laboratory output using ICPMS, IRMS, the combination of these techniques with each other and with the GCMS results.

The IRMS data set provided the best performance overall indicating full route and precursor specificity using both PCA and the more sophisticated SOFM analysis. This is a different result to that obtained in previous studies where GCMS data has been better at discriminating between synthetic routes and has most likely arisen because of the similarity in the organic impurity profiles of the Moscow and Hypophosphorous samples.

8.7 References

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Chapter 9 Conclusions and Future Work

9.1 Research conclusions

Batches of methylamphetamine were synthesised using two popular synthetic routes frequently employed by clandestine chemists. Both the Moscow and Hypophosphorous routes are variations of the Nagai route utilising iodine and red phosphorous in the synthetic phase. To mimic exact clandestine conditions, batches of *pseudo*ephedrine HCl were extracted from proprietary cold medication sourced from United Kingdom and Malaysia. Three different solvent systems and acid-base extractions used extensively in clandestine laboratories in the extraction of *pseudo*ephedrine HCl were utilised. Essential chemicals such as iodine and red phosphorous were extracted from tinctures and matchboxes using clandestine literature methods.

In total 53 batches of methylamphetamine HCl were prepared from laboratory and pharmaceutical grade *pseudo*ephedrine HCl. These samples were analysed using GCMS, IRMS and ICPMS. The data generated from the different techniques were assessed using four different mathematical data processing methods (HCA, PCA, SOFM and Pearson correlation analysis).

For the organic impurity profiling, impurities were extracted and analysed using GCMS. For this study, extraction of impurities from the samples was performed using a single basic extraction process using a basic buffer and was analysed using a HP-5MS column. The impurity profiles generated were compared mathematically using Pearson correlation coefficients calculated for each pair of samples. A list of target impurities were generated from the chromatographic results and peak areas normalized and various data pre-treatments assessed. The selected target impurities normalized and pretreated to the fourth root provided complete discrimination of the samples allocating all samples to their appropriate synthetic route and particular precursor extracting solvent.

IRMS has been used to identify geographic origin and synthetic pathways of illicit drugs. The combination of carbon, nitrogen and hydrogen isotopic values discriminated

the samples into four groups according to routes and precursor origin. The hydrogen isotopic values discriminated the methylamphetamine samples synthesised via the Hypo and Moscow routes using laboratory grade precursors. Sample discrimination by geographic origin or regio-specificity was best illustrated using nitrogen isotopic values plotted against hydrogen isotopic values. Carbon and hydrogen isotopic values discriminated the samples according to synthetic route.

This study has identified the potential applicability of the ICPMS analysis for methylamphetamine profiling. Results obtained for ICPMS identified the possibility of comparison and discrimination between batches of precursor produced via different extracting solvents and subsequent methylamphetamine produced for each route. Elemental variation was observed for the methylamphetamine samples synthesised from precursors extracted from proprietary cold medication compared to the methylamphetamine synthesised from laboratory grade chemicals.

HCA, PCA and SOFM were used in the discrimination of the GCMS, IRMS and ICPMS datasets. SOFM proved to be the best chemometric technique compared to the HCA and PCA and successfully discriminated the batches of methylamphetamine by laboratory output using ICPMS, IRMS, the combination of these techniques with each other and with the GCMS results.

HCA had limited success in discriminating samples by both precursor and synthetic route however the samples were readily discriminated by precursor but not by synthetic route. PCA analysis produced similar results to the HCA analysis.

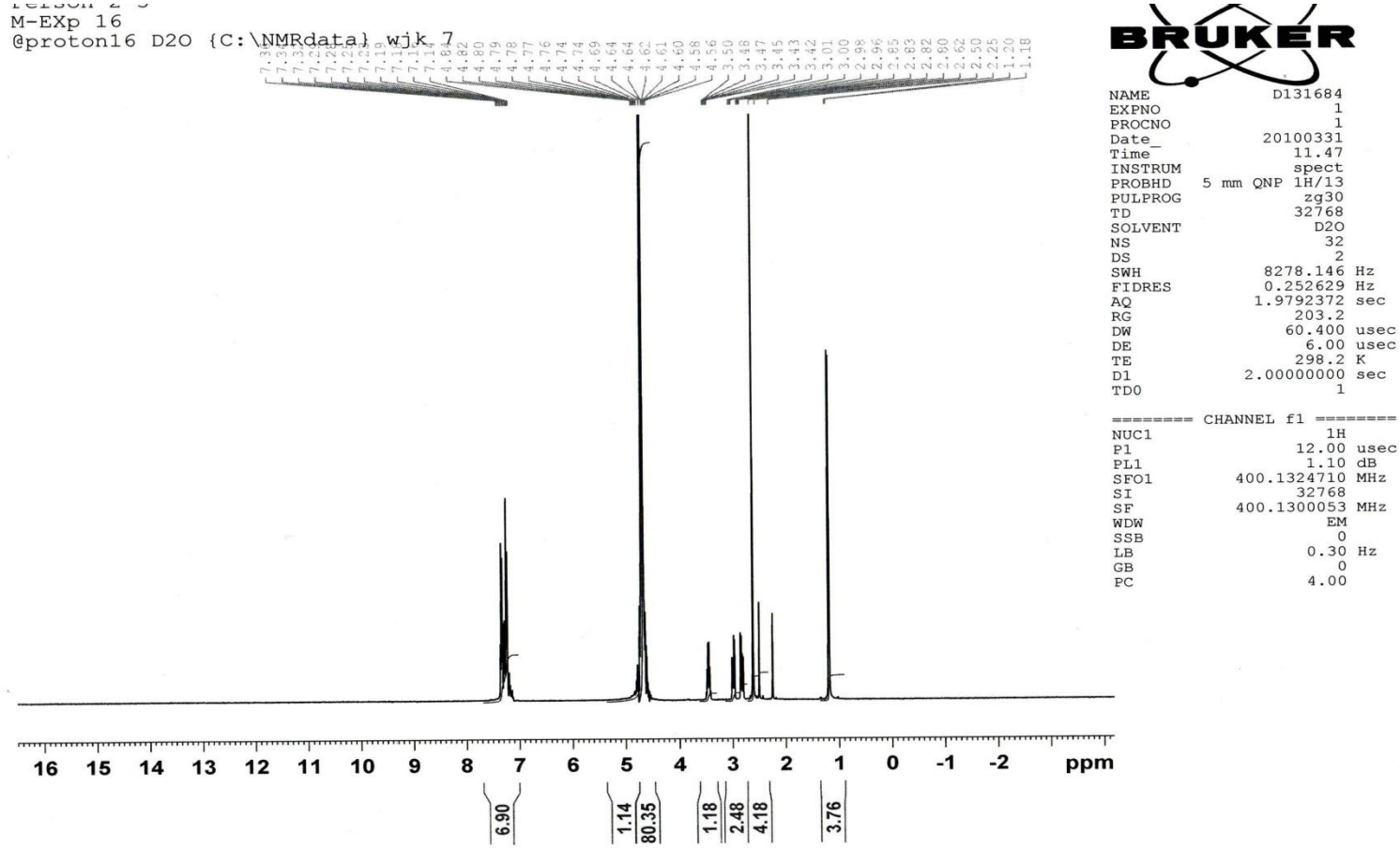
9.2 Recommendations of Future Work

Future work on methylamphetamine profiling could proceed in a number of areas. Firstly, repetitive synthesis methylamphetamine via other synthetic routes should be carried on precursors (*pseudo*ephedrine) extracted from cold medication using the three solvent systems. Further studies based on precursor sources from different regions such as the United States, Australia and Asia would be most useful to explore geographical origins, particularly in relation to IRMS analysis.

Besides using *pseudo*ephedrine extracted from cold medication it is also possible to extract the precursor from the Ephedra plant. The repetitive synthesis of methylamphetamine using the precursors extracted from the Ephedra plant would be a useful addition to the research literature. The effect of a natural precursor source rather than a synthetic or semi-synthetic source would be of significant interest.

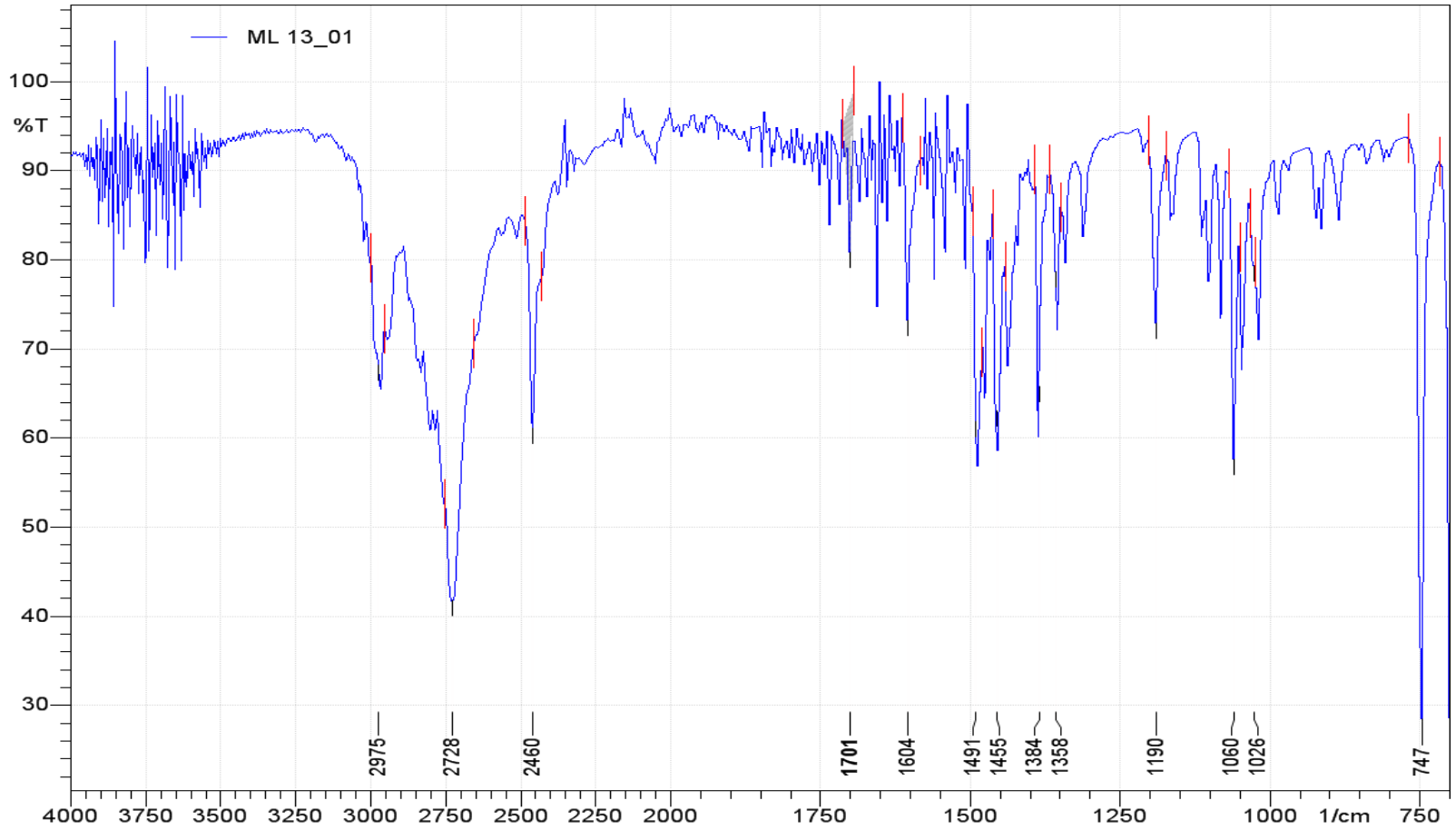
The batches of methylamphetamine synthesized in this study were from precursors sourced from cold medication and extracted using three different solvent systems and dried at room temperature. Isotopic variations were observed from drying experiments undertaken of the precursors. Further studies investigating the effect of drying on the isotopic variations would be advantageous. An extension of this work could also explore the effect of different batches and grades of precursor extracting solvent.

Appendix A



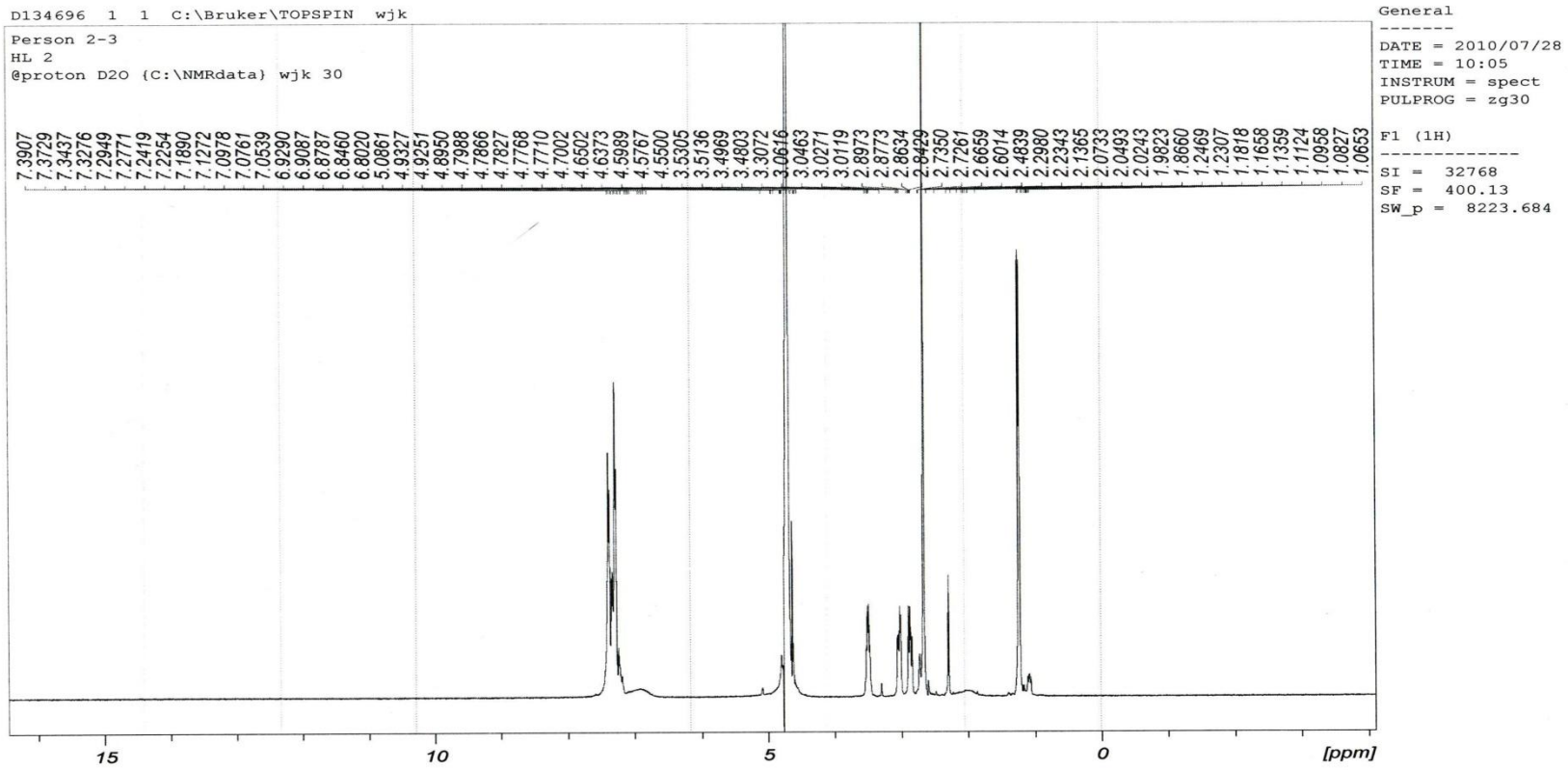
H NMR Spectra: Methylamphetamine HCl synthesised via the Moscow route using laboratory grade materials

Appendix B



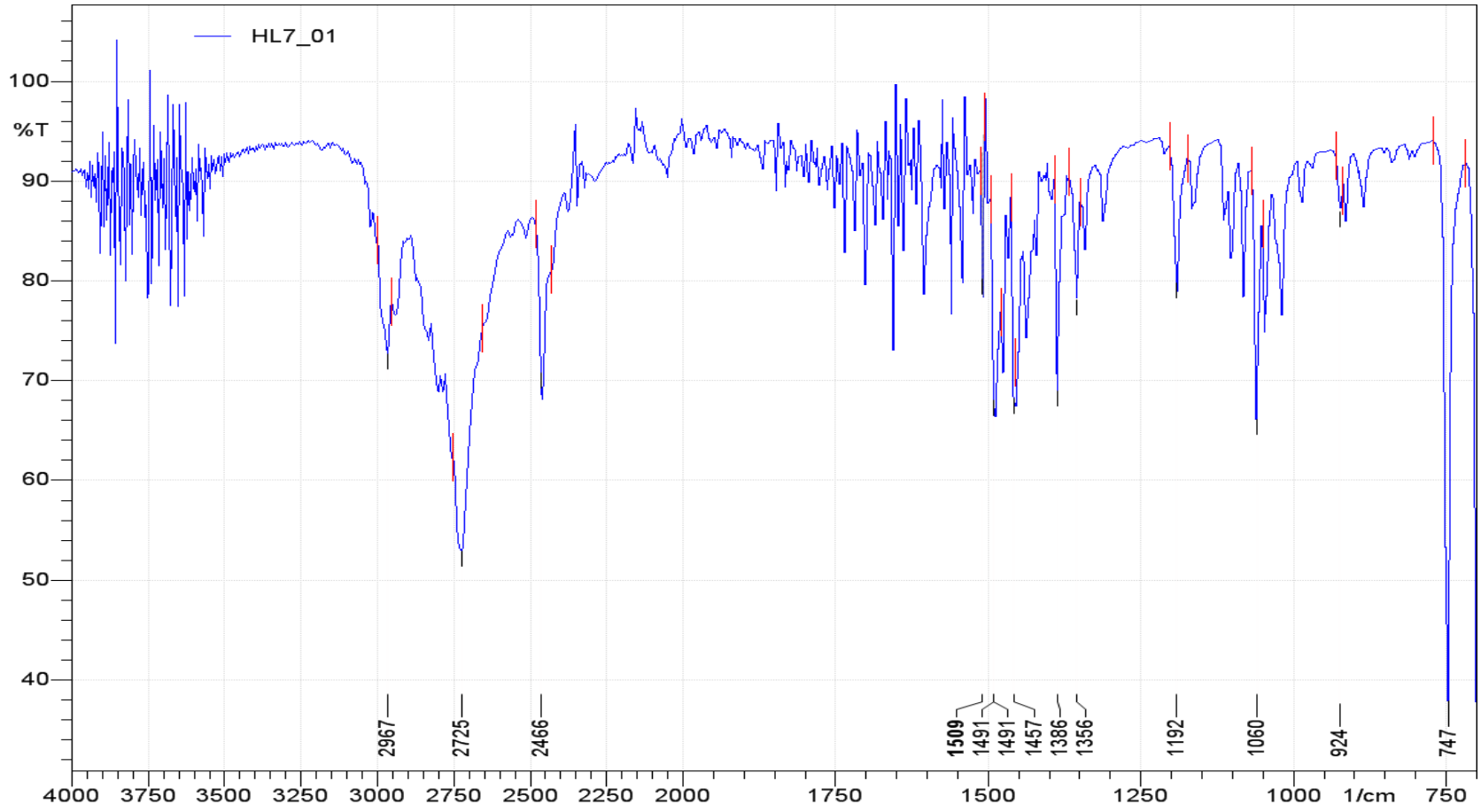
FTIR Spectra: Methylamphetamine HCl synthesised via the Moscow route using laboratory grade materials

Appendix C



H NMR Spectra: Methylamphetamine HCl synthesised via the Hypo route using laboratory grade materials

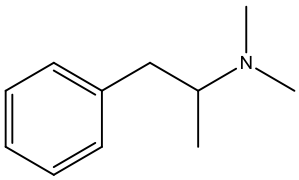
Appendix D



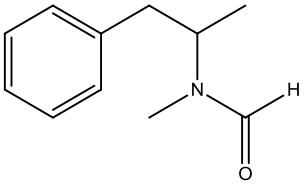
FTIR Spectra: Methylamphetamine HCl synthesised via the Hypo route using laboratory grade materials

Appendix E

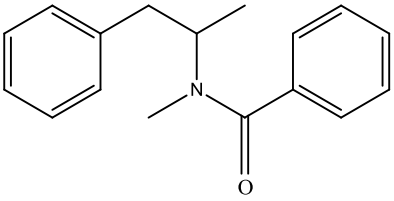
Structures of impurities identified in this study using HP5-MS column



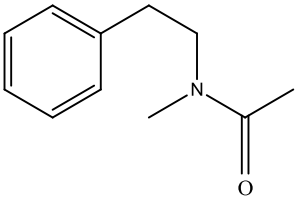
Dimethylamphetamine(DMA)



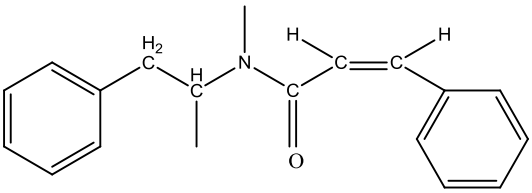
N-acetylmethamphetamine



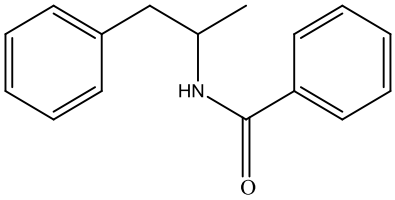
N-Benzoylmethamphetamine



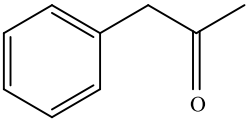
N-formylmethamphetamine



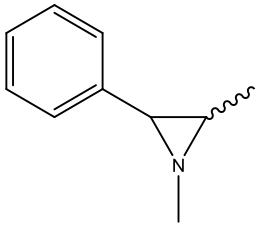
N-methyl-N(α-methylphenethyl)-3-



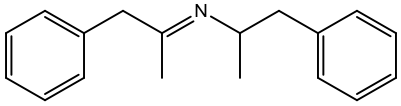
N-Benzoylamphetamine



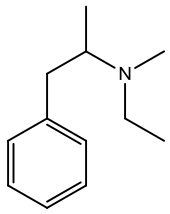
Phenyl-2-Propanone



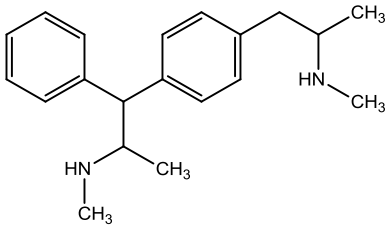
1,2-dimethyl-3-phenylaziridine



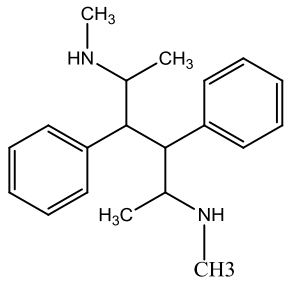
N-β-(phenylisopropyl)benzylmethylketimine



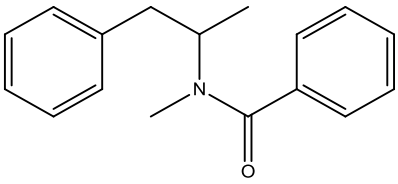
Ethyl-methamphetamine



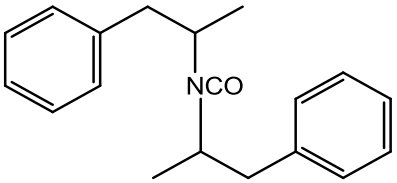
N-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropa-2-amine



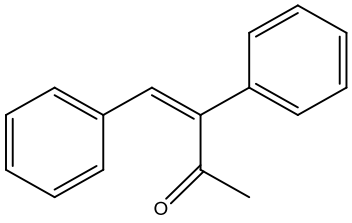
N, N' –dimethyl-3,4-diphenylhexane-2,5-diamine



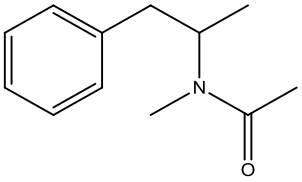
N-Benzoylmethamphetamine



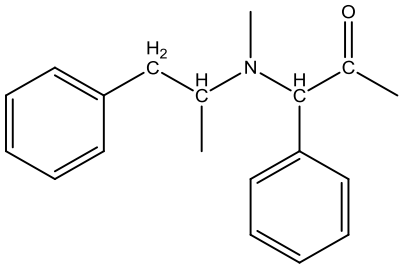
N,N,di β-phenylisopropylformamide



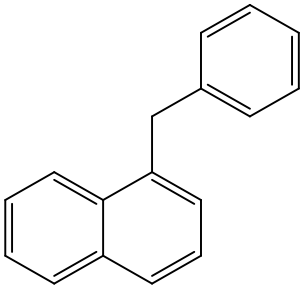
3,4-Diphenyl-3-buten-2-one



N-acetylmethamphetamine



N-methyl-N-(α-methylphenethyl)amino-1-phenyl-2-propane



1-benzyl-3-methyl-naphthalene