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Investigation of the thermal degradation products of bone

by

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Abstract

The understanding of the products generated by the combustion of bones is of significant importance in the identification of human remains and of interfering products in fire debris samples. The aim of this research was to document the thermal degradation products of fleshed and defleshed bone detected using gas chromatography-mass spectrometry (GC-MS).

The first phase of the study was based on the development of an experimental method for the combustion of defleshed bone that would produce a sufficient amount of pyrolysis products. The volatile compounds produced by the optimised experimental method were extracted using the passive headspace adsorption method, analysed by GC-MS, and identified by library and literature search. Finally, an investigation was carried out to determine the pyrolysis products of bone combusted in the presence of common interfering textile fibres which included acrylic, leatherette and wool.

The optimised experimental method provided good repeatability and involved burning bone pieces of at least 13 g for five minutes after the ignition of the bone fat. The pyrolysis of bone produced homologous series of n-alkanes and n-alkenes, aromatics, alkyl nitriles, alkyl cycloalkanes and alkyl cycloalkenes.

The pyrolysis products of the textile materials did not interfere with the identification of the volatile products of bone, but their detection was altered. Also, these materials absorbed and retained some of the bone fat as it was heated.

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Abbreviations

ACS	Activated carbon strip
EIC	Extracted ion chromatogram
EIP	Extracted ion profile
GC	Gas chromatography
MS	Mass spectrometry
m/z	Mass-to-charge ratio
PAN	Polyacrylonitrile
PET	Polyethylene terephthalate
PVC	Polyvinylchloride
%RSD	Percentage relative standard deviation
SIM	Selective ion monitoring
TCC	Target compound chromatography
TIC	Total ion chromatogram
C_n	n carbons
°C	degrees Celsius
Hz	Hertz
min	Minute
µL	Microliter
mL	Milliliter
mg	Milligram
g	Gram
µm	Micrometer
mm	Millimeter
m	Meter
✓	Present
✘	Absent

Glossary

Defleshed	Soft tissues were removed from the bone
Diaphysis	Long midsection of a long bone
Epiphysis	Rounded end of a long bone
Fleshed	Soft tissues were left on the bone

Thesis overview

Human remains found at a fire scene present specific features that can provide information about the intensity of the fire including the maximum temperature attained and the duration of the heat exposure [1]. Studies have been made to attempt to identify and characterise the effect of heat on bone [2-8]. In a fire, the bone undergoes heat-induced modifications including alterations to shape, size, weight, colour and microstructure changes, as well as developing fractures along its length. The exposure of a bone to heat flux will also instigate complex thermal decomposition reactions resulting in the production of pyrolysis products related to the materials being heated.

In order to understand the effect of heat flux on materials such as flesh and bone, one must first understand some of the basic concepts of fire and combustion as well as the pyrolysis process itself. This work focuses on the pyrolysis of defleshed and fleshed bone under the action of a controlled but realistic heat flux.

Chapter one provides a brief introduction to the principles of fire, combustion, pyrolysis and the analytical methods for the determination of pyrolysates. Also introduced is some of the relevant literature relating to the effect of heat flux on bone.

Chapter two provides the details of the experimental procedure used during both the extraction method validation and subsequent development of the analytical protocol followed in the various pyrolysis stages.

Chapter three gives information on the different pyrolysis products of bone detected by gas chromatography – mass spectrometry and on its chromatographic pattern.

Chapter four provides information about the effect of common interfering materials on the pyrolysis of bone.

Chapter 1 – Introduction

1.1 The combustion process

An understanding of the principles of fire is necessary to interpret the thermal effect of fire on bone decomposition. Fire is an exothermic oxidative reaction which produces light, smoke and heat [9]. In this process, also known as combustion, energy is released in the form of flames and heat.

1.1.1 Fire triangle

The fire triangle (Figure 1.1) is one of the two models used to describe how the fire phenomena occurs and is sustained. According to this model, the three essential components needed to create a fire are thermal energy (heat), fuel (combustible) and an oxidiser (oxygen) [10]. When a fuel reaches its ignition temperature, volatiles are released and fuel vapours are produced. The oxidiser and the fuel vapours must be present in the appropriate range of concentration to combust. If any of the three constituents is removed, the fire will extinguish [11].

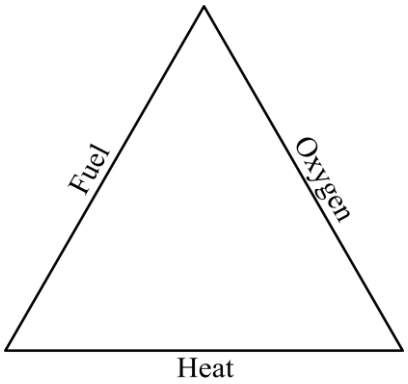

Figure 1.1 *Fire triangle model [10].*

1.1.2 Fire tetrahedron

The second model is the fire tetrahedron (Figure 1.2), which includes a self-sustained uninhibited chemical chain reaction between the fuel, oxygen and heat which causes the combustion to be self-sustained [10, 12]. If the tetrahedron is incomplete, the fire will extinguish. This model is now more utilised than the fire triangle.

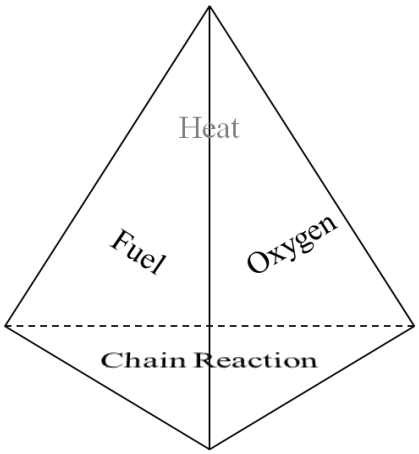

Figure 1.2 *Fire tetrahedron model [10].*

1.1.3 Fire types

Commonly, a fire can either be flaming or smouldering. The flaming combustion is a gas-to-gas reaction [13], where the burning of fuel gases or vapours in the presence of oxygen results in a visible flame. In contrast to this, a smouldering or glowing combustion, is an oxidation of a solid fuel, solid-to-gas reaction [13], and occurs without visible flame. Spontaneous and explosive combustions are also observed [1]. Spontaneous combustion is a slow chemical reaction that takes place in a porous or permeable to air material. During the reaction, self-heating occurs until the heat produced is sufficient to generate a flaming combustion and often transitions through a period of smouldering combustion depending on the ability of the material to sustain such combustion [1]. An explosive combustion usually occurs when vapours, dusts or gases premixed with the appropriate amount of oxygen are ignited, resulting in an extremely quick combustion process [9].

1.2 Heat transfer and fire development

In a fire, objects are ignited by heat transfer either by conduction, convection or radiation. Conduction heat is transferred by direct contact with a solid, along with the movement of molecules in a solid material from a warm to a cooler region [14]. Heat transfer by convection results from the physical movement of liquids or gases. Radiative heat is transferred from the emission of electromagnetic waves [14]; heat energy is transmitted in all directions. One of the principal sources of radiative heat in a fire is smoke. It is possible to have two or more of the heat transfer methods combined together, a process called superimposition [12]. However, in the developing stages of a compartment fire and once the fire is established, radiant heat transfer becomes the dominant heat transfer mechanism.

1.2.1 Fire development

The development of a fire can be divided into four different steps: incipient ignition, fire growth, fully developed fire and decay [1, 10]. In the first phase, a localised heat flux comes into contact with combustible materials such that the effect of the heat causes pyrolysis, generating flammable vapours which, when mixed with a sufficient supply of oxygen, supports combustion. This requires that both the time frame of the heat flux and the nature of the combustible materials are compatible with the generation of sufficient pyrolysates. Either a piloted source (i.e. a flame) or a non-piloted (spontaneous) ignition process result in the generation of small flames, and further heat flux. At this stage the heat transfer mechanism is predominantly convective. In the case of smouldering combustion, no flames are evident. Complete combustion, which rarely occurs outside of a laboratory, generates only carbon dioxide and water [10]. Incomplete combustion produces soot and hot gaseous materials (smoke) that rise within the compartment to form a layer once it impinges upon a horizontal surface such as a ceiling. The heat flux generated from the combustible material also affects nearby materials causing thermal decomposition and the evolution of pyrolysis gases, thus maintaining and furthering the combustion process and growth of fire.

During the growth period, the hot smoke layer also transfers heat to nearby materials by radiation which increases the heat flux within the compartment. The developing fire transitions through flashover when all combustible materials within the compartment experience sufficient heat flux to pyrolyse and become involved in the flaming combusting process. This usually occurs when the floor to ceiling temperature reaches or exceeds 600 °C and the further development of the fire becomes ventilation controlled.

When a fire is fully developed, or is in its steady-state phase, either the maximum burning rate is reached or the combustion cannot be sustained due to the lack of oxygen [12]. The decay phase takes place when little fuel remains [1], the heat flux decreases and the fire starts to extinguish if no oxygen is available.

1.2.2 The thermal degradation process

Pyrolysis is the chemical degradation of a material that occurs under the action of heat without oxygen, and produces vapours, gaseous products and residual char [12]. Heat causes the bonds binding atoms and molecules together to break, and to produce smaller compounds. Stauffer [10] stated that when more heat is provided pyrolysis products are produced faster. A flaming fire can occur if oxygen is present, where the volatile pyrolysis and evaporation products generated burn.

Pyrolysis products are usually a result of three common mechanisms which include random scission, side-group scission and monomer reversion [10]. Nevertheless, other mechanisms of pyrolysis can be found but they are not relevant to the analysis of fire debris samples [10]. It must also be noted that the production of pyrolysis products does not only depend on the mechanisms but also on external parameters such as the temperature, the heating rate, and the presence of oxygen, hydrogen, nitrogen or water [15].

1.2.2.1 Random scission

The random scission process, presented in Figure 1.3, is a random breakage of the carbon-carbon and carbon-hydrogen bonds that form the long carbon chain [10]. This mechanism results in the formation of free radicals, saturated and unsaturated molecules, and produces series of alkanes, alkenes and alkadienes.

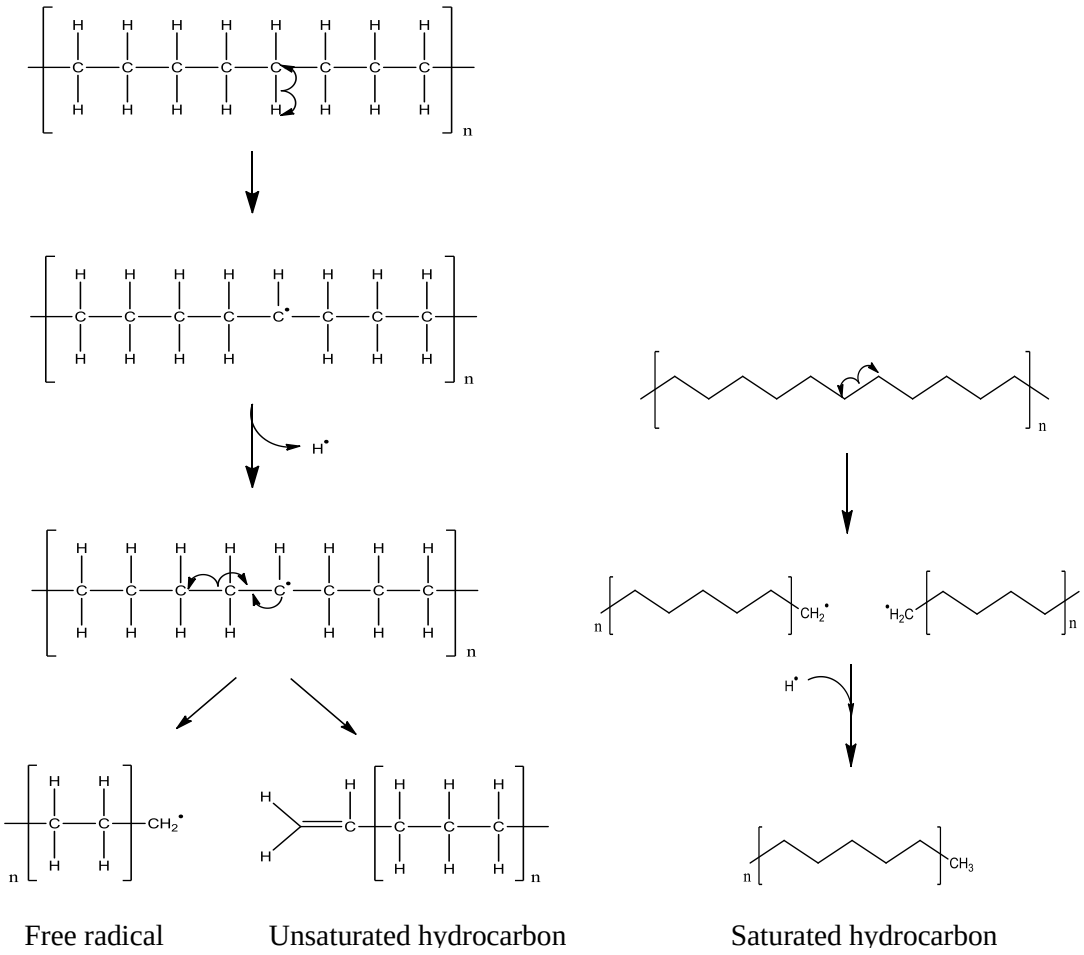

Figure 1.3 *Polyethylene random scission mechanism [10].*

1.2.2.2 Side-group scission

In the side-group scission mechanism, illustrated in Figure 1.4, the side groups located further away from the long carbon chain break first. For polyvinylchloride, for example, the carbon-chlorine bond breaks first since its dissociation energy is lower than the carbon-carbon and the carbon-hydrogen bonds [10]. The polymer chain then becomes polyunsaturated and undergoes rearrangements into aromatic and polyaromatic molecules.

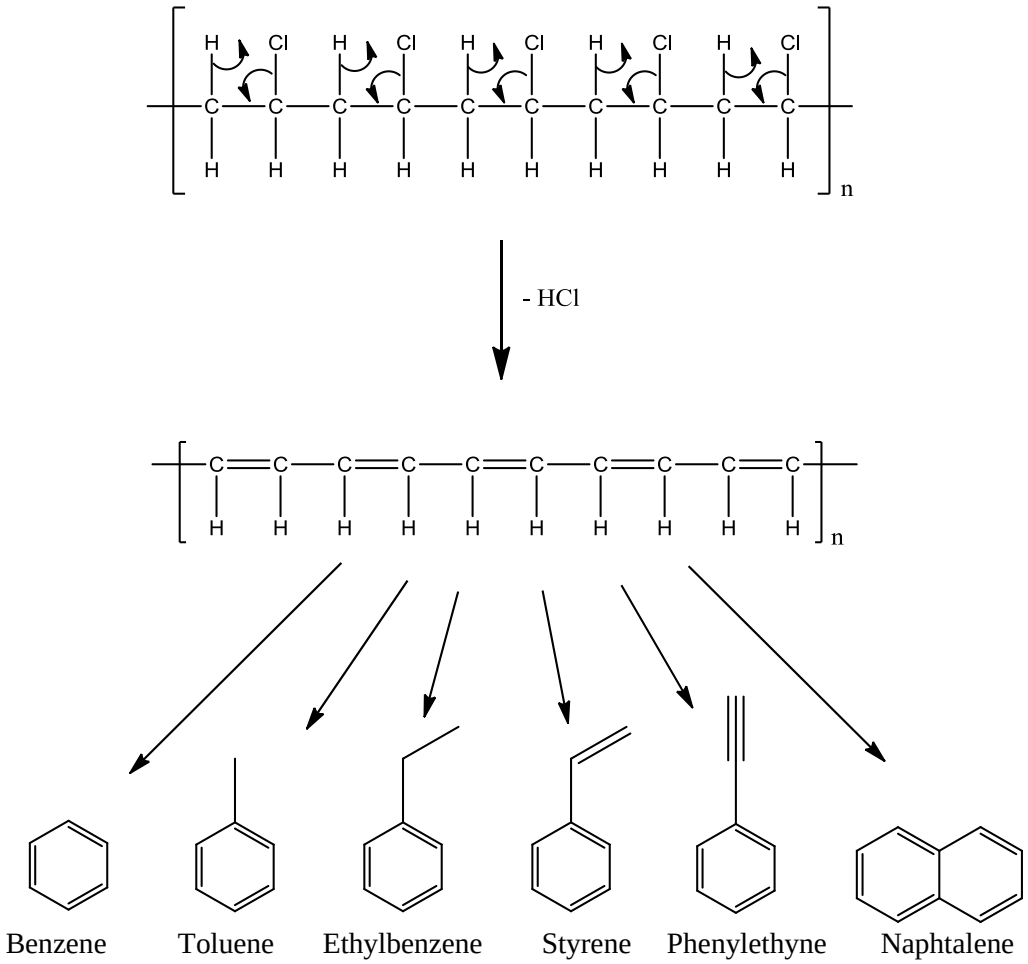

Figure 1.4 *Polyvinylchloride side-group scission mechanism [10].*

1.2.2.3 Monomer reversion

In the monomer reversion or depolymerisation process, the polymer returns to its monomeric version. This process only produces one compound that can be predicted if the polymer is known, as illustrated in Figure 1.5.

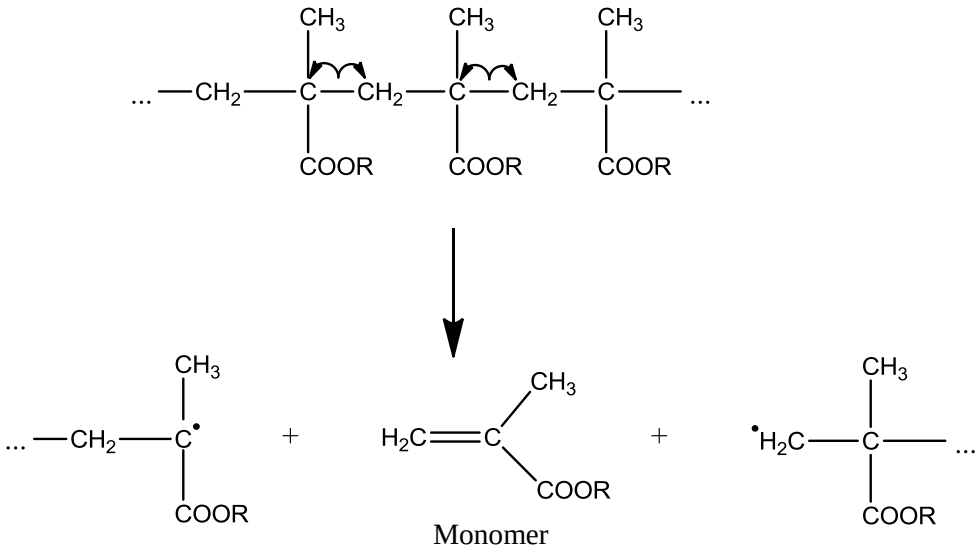

Figure 1.5 *Polymethylmethacrylate monomeric reversion mechanism [10].*

1.2.3 Interfering products

Interfering products are compounds present in a sample that interfere with the identification of a material by contributing to its chromatogram [16]. In the case of a sample found at a fire scene, products can interfere with the identification of ignitable liquid residues. Pyrolysis is one of the most important contributors of interfering products. It is hard to predict what the contribution of the interfering products is but by burning a sample pyrolysis products are generated that can be analysed to evaluate the contribution of interfering products to a fire debris sample.

1.3 Analysis of post-fire debris

1.3.1 The passive headspace extraction process

In forensic laboratories, the most common extraction process used for the isolation of thermal degradation products from fire debris is the passive headspace extraction technique [10]. It is a non-destructive, simple and sensitive method that has little to no contamination risk of the extracted sample.

The principle of this technique is to concentrate the volatile products on an activated carbon strip (ACS) or similar material that acts as an adsorbent (e.g. Tenax). Once the ACS has been introduced into the sample container, the sealed container is placed in an oven at a certain temperature for a certain amount of time. During this period, volatile components within the headspace above the fire debris sample are adsorbed on the ACS. Compounds are adsorbed until the equilibrium between the ACS, the headspace and the fire debris is reached [10]. At temperatures below 60 °C, the compounds heavier than C_{16} within the matrix of the debris may not volatise sufficiently for adsorption [17]. According to Newman *et al.* [18], above 60 °C and as the temperature increases, the rate of displacement in the lighter volatile compounds is increased and more balanced extraction of the volatile mixture can be obtained. Temperatures above 90 °C stimulate the degradation and the pyrolysis of residues and as such it is not recommended to work at those temperatures [18]. The incubation time depends on the temperature, it takes more time for heavier compounds to volatise and adsorb at low temperature than at a higher temperature. Long incubation times and high temperatures are often required for the adsorption of very small quantities of volatile compounds [17]. Stauffer [10] mentioned that for regular samples the container should be placed in an oven between 60 °C and 90 °C for twelve to sixteen hours. This is corroborated by the ASTM E1412 standard method for the analysis of ignitable liquid samples using this method of extraction, which recommends a sixteen hour incubation period [17].

For the desorption phase, the ACS is placed in a gas chromatography (GC) vial containing a small quantity of solvent such as carbon disulfide, n-pentane or dietylether [17], and an internal standard to facilitate semi quantitative analysis. The volatile residues are directly transferred into the solvent, and the sample is agitated to homogenise the sample and analysed.

1.3.2 Gas Chromatography - Mass Spectrometry

Gas chromatography coupled with mass spectrometry (MS) is a technique commonly used for the analysis of fire debris residues. GC is a technique that separates mixtures based on the difference of boiling point and polarity of the compounds. The mass spectrometer is a detector that provides information to identify the separated components. A compound can be analysed and identified by GC-MS only if it is sufficiently volatile and thermally stable [19].

1.3.2.1 Gas chromatography

GC systems utilise inert gaseous mobile phase and a liquid stationary phase to separate mixtures. The mobile phase, or carrier gas, can either be nitrogen (N_2), hydrogen (H_2), or helium (He) [20]. The sample is injected into the GC, vaporised in the injection port and is carried by the mobile phase into the column [19]. As the sample is carried through the column contained within an oven, separation of the different components takes place. The separation occurs due to the difference in affinities of the analytes with the mobile phase and the stationary phase. This results in a difference in the elution time and hence the detection time between compounds. Figure 1.6 represents a typical gas chromatography instrumental setup.

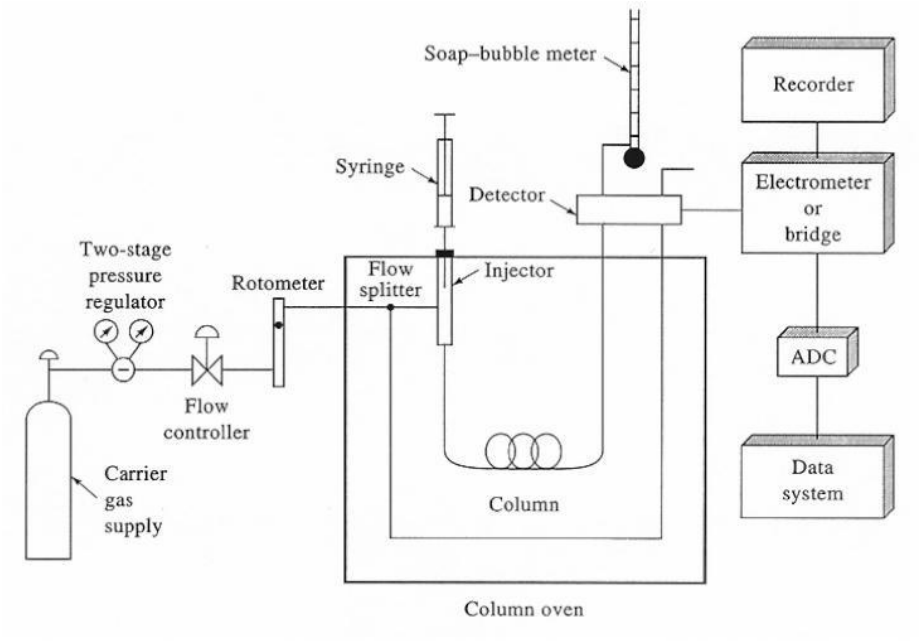

Figure 1.6 *Diagram of a typical gas chromatography instrumental setup [21].*

The retention time of a compound is measured from the time of injection into the GC to the time it elutes from the column. The relative measure of the amount of compound present gives the abundance, which can be illustrated by the peak height or peak area. This abundance is measured by the GC detector that produces an electronic signal when a compound elutes. A chromatogram (Figure 1.7) is commonly represented by the retention time (x-axis) against the abundance (y-axis).

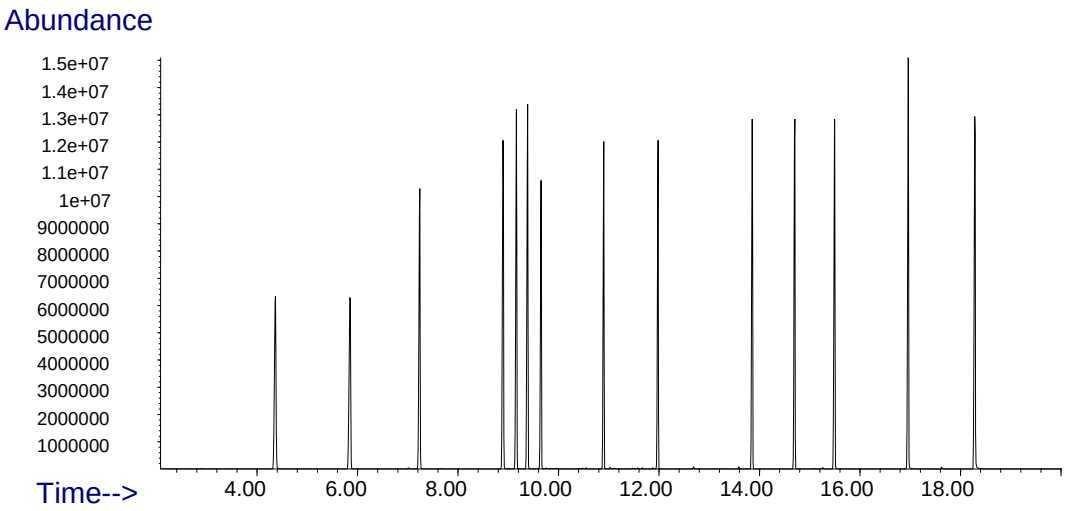

Figure 1.7 *Example of a chromatogram of a sample mixture. The mixture is composed of toluene, octane, 1,4-dimethylbenzene, m-ethyltoluene, o-ethyltoluene, 1,2,3-trimethylbenzene, decane, undecane, dodecane, tetradecane, pentadecane, hexadecane, octadecane, and eicosane.*

1.3.2.2 Mass spectrometry

The sample (in a gaseous state) is introduced in the ion source of the MS directly from the outlet of the GC column (when coupled to a GC) and ionised. A high energy electron beam is accelerated into the ionization chamber where the electrons collide with the gaseous molecules. During collision, an electron is removed from the molecules producing positive ions, known as molecular ions [10]. Due to the excess of energy, fragmentation of the molecular ions occurs which produces fragment ions. This type of ionisation used in fire debris analysis is called electron impact ionisation [10]. A quadrupole mass spectrometer (i.e. mass filter) is used to separate and sort the molecular and fragment ions according to their mass-to-charge ratio (m/z) [10]. An electromagnetic field is created so as to let through the quadrupole to the detector only fragment ions of a particular m/z range. When the ions of a specific m/z value enter the detector, the signal resulting from the ions is converted into an electric current that represents the abundance of the ions. The data collection can either be done in a scan acquisition mode which provides a wide range of ions detected or in a selective ion monitoring mode (SIM) where only the pre-selected ions of interest are

detected. Figure 1.8 illustrates the different components of a MS. A mass spectrum illustrates for each compound the relative amount of ions for different m/z values. The m/z values are represented on the x-axis against the abundance of the ions on the y-axis. This information provided by the MS detector allows the identification of compounds.

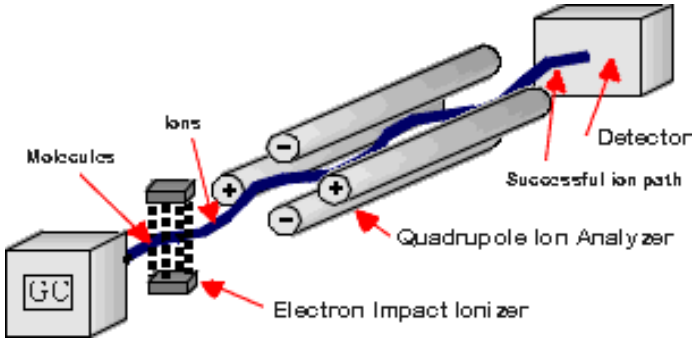

Figure 1.8 *Diagram of a quadrupole mass spectrometer detector [22].*

1.3.2.3 Data analysis and interpretation by GC-MS

The GC-MS instrumentation provides a combination between retention times and structural data which is an advantageous feature of this technique.

The structural data (i.e. fragment ions m/z) specific to a chemical class of compounds can be used to extract the chromatogram of a specific fragment ion or set of ions from the total ion chromatogram (TIC). The extracted ion chromatogram (EIC) filters an individual ion, whereas the extracted ion profile (EIP) filters summed ions [10]. These techniques produce simpler chromatograms by screening out interferences or background peaks and detecting specific ions of a given class of compounds. The typical ions indicative of a class of compounds are listed in Table 1.1.

Chemical class	Fragment ions m/z
Alkanes	29, 43, 57, 71, 85, 99
Alkenes	41, 55, 69, 83, 97
Cycloalkanes	41, 55, 69, 83
Alkylbenzenes	91, 92, 105, 106, 119, 120
Alcohols	31, 45, 59
Ketones	44, 58, 72, 86

Table 1.1 *Typical fragment ions m/z extracted for a class of compounds [23].*

For target compound chromatography (TCC), information about the target compound is provided which includes the retention time and the significant fragment ions m/z with their relative abundances. This technique is used to determine if a specific compound is present in a sample even in the presence of excessive interferences and background peaks, thus the presence of an ignitable liquid or a material in a fire debris sample can be detected.

1.4 Bone composition

1.4.1 Bone structure

A bone is an organ constituted of bone tissue, cartilage, bone marrow and fat [24], which can be long, short, flat and irregular. It is a highly dynamic porous tissue that changes in its composition and microscopic structure to adapt to its environment [25]. Bones have mechanical, synthetic and metabolic properties, they provide support, protection and movement, produce blood cells, and act as a storage reserve for calcium, phosphorus and fat.

Bones are composed of compact and cancellous tissues which differ in porosity. The compact, or cortical, bone forms the external layer of a bone. The long bone shaft, or diaphysis, is mostly composed of cortical bone. This dense cortical bone has a porosity between 5% and 10% [25]. The cancellous tissue can be found at the ends of

long bones, in flat bones and in sesamoid bones where the tendons are attached [26]. This trabecular or spongy bone is more porous than the compact bone, its porosity is between 75% and 95% [25]. The epiphysis is the rounded end of a long bone and is mainly composed of trabecular bone. Figure 1.9 illustrates the structure of a long bone.

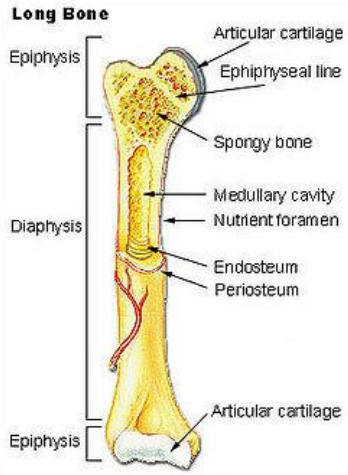

Figure 1.9 *Illustration of the structure of a long bone [27].*

Bone marrow is a tissue composed of blood vessels, cells and nerves. Yellow marrow which is surrounded by compact bone (diaphysis) acts as a fat storage, whereas red marrow found in trabecular bone (epiphysis) produces blood cells and platelets [26].

1.4.2 Bone components

Bones are composed of water, an organic matrix and an inorganic mineral [24]. The organic matrix is mainly composed of collagen, a structural protein that gives the bone flexibility and tensile strength, and of non-collagenous proteins [25]. The inorganic mineral consists predominantly of hydroxyapatite crystals, $Ca_{10}(PO_4)_6(OH)_2$, with traces of magnesium, fluoride and other ions. The high affinity between calcium and phosphate brings stability to the inorganic matrix [26], which provides rigidity and compressive strength to the bone [25]. Kerr considered

as an example to illustrate the distribution of the bone components, the weight proportion of an adult cortical bone components: 63% mineral, 23% collagen, 10% water, and 2% non-collagenous proteins [24].

1.5 Heat-induced alterations to bone

1.5.1 Stages of heat-induced modification in bone

Correia [2] divided the heat-induced modification process in bone into four stages, depending on temperatures and observations made, as dehydration, decomposition, inversion and fusion. Thompson [3] later reviewed the four stages and modified the temperature ranges of three steps based on his experiments (Table 1.2).

Temperature range (°C)	Stage of transformation	Observations
100 - 600	Dehydration	Fracture patterns; weight loss
300 - 800	Decomposition	Colour change; weight loss; reduction in mechanical strength
500 - 1100	Inversion	Increase in crystal size
above 700	Fusion	Reduction in dimensions; increase in crystal size

Table 1.2 *Stages of heat-induced modification in bone according to the bone temperature [3].*

The dehydration phase is characterised by the loss of adsorbed water (physisorbed) [28] and bonded water (chemisorbed) [2] from the apatite mineral, and by the hydroxyl bonds breaking in the crystal [29]. Adsorbed water is loosely-bound to the crystal and is present at the surface of the mineral. When water is removed, the crystals lose their conformation.

The decomposition process is defined as being the pyrolysis of organic components of bone [2]. During this stage, due to the elimination of organic material, the weight of the bone reduces. This process is discussed to a great degree in Chapter 2.

The inversion phase is identified by the modification of the hydroxyapatite crystal into β-tricalcuim phosphate $Ca_3(PO_4)_2$ [30], and the elimination of carbonates $CaCO_3$ that produce CO_2 and CaO [2]. The conversion of hydroxyapatite crystals to β-tricalcium phosphate results in the formation of a structure that is smaller than the original crystal. Recrystallisation occurs during the conversion, resulting in the formation of larger crystals [1].

Finally, the fusion phase is characterised by the melting of apatite crystals, which results in the shrinking of the bone.

1.5.2 Heat-induced colour alterations

During burning, the bone undergoes colour changes which indicate the development of thermal degradation, the heat flux and duration of exposure. These colour alterations can be related to the elimination of both the inorganic and organic components of the bone. Moreover, during a fire the colour range may also alter due to the amount of fuel present, the availability of oxygen, and the contact of bone with a metal [1] .

A black colour suggests that the bone has reached a temperature of approximately 300 °C [7], and that the inorganic compounds and bone marrow are lost. Symes stated that according to Herrmann [5] carbonisation of the bone occurs by direct contact with heat and flames [4]. A black bone is categorised by Symes [4] as charred.

During the calcination phase, pyrolysis of the organic components occurs to produce volatile species [29]. The colour of the bone turns gray when its temperature reaches at least 600 °C [7]. Its colour then turning white, an endpoint colour, suggests that its temperature has attained at least 800 °C. At this stage the calcination of the bone is

complete, a total loss of the organic components and the fusion of bone salts are observed [2]. Symes [4] classified the gray-white bone as calcined.

Temperature (°C)	Colour	Interpretation
300	Black	Carbonisation, loss of inorganic components and bone marrow [1]
600	Gray-blue, gray	Calcination, pyrolysis of the organic compounds [29]
800	White	Complete calcination [2]

Table 1.3 *Summary of the heat-induced colour alterations to bone.*

The border in a heat-altered bone can be identified by the difference in colour between an unaltered translucent bone and a heat-altered discoloured opaque bone [31]. Discoloration and dehydration occurs although the border is protected from heat and flames by soft tissues. A heat line is observed at the limit between unburned and burned bone, and represents the initial transition from unaltered to heat-altered bone [4].

1.5.3 Heat-induced dimensional alterations

It is important to understand the head-induced dimensional changes a bone can undergo since shrinkage, warping and sometimes expansion can influence the conclusions made concerning the age, sex and stature of an individual [2]. Shrinkage and warping are observed during the fusion stage, where melting and coalescence of the bone matrix occur [3]. A critical temperature has been set at 800 °C at which the degree of shrinkage considerably increases [8, 32, 33].

Two statements that explain why warping occurs are widely accepted but are not confirmed by quantitative data [8]. The first concept indicates that warping is more apparent in bone that is fleshed at the time of burning [34] and that is a result of the

heat-induced contraction of the muscle fibres. The second precept described by Spennemann and Colley [35] stated that heat causes expansion of air in the marrow cavity which is observed with the increase in size of the diaphysis [8]. Thompson [8] did not support these statements, he argued that contracting muscles alone cannot cause to bone to warp or shrink. Also warping cannot be a result of the expansion of air since the porosity of the bone does not allow the air to remain trapped in the marrow cavity under such pressure.

The amount of heat-induced shrinkage is expected to vary as burning conditions vary; Shipman *et al.* [29] defended that the amount of shrinkage and temperature are associated. Fairgrieve [1] described Herrmann's findings [36, 37] who was able to determine three stages of shrinkage (Table 1.4).

Temperature (°C)	Degree of shrinkage
150 - 300	1 - 2%
750 - 800	1 - 2%
1000 - 1200	14 -18%

Table 1.4 *Phases of shrinkage observed on 20mm x 5mm compact bones [36, 37].*

According to Fairgrieve [1], Herrmann [36] affirmed that the degree of shrinkage of a bone in a fire is influenced by four conditions which include the temperature of the bone exposure, the inorganic content of bone, the aspect of the mineral content, and the distribution of the compact and spongy bones types in a bone which is supported by others [29, 38]. Herrmann found that as the amount of inorganic portion of bone increased, the degree of shrinkage increased.

Gejvall [39] and Gilchrist [38] declared that compact bone shrinks more than spongy bone whereas, as mentioned by Thompson [8] and Fairgrieve [1], McKinley [40] and Van Vark [41] argued that the spongy bone shrinks at a higher degree. From his experiments Thompson [8] proposed that the compact bone shrinks to the greatest degree considering its thickness (absolute shrinkage), but the spongy bone shrinks at

a higher degree compared to actual measurements (relative shrinkage). Thompson concluded that the greatest occurrence of heat-induced shrinkage occurs in the trabecular bone [8] which is more rigid and absorbs forces from different directions and sources, and has less structural support than compact bone as a consequence of the random orientation of collagen. This finding supports McKinley [40] and Van Vark [41] who concluded that spongy bone shrinks most.

Another potential influence to the degree of shrinkage is the biological age of the bone [8]. Holden [7] suggested that shrinkage is restricted in older bones due to the bone mineralisation, hence collagen cross-linkage, which is greater than in younger bones. However, as the collagen is eliminated due to an increasing temperature, the crystal size difference decreases until the crystal reaches a constant size for all ages.

1.5.4 Heat-induced fractures

During the dehydration and decomposition phases of a bone, collagen is eliminated, and the elasticity and tensile strength of the bone are lost. Fractures appear as the bone shrinks and warps along the lines of heat-induced stress [42].

Herrmann and Bennett [6] described five different types of fractures seen on a long bone which include longitudinal, curved transverse, straight transverse, patina and delamination fractures. Longitudinal fractures develop along the long axis of the bone, vary in depth and can reach the marrow cavity of the bone. Curved transverse or curvilinear fractures surround the bone from one side to the other. They appear during burning with the shrinkage of soft tissue. Straight transverse fractures are perpendicular extensions of the longitudinal fractures. Patina fractures are usually situated on the surface of the cortical bone and are not deep enough to reach the marrow cavity. Finally, delamination fractures are a peeling or flaking of the cortical bone when it separates itself from the spongy bone.

A bone that has recently been defleshed is referred to as a dry bone whereas a bone covered with soft tissue is a green bone [1]. Fairgrieve [1] mentioned that Baby [43] noticed that dry bone did not display the warping seen on green bone, but that it

shows patina and longitudinal cracks, and transverse splintering. Binford [44] supported this conclusion and added that straight transverse fractures are observed in dry bone in comparison to curved transverse fractures seen in green bone [1]. Buikstra and Swegle [45] through their studies of bovid, human, and canine bone found that warping occurs in both dry and green bones, they did not support the conclusions made by Baby and Binford.

1.6 Conclusions and aims

In a fire bones undergo, in addition to heat-induced modifications, thermal degradation that produces volatile species. These compounds can act as interfering products for the identification of ignitable liquid residues. The understanding of the products generated by the combustion of bones and tissue could be of significant importance in the determination of interfering products in fire debris samples. The aim of this study was to investigate the pyrolysis products of bone, thus providing a robust methodology by which such products can be derived and analysed. Secondly, we aimed to establish a database of pyrolysis products from bone and investigated whether these products could be isolated from interfering products in order to facilitate the chemical identification of human remains in fire debris.

These aims were achieved by developing an experimental method to produce the thermal degradation products of bone and passive headspace extraction methods to facilitate the analysis of the resultant pyrolysis products by GC-MS. The total ion chromatogram of bone was then studied to identify the different compounds present and understand the bone chromatographic pattern.

The second stage involved the investigation and determination of these pyrolysis products in the presence of common interfering materials including acrylic, cotton, leatherette, nylon-lycra, polyester and wool, and evaluating the feasibility of using selective ion monitoring to isolate the bone pyrolysis products from these background matrices.

Chapter 2 – Experimental methodology and method development

2.1 Introduction

The first phase of the study was to develop an experimental method for the combustion of defleshed bone that would produce a sufficient amount of pyrolysis products.

Pork long bones and tissues, more precisely the femur, were used for the experiments due to their similarities in composition with human bones as demonstrated in previous studies [46, 47].

2.2 Experimental method

2.2.1 Gas Chromatography Mass Spectrometry instrumental repeatability

The samples were analysed according to the ASTM E1387 [20] standard method using a Hewlett Packard 6890 Series Gas Chromatograph system coupled with a HP 5973 Mass Selective Detector. The column used was a 128-0122 DB-IMS column of 25 m length x 0.2 mm inner diameter and 0.33 µm film thickness. 1 µL was injected onto the GC and swept into the column at 1 mL/min by helium the carrier gas. The oven temperature was programmed from 40 °C to 280 °C at 15 °C/min, and held at 40 °C for five minutes and for two minutes at 280 °C. The injector and detector temperatures were both set at 250 °C. The Hewlett Packard Chemstation software was used for data acquisition and peak integration. The data acquisition rate was of 20 Hz from zero to twenty-two minutes. The instrument was tuned using perfluorotributylamine (PFTBA), and an air and water check was carried out on a weekly basis.

Repeatability tests were carried using a known standard mixture to assess the instrument precision and the column performance. Fourteen high purity 99% analytical grade compounds (BDH Chemicals and Sigma, UK) were used to prepare the standard mixture: toluene, octane (C_8), 1,4-dimethylbenzene (p-xylene), m-ethyltoluene, o-ethyltoluene, 1,2,3-trimethylbenzene, decane (C_{10}), undecane (C_{11}), dodecane (C_{12}), tetradecane (C_{14}), pentadecane (C_{15}), hexadecane (C_{16}), octadecane (C_{18}), and eicosane (C_{20}).

A 100 mg/mL stock solution of each compound was prepared in pentane (HPLC grade, BDH Prolabo, UK) in class A volumetric flasks using the appropriate weight or volume of sample. From these solutions, a combined 1 mg/mL standard mixture was prepared in pentane from the stock solutions. Six replicate injections of the standard mixture were made and the peak areas of the compounds were assessed.

The column efficiency was assessed by analysing the peak resolution and the asymmetrical factor values. To assess the instrument precision or repeatability, the peak area relative standard deviation of each component of the mixture was calculated among the six injections.

The peak resolution (Rs) measures the separation of two adjacent peaks considering their average peak width at base [10].

$$\boldsymbol{Rs = \frac{trB - trA}{{}^{1}/_{2}\,(wA - wB)}} \quad \textbf{Equation 2.1}$$

trA: retention time of peak A
trB: retention time of peak B (trB > trA)
wA: width at base of peak A
wB: width at base of peak B
The peaks are well separated and have a good resolution if Rs is above or equal to one. A baseline resolution is achieved when Rs is equal to 1.5.

The asymmetrical factor (A), also called tailing factor, is used to measure peak tailing; when the peak deviates from the ideal symmetrical shape [48].

$$\boldsymbol{A = b/a} \quad \textbf{Equation 2.2}$$

The terms a and b are respectively the left and right half-widths at 10% of the peak height. The ideal peak shape is observed when A is equal to one. Peak tailing occurs when compounds are strongly retained on the column. If A is greater than one, the peak is characterised as tailing or asymmetrical, whereas when A is less than one the peak is defined as fronting.

The standard deviation (s) measures the spread about the mean of a set of values [49].

$$\boldsymbol{s = \sqrt{\frac{\sum_{i=1}^{n}(xi - Mean)^2}{n-1}}} \quad \textbf{Equation 2.3}$$

The relative standard deviation (RSD), also known as the coefficient of variation, calculates the standard deviation relative to the mean, and is expressed as a percentage [49]. As the %RSD increases, the variation between the different values is greater.

$$\boldsymbol{\%RSD = \frac{s}{Mean} \times 100} \quad \textbf{Equation 2.4}$$

2.2.2 Bone sample preparation

The long bones obtained from The Country Shop, Glasgow, UK, were less than twelve months old pork femurs with soft tissues attached, and were stored in the freezer before each experiment. Before sample preparation, bones were left to defrost for approximately thirty minutes at room temperature. Experiments were carried out using only the diaphysis of the bone to reduce variations in bone composition. The epiphysis was detached from the diaphysis by placing the bone on a vice and cutting

it with a hacksaw. Then, soft tissues were removed with scissors and tweezers, and the bone was cut across its width into small pieces of 3 g to 20 g.

2.2.3 The passive adsorption extraction process

The pyrolysis products of bone were extracted using a passive headspace adsorption method. The method was adapted from the ASTM standard practice E1412 [17] and validated in-house. The closed 125 mL tin can (WA products, UK), containing the activated carbon strip (ACS, 3M corporation, USA) which was hooked on a paper clip held on the lid with a magnet placed on the other side of the lid, was incubated in an oven at 80 °C for sixteen hours. During this period, the volatile components of the sample were adsorbed onto the ACS. At the end of the incubation time, the adsorbed volatile components were desorbed by placing the ACS in a GC vial (Agilent, UK) containing 1 mL of pentane which included 0.5 mg/mL of tetrachloroethylene (Sigma, UK) as the internal standard.

2.2.4 Generation of pyrolysis products

2.2.4.1 General experimental method

The bone sample (of varying weight) was placed in a tin can, which was then fixed on a retort stand. The tin can was heated over a Bunsen flame for varying periods of time to develop the experimental method. The temperature inside the tin was monitored using a k thermocouple linked to a PicoLog TC-08 data logger (Pico Technology Limited, UK) linked to a laptop computer. The data logger recorded the temperature of the thermocouple every second and this information was recorded on the laptop using PLW Recorder software 5.16.2. The software facilitated the transfer of the data into a Microsoft Office Excel spreadsheet (version 2007) for processing. Figure 2.1 illustrates the experimental setup.

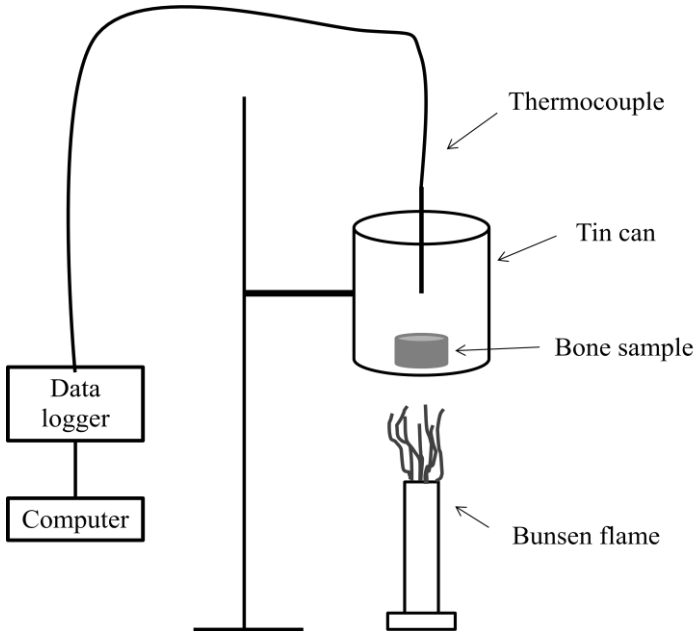

Figure 2.1 *Diagram of the experimental setup.*

The thermocouple was removed ten seconds prior to the desired heating time so that the lid of the tin could be placed on the tin at the required time in order to extinguish any flame by depriving it of oxygen. The lid was removed immediately after flame extinction. After burning, the tin was allowed to cool to room temperature for one minute, and then sealed using the lid on to which a 0.5 x 20 mm ACS was attached as previously described. Each experiment was carried out six times to determine the repeatability of the method.

2.2.4.2 Preliminary burning experiments

Pieces of bone of approximately 6 g to 10 g were combusted according to the general experimental method for ten minutes. The combustion generated smoke, and the sample ignited around 350 °C. The flame was sustained for two to four minutes before it self-extinguished. This experiment was carried out in order to determine the general characteristics of the burning process for the bone sample in terms of its temperature profile.

2.2.4.3 Preliminary studies of pyrolysis products generation

Pieces of bone between 4 g to 8 g were combusted as described in the general experimental method. Samples were combusted for two minutes in total, two minutes after the ignition of the sample and finally until self-extinction of the flame occurred. When the bone was combusted until self-extinction, the flame was sustained for two to four minutes.

The samples were extracted as described in Section 2.2.3 and analysed by GC-MS. These experiments were carried out to crudely identify the time range for more specific refinement for the method so that a final sample preparation method could be established.

2.2.4.4 Preliminary studies of the variation and optimisation of the sample exposure time and weight

Initially, six bone samples with average weight of 4.36 g (± 1.18 g) were prepared according to the general experimental method described previously. Each sample was allowed to continue to burn for two minutes after ignition. Samples were prepared for passive headspace analysis and subsequently extracted and analysed using the GC-MS method previously detailed.

The sample exposure time was further refined based on the results obtained in the initial studies. It became obvious that the sample weight and combustion conditions (length of time) had an effect on the resultant chromatogram. In order to explore this further, samples between 3 g and 21 g were exposed to each of the combustion conditions listed in Table 2.1. For each condition, six repetitive samples of varying weight were prepared according to the general experimental method described previously. Samples were prepared for passive headspace analysis and subsequently extracted and analysed using the GC-MS method previously detailed.

Experiment	Combustion conditions	Weight range
1	Temperature reaches 150 °C – 180 °C	3 – 6 g
2	Temperature reaches 250 °C – 290 °C	3 – 8 g
3	Ignition of the bone	2 – 8 g
4	1 minute after ignition	3 – 7 g
5	2 minutes after ignition	3 – 7 g
6	3 minutes after ignition	4 – 10 g
7	4 minutes after ignition	9 – 13 g
8	5 minutes after ignition	12 – 21 g

Table 2.1 *Sample exposure conditions for the eight experiments.*

2.3 Discussion and results

2.3.1 Instrumental repeatability

The standard mixture was used to determine the parameters such as the peak resolution, peak asymmetry and repeatability of the instrumental analysis and the results are presented in Figure 2.2 and Table 2.2.

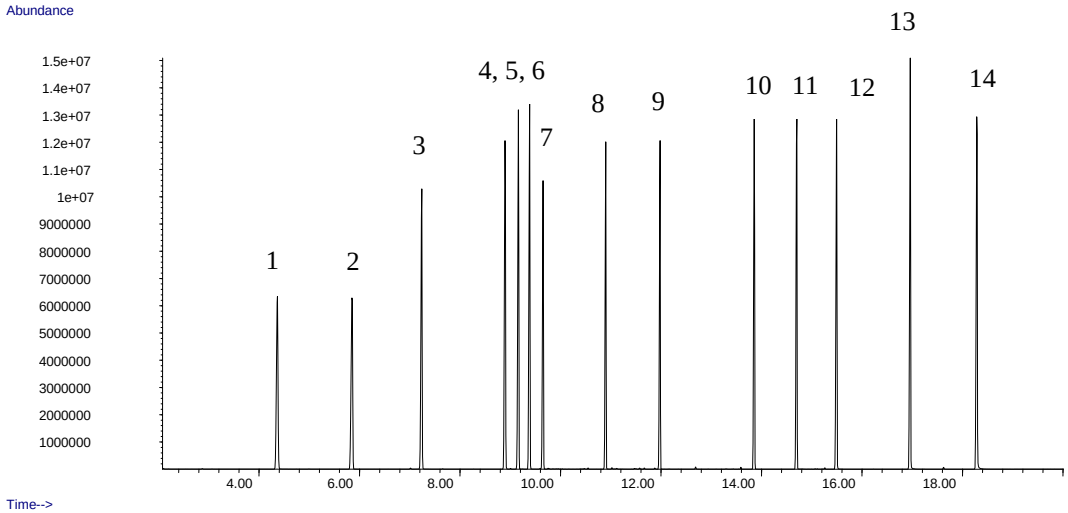

Figure 2.2 *Total ion chromatogram of 1 mg/mL standard mixture in pentane. The numbered peaks are listed in Table 2.2.*

Peak	Compound	Retention time (min)	Average peak resolution	Average asymmetric factor	%RSD	
					Original data	Normalised data
1	Toluene	4.365	14.21	0.72	0.80	0.50
2	C_8	5.853	14.21	0.54	1.00	0.71
3	p-Xylene	7.239	14.78	0.48	0.54	0.25
4	m-Ethyltoluene	8.895	20.38	0.72	0.52	0.28
5	o-Ethyltoluene	9.161	3.75	0.60	0.63	0.38
6	1,2,4-Trimethylbenzene	9.386	3.9	0.49	0.55	0.33
7	C_{10}	9.652	3.98	0.61	0.92	0.71
8	C_{11}	10.901	19.53	0.51	0.77	0.62
9	C_{12}	11.982	17.77	0.47	0.65	0.64
10	C_{14}	13.856	30.17	0.62	0.94	0.93
11	C_{15}	14.701	13.05	0.56	0.50	0.57
12	C_{16}	15.496	11.63	0.50	0.59	0.59
13	C_{18}	16.960	19.77	0.55	1.01	1.32
14	C_{20}	18.284	15.51	0.68	0.75	1.02

Table 2.2 *Retention time, peak resolution, asymmetric factor and percent relative standard deviation of the peak areas original data and the normalised to the total peak area data of the fourteen compounds present in the standard mixture (n=6).*

The calculated resolution demonstrated that all the peaks were completely resolved. The values of the asymmetric factor for each peak were evaluated using the HP Chemstation software. The values showed that all the peaks had a tailing factor under one, which suggested that none of the peaks were tailing. These results illustrated the good efficiency of the column. The percentage relative standard deviation (%RSD) of each compound across six injections varied from 0.50% to 1.01%, which revealed a good repeatability of the instrument. When the individual peak areas were normalised to the total peak area the %RSD generally improved slightly as expected.

Burning experiments were carried out sequentially in order to understand the general burning behaviour of bones including the weight of material and the time of burning required to produce pyrolysis products.

2.3.2 Determination of the bone burning profile

Dehaan *et al.* [50] stated that animal fat melts at relatively low temperatures and as a consequence can contribute to the fuel of a compartment fire. This was corroborated in the experiments carried out examining the general characteristics of burning of a bone sample. In each case when the temperature of the sample reached the auto-ignition temperature of pork fat, (355 °C [47]), ignition of the fat occurred. The underlying bone was in direct contact with the flames which, in turn, were sustained by the melted fat that acted as a fuel. Self-extinction of the flame occurred when no fuel, or fat, was left to sustain the flame. This generally occurred after two to four minutes. Figure 2.3 presents the typical variation of the bone temperature over a ten minute period.

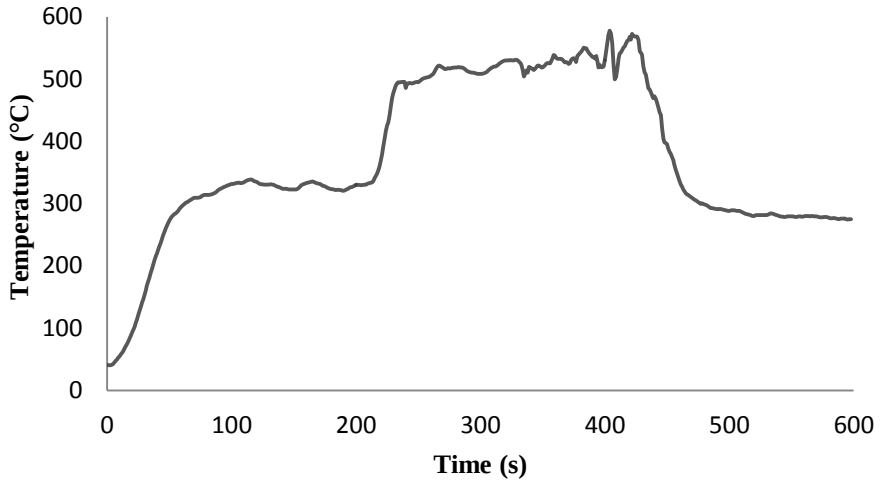

Figure 2.3 *Variation of the bone temperature over ten minutes. The flame was sustained after ignition for over three minutes.*

The temperature rapidly increased, from 40 °C to 300 °C within one minute, and then stabilised around 330 °C for approximately two minutes, time during which the fat associated with the bone was observed to melt. The ignition of the fat was characterised by a sharp rise in temperature, from 330 °C to 490 °C within twenty seconds. The temperature then levelled off between 500 °C and 550 °C as the fat sustained the flame for over three minutes. Once the flame self-extinguished, the temperature of the bone gradually decreased and then stabilised around 300 °C.

When the samples were extracted and analysed by GC-MS no pyrolysis products were detected by GC-MS in any sample.

2.3.3 Production of pyrolysis products

2.3.3.1 Preliminary studies of pyrolysis product generation

Pyrolysis products were extracted when the bone was left to burn for two minutes only (Figure 2.4) and for two minutes after ignition (Figure 2.5) which was approximately four to seven minutes total burning time. These products were produced in each of the six repeat samples. No peaks were detected when the bone was combusted until self-extinction of the flame (approximately six to eight minutes total burning time).

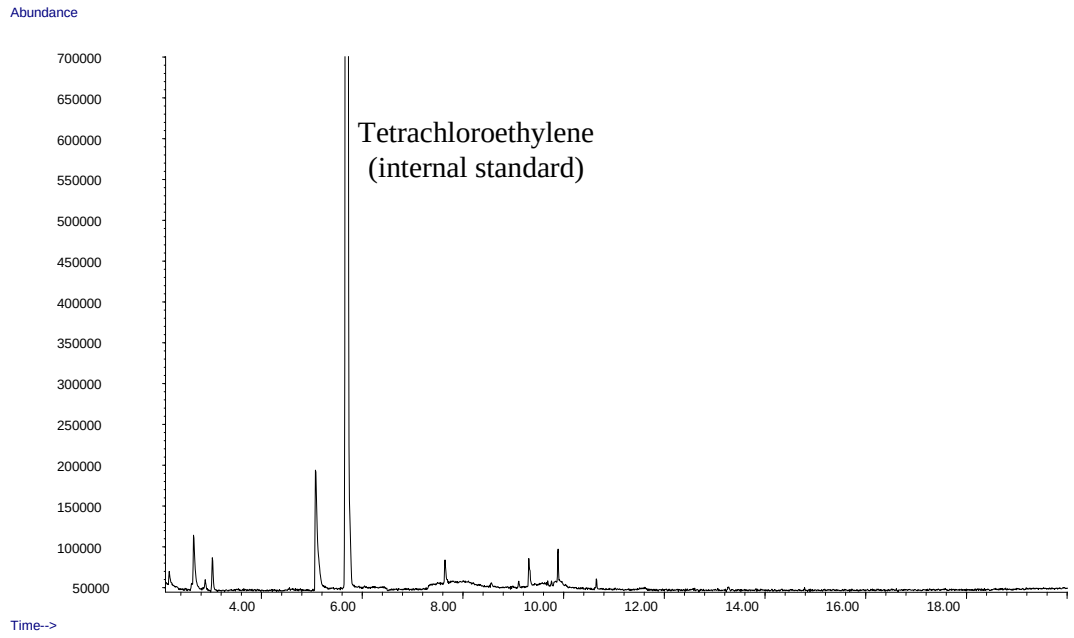

Figure 2.4 *GC-MS total ion chromatogram of bone (5.89 g) combusted for two minutes with tetrachloroethylene as the internal standard.*

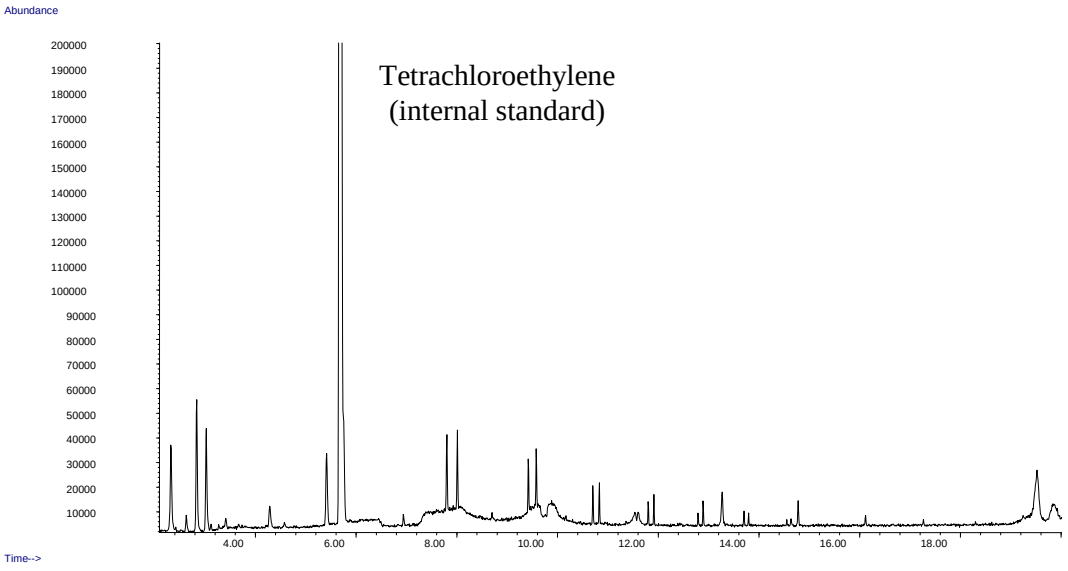

Figure 2.5 *GC-MS total ion chromatograph of bone (5.64 g) combusted for two minutes after ignition.*

The lack of result for the self extinguishing samples may be explained by the fact that when the bone was combusted in these cases, all organic molecules were consumed and as such pyrolysis products were no longer generated. In addition to this, it may be the case that the metallic surface of the sample tin was not contusive to the retention of pyrolysis product and this was further compounded by the fact that the sample was left exposed to the air for one minute before sealing the tin, which may have contributed to the loss of pyrolysis products.

These experiments demonstrated that it was possible to produce pyrolysis products from the bone samples using the devised experimental setup. The next set of experiments refined the pyrolysis product generation process in terms of the heat exposure time and weight of the bone samples.

2.3.3.2 Preliminary studies of the variation of the sample exposure time

The temperature profiles for six bone samples (average 4.36 ± 1.18 g) which were allowed to continue to burn for two minutes after ignition are presented in Figure 2.6. The actual ignition time as a function of the individual sample weight is provided in Table 2.3.

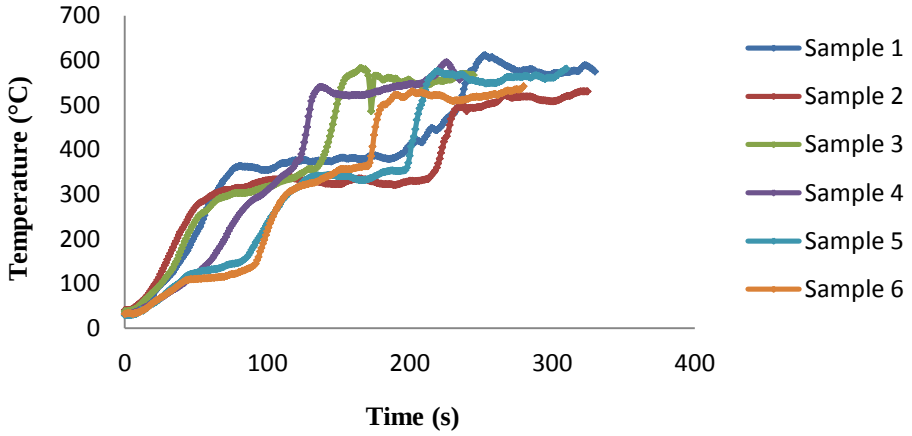

Figure 2.6 *Time temperature profiles of six samples combusted for two minutes after ignition.*

The same general burning behaviour was observed for all six samples with a stabilisation of the temperature around 350 °C before ignition of the bone fat and at 550 °C after ignition.

Sample	Bone weight (g)	Ignition time (min)
1	5.43	3.67
2	6.00	3.58
3	4.36	2.25
4	3.85	2.08
5	2.81	3.33
6	3.71	2.83

Table 2.3 *Quantities and ignition times of the six bone samples.*

Samples 1 and 2 involved a greater sample weight and were slightly slower to ignite than the other samples. Samples 3, 4 and 6 used smaller quantities of sample and this was reflected in shorter ignition times. Sample 5 did not follow the general trend of the other samples. According to the results, it can be suggested that the variations in the amount of time needed to reach the ignition of bone fat between samples may partly be due to quantity of sample present.

This difference could also be due to the variation of bone composition between samples. Although the variation in composition was reduced by using only the diaphysis of the femur and pieces from the same bone in all experiments, variations in composition still occurred between pieces of the same femur. The extremities of the diaphysis contained more marrow and less cortical bone than the middle part of the diaphysis.

2.3.3.3 Preliminary studies of the variation and optimisation of the sample exposure time and weight

As a result of the variation in the time of ignition, a set of time and sample weight optimisation experiments were undertaken which varied in the length of time post ignition for which bone samples of different weight were allowed to continue to burn. The time variation and the bone weight range for each experiment are presented in Table 2.4.

Experiment	Combustion conditions	Weight range
1	Temperature reaches 150 °C – 180 °C	3 – 6 g
2	Temperature reaches 250 °C – 290 °C	3 – 8 g
3	Ignition of the bone	2 – 8 g
4	1 minute after ignition	3 – 7 g
5	2 minutes after ignition	3 – 7 g
6	3 minutes after ignition	4 – 10 g
7	4 minutes after ignition	9 – 13 g
8	5 minutes after ignition	12 – 21 g

Table 2.4 *List of the temperatures and the post ignition timings investigated.*

The volatile compounds produced at each stage of the combustion process described in Table 2.4 were studied in order to determine the burning length and sample weight that would generate the greatest amount of pyrolysis products. The bone was heated until it reached the desired temperature or time after ignition. For each experiment the presence or absence of volatile compounds detected as a function of the sample weight is provided in Table 2.5.

Experiment 1						
Sample	1	2	3	4	5	6
Weight (g)	3.35	4.62	4.13	5.79	5.45	5.81
Compounds detected	✓	✓	✓	✓	✓	✓
Experiment 2						
Sample	1	2	3	4	5	6
Weight (g)	7.62	7.65	5.89	3.36	4.29	4.12
Compounds detected	✓	✓	✓	×	×	×
Experiment 3						
Sample	1	2	3	4	5	6
Weight (g)	4.28	3.47	7.48	4.87	3.94	3.73
Compounds detected	✓	✓	✓	✓	✓	✓
Experiment 4						
Sample	1	2	3	4	5	6
Weight (g)	5.43	6.60	4.36	3.85	2.81	3.71
Compounds detected	✓	✓	✓	✓	×	✓
Experiment 5						
Sample	1	2	3	4	5	6
Weight (g)	5.42	6.00	4.52	3.54	3.78	4.10
Compounds detected	✓	✓	×	×	×	×
Experiment 6						
Sample	1	2	3	4	5	6
Weight (g)	6.36	4.74	4.24	8.44	9.89	9.74
Compounds detected	✓	×	×	✓	✓	✓
Experiment 7						
Sample	1	2	3	4	5	6
Weight (g)	12.74	10.24	10.13	10.90	9.41	10.25
Compounds detected	✓	✓	×	✓	×	✓
Experiment 8						
Sample	1	2	3	4	5	6
Weight (g)	12.81	19.14	20.22	12.94	17.86	19.27
Compounds detected	✓	✓	✓	×	✓	✓

Table 2.5 *Determination of the presence (✓) or absence (×) of volatile compounds of the six bone samples from each of the eight experiments.*

Volatile compounds were detected under the conditions used in experiment 1 for bone samples weighing between 3 g and 6 g. For experiment 2, no compounds were detected when the bone sample weighed less than 4.29 g. When the experiments were carried using the combustion conditions of experiment 3, volatile species of bone samples weighing between 3 g and 8 g were detected. For experiment 4 no compounds were detected when the bone sample weighed 2.81 g. Volatile compounds were not detected for experiments 5 and 6 when the bone sample respectively weighed less than 4.52 g and 4.74 g. Finally, compounds were present for experiments 7 and 8 when the bone sample respectively weighed above 10.13 g and 12.94 g. The volatile compounds detected from bone samples obtained for each experiment are presented in the Figures 2.7 to 2.14.

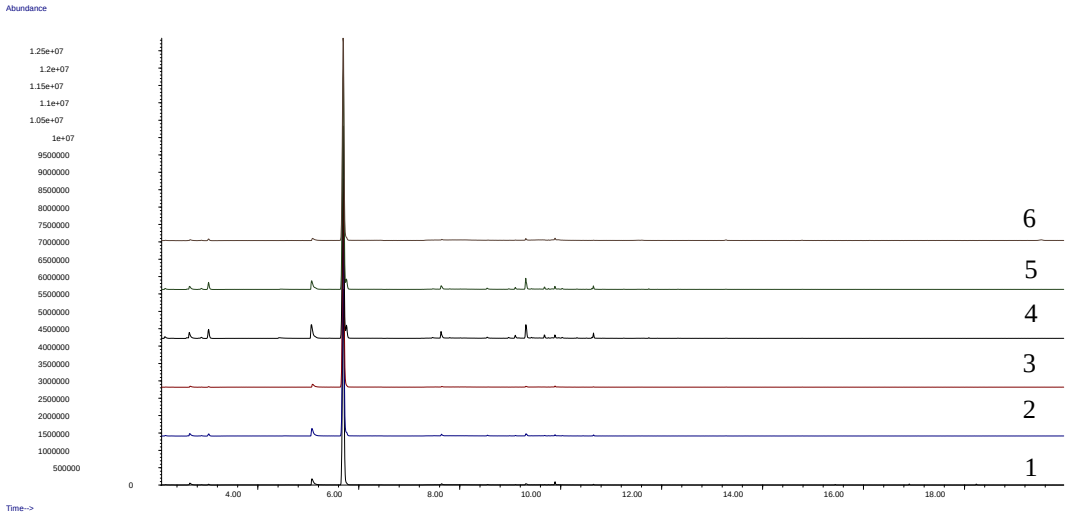

Figure 2.7 *Overlay of the six chromatograms of bones combusted until their temperature reached 150 °C to 180 °C (experiment 1).*

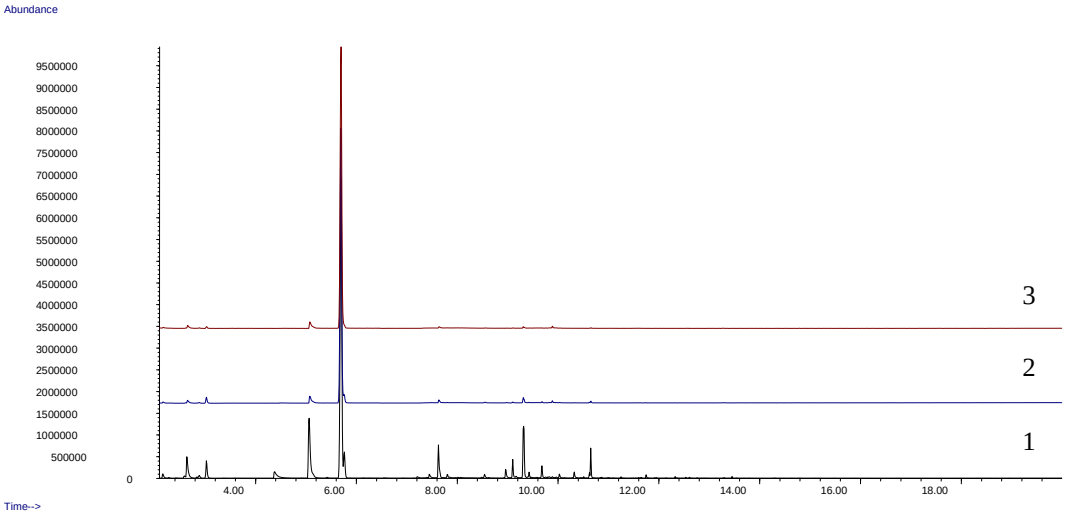

Figure 2.8 *Overlay of the three chromatograms of bones combusted until their temperature reached 250 °C to 290 °C (experiment 2).*

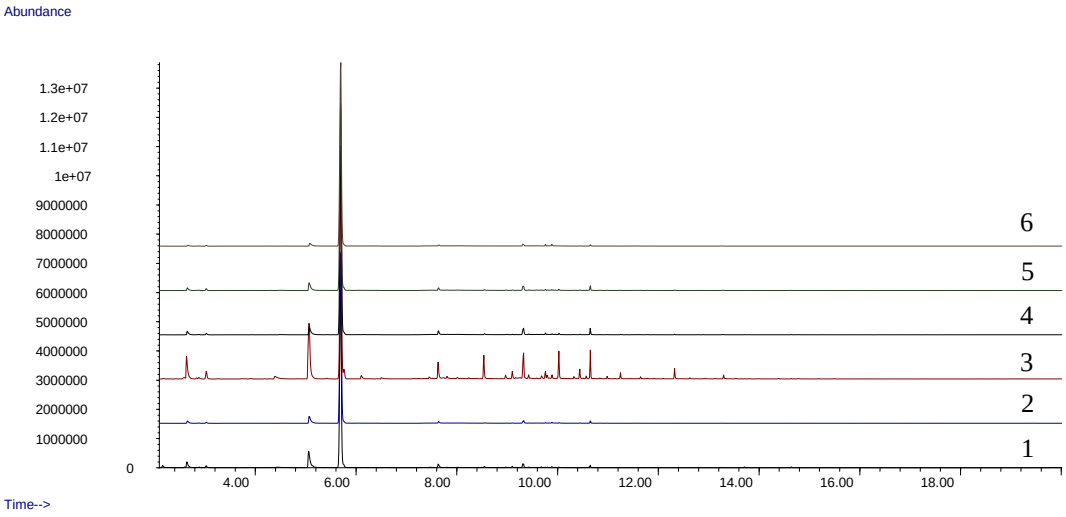

Figure 2.9 *Overlay of the six chromatograms of bones combusted until ignition occurred (experiment 3).*

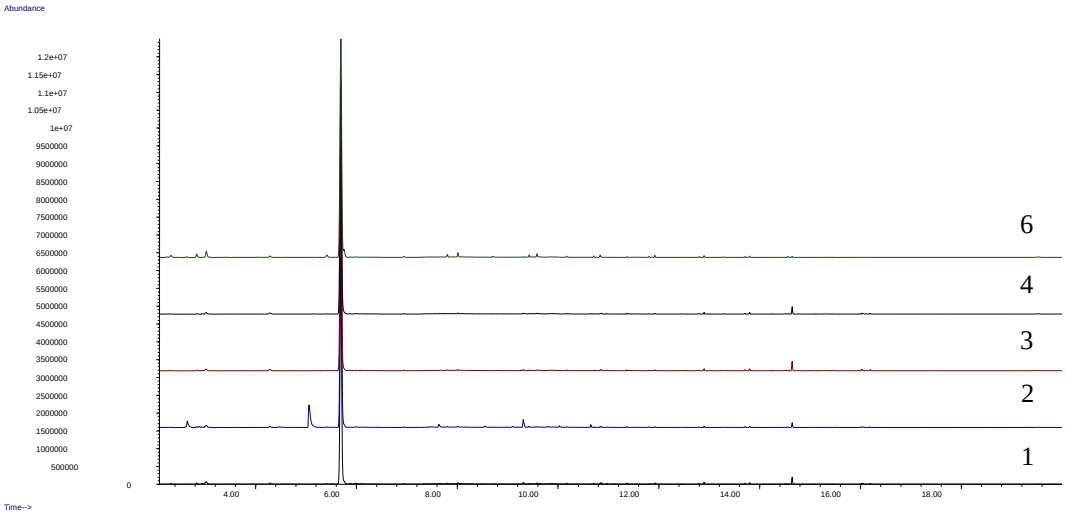

Figure 2.10 *Overlay of the five chromatograms of bones combusted for one minute after ignition (experiment 4).*

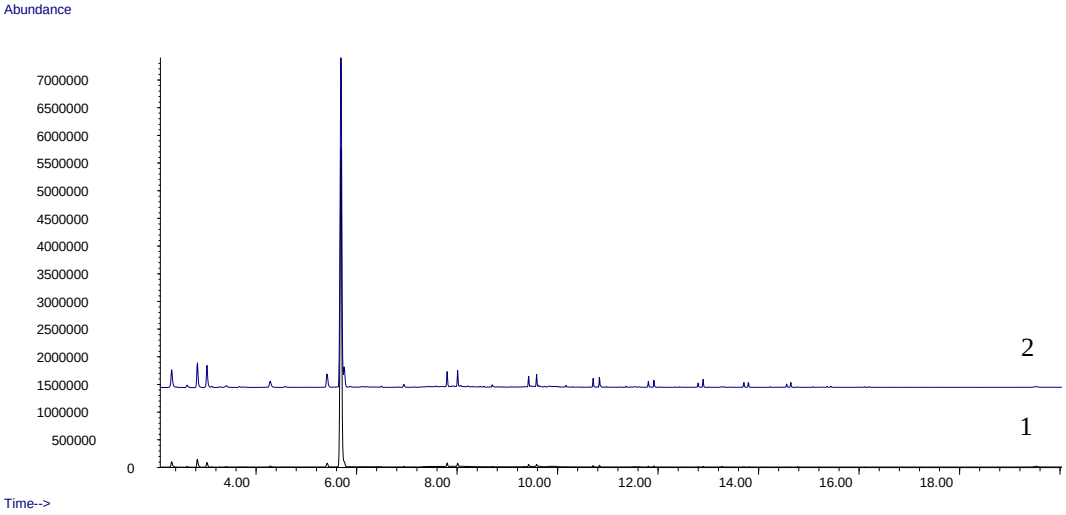

Figure 2.11 *Overlay of the two chromatograms of bones combusted for two minutes after ignition (experiment 5).*

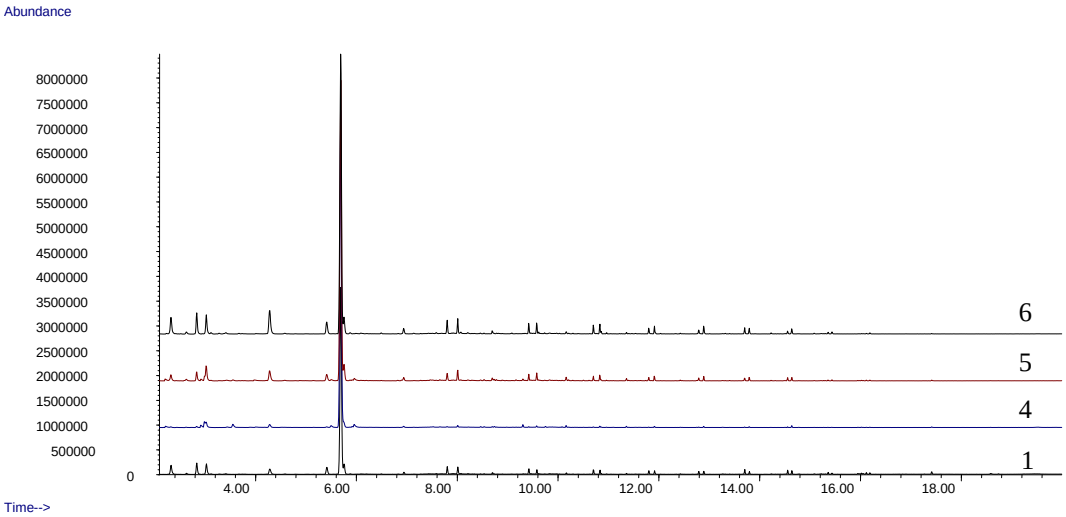

Figure 2.12 *Overlay of the four chromatograms of bones combusted for three minutes after ignition (experiment 6).*

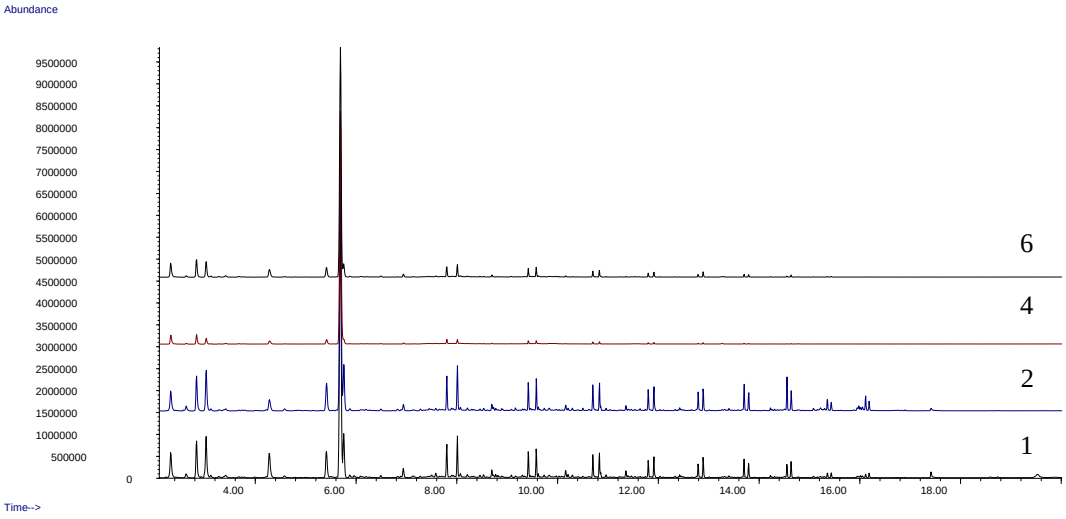

Figure 2.13 *Overlay of the four chromatograms of bones combusted for four minutes after ignition (experiment 7).*

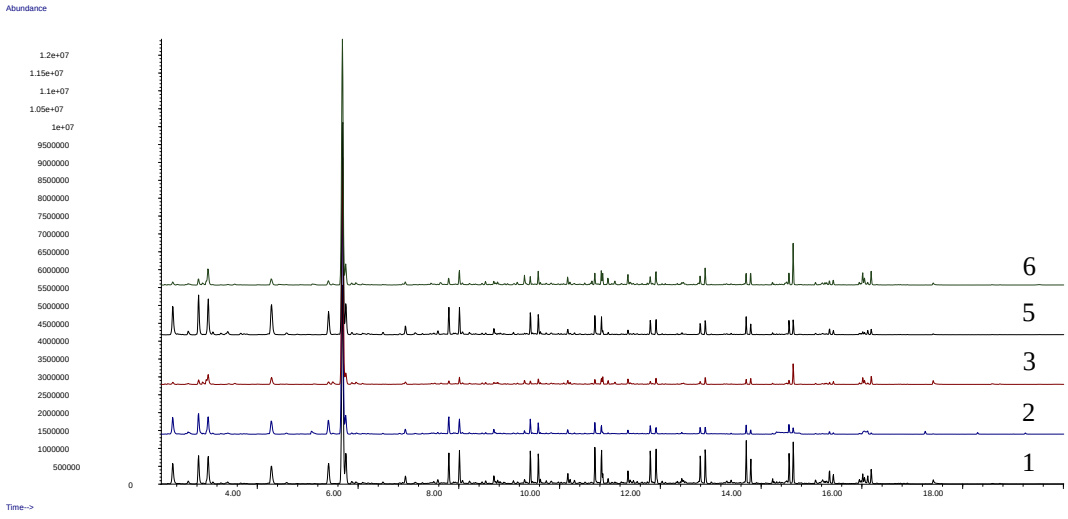

Figure 2.14 *Overlay of the five chromatograms of bones combusted for five minutes after ignition (experiment 8).*

The weight of sample used had an influence on the generation of pyrolysis products. The weight used also had an influence on the duration of burning between the ignition of fat and self-extinction of the flame. As the burning time increased, the amount of fat needed to sustain the flame for a longer period of time had to be greater, thus the weight of sample required was greater. When the weight of the sample was insufficient, the flame self-extinguished before the desired time, and no pyrolysis products were extracted.

Table 2.6 illustrates the final minimum weight of sample required for each set of experiments in order to generate measureable pyrolysis products. Samples were not combusted for longer than five minutes after ignition as it required a sample size which was too large to be accommodated within the sampling tin.

Experiment	Combustion phase	Minimum weight (g)
1	Temperature reaches 150°C - 180°C	3
2	Temperature reaches 250°C - 290°C	5
3	Ignition of the bone	5
4	1 minute after ignition	5
5	2 minutes after ignition	6
6	3 minutes after ignition	7
7	4 minutes after ignition	11
8	5 minutes after ignition	13

Table 2.6 *Minimum quantity of bone required to generate pyrolysis products for each experiment.*

The resultant chromatograms obtained at each optimised weight for the relevant temperature and post ignition time are provided in Figure 2.15.

It is important to note that due to the overlay of the chromatograms the abundance (y-axis) is not representative of the peak areas. Nevertheless, the overlay of the different chromatograms clearly indicates that the peaks areas and the amount of pyrolysis products produced depended on the length of time the samples had been allowed to burn.

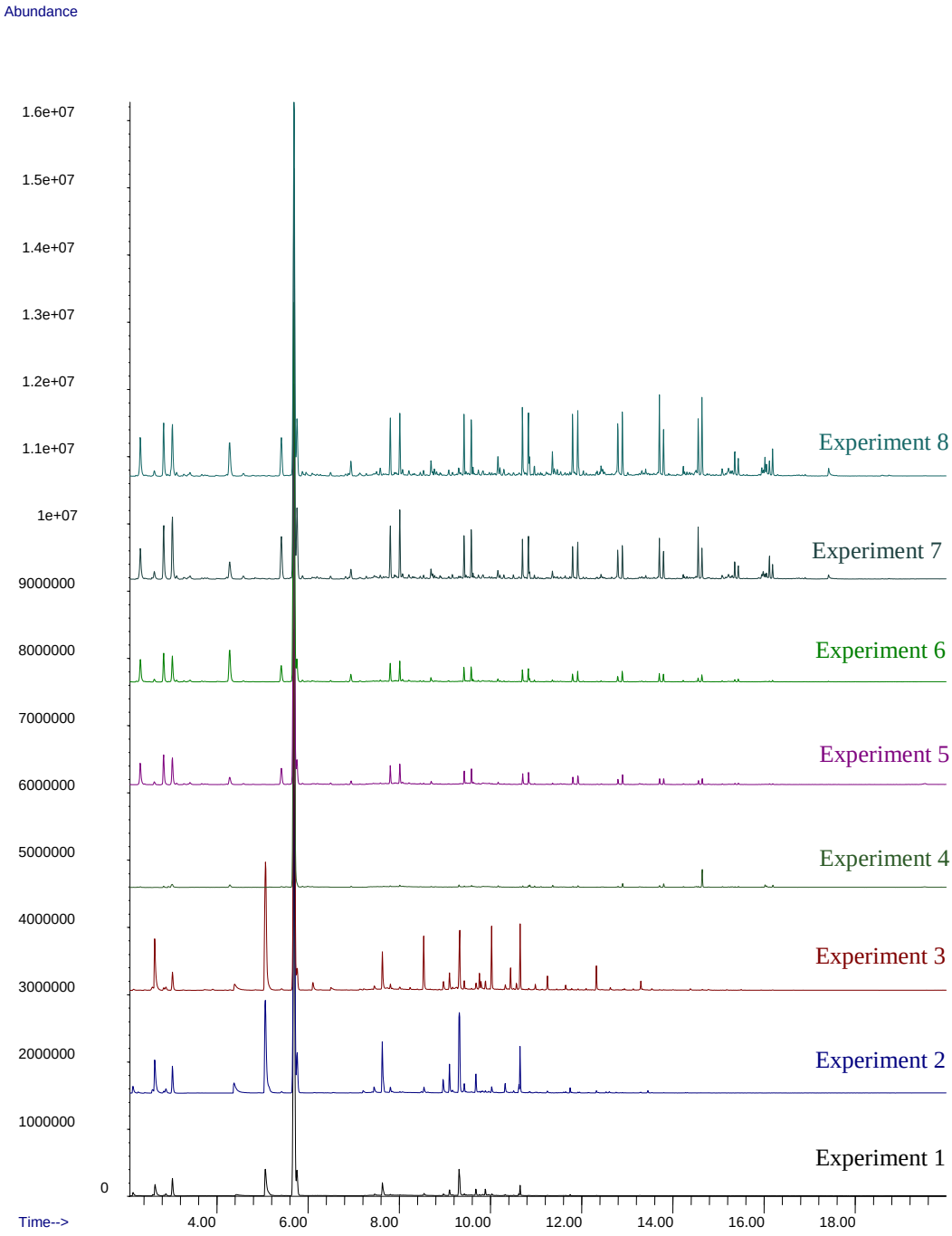

Figure 2.15 *Overlay of the GC-MS total ion chromatograms for samples from each of the eight experiments.*

The chromatograms of the samples obtained before ignition (experiments 1 to 3) and after ignition (experiments 4 to 8) were clearly different. The chromatogram of bone combusted after ignition at 550 °C may correspond more to that recovered from fire debris where the burning temperature can reach 600 °C [10], than the chromatogram obtained before ignition at 350 °C, though this is very case specific. For this reason, the chromatogram obtained when the bone was combusted after ignition was considered for the investigation of the thermal degradation products.

Experiments 7 and 8 generated a higher concentration of compounds eluting after fifteen minutes which can clearly be seen in Figure 2.16. Thus, the high-boiling point compounds present at the end of the chromatogram took more time to volatilise than the low-boiling point compounds at the front end of the chromatogram, and were detected when the sample was combusted for a certain period of time [10].

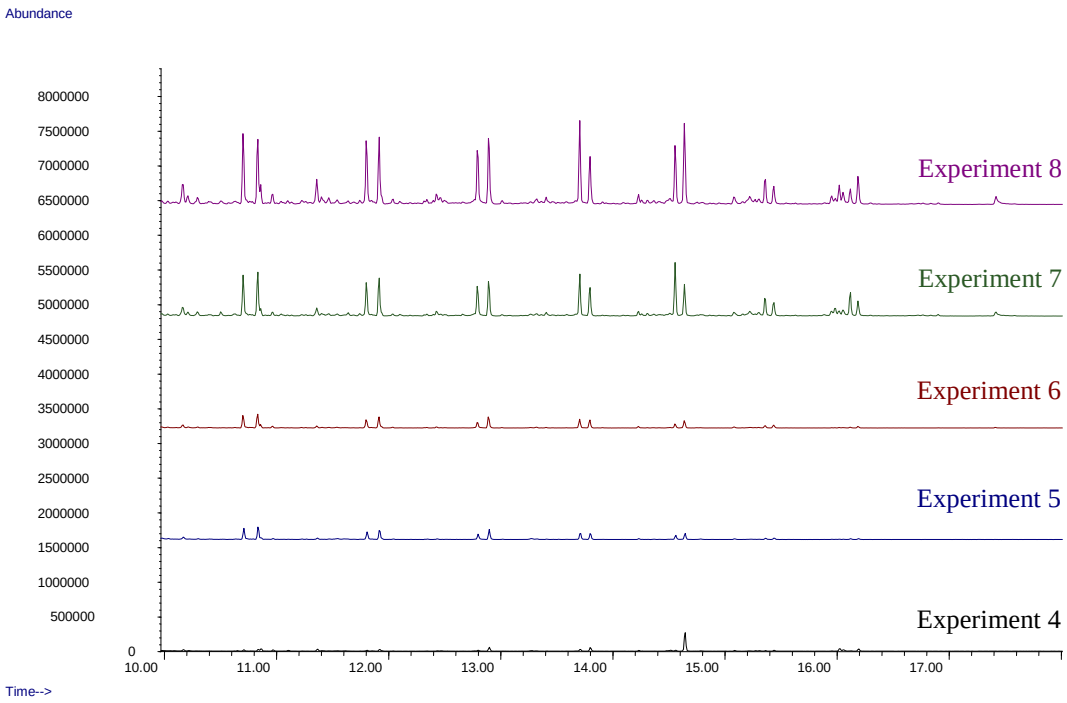

Figure 2.16 *Overlay of the GC-MS total ion chromatograms from ten minutes to eighteen minutes of experiments 4 to 8.*

As the burning time after ignition increased, more volatile compounds were produced and at a higher intensity as would be expected with the TIC response being optimum when the bone sample was combusted for five minutes after ignition (experiment 8). If the sample was allowed to burn for longer than this time (until self-extinction of the flame) no TIC response was observed.

Thus, the samples which provided the best chromatographic results occurred when approximately 13 g to 20 g of sample was allowed to burn for five minutes post ignition which usually occurred within three or six minutes providing a total heat exposure time of approximately eleven minutes.

2.3.3.4 Determination of the repeatability of the optimised experimental method

The experimental conditions of the six bone samples combusted for five minutes after ignition are listed in Table 2.7 below.

Sample	Weight (g)	Total burning time (min)
1	15.50	7.7
2	15.15	6.5
3	15.66	7.8
4	20.02	8.0
5	18.19	7.4
6	20.51	8.7

Table 2.7 *Experimental conditions of the six defleshed bone samples combusted for five minutes after ignition.*

The compounds investigated to determine the repeatability of the experimental method were benzene, 1-heptene, toluene, 1-nonene, nonane, 1-decene, decane, 1-undecene, 1-dodecene, 1-tridecene, tridecane, 1-tetradecene, tetradecane, 1-pentadecene, pentadecane, 1-hexadecene, hexadecane, 1-heptadecene, heptadecane and tetrachloroethylene (internal standard), as shown in Figure 2.17.

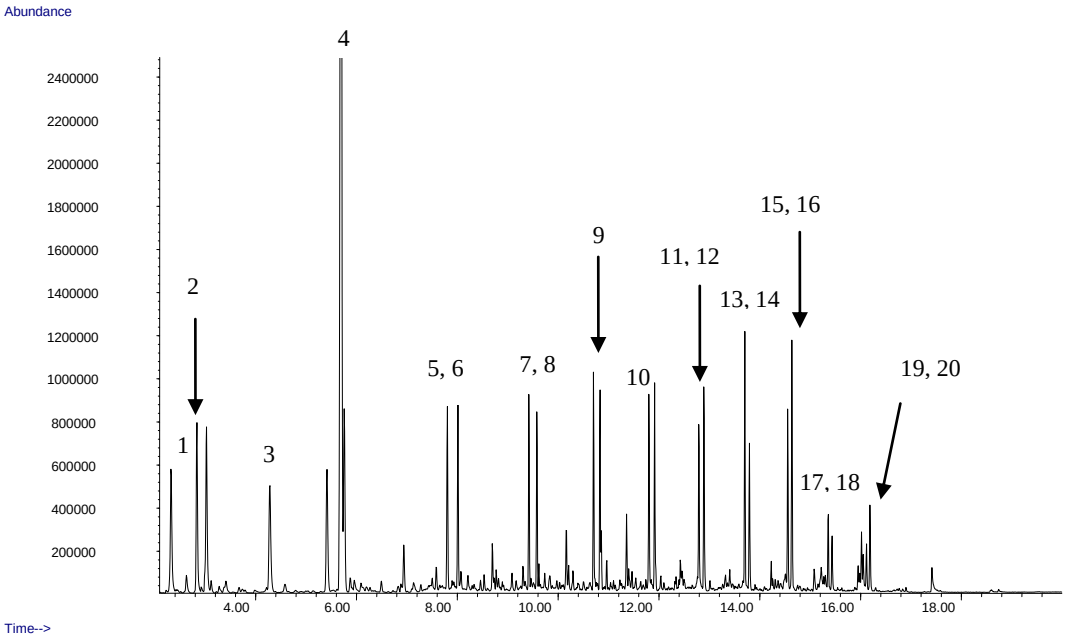

Figure 2.17 *Total ion chromatogram of combusted bone. The numbered peaks are listed in Table 2.8.*

The qualitative repeatability of the experimental method was evaluated by the presence or absence of the selected compounds in the six chromatograms. The overlay of the chromatograms of bone shown in Figure 2.18 illustrated the consistency of the six chromatograms that presented the same peaks at different intensities as shown in Table 2.8. This observation demonstrated the good qualitative repeatability of the final experimental conditions.

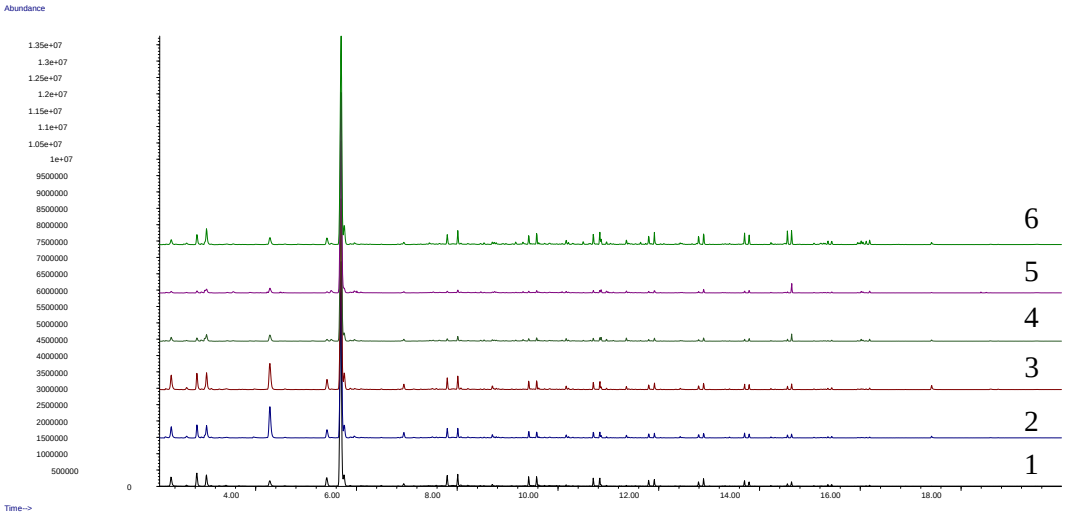

Figure 2.18 *Overlay of the chromatograms of the six defleshed bone samples combusted for five minutes after ignition.*

Peak	Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
1	Benzene	✓	✓	✓	✓	✓	✓
2	1-Heptene	✓	✓	✓	✓	✓	✓
3	Toluene	✓	✓	✓	✓	✓	✓
4	Tetrachloroethylene	✓	✓	✓	✓	✓	✓
5	1-Nonene	✓	✓	✓	✓	✓	✓
6	Nonane	✓	✓	✓	✓	✓	✓
7	1-Decene	✓	✓	✓	✓	✓	✓
8	Decane	✓	✓	✓	✓	✓	✓
9	1-Undecene	✓	✓	✓	✓	✓	✓
10	1-Dodecene	✓	✓	✓	✓	✓	✓
11	1-Tridecene	✓	✓	✓	✓	✓	✓
12	Tridecane	✓	✓	✓	✓	✓	✓
13	1-Tetradecene	✓	✓	✓	✓	✓	✓
14	Tetradecane	✓	✓	✓	✓	✓	✓
15	1-Pentadecene	✓	✓	✓	✓	✓	✓
16	Pentadecane	✓	✓	✓	✓	✓	✓
17	1-Hexadecene	✓	✓	✓	✓	✓	✓
18	Hexadecane	✓	✓	✓	✓	✓	✓
19	1-Heptadecene	✓	✓	✓	✓	✓	✓
20	Heptadecane	✓	✓	✓	✓	✓	✓

Table 2.8 *Determination of the presence (✓) or absence (✗) of the selected compounds from six defleshed bones.*

The quantitative reproducibility of the method was assessed by reviewing the peak area relative standard deviations of the twenty compounds previously mentioned identified on the TIC of combusted bone calculated across six repeat samples. The relative standard deviation was calculated from the peak areas of the original data, peak areas normalised to the internal standard data and peak areas normalised to the total peak area data (Table 2.9).

Peak	Compound	%RSD		
		Original data	Normalised to internal standard	Normalised to total peak area
1	Benzene	65.87	69.21	62.02
2	1-Heptene	61.18	63.80	58.41
3	Toluene	90.49	96.16	87.77
4	Tetrachloroethylene	6.55	0.00	13.50
5	1-Nonene	58.87	60.28	56.22
6	Nonane	51.16	51.12	45.13
7	1-Decene	56.03	56.37	52.13
8	Decane	55.26	53.82	49.34
9	1-Undecene	53.28	51.54	46.47
10	1-Dodecene	62.09	59.28	55.49
11	1-Tridecene	67.89	63.33	59.11
12	Tridecane	49.99	46.54	40.89
13	1-Tetradecene	65.96	61.68	57.53
14	Tetradecane	57.21	52.20	46.89
15	1-Pentadecene	115.83	108.84	105.59
16	Pentadecane	52.45	46.72	48.12
17	1-Hexadecene	85.64	79.96	76.40
18	Hexadecane	53.21	48.46	43.11
19	1-Heptadecene	128.15	121.32	117.97
20	Heptadecane	72.51	66.95	63.64

Table 2.9 *Peak area relative standard deviations of twenty compounds among six samples. The %RSD was calculated from the original, the normalised to the internal standard (tetrachloroethylene), and the normalised to the total peak area values.*

The relative standard deviations from the three data sets were high, which demonstrated that the quantity of pyrolysis products varied considerably among the samples.

Even though the samples were combusted and analysed under the same conditions, it was not possible to reproduce the same amount of pyrolysis products among the six repeat samples. The parameters that could have affected the pyrolysis were the oxygen flow, the temperature and the quantity of sample. After ignition, temperatures varied between 500 °C and 600 °C despite the fact that each sample was placed at the same distance from the Bunsen flame. This is not surprising given the nature of the combustion process even under controlled conditions and emphasises the fact that analysis of pyrolysis products from the burning of bone cannot realistically be considered for quantitative analysis.

2.4 Conclusion

An experimental method was developed to produce a significant amount of pyrolysis products of bones. The method involved burning bone pieces of at least 15 g for five minutes after the ignition of the fat present in the bone, which occurred at 350 °C. The flame in the tin was extinguished after five minutes using a lid rather than letting it self-extinguish which did not produce any volatiles. This method gave a good qualitative reproducibility.

Chapter 3 – Interpretation of pyrolysis results from combusted bone samples

3.1 Introduction

When a human body is exposed to heat and flames, it undergoes thermal degradation that produces volatile species. Dehaan *et al.* [47] identified the volatile compounds produced by the combustion of animal and human fat. A CDS Pyrolysis Probe operating in air was used and the volatile products were retained in a vial containing the carbon-strip that was placed over the ribbon heater. The temperature was ramped from ambient to 300 °C, 500 °C or 700 °C at 5 °C/ms, and was held there for ten seconds. Significant pyrolysis products were produced when the furnace was set above the auto-ignition temperature of animal fat, at 355 °C. Homologous series of C_5 to C_{15} n-alkanes, n-alkenes, dienes and n-aldehydes, and also light aromatics were produced by the combustion of animal and human fat. Most of the compounds generated were the same as those produced by the combustion of polyethylene. The difference in compound ratios was used to differentiate fat from polyethylene. For polyethylene, the major peak was the alkene peak, followed by the alkane, diene and aldehyde peaks. The dominant peak for animal and human fat was also the alkene peak, followed by equally sized alkane and aldehyde peaks. The diene peak was either very small or absent. Furthermore, additional fragmentation and oxidation were observed at temperatures above 700 °C, which gave less straight-chain aldehydes. Finally, no differences between pork and human fat volatiles were seen, which confirmed that pork fat could be substituted for human fat in research and forensic casework.

Moreover, Purevsuren *et al.* [51] studied the composition of tar from animal bone pyrolysis produced in Mongolia. Fatty residues were removed from the bone after they were crushed into small pieces. For the pyrolysis of bone, a vertical steel retort was equipped with an air-cooled iron tube and a glass condenser, and was placed in

an electric furnace. The limit temperature was set to 700 °C and ramped at 20 °C/min. The pyrolysis of bone lasted for approximately four to five hours until liquid products, which included tar and pyrolysis water, stopped condensing. The tar was composed of the pyrolysis products of collagen, but not of the fatty residues. The identification of the GC-MS total ion chromatogram (TIC) showed that bone tar produced similar compounds to fat and tissues such as n-alkanes, n-alkenes and light alkyl benzenes, but were also composed of alkyl nitriles, pyridines, pyrroles and amides.

The volatile compounds of bone produced by the optimised experimental method were analysed by GC-MS and identified by library and literature search. Moreover, the species produced by the combustion of fleshed bone using the aforementioned experimental method were compared to the volatiles produced by defleshed bone.

3.2 Experimental conditions

Defleshed bone samples (weighing 4 g to 21 g) were prepared as described in Chapter 2 (Subsection 2.2.2) and combusted under different conditions as described in Table 3.1. After ignition of the fat present in the bone occurred, the flame was extinguished by placing a lid on the tin to deprive it of oxygen. The lid was removed immediately after flame extinction. After burning, the tin was allowed to cool to room temperature for one minute, and then sealed using the lid on to which an ACS was attached. The samples were extracted as described in Chapter 2 (Subsection 2.2.3) and analysed by GC-MS.

Experiment	Combustion phase	Minimum weight (g)
1	Temperature reaches 150 °C – 180 °C	3
2	Temperature reaches 250 °C – 290 °C	5
3	Ignition of the bone	5
4	1 minute after ignition	5
5	2 minutes after ignition	6
6	3 minutes after ignition	7
7	4 minutes after ignition	11
8	5 minutes after ignition	13

Table 3.1 *Combustion conditions for the eight experiments.*

For fleshed bones, the idealised sample preparation was repeated but in this case the flesh was not removed from the bones prior to combustion and subsequent analysis. The bone samples were combusted for five minutes after ignition of the fat present in the bone. Each experiment was carried out six times to determine the repeatability of the method.

3.3 Results and discussion

As it has been mentioned in Chapter 2 (Subsection 2.3.3.3), the chromatograms of the pre ignition and ignition samples were different from those of the post ignition samples. The chromatographic peaks were identified in both cases. However, a further investigation of the pyrolysis products of bone was carried out when combusted was continued after ignition, as the chromatogram obtained may correspond more to that recovered from fire debris.

3.3.1 Peak identification of the pre ignition and ignition samples

The compounds detected were identified by library search with NIST library, and by reference to the previous work done by Dehaan and Purevsuren [47, 49]. Figure 3.1 shows the representative TIC of the pyrolysis compounds of bone obtained at

ignition of the bone fat that presented more volatile compounds than the pre ignition samples (Figure 2.15).

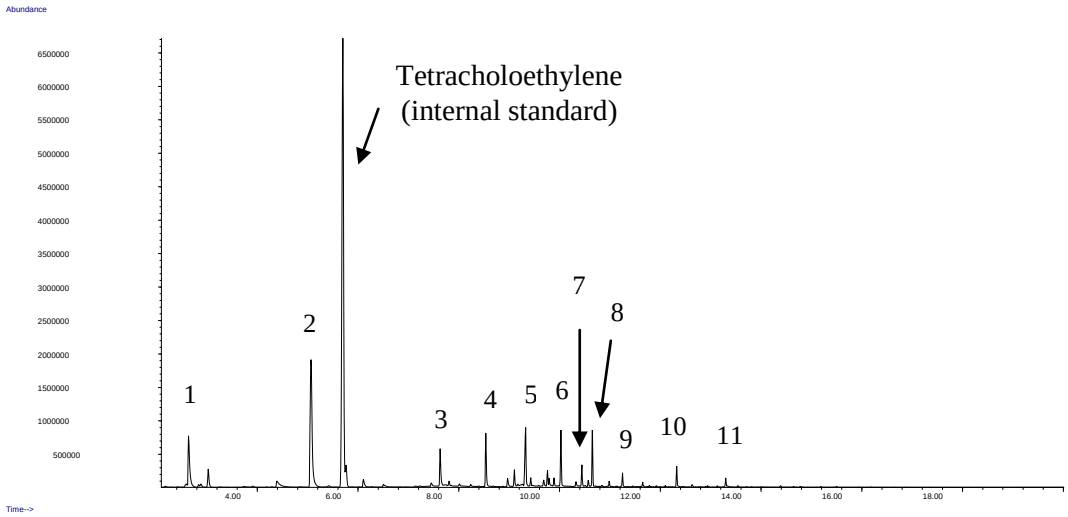

Figure 3.1 *TIC of the bone ignition sample. The numbered peaks are listed in Table 3.2.*

Peak	Retention time (min)	Compound
1	2.635	Pentanal
2	5.065	Hexanal
3	7.621	Heptanal
4	8.528	2-Heptenal
5	9.319	Octanal
6	10.035	2-Octenal isomer
7	10.442	2-Octenal isomer
8	10.659	Nonanal
9	11.250	2-Nonenal
10	12.323	2-Decenal
11	13.297	2-Undecenal

Table 3.2 *Identification of the significant thermal degradation products of bone obtained at ignition from the total ion chromatogram in Figure 3.1.*

Some peaks that were present before the ignition and at the ignition of the bone fat at around 350 °C were no longer present after a temperature of between 500 °C and 600 °C had been achieved (post ignition). These peaks were identified using the NIST library as being n-aldehydes and n-alkenal in Table 3.2. Dehaan [47] identified the n-aldehydes in the TIC of volatile products of animal fat. The other peaks still present post ignition were n-alkanes and n-alkenes, and are listed in Table 3.3. They were also identified by Dehaan in his work. These results suggested that the melted fat produced volatile products before ignition that were similar to those observed by Dehaan.

3.3.2 Peak identification – post ignition samples

Again, the compounds detected in the chromatogram were identified by library search with NIST library, and by reference to the relevant literature. Figure 3.2 shows the representative TIC of the pyrolysis compounds of bone combusted for five minutes after ignition (optimised experimental method).

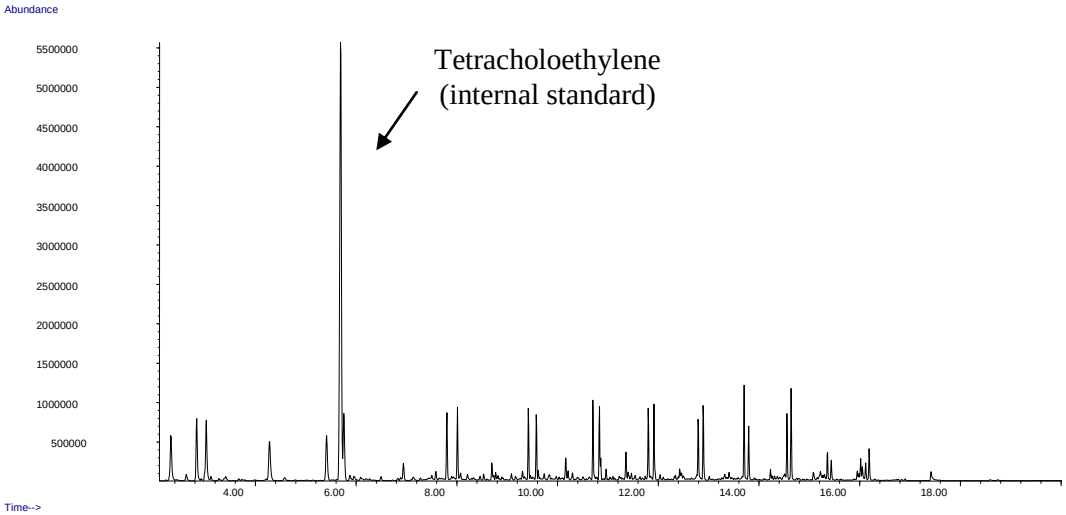

Figure 3.2 *GC-MS total ion chromatogram of bone combusted for five minutes after ignition.*

The pyrolysis of bone post ignition produced C_7 to C_{17} n-alkanes and n-alkenes, aromatics, and nitriles, which have been previously identified in animal fat and bone tar [47, 51]. However, C_1 to C_9 n-alkyl cycloalkanes and cycloalkenes were also produced and have not been previously identified in the literature. Also, when the sample experienced temperatures at approximately 550 °C, the resultant chromatogram illustrated the absence of n-aldehydes, which were presumably consumed at these temperatures. Figures 3.3 to 3.7 represent the extracted ion chromatograms (EIC) of ions with mass-to-charge ratio (m/z) of 55 indicative of alkenes, 57 indicative of alkanes, 83 and 69 indicative of cycloalkanes, and 77, 91 and 104 indicative of aromatics. All identified compounds are presented in Table 3.3.

Homologous series of n-alkanes and n-alkenes were repeated within the chromatogram with a separation conforming to the addition of a methyl group, which, according to Stauffer [10] may be as a result of the random scission pyrolysis process. The presence of n-alkyl cycloalkanes was verified by extracting ions m/z 69 and 83 (Figure 3.5). The major peaks in both the EICs were alkene peaks. This can be explained by the fact that cycloalkanes and alkenes share some indicative ions and that alkenes are present at a greater quantity than cycloalkanes. The EIC of ion m/z 91 in Figure 3.6 clearly showed the presence of C_1 to C_9 alkylbenzenes. The presence of benzene and styrene was noted in the respective EICs of ions m/z 77 and 104 in Figure 3.7. These compounds may be generated by the side group scission of the molecular backbone. The C_4 to C_{17} n-alkyl nitriles were identified by library search. These compounds can be characterised by the fragments ions m/z 41, 82, 110, 124 and 138.

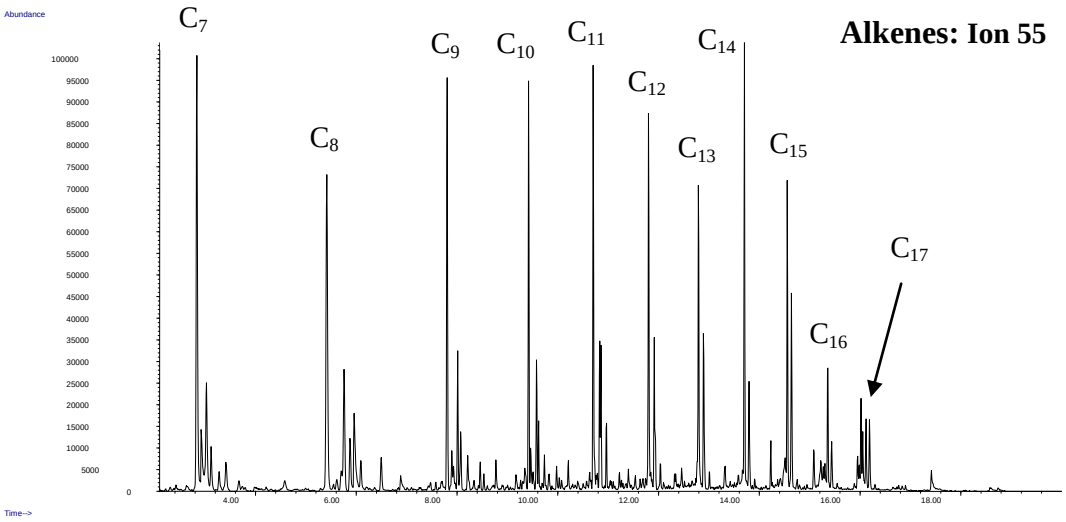

Figure 3.3 *Extracted ion m/z 55 chromatogram of combusted bone.*

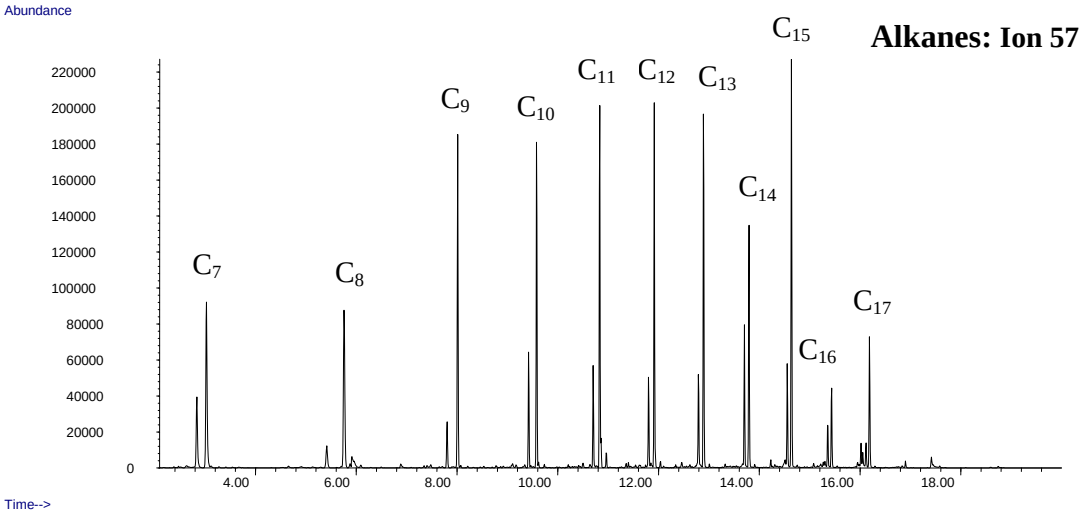

Figure 3.4 *Extracted ion m/z 57 chromatogram of combusted bone.*

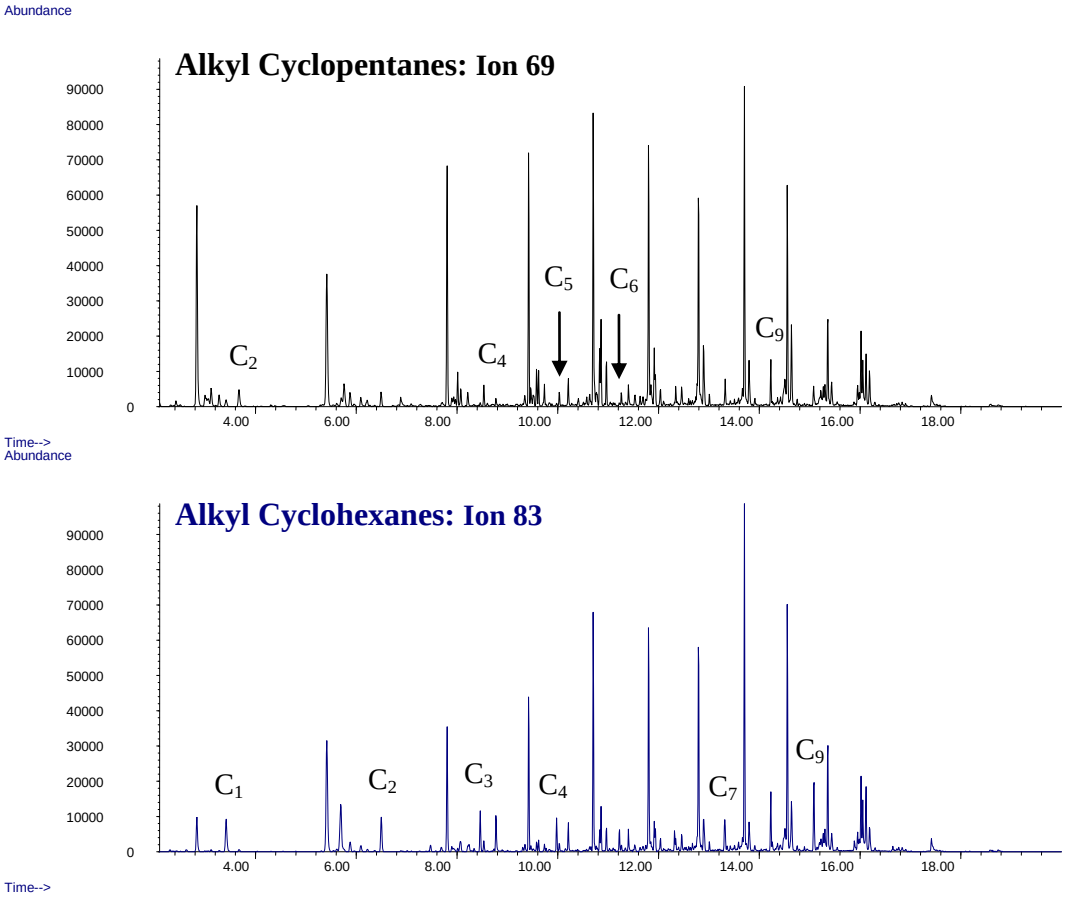

Figure 3.5 *Extracted ions m/z 69 and 83 chromatograms of burn bone.*

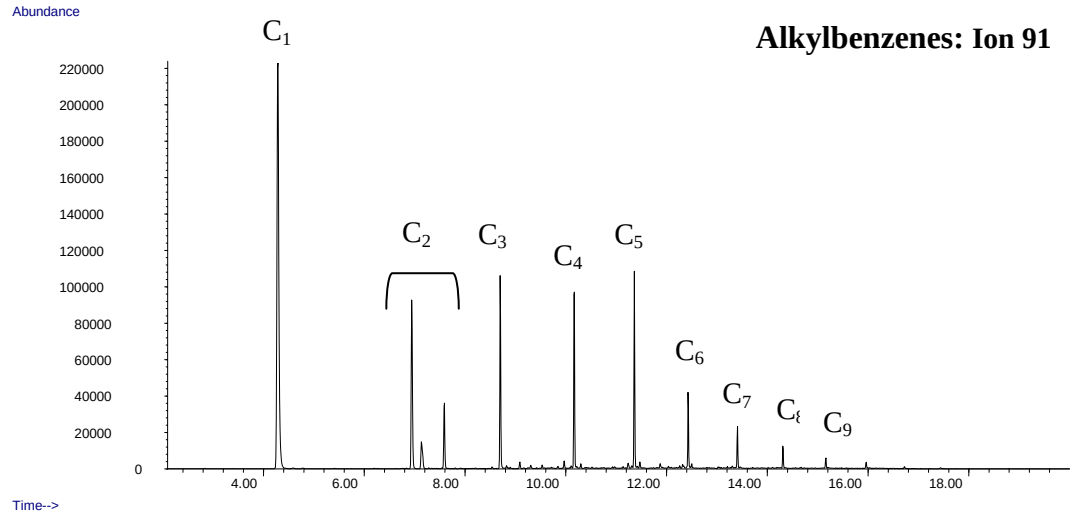

Figure 3.6 *Extracted ion m/z 91 chromatogram of combusted bone.*

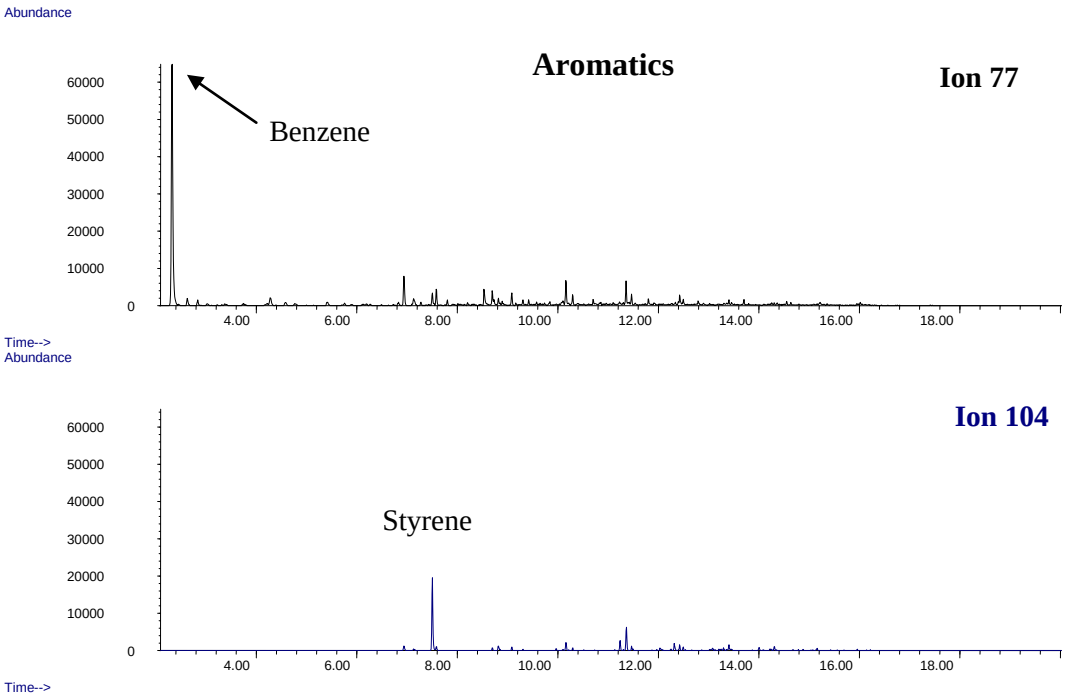

Figure 3.7 *Extracted ions m/z 77 and 104 chromatograms of combusted bone.*

Retention time (min)	Compound	Molecular ion m/z	Fragment ions m/z
2.219	Butanenitrile		41, 29, 39
2.319	Benzene	78	52, 39
2.419	Cyclohexane	84	56, 41, 27
2.627	Cyclohexene	82	67, 54, 39
2.836	1-Heptene	98	56, 41, 29, 70
3.028	Heptane	100	43, 29, 57, 71
3.118	Heptene isomer	98	55, 41, 69
3.276	Heptene isomer	98	41, 56, 69
3.409	Methylcyclohexane	98	55, 83, 41
3.667	Ethylcyclopentane	98	69, 41, 55
4.217	Ethylcyclopentene	96	67, 39, 41
4.283	Toluene	92	91, 65, 39
4.583	Methylcyclohexene	96	81, 68, 55
5.415	1-Octene	112	43, 55, 70, 41
5.607	Octene isomer	112	55, 41, 70, 39

Retention time (min)	Compound	Molecular ion m/z	Fragment ions m/z
5.696	Tetracholoroethylene (internal standard)	164	166, 129, 131
5.756	Octane	114	43, 57, 29, 85
5.873	Octene isomer	112	55, 41, 70, 39
5.956	4-Methylpentanenitrile	97	55, 41, 27, 39
6.089	Octene isomer	112	55, 41, 70, 39
6.497	Ethylcyclohexane	112	83, 55, 41
6.880	Hexanenitrile	97	41, 54, 27
6.938	Ethylbenzene	106	91, 51, 65, 77
7.130	Xylene isomer	106	91, 77, 51
7.280	Ethylcyclohexene	110	81, 67, 41, 79
7.504	Styrene	104	103, 78, 51
7.579	Xylene isomer	106	91, 77, 51
7.804	1-Nonene	126	43, 55, 41, 69
7.896	Nonene isomer	126	55, 41, 69, 43
8.016	Nonane	128	43, 57, 85, 71
8.070	Nonene isomer	126	55, 41, 69, 43
8.212	Nonene isomer	126	55, 41, 69, 43
8.426	Propylcyclohexane	126	83, 55, 41, 67
8.528	Butylcyclopentane	126	41, 69, 55, 39
8.695	Propylbenzene	120	91, 65, 78
8.728	Butylcyclopentene	124	67, 82, 41
8.770	Heptanenitrile	111	41, 82, 27
8.894	Propylcyclohexene	124	81, 67, 95, 41
9.086	Ethyltoluene	120	105, 91, 77
9.308	2-Pentylfuran	138	81, 53, 27, 41
9.344	Decene isomer	140	55, 41, 69
9.424	1-Decene	140	41, 55, 43, 70
9.460	Decene isomer	140	55, 41, 69
9.583	Decane	142	43, 57, 29, 71
9.619	Decene isomer	140	55, 41, 69
9.735	Decene isomer	140	55, 41, 69, 43
9.976	Butylcyclohexane	140	83, 55, 41, 67
10.026	Pentylcyclopentane	140	69, 41, 55, 83
10.168	Butylbenzene	134	91, 65, 105, 39

Retention time (min)	Compound	Molecular ion m/z	Fragment ions m/z
10.209	Octanenitrile	125	82, 41, 96, 54
10.293	Propyltoluene	134	105, 77, 91
10.701	1-Undecene	154	41, 55, 70, 29
10.759	Undecene isomer	154	41, 55, 69, 43
10.834	Undecane	156	57, 43, 71, 85
10.859	Undecene isomer	154	55, 69, 41, 83
10.959	Undecene isomer	154	55, 69, 41, 83
11.100	Hexylcyclopentene	152	67, 82, 41, 95
11.258	Hexylcyclopentane	154	69, 41, 55, 83
11.358	Pentylbenzene	148	91, 65, 105, 41
11.400	Decanenitrile	153	41, 29, 82, 96
11.466	1-Methylbutylbenzene	148	105, 91, 77
11.799	1-Dodecene	168	55, 41, 83, 69
11.849	Dodecene isomer	168	41, 69, 55, 83
11.916	Dodecane	170	57, 43, 71, 85
12.041	Dodecene isomer	168	41, 55, 69, 83
12.424	Hexylbenzene	162	91, 65, 43, 77
12.457	Undecanenitrile	167	41, 29, 55, 97
12.790	1-Tridecene	182	55, 41, 83, 69
12.890	Tridecane	184	57, 43, 71, 85
13.314	Heptylcyclohexane	182	83, 55, 41, 67
13.356	Octylcyclopentene	180	67, 82, 41 95
13.406	Heptylbenzene	176	91, 43, 65, 41
13.664	Tetradecene isomer	196	41, 55, 69, 83
13.705	1-Tetradecene	196	41, 55, 29, 69
13.797	Tetradecane	198	57, 43, 71, 85
14.230	Nonylcyclopentane	196	69, 41, 55, 83
14.305	Octylbenzene	190	91, 41, 57, 65
14.554	1-Pentadecene	210	43, 55, 83, 69
14.638	Pentadecane	212	57, 43, 71, 85
15.079	Nonylcyclohexane	210	83, 55, 41, 67
15.154	Nonylbenzene	204	91, 41, 65, 105
15.270	Hexadecene isomer	224	41, 55, 69, 83
15.303	Hexadecene isomer	224	41, 55, 69, 83

Retention time (min)	Compound	Molecular ion m/z	Fragment ions m/z
15.362	1-Hexadecene	224	41, 55, 83, 69
15.437	Hexadecane	226	57, 43, 71, 85
15.628	Nonylcyclohexene	208	81, 96, 67, 55
16.019	Heptadecene isomer	238	55, 41, 69, 83
16.053	Heptadecene isomer	238	55, 41, 69, 83
16.119	Heptadecene isomer	238	43, 55, 83, 69
16.186	Heptadecane	240	57, 43, 71, 85
17.418	Hexadecanenitrile	237	43, 97, 110, 124
18.600	Hexadecenenitrile	235	43, 97, 122, 192
18.741	Heptadecanenitrile	251	57, 97, 110, 124

Table 3.3 *Identification of the thermal degradation products of bone from the total ion chromatogram in Figure 3.2.*

3.3.3 Fleshed versus defleshed bone

The overlay of the chromatograms of fleshed and defleshed bones shown in Figure 3.8 illustrate that the two chromatograms were similar. No additional pyrolysis products were generated by the presence of extra amounts soft tissues. However, unlike Dehaan [47], no dienes were found in the combustion of the fat and tissues on the fleshed samples.

Again, this is not surprising since Dehaan described the dienes peaks to be small or absent. The absence of n-aldehydes from fat and tissues consolidated the hypothesis made above regarding their possible decomposition or consumption at higher temperatures where the combustion process proceeded past initial ignition.

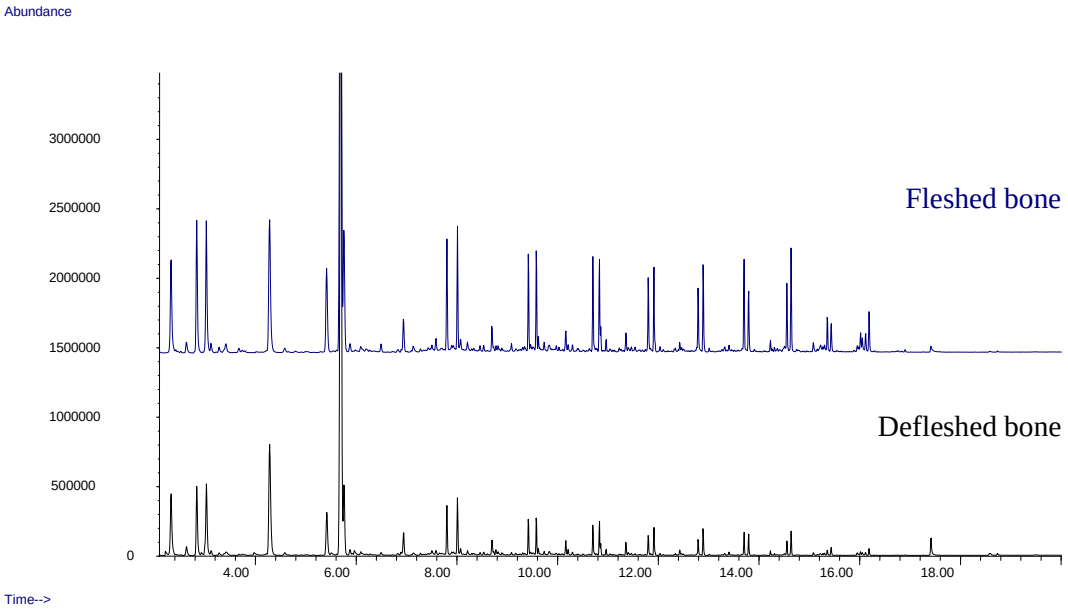

Figure 3.8 *Overlay of the chromatograms of fleshed and defleshed bones.*

The average peak area ratios of selected compounds in the fleshed and defleshed bone samples, which included benzene, 1-heptene, toluene, 1-nonene, nonane, 1-decene, decane, 1-undecene, 1-dodecene, 1-tridecene, tridecane, 1-tetradecene, tetradecane, 1-pentadecene, pentadecane, 1-hexadecene, hexadecane, 1-heptadecene, heptadecane and tetrachloroethylene (internal standard), were calculated across six samples in Table 3.4. These ratios were calculated from the peak areas of the original data, normalised to the internal standard, and normalised to the total peak area.

Peak	Compound	Ratio fleshed to defleshed		
		Original data	Normalised to internal standard	Normalised to total peak area
1	Benzene	1.39	1.31	1.16
2	1-Heptene	1.46	1.39	1.22
3	Toluene	0.94	0.87	0.76
4	Tetrachloroethylene	1.03	1.00	0.94
5	1-Nonene	1.62	1.54	1.34
6	Nonane	1.52	1.46	1.27
7	1-Decene	1.69	1.61	1.39
8	Decane	1.61	1.55	1.34
9	1-Undecene	1.65	1.58	1.35
10	1-Dodecene	1.78	1.72	1.46
11	1-Tridecene	1.65	1.60	1.37
12	Tridecane	1.59	1.54	1.31
13	1-Tetradecene	1.98	1.93	1.64
14	Tetradecane	1.55	1.51	1.27
15	1-Pentadecene	1.60	1.58	1.36
16	Pentadecane	1.54	1.51	1.23
17	1-Hexadecene	2.35	2.29	1.86
18	Hexadecane	1.70	1.65	1.34
19	1-Heptadecene	1.97	1.95	1.61
20	Heptadecane	2.13	14.83	1.65

Table 3.4 A*verage peak area ratios of fleshed to defleshed bones of twenty compounds among six samples. These ratios were calculated from the original, the normalised to the internal standard (tetrachloroethylene), and the normalised to the total peak area values.*

The ratios obtained from the original data varied from 1.39 to 2.35, apart from toluene with a ratio of 0.94 and tetrachloroethylene (internal standard) with a ratio of 1.03. These results suggested that toluene had approximately the same intensity in fleshed and defleshed bones, but that the peak intensities of fleshed bone were generally one or two times higher than those of defleshed bone which as expected given the extra amount of tissue and fat present. The experimental conditions of the six fleshed bone samples combusted for five minutes after ignition of the bone fat are listed in Table 3.5 and the chromatograms are represented in Figure 3.9.

Sample	Weight (g)	Total burning time (min)
1	18.80	9.6
2	19.60	18.9
3	15.73	7.7
4	21.75	11.1
5	25.45	21.9
6	20.47	10.8

Table 3.5 *Experimental conditions of the six fleshed bone samples combusted for five minutes after ignition.*

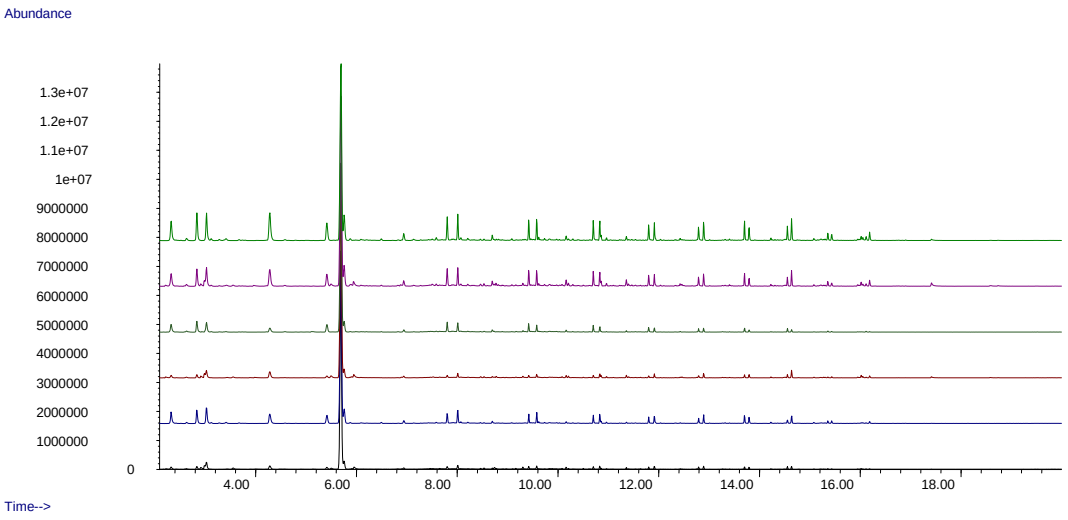

Figure 3.9 *Overlay of the chromatograms of the six fleshed bone samples combusted for five minutes after ignition.*

The overlay of the chromatograms indicated that the chromatographic patterns were similar for each samples. The qualitative repeatability of the experimental method for the production of pyrolysis products of fleshed bone was evaluated by the presence or absence of the selected compounds aforementioned among the six chromatograms, illustrated in Table 3.6.

Peak	Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
1	Benzene	✓	✓	✓	✓	✓	✓
2	1-Heptene	✓	✓	✓	✓	✓	✓
3	Toluene	✓	✓	✓	✓	✓	✓
4	Tetrachloroethylene	✓	✓	✓	✓	✓	✓
5	1-Nonene	✓	✓	✓	✓	✓	✓
6	Nonane	✓	✓	✓	✓	✓	✓
7	1-Decene	✓	✓	✓	✓	✓	✓
8	Decane	✓	✓	✓	✓	✓	✓
9	1-Undecene	✓	✓	✓	✓	✓	✓
10	1-Dodecene	✓	✓	✓	✓	✓	✓
11	1-Tridecene	✓	✓	✓	✓	✓	✓
12	Tridecane	✓	✓	✓	✓	✓	✓
13	1-Tetradecene	✓	✓	✓	✓	✓	✓
14	Tetradecane	✓	✓	✓	✓	✓	✓
15	1-Pentadecene	✓	✓	✓	✓	✓	✓
16	Pentadecane	✓	✓	✓	✓	✓	✓
17	1-Hexadecene	✓	✓	✓	✓	✓	✓
18	Hexadecane	✓	✓	✓	✓	✓	✓
19	1-Heptadecene	✓	✓	✓	✓	✓	✓
20	Heptadecane	✓	✓	✓	✓	✓	✓

Table 3.6 *Determination of the presence (✓) or absence (✗) of the selected peaks from six fleshed bones.*

The selected compounds were detected in all the chromatograms, which illustrated the good qualitative repeatability of this experimental method.

The quantitative repeatability was determined in a manner similar to that detailed in Chapter 2 (Subsection 2.3.3.4) and the values are presented in Table 3.7.

Peak	Compound	%RSD		
		Original data	Normalised to internal standard	Normalised to total peak area
1	Benzene	71.35	70.67	54.61
2	1-Heptene	74.86	74.23	57.01
3	Toluene	85.13	84.21	60.21
4	Tetrachloroethylene	4.34	0.00	21.50
5	1-Nonene	75.39	73.94	57.66
6	Nonane	70.99	69.90	50.16
7	1-Decene	78.67	76.87	58.55
8	Decane	75.00	73.58	52.27
9	1-Undecene	79.31	77.44	58.13
10	1-Dodecene	84.55	83.09	61.59
11	1-Tridecene	80.52	79.12	55.24
12	Tridecane	76.14	75.19	52.25
13	1-Tetradecene	81.90	80.85	58.56
14	Tetradecane	78.46	77.61	53.78
15	1-Pentadecene	79.05	77.76	51.72
16	Pentadecane	81.80	80.55	60.33
17	1-Hexadecene	102.20	101.21	81.06
18	Hexadecane	98.09	97.61	75.68
19	1-Heptadecene	102.09	101.25	78.41
20	Heptadecane	103.45	216.57	83.65

Table 3.7 *Peak area relative standard deviations of twenty compounds detected over six defleshed bone samples. The %RSD was calculated from the original, the normalised to the internal standard (tetrachloroethylene), and the normalised to the total peak area values.*

The relative standard deviations from the three data sets were high, and varied from 50.16% to 83.65% when calculated from the data normalised to the total peak area (the range does not include the internal standard). These results illustrated that the quantity of pyrolysis products varied significantly among the samples.

This experimental method failed to give repeatable quantitative results, but produced the same chromatographic over the samples.

3.4 Conclusion

The pyrolysis of bone produced homologous series of C_7 to C_{17} n-alkanes and n-alkenes, aromatics, nitriles, and C_1 to C_9 n-alkyl cycloalkanes and cycloalkenes. No n-aldehydes were detected which was different to the finding of Dehaan. However, n-aldehydes were present in the pre ignition and ignition samples. It was suggested that they might have decomposed or been consumed after the sample was left to burn at around 550 °C for at least one minute. Moreover, no dienes were found in the TIC of fleshed bone, but it is not surprising since Dehaan described the diene peaks as being small or non-existent.

The comparison of fleshed and defleshed bones demonstrated that they produced similar volatile compounds, but that the peak intensities of fleshed bones were generally one to two times higher than those of defleshed bones. For this reason, fleshed bones were used for following experiments.

Chapter 4 – Investigation of pyrolysis products of bone combusted in the presence of textile fibres

4.1 Introduction

An understanding of the volatile compounds produced by the combustion of a material is necessary to determine the interfering products in fire debris samples and to detect the presence of that material. The thermal degradation of common materials (cotton, nylon, polyester, acrylic, leatherette and wool) used in the textile industry were investigated as these materials are commonly used in clothing and as a consequence may have relevance as potential background pyrolysis products in the analysis of suspected combusted bone.

Acrylic is a synthetic fibre that contains at least 85% of acrylonitrile, and not more than 15% of additives and comonomers such as vinylacetate and methylacrylate [52]. Causin *et al.* [52] pyrolised different polyacrylonitrile (PAN)-based samples using a CDS Pyroprobe coupled to a gas chromatography with a mass analyser, at 700 °C for five seconds. The PAN-based fibres generated hydrocyanic acid, acetonitrile, acrylonitrile and methacrylonitrile, but also produced dicyanoalkenes, tricyanoalkenes and tricyanoalkanes. These volatile products were also found by Surianarayanan *et al.* [53] who pyrolised PAN-based samples at 200 °C, 325 °C, 425 °C and 590 °C for five seconds using a Curie point pyrolyser. They demonstrated that a raise of the pyrolysis temperature contributed to the increase of the formation of low molecular weight compounds.

Cotton fibres are essentially composed of cellulose [54], which is a polymer of glucose units [55]. Significant pyrolysis products of cotton have been generated when using a CDS Pyrolysis Autosampler at 750 °C for fifteen seconds, the cellulose produced many organic materials including aldehydes and ketones [54]. Zhu *et al.* [56] pyrolised cotton using a CDS Pyrolyzer at 600 °C, which generated aldehydes, ketones, furans, alcohols, esters and ethers.

Leatherette is a synthetic leather made of a synthetic or natural fibre covered with a thin layer of polyvinylchloride (PVC) producing a non-porous material [57]. Aracil *et al*. [58] placed PVC samples into a quartz-tube type reactor which was situated into a furnace to produce combustion and pyrolysis products. The leatherette samples were left in the reactor for 100 seconds at 500 °C, 700 °C, 850 °C and 1000 °C. Substituted monoaromatic compounds were detected, as well as polyaromatic hydrocarbons from two to six aromatic rings, oxygenated compounds (like carboxylic acids, benzaldehyde, phenol and benzofuran), chloroalkanes, chloronaphthalenes, and chloroalkylaromatic compounds. The total yield of pyrolysis compounds reached a maximum at 700 °C, and only oxygenated combustion compounds were detected at temperatures above 500 °C.

Nylon, a synthetic polyamide, produced a considerable amount of volatile compounds when the samples were rapidly heated using a CDS Pyroprobe at 850 °C. The pyrolysis products included mononitriles, amides, mononitriles with an amide group, and hydrocarbons [59]. Almirall *et al.* [60] also detected the presence of substituted aromatics, branched alkenes, benzaldehyde, indene, acetophenone, and naphthalene, when nylon samples were ignited inside a can and left to burn freely for two minutes or until two-thirds of the sample was charred.

Polyethylene terephthalate (PET) is almost always used in polyester clothing. Significant pyrolysis products of PET have been generated when using a CDS Pyrolysis Autosampler at 750 °C for fifteen seconds [54]. Its thermal degradation produced benzene, benzoic acid and oligomeric fragments. Stauffer [16] demonstrated that polyester produced substituted aromatics, branched alkenes, naphthalene, benzaldehyde and acetophenone when pyrolised at 800 °C for twenty-five seconds using a Pyrolyzer CDS Analytical.

Wool is a natural fibre that was found to produce substituted aromatics, alkanes, branched alkenes, substituted naphthalene, indene, acetophenone, benzonitrile and benzaldehyde when it is left to burn freely for two minues or until two-thirds is charred [60].

The aim of this study was to investigate and determine the pyrolysis products of bone combusted in the presence of materials. The variation of the chromatographic pattern and the detection of the presence of unidentified peaks were evaluated by comparisons between chromatograms and the presence of interfering products generated by textile fibres was determined using target compound chromatography.

4.2 Experimental method

4.2.1 Textile fibres samples

4.2.1.1 Preliminary experiments to assess pyrolysis product generation of textile fibres

For each sample, 1 g of material was placed into a 125 mL tin can, which was then fixed on a retort stand. The tin can was heated over a Bunsen flame for one minute. The temperature inside the tin was monitored with a thermocouple as described in Chapter 2 (Subsection 2.2.4.1). After burning, the tin was cooled down at room temperature for one minute, and then sealed using its lid with an ACS attached to it as previously described.

The volatile components were extracted using the passive adsorption extraction method as described in Chapter 2 (Subsection 2.2.3), and the extract was analysed by GC-MS. All experiments for each sample were repeated six times.

4.2.1.2 Variation of volatiles products as a factor of exposure to air immediately post combustion

Some pyrolysis products were detected of acrylic and leatherette using the mechanism previously described in Subsection 4.2.1.1. No pyrolysis products of wool were produced, but after one minute it was observed that the combustion of the sample was not complete. No pyrolysis products of cotton, nylon, polyester were

generated using the aforementioned experimental method. As a consequence only acrylic, leatherette and wool were subjected to more specific testing.

To determine the variation of the volatiles produced as a factor of exposure to air after combustion, 1 g of acrylic, leatherette and wool were combusted for one minute and allowed to cool at room temperature for fifteen seconds, thirty seconds and sixty seconds respectively before the container was sealed with an ACS attached to the lid.

4.2.1.3 Variation in the quantity of pyrolysis products generated as a function of duration of combustion

Acrylic, leatherette and wool (1 g of material) were combusted over different durations to determine the effect this would have on the subsequent production of pyrolysis products. Both acrylic and wool samples were combusted for thirty seconds, and one to five minutes with one minute intervals. Leatherette was combusted for thirty seconds, one and two minutes, and combusted completely after two minutes. After combustion the tin was allowed to cool in air for fifteen seconds.

4.2.2 Bone samples

4.2.2.1 Bone sample preparation

Bones were stored in the freezer before each experiment. Before sample preparation, bones were left to defrost for approximately thirty minutes at room temperature. The epiphysis was detached from the diaphysis by placing the bone on a vice and cutting it with a hacksaw. The tissues were left on the bone and it was cut across its width into small pieces of approximately 15 g to 30 g. Each piece of bone was wrapped twice around its circumference in about 1 g to 3 g of the textile fibre under test.

4.2.2.2 Generation of pyrolysis products

The bone sample was placed in a tin can (125 mL) and heated over a Bunsen flame for five minutes after the ignition of the bone fat. This combustion process was used because it was the optimised condition for the production of pyrolysis products for the bones rather than the textile materials. The tin was then left to cool at room temperature without the lid for forty-five seconds and then was sealed using its lid with an ACS attached. The experiments carried out in Subsection 4.2.1.3 demonstrated that more volatile products of the textile fibres were detected when the sample was exposed to air for fifteen seconds after combustion, so in order to recover as much pyrolysis products of textile fibres as possible when combusted in the presence of bone, the tin was sealed after forty-five seconds instead of one minute as applied when only the bone was combusted. Each experiment was repeated six times to determine the repeatability of the experimental method.

The volatile components were extracted using the passive adsorption extraction method as described in Chapter 2 (Subsection 2.2.3), and the extract was analysed by GC-MS.

4.3 Results and discussion

4.3.1 Preliminary pyrolysis product generation

Preliminary studies demonstrated that using the experimental methodology which successfully produced pyrolysis products from bone samples, only two (acrylic and leatherette) of the tested textiles produced any detectable pyrolysis products. An example chromatogram of each material is provided in Figures 4.1 to 4.3.

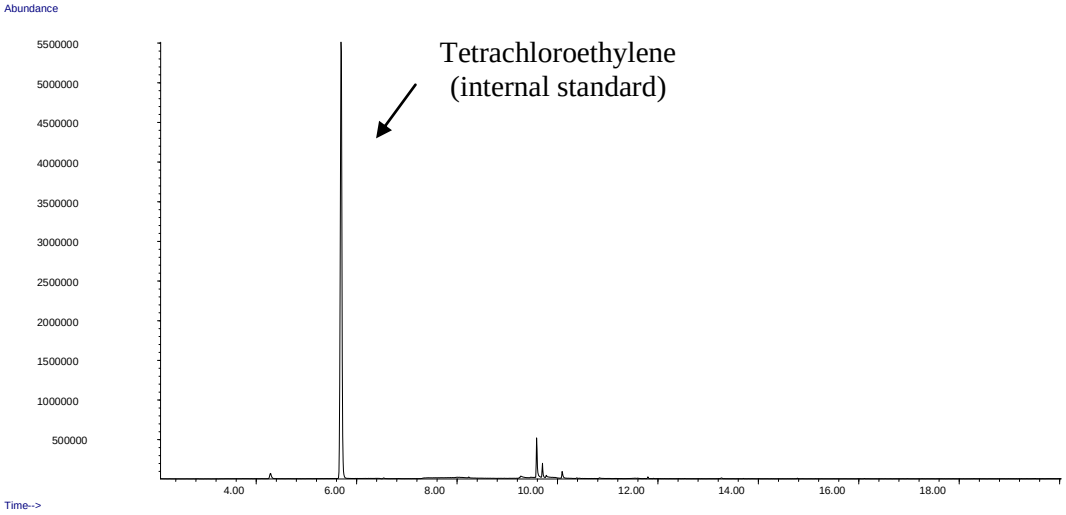

Figure 4.1 *Chromatogram of acrylic combusted for one minute.*

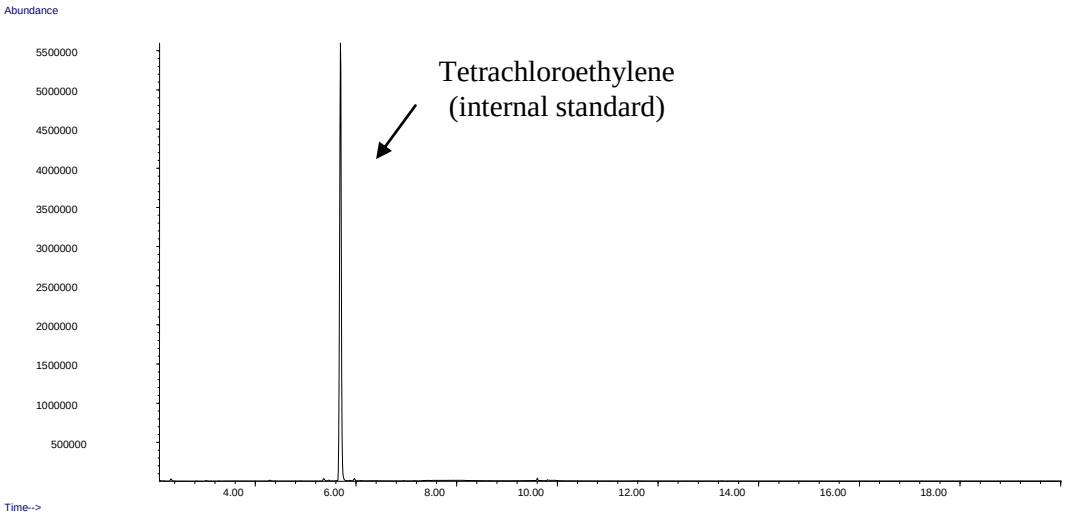

Figure 4.2 *Chromatogram of leatherette combusted for one minute.*

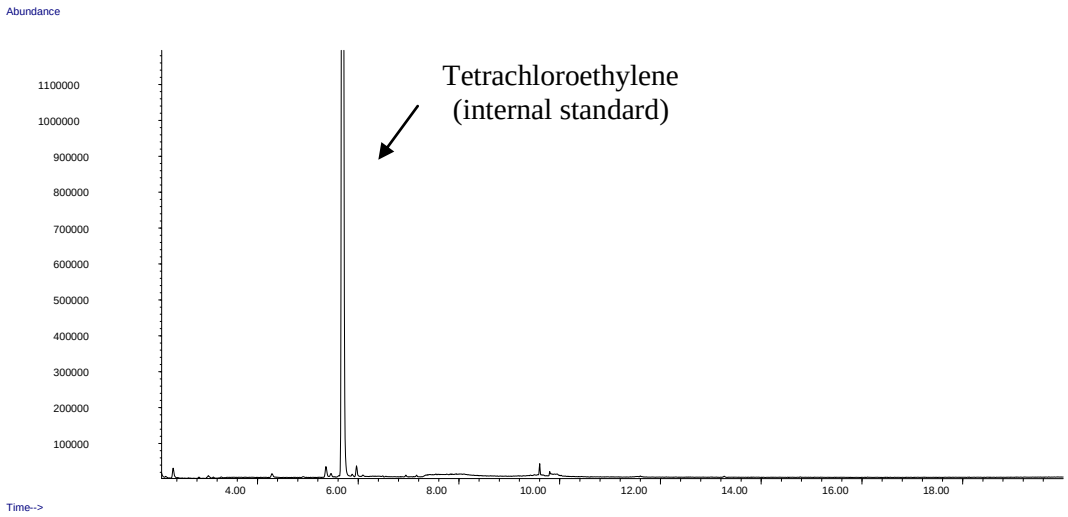

Figure 4.3 *Close-up of the chromatogram of leatherette combusted for one minute.*

Wool, cotton, nylon and polyester did not produce pyrolysis products under these conditions. The absence of pyrolysis products could have been a consequence of a low pyrolysis temperature (below 400 °C) or of an unsuitable combustion length. Stauffer [61] suggested that the most influential parameter concerning the production of pyrolysis products is the temperature at which pyrolysis occurs. When the temperature increases, small radicals and molecules are produced resulting in a greater degree of pyrolysis. Stauffer used as an example the pyrolysis of polyethylene proposed by Madorsky [62], which gave 0.03% of monomer at 500 °C, 5.5% at 800 °C and 26.4% at 1200 °C.

The fact that no pyrolysis products were generated for cotton, polyester and nylon is not in itself an issue as it demonstrated that the sample preparation method for suspected bone samples will not facilitate the generation and extraction of these background materials. However, after one minute the combustion of wool was not complete, so it was further investigated along with acrylic and leatherette.

4.3.2 Variation of volatiles produced as a factor of exposure to air immediately post combustion

The sampling tin was not sealed immediately after combustion as this could have led to the failure of the ACS. In order that the subsequently inserted ACS would function correctly a minimum time of fifteen seconds was fixed where the sample was exposed to air at room temperature. After this time the tin was sealed with the ACS inside and the sample was incubated as described previously.

Subsequent analysis by GC-MS revealed that more volatile products were extracted and detected for the acrylic, leatherette and wool materials when the tin was sealed within thirty seconds post combustion and best results were obtained when the tin was sealed after fifteen seconds.

Figures 4.4 to 4.6 show the overlay chromatograms of acrylic, leatherette and wool obtained when the tin was secured after fifteen seconds, thirty seconds and one minute.

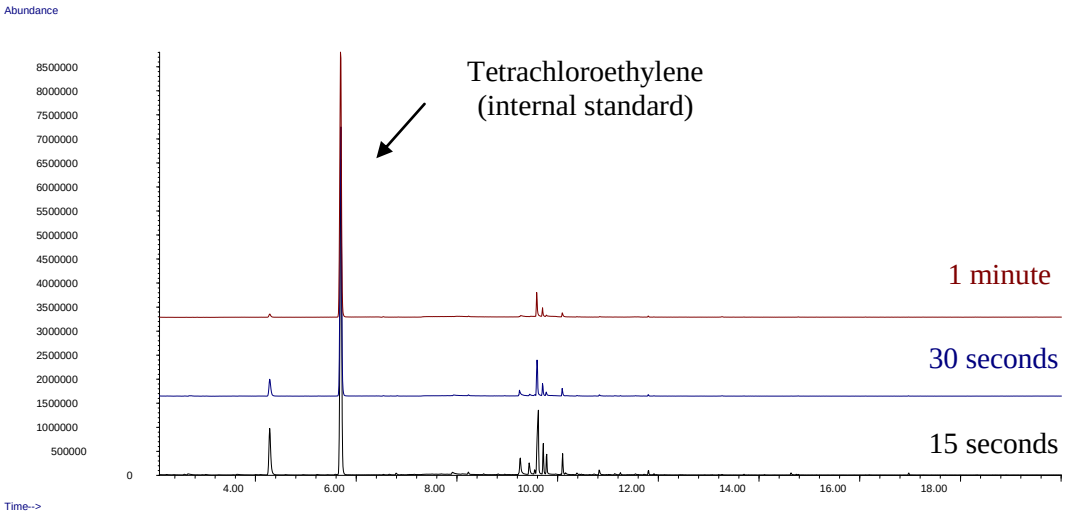

Figure 4.4 *Overlay of the chromatograms of acrylic combusted for one minute and the tin was allowed to cool in air for fifteen seconds, thirty seconds and one minute.*

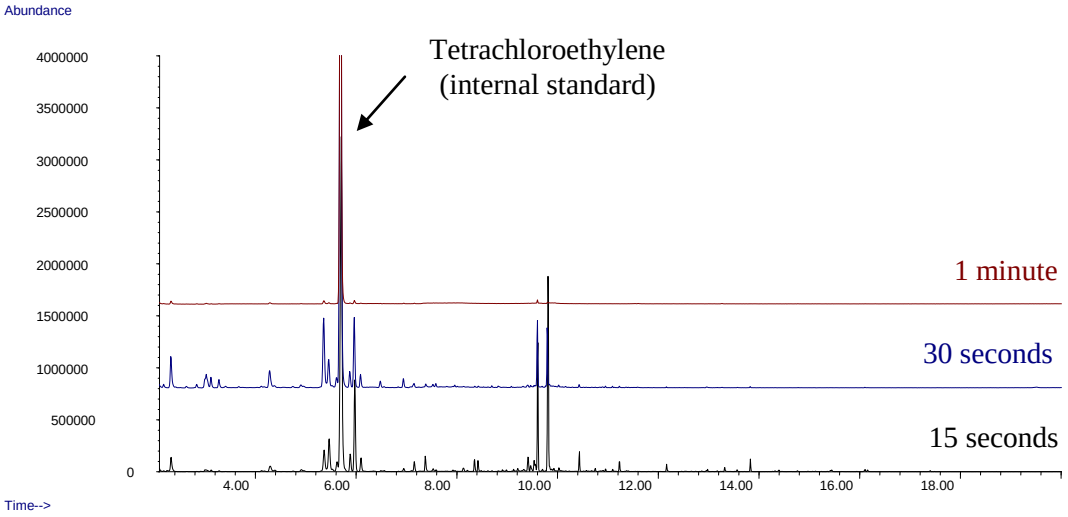

Figure 4.5 *Overlay of the chromatograms of leatherette combusted for one minute and the tin was allowed to cool in air for fifteen seconds, thirty seconds and one minute.*

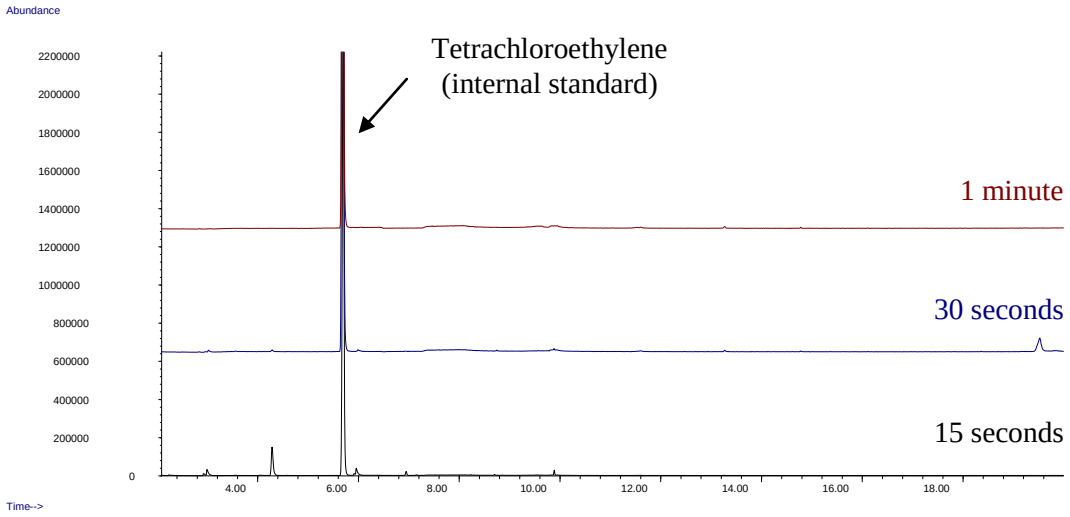

Figure 4.6 *Overlay of the chromatograms of wool combusted for one minute and the tin was allowed to cool in air for fifteen seconds, thirty seconds and one minute.*

The TIC of wool obtained when the sample was allowed to cool in air for fifteen seconds post sealing produced small peaks that were not present on the other chromatograms generated for this material. The overlay of the chromatograms of acrylic and leatherette also clearly demonstrated that a greater quantity of pyrolysis products was obtained when the tin was sealed fifteen seconds after the end of the combustion process. Thus, for the following experiments the tin was sealed fifteen seconds post combustion.

4.3.3 Variation in the quantity of pyrolysis products generated as a function of the duration of the combustion

Both acrylic and wool samples were combusted for thirty seconds, and one to five minutes with one minute intervals, and leatherette was combusted for thirty seconds, one and two minutes.

The overlay chromatograms for each material are provided in Figures 4.7 to 4.9. In each case the samples were allowed to cool in air for fifteen seconds post combustion.

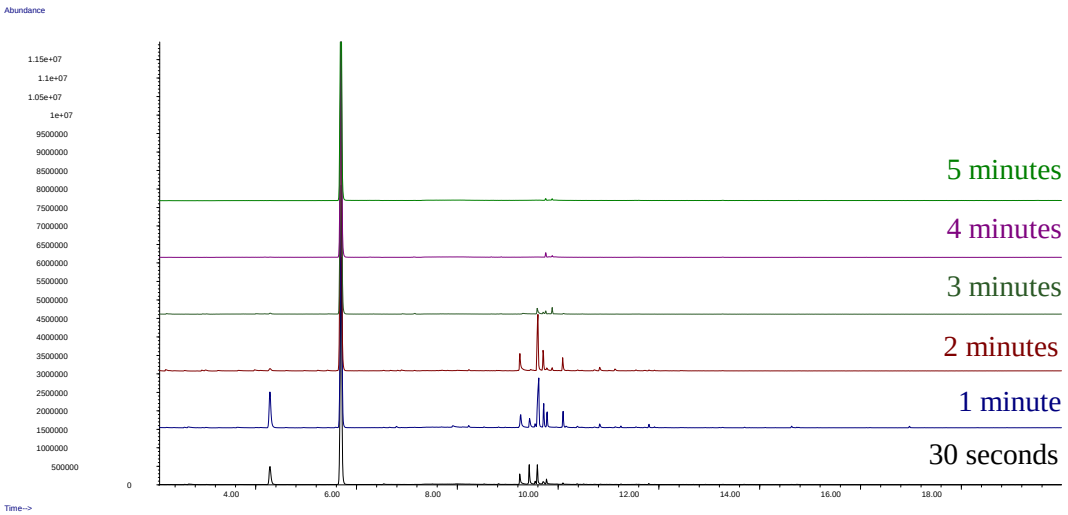

Figure 4.7 *Overlay chromatograms of acrylic combusted for thirty seconds, one, two, three, four and five minutes.*

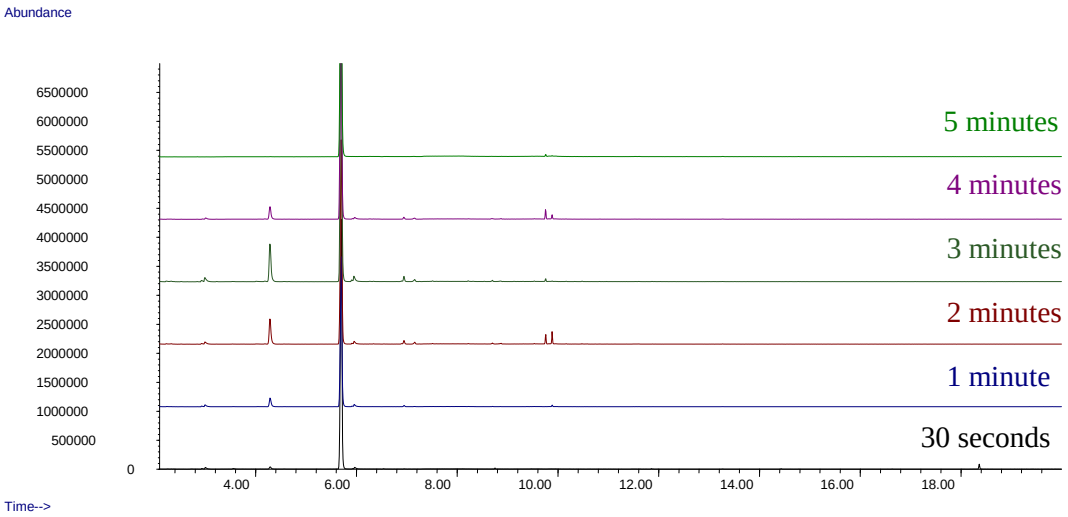

Figure 4.8 *Overlay chromatograms of wool combusted for thirty seconds, one, two, three, four and five minutes.*

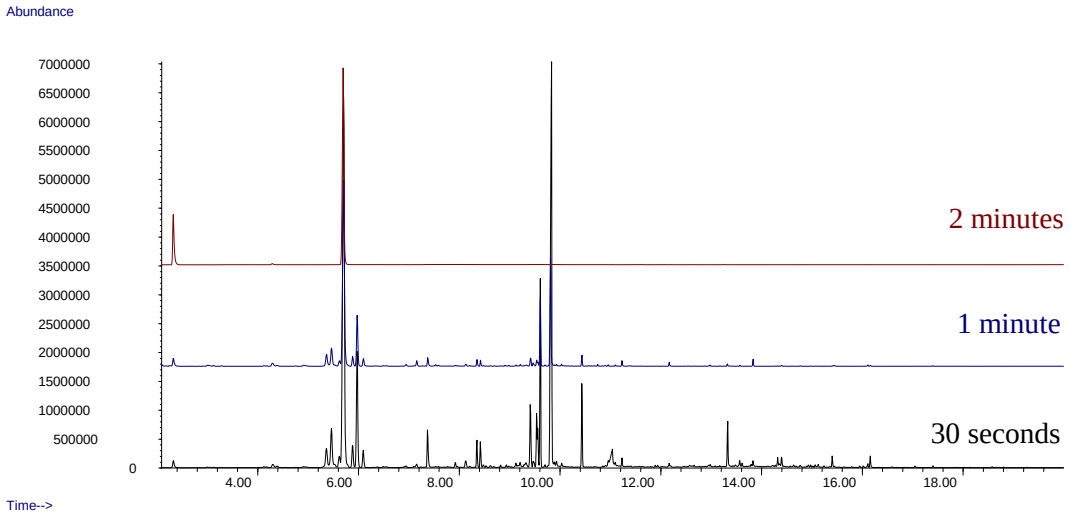

Figure 4.9 *Overlay chromatograms of leatherette combusted for thirty seconds, one and two minutes.*

For acrylic (Figure 4.7), more volatile products were produced when the material was combusted for one minute. As the combustion length increased, the amount of volatiles recovered decreased. No peaks were detected in the extract from the sample allowed to combust for five minutes. Most pyrolysis products from the wool sample

were evident in the extract from the material combusted for between two and three minutes as illustrated in Figure 4.8. Leatherette produced a large quantity of products when it was combusted for thirty seconds (Figure 4.9); however after two minutes of combustion, only one peak was detected at the front end of the TIC and the material was completely combusted.

The volatile compounds present in the TICs of acrylic combusted for one minute, wool combusted for two minutes, and leatherette combusted for thirty seconds were further investigated and are discussed in the next section.

4.3.4 Identification of the thermal degradation products of acrylic, leatherette and wool

The identification of the pyrolysis products of the three materials was completed using the NIST library associated with the GC-MS instrument and reference to literature. In each case six repeat samples were prepared, extracted and analysed. The overlay chromatograms of these are presented in each case. The accompanying tables (Tables 4.1 to 4.6) present the identified compounds, their %RSD values for their peak areas (unnormalised, normalised to the internal standard and normalised to the total peak area) to determine repeatability of the analysis, as well as a semi-quantitative determination of their concentration in the sample calculated using a single point estimate based on the concentration of the internal standard (0.5 mg/mL).

4.3.4.1 Acrylic

The compounds identified from Figure 4.10 are listed in Table 4.1 together with the most abundant fragment ions (m/z). Dinitriles, diidopropylcyanamide and isopropenylpyrazine have not been previously mentioned in the literature but were repeatedly generated under the conditions used. The overlay of the six chromatograms of acrylic is represented in Figure 4.11.

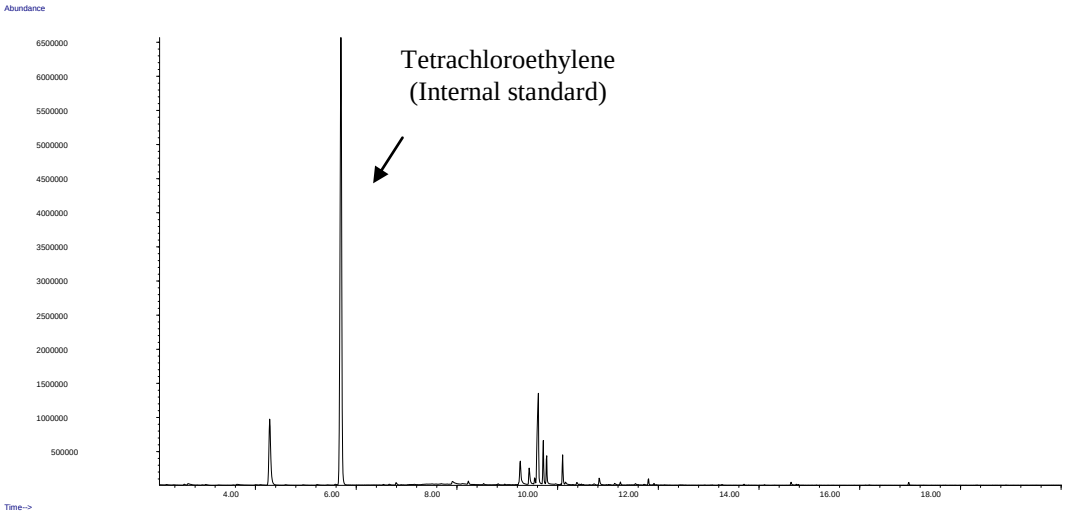

Figure 4.10 *Total ion chromatogram of acrylic combusted for one minute.*

Retention time (min)	Compound	Fragment ions m/z : abundance
4.277	Toluene	91:99, 65:10, 39:7, 51:5, 89:4
9.230	Pentanedinitrile	54:99, 41:79, 52:13, 66:6, 207:4
9.438	2-Methylenepentanedinitrile	66:99, 106:32, 39:18, 52:15, 51:7
9.546	Diisopropylcyanamide	69:99, 43:37, 126:21, 41:20, 207:17, 281:16
9.596	2-Methylpentanedinitrile	68:99, 54:55, 41:49, 39:10, 80:7
9.704	Hexanedinitrile	68:99, 55:84, 41:40, 54:40, 281:19, 207:17
9.771	Isopropenylpyrazine	66:99, 67:89, 120:39, 119:37, 39:36, 54:32
10.096	2-Methylpentanedinitrile	68:99, 55:98, 54:47, 41:46, 207:33, 281:20
10.832	Isopropylacrylonitrile	80:99, 53:44, 41:23, 68:13, 32:12
11.248	Acetoxytoluene	108:99, 91:76, 150:37, 32:27 , 43:25, 79:25
11.806	3-Dodecene	55:99, 41:73, 70:63, 83:60, 97:46, 32:40

Table 4.1 *Identification of the thermal degradation compounds of acrylic, and their most abundant fragment ions.*

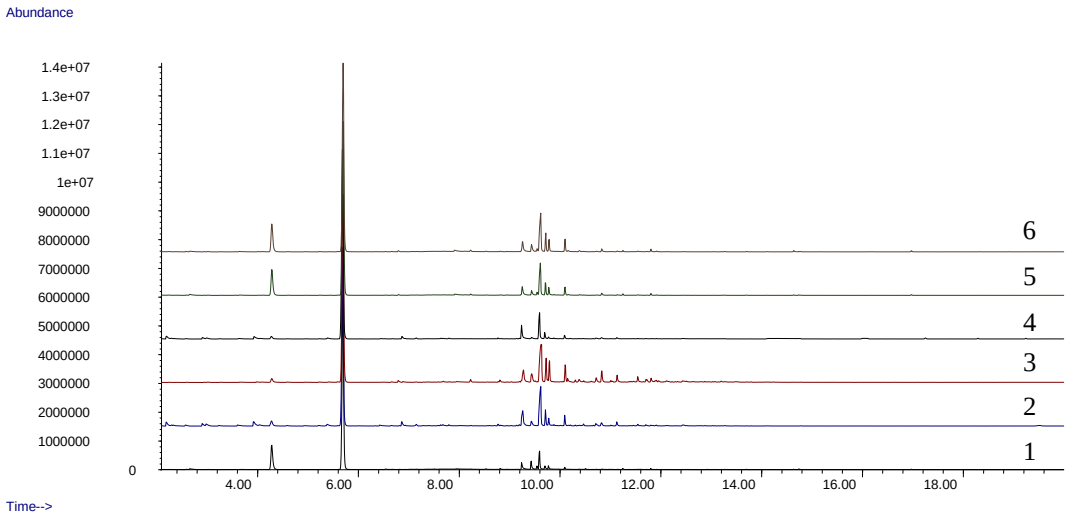

Figure 4.11 *Overlay of the chromatograms of the six acrylic samples combusted for one minute.*

Compound	%RSD			Concentration (mg/mL)
	Original data	Normalised to internal standard	Normalised to total peak area	
Toluene	82.35	83.21	83.05	0.0468 ± 0.0390
Tetrachloroethylene	5,06	0,00	10.96	0.5000 ± 0.0000
Pentanedinitrile	42.40	42.73	40.68	0.0196 ± 0.0084
2-Methylpentanedinitrile	47.34	46.85	40.14	0.0765 ± 0.0358
Hexanedinitrile	59.81	58.85	52.43	0.0182 ± 0.0107
Isopropenylpyrazine	87.14	84.65	78.10	0.0112 ± 0.0095
2-Methylpentanedinitrile	68.31	66.91	60.25	0.0121 ± 0.0081
Isopropylacrylonitrile	103.30	101.36	94.24	0.0061 ± 0.0062
3-Dodecene	90.86	88.40	82.19	0.0025 ± 0.0022

Table 4.2 *Peak area relative standard deviations of nine compounds among the six acrylic samples. The %RSD was calculated from the original, the normalised to the internal standard (tetrachloroethylene), and the normalised to the total peak area values.*

The %RSD values are high for the three data sets (Table 4.2). The values varied from 40.14% to 94.24% (excluding the internal standard) for the normalised to the total peak area data set. Also, the concentration of the different compounds varied significantly among the samples. This experimental method failed to give repeatable results for acrylic.

4.3.4.2 Wool

The volatile compounds of wool illustrated in Figure 4.12 are listed in Table 4.3. Only aromatics and nitriles were detected and identified. This experimental method failed to produce branched alkanes and alkenes which have been detailed in previous literature [60]. Figure 4.13 illustrates the overlay of the six chromatograms of wool combusted for two minutes.

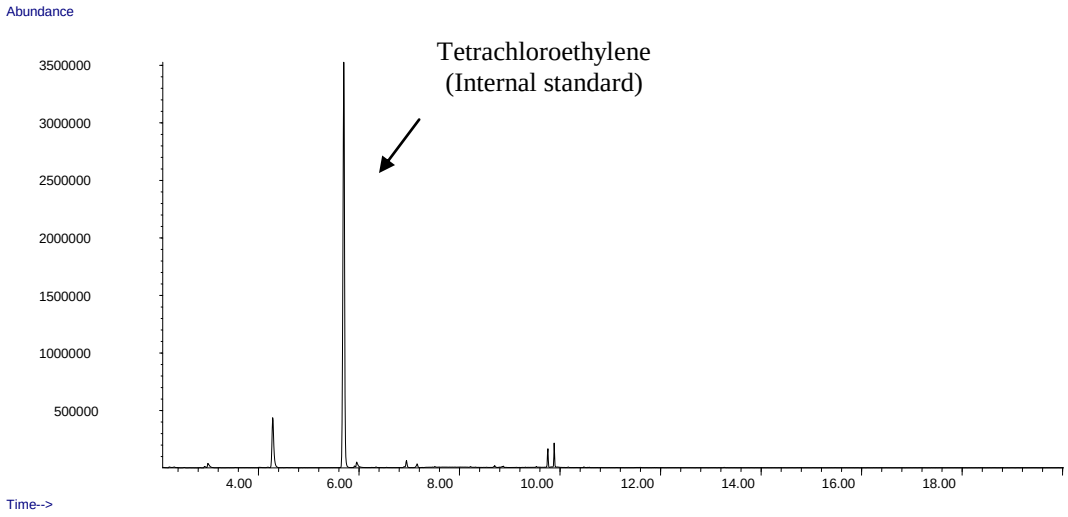

Figure 4.12 *Total ion chromatogram of wool combusted for two minutes.*

Retention time (min)	Compounds	Fragment ions m/z : abundance
3.000	3-Methylbutanenitrile	43:99, 41:60, 39:18, 68:7, 40:6
4.282	Toluene	91 :99, 92 :60, 65 :8, 39 :6, 51 :4, 89 :4
5.963	4-Methylpentanenitrile	55:99 , 41:36, 54:27, 57:1, 82:17, 32:11
6.937	Ethylbenzene	91:99, 106:33, 51:8, 92:8, 65:8
7.153	Xylene isomer	91:99, 106:50, 32:16, 77:12, 51:7
9.759	Isopropyltoluene isomer	119:99, 134:28, 91:19, 117:14, 77:6
9.884	Limonene	68:99, 93:83, 67:75, 79:41, 121:30, 136:28

Table 4.3 *Identification of the thermal degradation compounds of wool, and their most abundant fragment ions.*

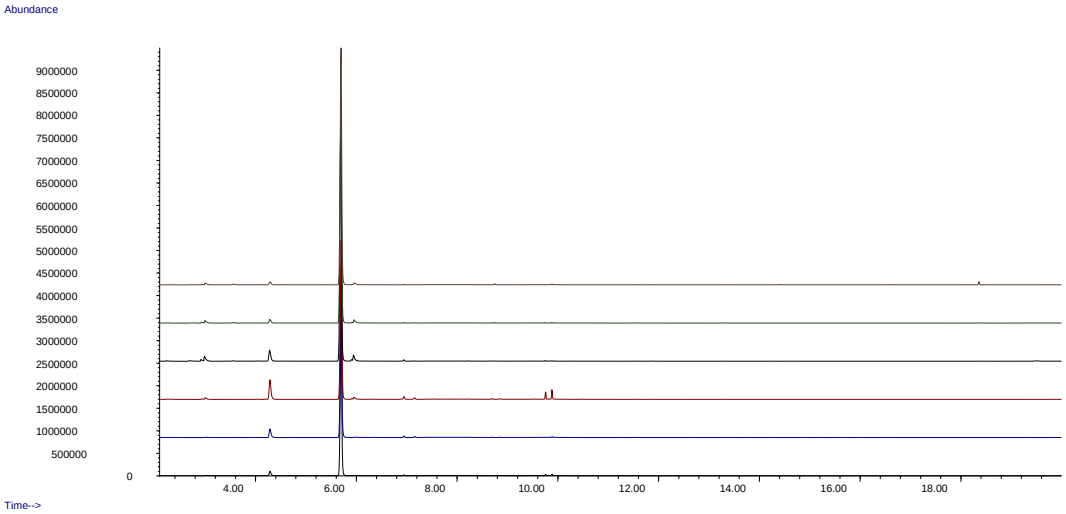

Figure 4.13 *Overlay of the chromatograms of the six wool samples combusted for two minutes.*

Compound	%RSD			Concentration (mg/mL)
	Original data	Normalised to internal standard	Normalised to total peak area	
Toluene	90.05	88.06	80.34	0.0270 ± 0.0238
Tetrachloroethylene	3.90	0.00	5.74	0.5000 ± 0.0000
Isopropyltoluene isomer	176.25	174.44	167.60	0.0026 ± 0.0045
Limonene	172.26	169.82	162.28	0.0032 ± 0.0054

Table 4.4 *Peak area relative standard deviations of four compounds among six wool samples. The %RSD was calculated from the original, the normalised to the internal standard (tetrachloroethylene), and the normalised to the total peak area values.*

The %RSD values of the three data sets were very high varying from 80.34% to 167.60% (excluding the internal standard) for the normalised to the total peak area data set, and the concentration variation of selected compounds among the samples was also high (Table 4.4). Again, this experimental method failed to give good repeatability.

4.3.4.3 Leatherette

The TIC of leatherette combusted for thirty seconds is presented in Figure 4.14 and the most abundant fragments ions (m/z) of the volatile compounds identified are provided in Table 4.5. The major compounds detected in the TIC of leatherette were alkenes and esters, and chloroalkanes and carboxylic acids which were produced from the PVC used in the manufacture of the material [58]. The overlay of the six chromatograms of leatherette combusted for thirty seconds is represented in Figure 4.15.

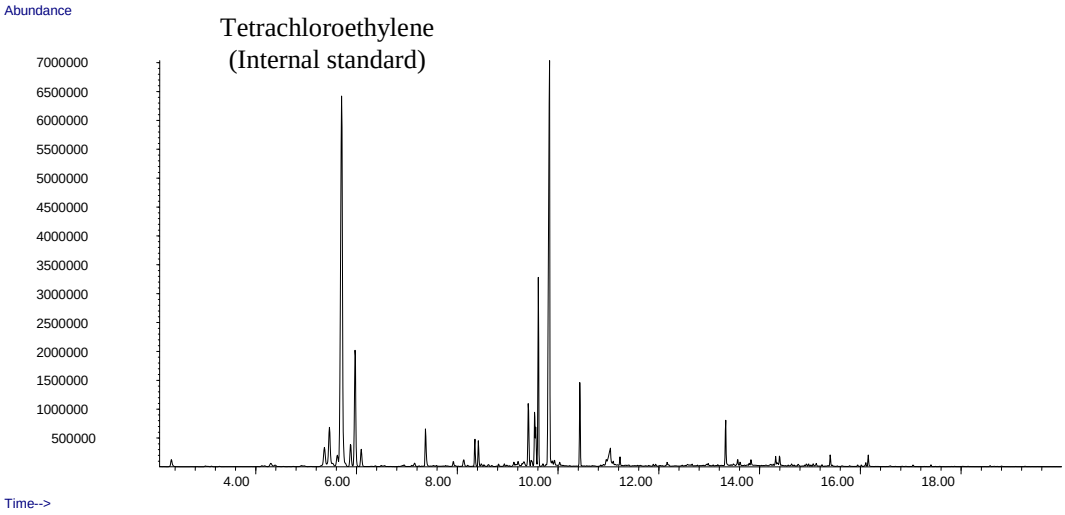

Figure 4.14 *Total ion chromatogram of leatherette combusted for thirty seconds.*

Retention time (min)	Compounds	Fragment ions m/z : abundance
2.334	Benzene	78:99, 77:23, 51:13, 52:13, 39:6, 74:4
5.364	2-Ethylhexene	70:99, 55:78, 41:34, 112:16, 39:13
5.463	3-Methyl-3-heptene	55:99, 83:65, 112:33, 41:26, 70:13, 39:10
5.622	Octene isomer	55:99, 41:59, 112:45, 70:41, 83:26
5.888	Octene isomer	55:99, 41:55, 70:52, 112:49, 83:22
5.980	3-Methyl-2-heptene	70:99, 55:75, 41:56, 112:47, 83:17
6.096	Octene isomer	55:99, 41:64, 70:59, 112:53, 83:25
7.370	3-Heptanone	57:99, 85:37, 72:21, 41:19, 114:17
7.919	4-Chloroheptane	56:99, 41:70, 69:64, 70:55, 98:20
8.352	Hydroxycyclohexanecarboxylic acid	99:99, 81:49, 55:40, 43:35, 41:18, 57:14
8.418	Hydroxycyclohexanecarboxylic acid	99:99, 81:52, 55:44, 43:40, 41:19, 57:14
9.409	4-Chlorooctane	70:99, 55:75, 41:73, 83:60, 56:60, 69:52
9.534	3-Chlorooctane	70:99, 55:87, 83:78, 41:72, 56:83
9.559	2-Chlorooctane	70:99, 55:68, 83:64, 41:62, 56:58
9.609	Chloro-2-ethylhexane	57:99, 41:23, 55:23, 99:20, 119:4
9.817	2-Ethylhexanol	57:99, 41:32, 83:27, 70:26, 98:9
10.433	2-Ethylhexylchloroacetate	57:99, 70:75, 55:46, 41:38, 83:30
11.040	2-Ethylhexanoic acid	88:99, 73:97, 57:30, 87:23, 110:20, 106:16
11.232	2-Ethylhexylacetate	43:99, 70:99, 57:54, 41:33, 83:31, 74:20
13.330	Chlorooctane	70:99, 57:59, 83:45, 75:43, 41:40
13.562	2-Chloroethylbenzoate	105:99, 122:64, 77:42, 51:15, 106:7, 32:6
13.829	2-Ethylhexylpentanoate	85:99, 70:77, 57:71, 41:37, 55:34, 112:27
14.320	Chlorododecane	91:99, 57:78, 43:73, 69:43, 105:26, 83:25
14.395	4-Methylnonanoic acid	101:99, 73:93, 57:72, 43:36, 113:34, 185:14
15.410	2-Ethylhexyl-2-ethylhexanoate	57:99, 70:69, 127:55, 112:51, 145:37, 88:33
16.159	2-Ethylhexylbenzoate	105:99, 70:48, 112:35, 77:33, 123:18, 83:13

Table 4.5 *Identification of the thermal degradation compounds of leatherette, and their most abundant fragment ions.*

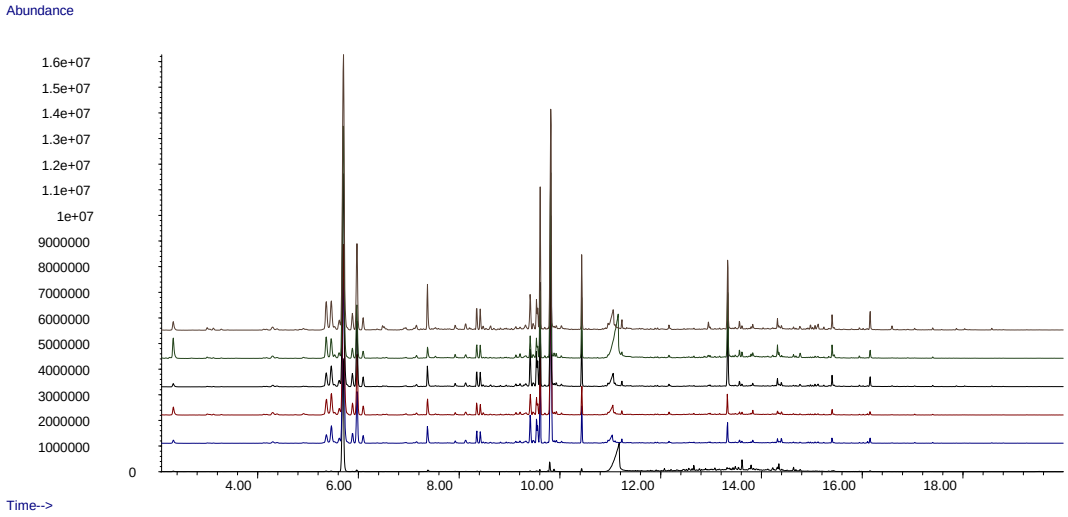

Figure 4.15 *Overlay of the chromatograms of the six leatherette samples combusted for thirty seconds.*

Compound	%RSD			Concentration (mg/mL)
	Original data	Normalised to internal standard	Normalised to total peak area	
2-Ethylhexene	57.72	49.18	50.77	0.0285 ± 0.0153
2-Methyl-3-heptene	51.16	44.52	43.96	0.0350 ± 0.0171
Tetrachloroethylene	8.61	0.00	45.28	0.5000 ± 0.0000
3-Methyl-2-heptene	50.80	44.34	43.43	0.0944 ± 0.0459
3-Heptanone	67.74	55.87	50.87	0.0242 ± 0.0148
Hydroxycyclohexanecarboxylic acid	49.79	43.08	41.50	0.0153 ± 0.0072
Hydroxycyclohexanecarboxylic acid	49.58	42.97	40.79	0.0135 ± 0.0064
4-Chlorooctane	53.69	49.38	48.24	0.0329 ± 0.0178
Chloro-2-ethylhexane	53.38	46.56	45.60	0.0854 ±0.0435
2-Ethylhexanol	52.24	45.98	43.10	0.2489 ± 0.1253
2-Ethylhexylchloroacetate	53.42	47.85	53.02	0.0414 ± 0.0217
Chlorooctane	63.77	60.73	62.53	0.0464 ± 0.0309
2-Ethylhexyl-2-ethylhexanoate	49.75	45.28	44.32	0.0073 ± 0.0036
2-Ethylhexylbenzoate	71.28	59.58	53.66	0.0074 ± 0.0048

Table 4.6 *Peak area relative standard deviations of fourteen compounds among six leatherette samples. The %RSD was calculated from the original, the normalised to the internal standard (tetrachloroethylene), and the normalised to the total peak area values.*

The high %RSD values demonstrate the poor repeatability of the experimental method. Also, the concentration of selected compounds significantly varies among the samples.

This experimental method failed to produce repeatable results for the three experiments and this method could not be used to determine the concentration of a compound in a sample, as the concentration significantly varied among samples with the same amount of material (1 g).

4.4 Interpretation of the results

4.4.1 Variation of the chromatographic pattern

The overlay of the six chromatograms of bone combusted in the presence of acrylic, leatherette and wool are shown in Figures 4.16 to 4.18 respectively, and the experimental conditions used for the bone samples combusted in the presence of acrylic, leatherette and wool are described in Table 4.7 to 4.9 respectively.

Sample	Weight bone (g)	Weight material (g)	Total burning time (min)
1	16.75	1.68	11.6
2	21.35	1.88	11.9
3	15.25	1.67	8.3
4	27.04	2.35	16.3
5	20.24	1.96	10.1
6	17.85	1.75	10.3

Table 4.7 *Experimental conditions for the six bone samples combusted for five minutes after ignition in the presence of acrylic.*

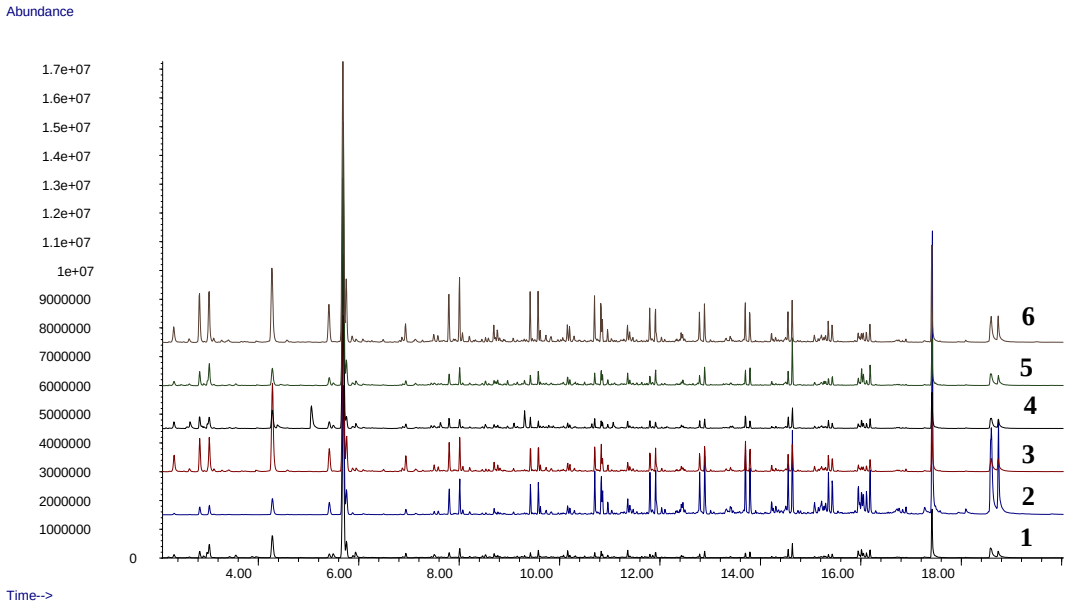

Figure 4.16 *Overlay of the chromatograms of the six samples of bone wrapped in acrylic.*

The TICs of samples 1 and 4 of the bone combusted in the presence of acrylic represented peaks with low intensities when compared with the other samples. In the chromatogram of sample 2 the peak intensities were greater for the heavier (less volatile) compounds than in the lighter (more volatile) compounds. However, not withstanding these differences, the general chromatographic pattern was consistent across five of the samples with only sample 4 producing a chromatogram with significant extra peaks (see Subsection 4.4.2).

Sample	Weight bone (g)	Weight material (g)	Total burning time (min)
1	16.52	1.48	12.6
2	17.82	2.50	8.3
3	15.36	1.62	6.7
4	19.14	1.24	8.4
5	13.87	1.17	9.5
6	21.601	2.09	11.3

Table 4.8 *Experimental conditions for the six bone samples combusted for five minutes after ignition in the presence of wool.*

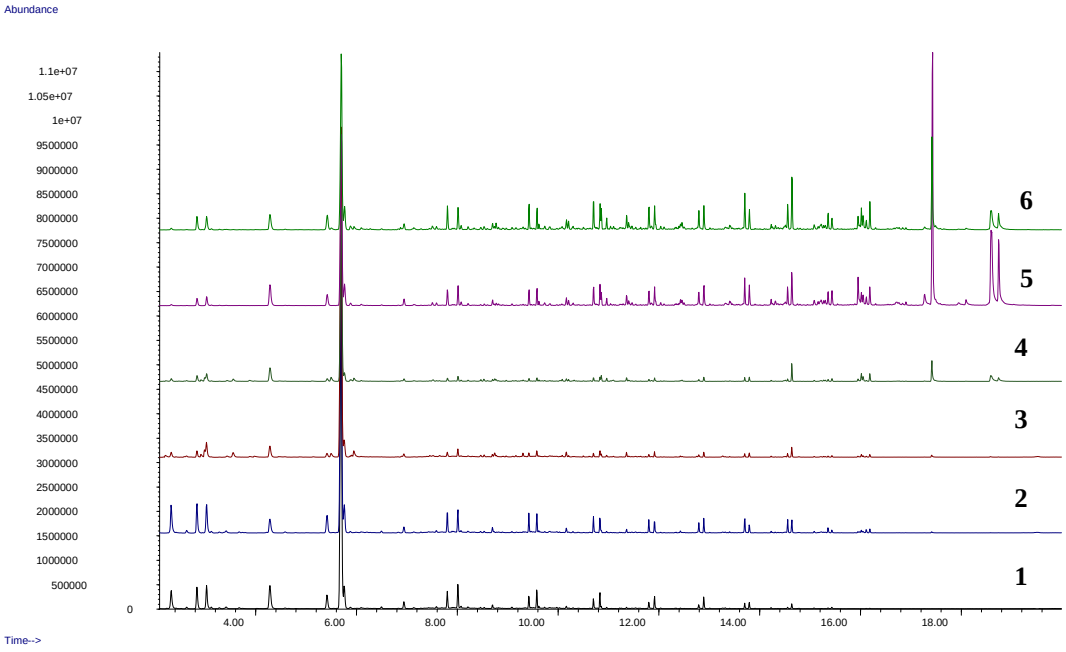

Figure 4.17 *Overlay of the chromatograms of the six samples of bone wrapped in wool.*

The TICs of samples 1, 2 and 3 of bone combusted in the presence of wool did not present peaks beyond 16.40 minutes. Furthermore, the TICs of samples 3 and 4 displayed low intensity peaks. Similarly, samples 1 and 2 reveal a decrease in the peak intensity could be seen along the chromatogram from more volatile to less volatile components and this trend is reversed in the chromatograms for samples 5 and 6.

Sample	Weight bone (g)	Weight material (g)	Total burning time (min)
1	30.82	2.79	12.6
2	16.30	2.44	6.5
3	14.71	1.89	6.7
4	28.46	2.38	21.7
5	24.34	2.45	17.4
6	16.39	2.34	7.7

Table 4.9 *Experimental conditions for the six bone samples combusted for five minutes after ignition in the presence of leatherette.*

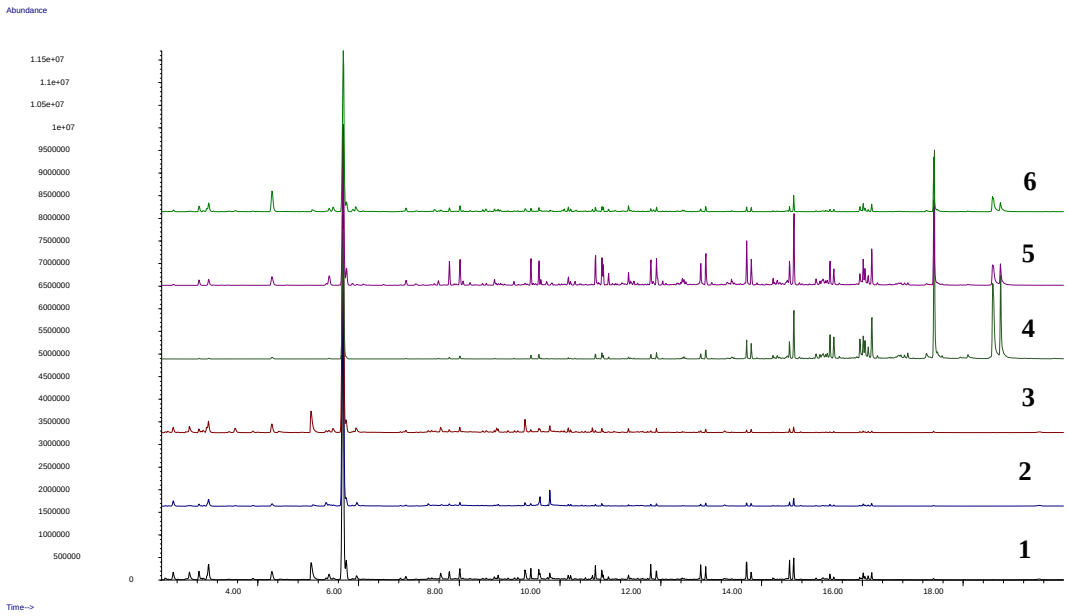

Figure 4.18 *Overlay of the chromatograms of the six samples of bone wrapped in leatherette.*

The TICs obtained for the samples of leatherette clearly demonstrated the variability within the results obtained. The TICs of samples 4 and 5 of combusted bone wrapped in leatherette gave few or no peaks at the early part of the chromatogram and the peak intensities gradually increased as heavier (less volatile) compounds eluted. In contrast, the TICs of samples 2, 3 and 6 however, contained very low concentrations of the various compounds present. The TIC of sample 1 illustrated peaks on the chromatogram at relatively low intensities across the retention time frame.

The shift of the chromatographic pattern toward the heavy less volatile end of the chromatogram is usually a result of evaporation of an ignitable liquid [10]. The lighter more volatile compounds evaporate faster than heavier compounds, causing the peaks on the right side of the chromatogram (in the figures above) to be enhanced. The presence of textile fibres could have caused the lighter compounds to evaporate faster as seen in the TIC of sample 2 in Figure 4.16, samples 5 and 6 in Figure 4.17, and samples 4 and 5 in Figure 4.18. On the other hand, the different fabrics could also retard the pyrolysis of the bone by protecting it from heat, resulting in less pyrolysis materials being present within the resultant extracts and chromatograms (e.g. TIC of sample 1 in Figure 4.16, sample 3 in Figure 4.17 and

sample 2 in Figure 4.18), and the constant decrease of the peaks intensities as the elution time increases in the TICs of samples 1 and 2 in Figure 4.17.

Furthermore, in cases where the light compounds evaporated and the more volatile compounds were more pronounced, an increase of the hexadecanenitrile (17.418 minutes), hexadecenenitrile (18.600 minutes) and heptadecanenitrile (18.741 minutes) peaks was observed.

4.4.2 Detection of the presence of aldehydes

Dehaan *et al.* [47] reported that animal fat and tissues produced homologous series of n-alkenes, n-alkanes and n-aldehydes, however no aldehydes were detected in the TIC of samples of fleshed or defleshed bone in this work after the ignition of the bone fat, although they were observed prior to combustion of the bone. However, the TIC of sample 4 of the bone wrapped in acrylic clearly displayed the presence of additional peaks at 2.638 and 5.052 minutes identified as pentanal and hexanal using the NIST library, as shown in Figure 4.19.

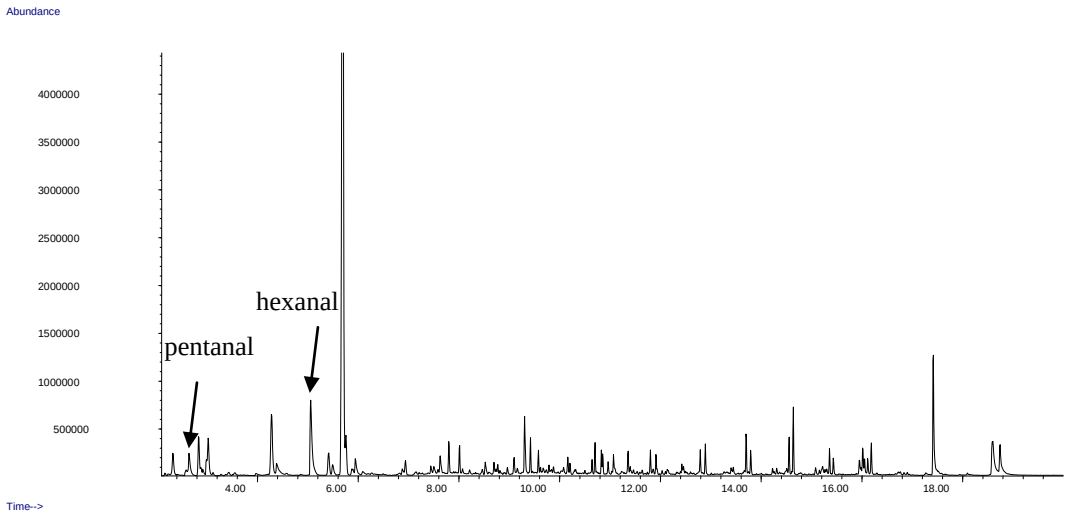

Figure 4.19 *Chromatogram of the bone sample 4 wrapped in acrylic presenting extra peaks.*

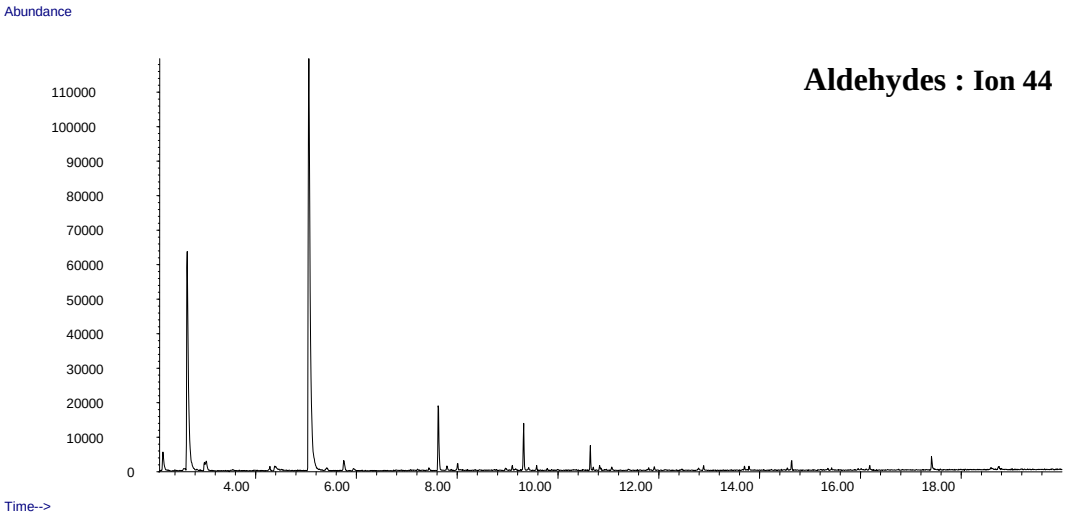

Figure 4.20 *Extracted chromatogram of ion m/z 44 from the total ion chromatogram of sample 4 of bone combusted in the presence of acrylic.*

The extracted chromatogram of ion m/z 44 represented in Figure 4.20 indicated the presence of other aldehydes such as 3-methylbutanal (2.163 minutes), heptanal (7.632 minutes), octanal (9.322 minutes) and nonanal (10.645 minutes), and also revealed the presence of 2-ethylhexanol at 9.788 minutes. The extracted ion chromatogram of ion m/z 44 of the samples of bone wrapped in leatherette also revealed the presence of the aforementioned aldehydes in samples 1, 2, 3 and 6 (Figure 4.21), and also 2-ethylhexanol in samples 1, 2 and 3. 3-methylbutanal and 2-ethylhexanol have been previously identified as volatile products of clothing soiled with body oils by Dehaan *et al*. No aldehydes were detected when the bone was combusted in the presence of wool.

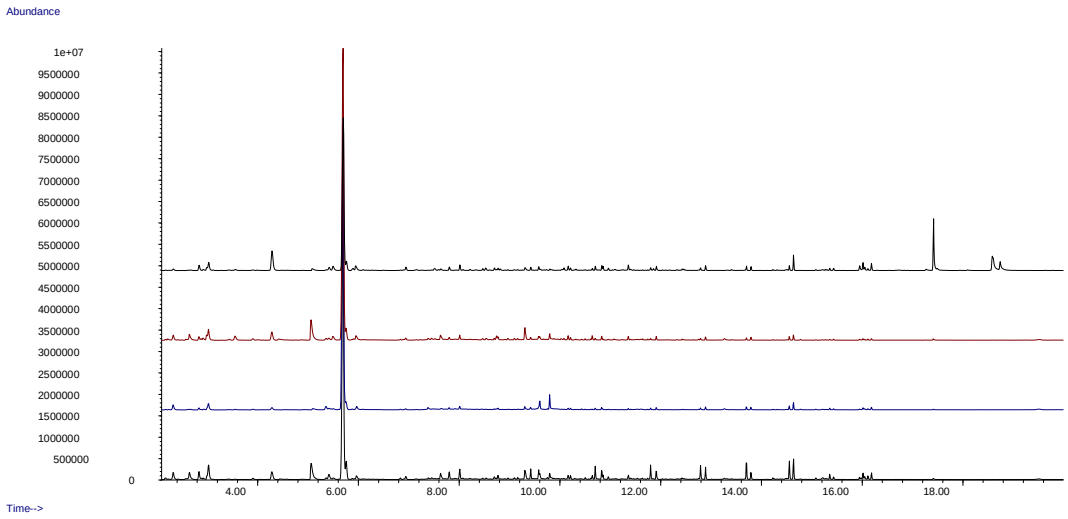

Figure 4.21 *Overlay of the chromatograms of bone wrapped in leatherette that presented aldehyde compounds.*

The presence of the aldehydes in the combusted samples suggests that the melted bone fat and soft tissues were absorbed by the acrylic and leatherette during the combustion process facilitating their subsequent presence in the chromatograms. The fat from the marrow and tissues could have melted and soaked into the textile materials as an example of the wick effect. When the flame was extinguished the products absorbed in the fabric were trapped and released during incubation in the extraction process.

4.4.3 Identification of the presence of textile fibres

The components previously identified for each material (and not present in the chromatogram of the bone sample) were used as target compounds to determine whether the pyrolysis products of the textile fibres were present in the chromatogram of the bone combusted with that material. Each target compound of a material was identified by retention time and mass spectral comparisons. Figures 4.22 to 4.24 represent the pyrolysis product chromatogram of the textile at the top, the chromatograms of the six repetitive samples underneath, and the chromatogram of bone below.

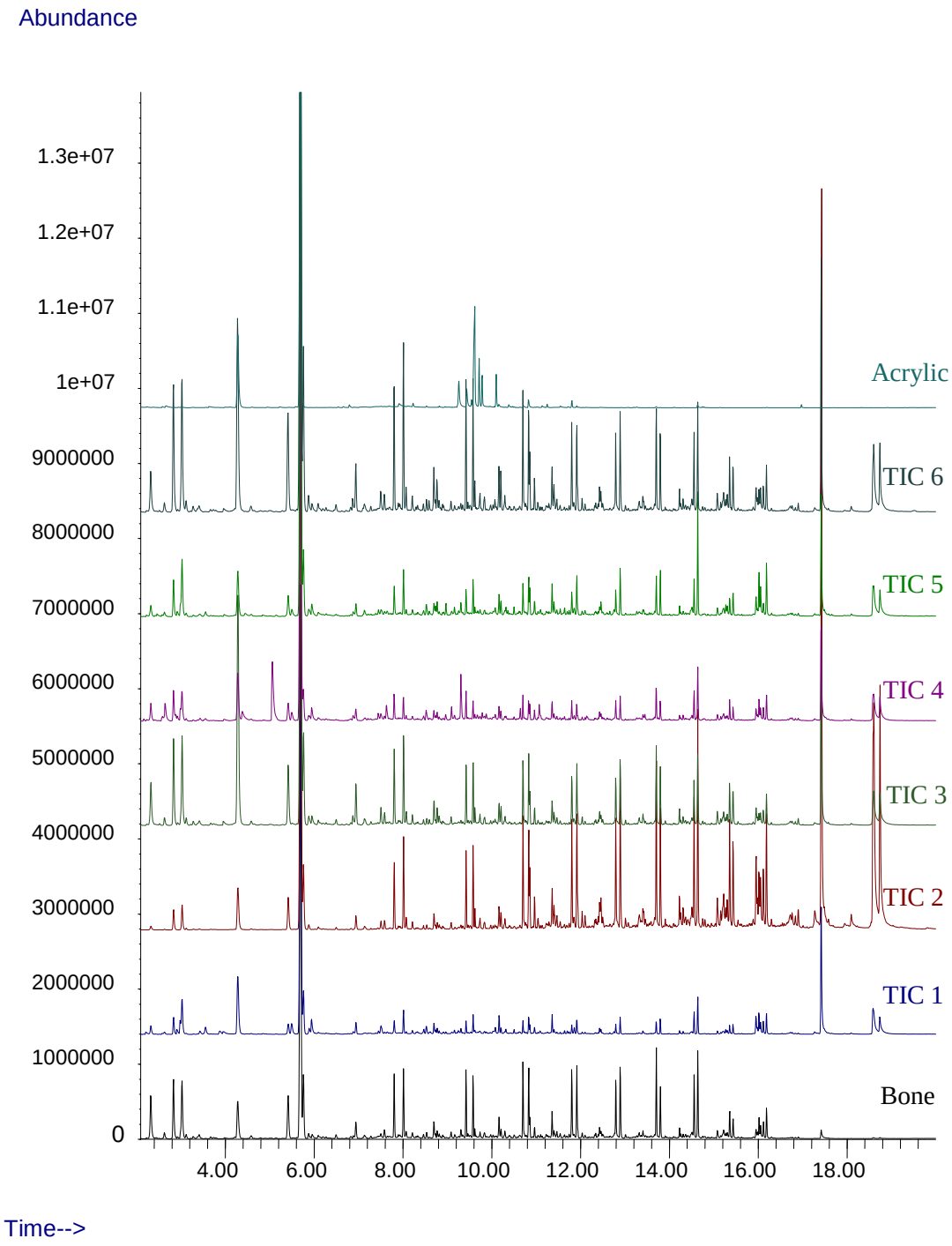

Figure 4.22 *Overlay of the chromatograms of bone, acrylic and the six samples of bone wrapped in acrylic.*

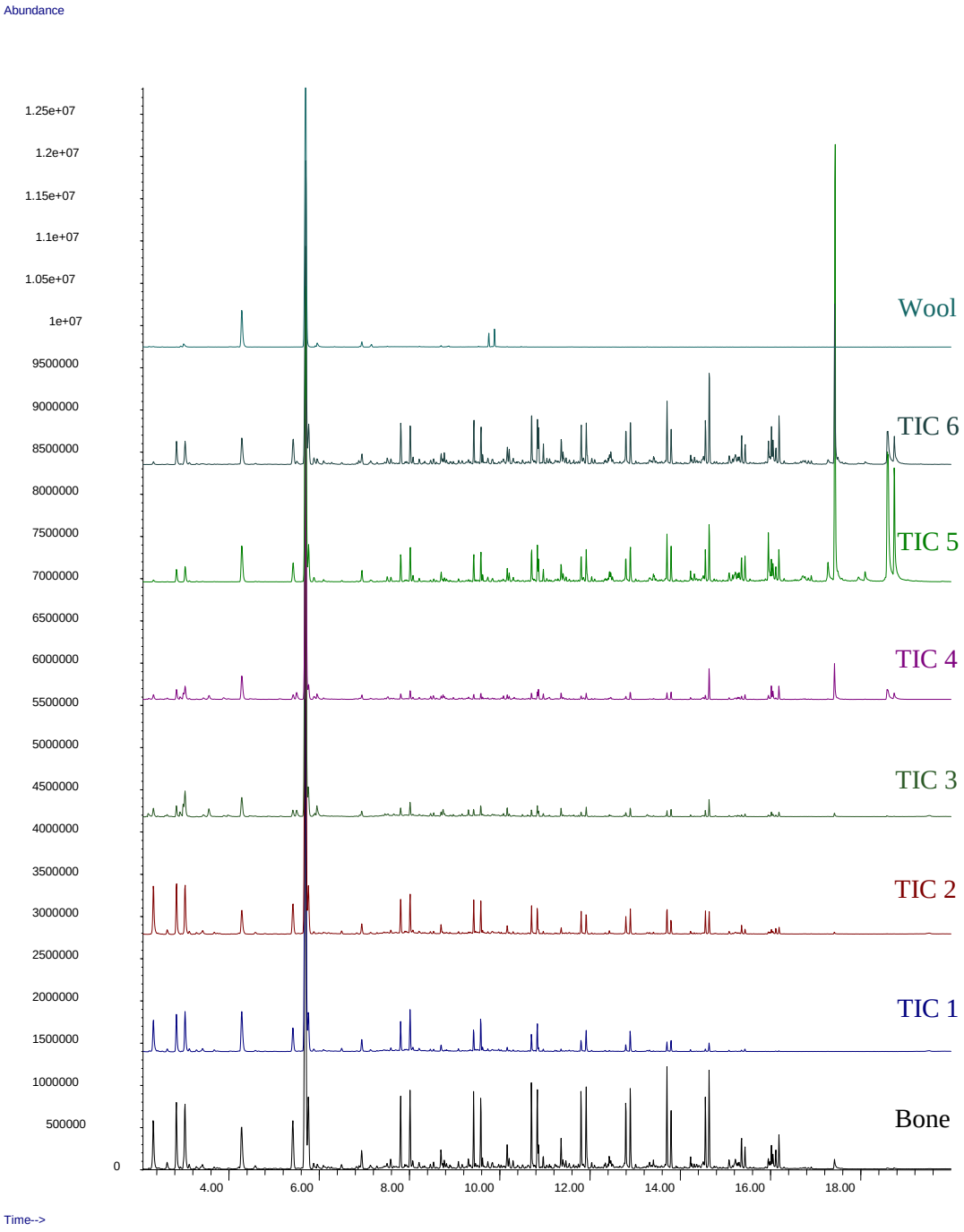

Figure 4.23 *Overlay of the chromatograms of bone, wool and the six samples of bone wrapped in wool.*

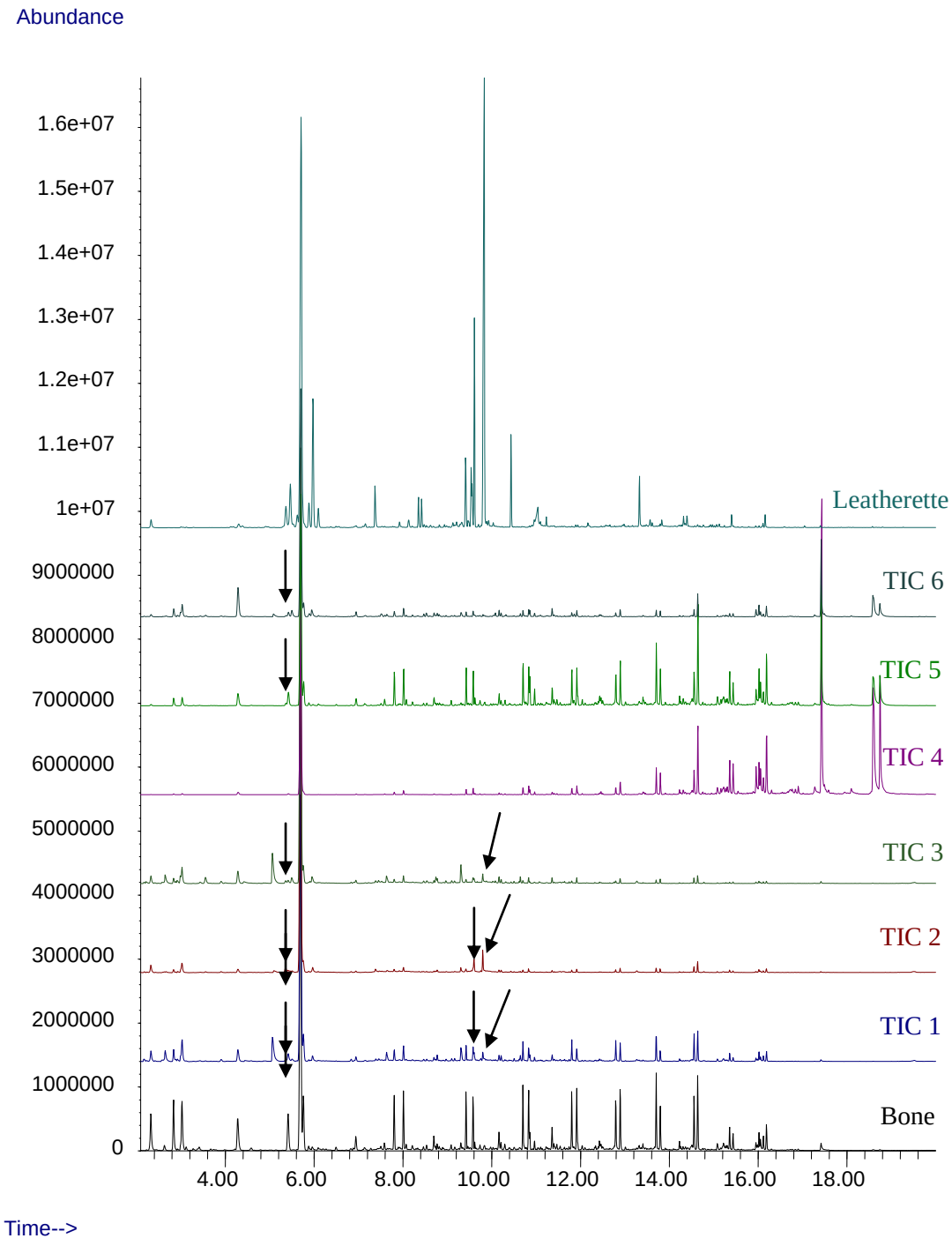

Figure 4.24 *Overlay of the chromatograms of bone, leatherette and the six samples of bone wrapped in leatherette.*

No acrylic or wool volatile components were detected in any of the relevant samples, which is unsurprising given the duration of combustion and the previous results relating to the generation of pyrolysis products from these materials under the test conditions used. Also, when the bone was combusted in the presence of leatherette, 2-ethylhexene was detected in samples 1, 2, 3, 5 and 6, chloro-2-ethylhexane was identified in samples 1 and 2, and 2-ethylhexanol was identified in samples 1, 2 and 3 (the compounds are indicated in the relative chromatograms in Figure 4.24). No other pyrolysis products of leatherette were identified.

The pyrolysis products of the materials did not interfere with the identification of the pyrolysis products of bone; hence the SIM technique was not needed to eliminate the background matrices produced by the materials.

4.4.4 Consequence on the repeatability of the results

The qualitative repeatability of the results was evaluated by the presence or absence of peaks among the six chromatograms of each material. The benzene peak (2.319 minutes) and the 1-heptene peak (2.836 minutes) were absent in the chromatogram of the bone sample 4 combusted in the presence of leatherette. Moreover, the heptadecene peak (16.119 minutes) and the heptadecane peak (16.186 minutes) were not detected in the chromatogram of the bone sample 1 wrapped in wool. Finally, aldehyde compounds were detected in the chromatograms of one bone sample covered with acrylic and four samples covered with leatherette (Subsection 4.4.2). These observations demonstrate that the experimental method did not give repeatable qualitative results.

The quantitative repeatability was determined by considering the peak intensity differences between each chromatogram for a given material. This repeatability was determined in a manner similar to that detailed in Chapter 2 (Subsection 2.3.3.4) and the values are presented in Tables 4.10 to 4.12.

Peak	Compound	%RSD			Concentration (mg/mL)
		Original data	Normalised to internal standard	Normalised to total peak area	
1	Benzene	90.12	84.47	65.17	0.0153 ± 0.0129
2	1-Heptene	80.98	74.81	55.04	0.0316 ± 0.0236
3	Toluene	88.79	82.97	64.79	0.0942 ± 0.0782
4	Tetrachloroethylene	7.94	0.00	28.91	0.5000 ± 0.0000
5	1-Nonene	75.27	70.34	50.52	0.0253 ± 0.0178
6	Nonane	71.33	66.78	46.60	0.0314 ± 0.0210
7	1-Decene	74.75	70.66	50.79	0.0207 ± 0.0146
8	Decane	72.29	68.37	48.02	0.0214 ± 0.0146
9	1-Undecene	75.80	73.56	52.07	0.0205 ± 0.0151
10	1-Dodecene	85.82	85.08	64.30	0.0172 ± 0.0147
11	1-Tridecene	88.71	88.49	66.22	0.0157 ± 0.0139
12	Tridecane	83.45	83.10	60.52	0.0258 ± 0.0214
13	1-Tetradecene	93.03	93.07	70.02	0.0257 ± 0.0239
14	Tetradecane	85.25	84.99	62.00	0.0241 ± 0.0205
15	1-Pentadecene	78.66	77.47	50.96	0.0182 ± 0.0141
16	Pentadecane	71.07	70.18	49.87	0.0356 ± 0.0250
17	1-Hexadecene	92.85	92.85	69.56	0.0140 ± 0.0130
18	Hexadecane	90.36	90.30	66.26	0.0144 ± 0.0130
19	1-Heptadecene	100.77	99.70	72.16	0.0077 ± 0.0077
20	Heptadecane	81.73	80.84	56.27	0.0183 ± 0.0148

Table 4.10 *Peak area relative standard deviations of twenty compounds among six bone samples wrapped in acrylic. The %RSD was calculated from the original, the normalised to the internal standard (tetrachloroethylene), and the normalised to the total peak area values.*

Peak	Compound	%RSD			Concentration (mg/mL)
		Original data	Normalised to internal standard	Normalised to total peak area	
1	Benzene	105.52	106.76	105.55	0.0145 ± 0.0155
2	1-Heptene	52.15	52.99	52.76	0.0213 ± 0.0113
3	Toluene	42.70	43.61	33.42	0.0399 ± 0.0174
4	Tetrachloroethylene	3.01	0.00	23.38	0.5000 ± 0.0000
5	1-Nonene	72.82	72.55	53.60	0.0172 ± 0.0125
6	Nonane	57.98	58.19	41.25	0.0199 ± 0.0116
7	1-Decene	82.96	82.57	61.72	0.0146 ± 0.0121
8	Decane	65.58	65.59	47.14	0.0150 ± 0.0099
9	1-Undecene	97.07	96.54	74.56	0.0152 ± 0.0146
10	1-Dodecene	103.67	103.18	82.80	0.0113 ± 0.0116
11	1-Tridecene	108.29	107.98	88.87	0.0089 ± 0.0097
12	Tridecane	89.93	89.73	67.64	0.0158 ± 0.0142
13	1-Tetradecene	106.27	105.85	87.50	0.0151 ± 0.0160
14	Tetradecane	88.66	88.87	68.51	0.0110 ± 0.0097
15	1-Pentadecene	109.50	109.27	91.51	0.0104 ± 0.0114
16	Pentadecane	105.26	104.97	84.19	0.0269 ± 0.0282
17	1-Hexadecene	122.72	122.44	109.00	0.0076 ± 0.0093
18	Hexadecane	115.77	115.94	102.47	0.0075 ± 0.0087
19	1-Heptadecene	113.71	113.59	99.10	0.0037 ± 0.0042
20	Heptadecane	116.01	115.72	98.92	0.0120 ± 0.0138

Table 4.11 *Peak area relative standard deviations of twenty compounds among six bone samples wrapped in wool. The %RSD was calculated from the original, the normalised to the internal standard (tetrachloroethylene), and the normalised to the total peak area values.*

Peak	Compound	%RSD			Concentration (mg/mL)
		Original data	Normalised to internal standard	Normalised to total peak area	
1	Benzene	66.81	67.98	77.06	0.0055 ± 0.0037
2	1-Heptene	73.65	72.01	62.90	0.0074 ± 0.0053
3	Toluene	107.67	102.73	109.42	0.0242 ± 0.0249
4	Tetrachloroethylene	7.11	0.00	29.19	0.5000 ± 0.0000
5	1-Nonene	152.28	151.75	111.01	0.0110 ± 0.0166
6	Nonane	129.26	128.64	86.17	0.0131 ± 0.0168
7	1-Decene	142.95	141.27	101.96	0.0111 ± 0.0157
8	Decane	130.01	128.32	91.78	0.0109 ± 0.0140
9	1-Undecene	141.85	139.67	103.73	0.0126 ± 0.0176
10	1-Dodecene	139.70	136.64	104.49	0.0108 ± 0.0147
11	1-Tridecene	131.37	128.93	94.54	0.0094 ± 0.0121
12	Tridecane	145.17	143.52	103.77	0.0140 ± 0.0202
13	1-Tetradecene	121.74	119.35	90.50	0.0184 ± 0.0220
14	Tetradecane	112.42	112.70	88.42	0.0137 ± 0.0155
15	1-Pentadecene	94.16	94.75	69.69	0.0141 ± 0.0134
16	Pentadecane	112.36	113.44	93.06	0.0430 ± 0.0488
17	1-Hexadecene	127.04	129.45	120.51	0.0137 ± 0.0178
18	Hexadecane	132.68	137.94	135.11	0.0125 ± 0.0173
19	1-Heptadecene	109.30	115.58	104.43	0.0063 ± 0.0073
20	Heptadecane	127.57	132.31	127.18	0.0262 ± 0.0346

Table 4.12 *Peak area relative standard deviations of twenty compounds among six bone samples wrapped in leatherette. The %RSD was calculated from the original, the normalised to the internal standard (tetrachloroethylene), and the normalised to the total peak area values.*

The %RSD values of the three data sets for the three materials were high, varying from 46.60% to 72.16%, 33.42% to 109.00% and 62.90% to 135.11% (excluding the internal standard) for the normalised to the total peak area data set of acrylic, wool and leatherette respectively. Hence the experimental method failed to produce repeatable quantitative results.

It is important to note that prior to burning the bone samples in the presence of the textile materials, the experimental method gave good qualitative repeatability of the results as shown in Chapter 2 (Subsection 2.3.3.4). The presence of textile materials demonstrated a significant decrease in this repeatability, which could have been due to the contact of the material with the bone. This is an important finding as it suggests that some of the expected pyrolysis products from bone samples may be missing as a consequence of the presence of clothing.

4.5 Conclusion

The study demonstrated that more pyrolysis products of textile fibres were successfully extracted when the sample was allowed to cool in air for fifteen seconds after combustion. Using the method described only acrylic, leatherette and wool produced volatile compounds, unlike cotton, nylon and polyester.

Significant volatile compounds were produced when acrylic was combusted for one minute and these included dinitriles, diidopropylcyanamide and isopropenylpyrazine. Leatherette generated a greater quantity of pyrolysates including alkenes, esters, chloroalkanes and carboxylic acids when combusted for thirty seconds. Finally, aromatic and nitrile compounds were noted as pyrolysis products of wool when combusted for two minutes.

In the presence of acrylic, leatherette and wool the detection of pyrolysis products of bone were altered which resulted in the decrease of peak intensities as the elution time increased and in the presence of compounds with very low peak intensities in some cases.

However, aldehyde compounds previously absent in the pyrolysis products of combusted bone were present as pyrolysis products in some of the fabric covered samples (one bone sample covered with acrylic and four samples covered with leatherette). The textile fibres may have absorbed some of the fat and oil as it was heated and were subsequently retained by the fabric.

The compounds targeted from the different textile materials did not reveal the presence of the fibres when combusted with bones. Hence, the pyrolysis products of the textile materials examined did not interfere with the identification of the pyrolysis products of bone.

With the presence of aldehyde compounds, the low peak intensities and the absence of the benzene peak, and the heptadecene and heptadecane peaks when the bone was respectively combusted in the presence of leatherette and wool, the experimental method failed to produce repeatable results.

Chapter 5 – Conclusion and further work

The aim of this study was to investigate the pyrolysis products of bone, as an understanding of the products generated by the combustion of bones and tissue could be of significant importance in the determination of interfering products and human remains in fire debris samples.

A methodology was developed by which a sufficient amount of thermal degradation products of bone were generated. The method involved burning fleshed or defleshed bone pieces of at least 13 g for five minutes after the ignition of the fat present in the bone. The volatile species were then extracted using the passive headspace adsorption method and the subsequent extract was analysed by GC-MS. This experimental method gave good qualitative repeatability.

A database of pyrolysis products from bone obtained post ignition was established by identifying the different compounds present in the total ion chromatogram of bone. It was demonstrated that the pyrolysis of bone produced homologous series of C_7 to C_{17} n-alkanes and n-alkenes, alkylbenzenes, n-alkyl nitriles, n-alkylcycloalkanes and n-alkylcycloalkenes. No aldehyde compounds were detected post ignition though they were present in the pre ignition and ignition samples. It was suggested that they might have decomposed or been consumed after the sample was left to burn at around 550 °C for at least one minute after ignition. It would be interesting to further investigate bone samples combusted between ignition and one minute post ignition of the bone fat to determine the cause of the absence of aldehydes post ignition.

The thermal degradation of common textiles fibres (cotton, nylon, polyester, acrylic, leatherette and wool) were investigated as these materials are commonly used in clothing and could interfere with the identification of pyrolysis products of bone in a fire debris sample. Only acrylic, leatherette and wool produced volatile compounds when the material samples were combusted for one minute and were allowed to cool in air for fifteen seconds after combustion. Further experiments could be carried out to develop a method that would generate the pyrolysis products of cotton, nylon and

polyester, so as to determine whether or not these materials could act as interfering products with the identification of pyrolysis products of bone.

When the bone was combusted in the presence of these materials using the aforementioned experimental method, it was observed that the pyrolysis products of the textile materials examined did not interfere with the identification of the pyrolysis products of bone, as the targeted compounds for each material were not did not reveal the presence of the fabric in the chromatograms. However, in the presence of these textile fibres the detection of pyrolysis products of bone was altered due to the evaporation of the lighter compounds which enhanced the peak areas of the heavier compounds, and also to the probable delay of the pyrolysis of bone caused by the materials that could have protected the bone from heat and flames, which was characterised by lower peak intensities. Furthermore, aldehyde compounds previously absent in the pyrolysis products of combusted bone were present as pyrolysis products in acrylic and leatherette covered samples. The textile fibres may have absorbed the melted bone fat during combustion and was subsequently retained by the fabric.

Finally, the combustion of bone in the presence of ignitable liquids using the optimised experimental method could provide information on the interfering products of bone and ignitable liquids for the respective identification of ignitable liquids residues and human remains in fire debris samples.

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