

An investigation into the recovery of ignitable liquid residues from entomological samples using solid-phase microextraction

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Somehow I can't believe that there are any heights that can't be scaled by a man who knows the secrets of making dreams come true. This special secret, it seems to me, can be summarized in four C s. They are curiosity, confidence, courage, and constancy, and the greatest of all is confidence. When you believe in a thing, believe in it all the way, implicitly and unquestionable.

Walt Disney

Certificate of Authorship and Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Lisa Mingari

25/03/2011

Signature of Candidate

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Abbreviations

Standard Abbreviations

ACS	Activated Charcoal Strip
ASTM	American Society for Testing and Materials
e.g.	Latin: <i>exempli gratia</i> , “for example”
EIC	Extracted Ion Chromatogram
<i>et al.</i>	Latin: <i>et alia</i> , “and others”
GC	Gas Chromatograph
GC-MS	Gas Chromatograph-Mass Spectrometer
i.e.	Latin: <i>id est</i> , “that is”
MS	Mass Spectrometer
<i>N.B.</i>	Latin: <i>nota bene</i> , “note well”
NFPA	National Fire Protection Authority
NSW	New South Wales
RT	Retention Time
SIM	Selected Ion Monitoring
TIC	Total Ion Chromatogram
UTS	University of Technology, Sydney
LPD	Light Petroleum Distillate
m/z	mass-to-charge ratio
MPD	Middle Petroleum Distillate

Unit and Quantity Abbreviations and Symbols

amu	atomic mass unit
°C	degrees Celsius
g	gram
hr	hour
L	litre
m	metre
km	kilometre
min	minute
mL	millilitre
s	second
eV	electron volts

Prefix Abbreviations and Symbols

α	alpha
β	beta
μ	micro (10^{-6})
m	milli (10^{-3})
c	centi (10^{-2})
k	kilo (10^3)
%	percentage
®	registered trademark

Definitions

There are words and phrases used in this thesis that have specific meanings in relation to this work. These have been described in the text; however, some of these definitions have been clarified below.

Absorption: The process in which a fluid permeates or is dissolved by a liquid or solid.

Accelerant: A substance, often an ignitable liquid, used to initiate a fire or increase the rate of growth or spread of fire.

Adsorption: Is the adhesion of atoms, ions, biomolecules or molecules of gas, liquid, or dissolved solids to a surface.

Ambient temperature: The fluctuating levels of heat in air.

Ant: An insect in the family Formicidae (order- Hymenoptera) which preys on carrion feeding arthropods.

Ante-mortem: Prior to death.

Arson: The act of deliberately and maliciously setting a fire to destroy property or to take a life.

Arthropod: Any of a large group of segmented invertebrate animals, such as insects and spiders, with jointed legs and sometimes a hard, external skeleton.

Blow fly: A higher fly in the family Calliphoridae, also known as bottle flies.

Calliphoridae: The insect order commonly known as blow flies.

Carrion: Decaying animal flesh.

Chromatography: Is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a mobile phase through a stationary phase, which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases.

Coleoptera: The insect order commonly known as beetles.

Combustible liquid: Is a liquid that vaporises and forms flammable mixtures with air when in an open container or when heated. An important characteristic of a flammable liquid is its flashpoint. Flashpoint is the minimum temperature at which the vapour concentration near the surface of the liquid is high enough to form an ignitable mixture. Any liquid with a flashpoint between 38°C to 93°C is considered combustible.

Decomposition: Post-mortem degenerative rotting of the corpse.

Desorption: The phenomenon whereby a substance is released from or through a surface. This process is the opposite of sorption (either adsorption or absorption).

Diptera: The insect order commonly known as true flies.

Egg: The characteristic reproductive unit of an adult female. Most carrion insects deposit eggs, but a few deposit first-instar larvae.

Entomology: The study of insects.

Entomotoxicology: The study of foreign substances in insects.

Flammable liquid: Any liquid with a flashpoint less than 38°C is considered to be a flammable liquid. (See combustible liquid).

Gas chromatography: The separation of organic liquids or gases into discrete compounds seen as peaks on a chromatogram (which indicates the relative amount of each specific component). Separation is done in a column that is enclosed in an oven held at a specific temperature or programmed to change temperature at a reproducible rate. The column separates the compounds according to their affinity for the material inside the column (stationary phase) and their boiling point.

Headspace: The gas phase in a container above the sample.

Ignitable liquid: Any liquid that is capable of promoting the spread of a fire, including a flammable liquid, combustible liquid, or any other material that can be liquefied and burned.

Incendiary fire: A fire demonstrated to be deliberately lit when referring to statistical data.

Instar: Larval growth stages.

Larva: Soft-bodied, sexually immature, feeding stage of an insect life cycle; otherwise known as a maggot for flies; plural is 'larvae'.

Larviposition: Eggs hatch within the adult female and are born as an active larva.

Maggot: See larva.

Maggot-mass: The collective, closely packed mass of higher fly larvae occurring in decomposing carrion.

Mass chromatograph: A representation of mass spectrometry data as a chromatogram, where the x-axis represents time and the y-axis represents signal intensity.

Mass spectrometry: A method of chemical analysis that vaporises then ionises, the substance to be analysed, and then accelerates the ions through a magnetic field to separate the ions by molecular weight. Mass spectrometry can result in the exact identification of an unknown compound, and is a very powerful analytical technique, especially when combined with chromatography.

Metamorphosis: Change in insects from larva to adult form.

Mobile phase: The part of the chromatographic system which carries the solutes through the stationary phase. The mobile phases are either liquids or gases.

Moult: Shedding of the skin of one life phase.

Mouth hooks: The paired maxillary oral structures of a maggot.

Muscidae: The insect order commonly known as house flies.

Necrophagous: Carrion-feeding insects; feeding on dead bodies.

Omnivorous: A tendency to eat anything and everything.

Oviposition: Egg-laying.

Post-mortem: After death.

Post-mortem interval (PMI): Time elapsed since death.

Prepuparium: Inactive post-feeding larval stage in which the body is contracted.

Pupa: That immature stage between the larva and adult in insects having complete metamorphosis. This is a stage of major transformation. Among higher flies the pupa is inside the puparium.

Pupariation: The immobilisation of the post-feeding maggot with the shrinking, hardening and darkening of its outer skin.

Puparium: A globular, barrel-shaped container formed from the retained, hardened skin of the third larval instar and inside which the pupa is formed.

Pupation: To change form into a pupa in the process of transforming from the larval stage to the adult.

Putrefaction: The foul smelling, anaerobic decomposition of moist or wet organic matter by micro-organisms.

Pyrolysis: A process where thermal energy (heat) breaks chemical bonds in polymeric materials. The resulting fragments are often volatile. Pyrolysis provides the fuel for matrices that do not undergo unassisted combustion. Wood burns because it pyrolyses into gas phase volatiles.

Pyrolysis products: The products formed as a result of the pyrolysis process.

Sarcophagidae: The insect order commonly known as fly flies.

Sheep strike: The condition produced by the development of blow fly maggots on living sheep.

Solid-phase microextraction (SPME): A sample preparation technique that involves the use of a fibre coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes (both volatile and non-volatile) from different kinds of media that can be in liquid or gas phase.

Species: A group of potentially interbreeding individuals that will produce reproductively viable offspring.

Stationary phase: In chromatography, the stationary phase is the non-mobile phase that is contained in the chromatographic bed.

Succession: Groups of species successively occupying a given habitat as the conditions of that habitat change.

Suspicious fire: A fire, demonstrated to be possibly deliberately lit, where no other cause is evident when referring to statistical data.

Volatility: The ease with which a substance passes from being a solid or liquid to being a vapour.

Wasp: Any winged insect in the order Hymenoptera, generally possessing a sting. These may be scavengers, predators or parasites of organisms at carrion.

Weathering: The evaporation of the more volatile compounds of an ignitable liquid resulting in a greater concentration of the less volatile compounds. This may be due to environmental conditions or due to exposure to extreme heat of a fire.

Abstract

The analysis of fire debris can indicate the presence of an ignitable liquid, but the volatility of these substances means that the likelihood of detecting them diminishes over time. It is proposed in this thesis that when a scene contains burnt human remains, entomological samples can be analysed for the detection of ignitable liquids, as an alternative to fire debris. It is hypothesised that a larva's ability to invade areas protected from the external environment, such as the natural body openings of cadavers, and accumulate substances present in the tissue in which they are feeding, will extend the period in which ignitable liquids can be detected.

In small-scale experiments conducted under controlled laboratory conditions, petrol and kerosene were detected in larvae of the blow fly *Lucilia cuprina*, (Wiedemann) (Diptera: Calliphoridae) that had been fed on meat burnt using these ignitable liquids. Four sample sets of meat, each with six replicates (24 meat samples in total) were prepared. The first and second sets were burnt using petrol and kerosene, respectively. The final two sets were control groups. Six larvae were collected daily from each of the 24 meat samples for a period of five days. Once the adults had emerged, six adults and six puparia were also collected from each meat sample. All of the entomological samples collected were analysed using solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS). It was found that larvae of the blow fly *Lucilia cuprina* can be used in a small-scale setting to detect both petrol and kerosene from burnt substrates for at least five days. Positive results for the ignitable liquids of interest were also obtained for a limited number of adult flies and puparia.

Given these findings, further research was conducted using a more realistic experiment (conducted in duplicate) that mirrored a casework scenario more closely. Sample sets identical to those in the small-scale experiments were prepared using 24 piglets, each approximately 1.39 kg in weight, instead of the meat samples. These piglets were placed a minimum of 51 m apart at the Holsworthy Military Area in New

South Wales, Australia, for three days. After this time, the piglets were transferred to a controlled laboratory. It was found during these fieldwork experiments that petrol and kerosene could be successfully detected in larvae for as long as eight days, and in the adult and puparia samples for at least one month. These findings confirm the significant advantage of using entomological samples as an alternative to fire debris, in that they extend the period available for sampling volatile ignitable liquids by at least one month. In particular, puparia can withstand changing climatic conditions, and unlike the larvae and adults, are immobile and hence could be found close to human remains even after considerable time has elapsed.