Title: Examination and Extraction of Fire Debris Evidence

1 Introduction

The analysis of fire debris evidence for the presence of ignitable liquids (whether residual or bulk) can play a large role in law enforcement's (or other similar agency's) investigations. The detection of such compounds at a fire scene does not necessarily lead to a conclusion that a fire was incendiary in nature. Certain chemicals and types of mixtures such as gasoline, fuel oils, charcoal lighter fluids, and paint thinners can be used to accelerate or help ignite fires.

The term ignitable liquid is a set of substances which fall within certain commonly accepted classification groups of chemicals and/or mixtures. While certain liquids may, in fact, be ignited under certain parameters or within particular environments (e.g., candle wax, motor/lamp oils), they are outside the classification scheme used within this discipline and will not be included within the ignitable liquid definition. Ignitable liquids are often mixtures that contain large numbers of hydrocarbon constituents. Gasoline, for example, has over 400 chemical constituents and can be considered one of the more complex ILRs. The collection and concentration of ignitable liquids takes advantage of the volatility of their constituents. A charcoal adsorbent material is used in conjunction with a heated container to capture such chemical constituents. Volatile chemicals are adsorbed onto the charcoal and then eluted with a solvent for further analysis.

Detection limits, volatility of liquid residues, and variations of sampling techniques/methods can influence detectability. Difficulties in identifying ignitable liquids arise from their complex composition, from matrix interferences caused by materials which contain ignitable liquids (e.g., pyrolysis products of styrene from carpet backing), and from the loss of volatile constituents due to weathering or other factors. Because most evidence is post-burn, very small amounts of ignitable liquids are trying to be detected – hence the term ignitable liquid residues (ILRs). Analysis of materials for ILRs is generally performed using gas chromatography-mass spectrometry (GC/MS). A technique within mass spectrometry called ion profile analysis (or target compound analysis) can be used to compare chemical data to reference materials and standards for identification purposes. Such an identification can be considered a characterization of a material based upon physical or chemical properties that sufficiently differentiates that material from similar substances.

2 Scope

This procedure serves as a general guideline for determining whether ignitable liquid(s) are within evidence by using certain sample preparations (e.g., extraction/elution) and tests (e.g., GC/MS). Authorized analysts who conduct such examinations within the fire debris category of testing are responsible for ensuring this procedure gets applied to applicable evidence and that results are placed within final reports. This procedure allows for the examination, extraction, and detection of ignitable liquids (ILs) within evidentiary materials. There are seven major classes of ignitable liquids (and one called miscellaneous/other) that chemicals may be identified as being part of by using gas chromatography, mass spectrometry, extracted ion profiling (or extracted ion profile analysis), or a combination thereof within recovered fire debris evidence (or related materials). The scope of this procedure will focus on the following classifications: gasoline, distillates (aka. petroleum distillates), isoparaffinic products, aromatic products, naphthenic-paraffinic products, normal-alkane products, oxygenated solvents, and miscellaneous (aka. others-miscellaneous)).

Certain low molecular weight chemicals (e.g., alcohols) may only be detected when in sufficient quantity and not at the residue level using this procedure.

3 Principle

The techniques employed within this procedure utilize physical examination, extraction, and instrument analysis to determine whether ILRs are present within evidentiary material. In general analytes are often captured and isolated by heating evidence within a container's headspace, trapped onto highly adsorbent material, extracted using an elution solvent, and then analyzed using instrumentation (e.g., GC/MS). Evidence may be examined without the need of an adsorbent material (e.g., when samples are in sufficient quantity/concentration) and may warrant dilution prior to analysis. When necessary, and if appropriate, alternate techniques can be used for analysis (e.g., passive headspace extraction, infrared spectrophotometry, headspace-GC/MS) even though they may not be specifically described in this procedure. Use of other unit/section procedures is allowed with reasonable modifications and if approved by the unit's FSE2 (or higher).

4 Specimens

Any type of material related to fire debris-type evidence should be able to be analyzed using this procedure. In order for heated headspace-type analyses to be performed, materials need to fit into a closed container (e.g., airtight-like metal can). Specimens needing to be diluted (e.g., suspected liquid gasoline) do not necessarily need to be in an airtight container for analyses to be performed. Evidence submitted which haven't been stored within airtight containers may need to be returned to submitting agencies due to quality control issues (e.g., contamination or loss of sample).

5 Equipment/Materials/Reagents

a. General laboratory glassware

b. Gas Chromatograph/Mass Spectrometer (GC/MS) (Agilent or equivalent)

c. Autosampler – Agilent (or equivalent)

d. GC Column - DB-5(MS), 30 m, 0.25 mm i.d., 0.25 µm film (or equivalent)

e. Heating mantles: capable of heating pint, quart, and gallon cans

f. Thermometer (reference only ; need not be certified/calibrated)

g. Vacuum source (mechanical pump or equivalent)

h. Cans: unused paint cans (e.g., pint, quart, one gallon, five gallon)

i. Pentane (reagent grade or equivalent)
j. Acetone (reagent grade or equivalent)
k. Appropriate solvent (reagent grade or equivalent)
l. Appropriate standards and controls (e.g., ASTM mix, Gasoline, Diesel, Lighter Fluid)
m. Activated charcoal tubes (SKC or equivalent)
n. Appropriate Gases (e.g., Nitrogen, Helium, Air)
o. Syringe (e.g., 0.5 mL – 2 mL, or equivalent)
p. Adhesive tape
q. Reference Standards (e.g., certified reference or purchased at store)

6 Standards and Controls

Positive and Negative Controls:

Standards/Controls can be purchased or generated in-house (e.g., evaporated gasoline) and stored appropriately. If known materials are purchased then their stabilities should be determined by the manufacturer (e.g., expirations dates). If stability information is not available, then known materials, when stored appropriately, will be valid for one (1) year since their last verification. All standards/controls/reagents will be verified prior to be used in casework analysis and documentation will be retained. Appropriate positive and negative controls will be used with each sample or batch of samples.

a. Positive Control

If necessary, one or more of the following can be used as a positive control.

i. Hydrocarbon Mixture (e.g., GC/MS Performance Check Solution)
ii. Gasoline Reference Standard (Prepared in-house at an appropriate concentration)
iii. Other ignitable liquid standards/materials (e.g., NCFS, ASTM, NIST, purchased)

b. Negative Control

One or more of the following will be analyzed with every case and prior to each batch of extract solutions that are analyzed to ensure that extractions and instrumentation are free from contamination. If samples are concentrated under $N_{2(g)}$ then a negative control (same solvent used for the evidence sample) will be concentrated and analyzed in the same manner at least once during the case in order to demonstrate that extractions are contamination-free.

i. Solvent(s) that may have been used with sample analysis (e.g., pentane)
ii. Empty Can

iii. Activated Charcoal Tube Extract

Note-01: The above can be combined to form a negative control (e.g., empty can's heated headspace purged through a charcoal tube and extracted with pentane)

c. Blanks

An appropriate blank solvent (e.g., extraction solvent) should be analyzed in-between each sample to ensure the instrument is free from carryover and is ready for the next analysis.

7 Sampling

All submitted specimens will be analyzed and sampling is not applicable. However, if multiple specimens are submitted and they are determined that they were sampled from the same/similar area and enough positive results have been found with respect to ignitable liquid/residues, it is up to the analyst's discretion to contact the FSE2 (or higher) and/or the submitting agency representative to possibly change the number of specimens to be analyzed in the submission.

8 Procedure

a. Initial Examination

i. Observe the evidence (i.e., contents of the can/packaging)

ii. Presence of odors within samples:

- Always work in well ventilated area.
- Wafting the contents in order to evaluate the odor of ignitable liquids is not recommended due to the nature of potential hazardous chemicals within fire debris samples. However, strong ignitable liquid odors may be noted when the can has been opened without purposely smelling the sample.
- Odor of strong ignitable liquids may influence recovery method choice.

iii. Note on the worksheet a description of the material and any other appropriate observations or characteristics.

iv. The lab case number, the item number, and the analyst's initials will be on the top or side of the can/packaging.

v. If materials are present which may require additional forensic analyses (e.g., latent print or DNA), the nature of the materials and the type of testing should be considered before heating and confirmed with appropriate section analyst(s).

b. Recovery and analysis of organic compounds (use one or more of the following techniques)

- Analyze a negative control (e.g., blank air, extraction solvent, or a can treated in the same manner as evidence) to ensure that the procedure and/or instrument is contamination-free.
- Analyze blanks (e.g., blank air, appropriate solvent) in-between all samples to ensure that there is no significant carryover.
- All instrumentation will have been successfully evaluated for quality prior to being used for casework.

i. Heated Headspace Technique

   A. Punch a hole in the lid of the submitted can.
   B. Cover the hole with a piece of plastic adhesive tape.
   C. Heat the can until a bulge appears under the tape (or reasonable time period)
   D. Remove the can from the heat source.
   E. Using an appropriate syringe (e.g., 3 mL), inject 0.5 mL (0.5 cc) of heated headspace into the GC/MS.
   F. Record appropriate information (e.g., approximate temperature, time period) within case notes.

ii. Adsorption/Elution (Dynamic) Technique

   A. Check the outside of the can for dirt or debris. If present, clean the can to ensure that it is contamination-free.
   B. Punch 1-4 evenly spaced holes around the circumference of the can near the bottom. If there is water in the can then place the holes approximately 1 inch above the water level.
   C. Replace the original lid with a lid adapted to hold a charcoal tube. Snip the ends of the charcoal tube and place it into the Swagelok fitting. Insert the tube with the ‘open’ end in the holder.
   D. Insert the can into the heating mantle (the temperature is approximate 80 °C. In some cases it may be necessary to insert a dial thermometer through the lid until it touches the contents of the can (determined by analyst).
   E. Connect the end of the charcoal tube to the vacuum line, and turn on the vacuum pump.
   F. After an appropriate period of time (i.e., when condensation appears in the open area of the tube or when the temperature of the contents reaches 40-50 °C) remove the charcoal tube. CAUTION: Avoid sample temperatures above 80 °C to minimize the stripping of ‘lighter’ components of flammable liquids.

G. Place the charcoal tube into an appropriate glass vial for collection of the eluate.

H. Using an appropriate pipette add solvent (e.g., 1.5 mL of pentane) through the top of the charcoal tube.

I. Elute the solvent through the charcoal tube. Repeat the procedure with a second wash, if necessary.

J. If the sample extract cannot be analyzed the same day then store the extract in the refrigerator or freezer under proper seal and record necessary information within LIMS.

K. If a petroleum distillate or gasoline odor was not discernible during initial observations (or if residual amounts are suspected) then concentrate the sample by using a stream of nitrogen gas ($N_{2(g)}$) to approximately 100 µL.

L. Inject extract solution into the GC/MS and analyze. Clean instrumentation appropriately in-between samples.

M. Thoroughly clean the bottom of the modified can lid by rinsing it and the interior of the Swagelok fitting with acetone. This removes condensed water and any organics from the lid.

N. Clean and/or ventilate sampling area to ensure it is free of any possible contamination of ignitable liquid residues (liquid or gaseous) prior to analyzing additional evidence.

O. Record appropriate notes in case file (e.g., differing solvents or extraction volumes, if used).

iii. Solvent Extraction (Debris, Liquids, Residues)

A. Debris:

1. While in a hood (or appropriate clean sampling area) rinse a suitable container with pentane (or other appropriate solvent), isolate the solvent, and treat as you would when analyzing an evidentiary sample. Label the extract as a negative control.

Note-02: At times a negative control may be labeled as a solvent blank.

2. Fill the same container (as above) half way with all or a portion of the contents to be analyzed. While in a hood (or appropriate clean sampling area) add an appropriate amount of solvent, such as pentane (the amount needed will vary with the amount of debris that was submitted). Add the solvent slowly, rinsing

the debris in the process of adding. Ensure the solvent comes into contact with all of the debris for a complete extraction to take place.

3. Filter the pentane extract solution and collect into a second clean container.
4. Concentrate (either by gentle heating or evaporation) until a small amount of solvent remains.
5. Transfer the extract solution into an appropriate container (e.g., glass vial or test tube) and concentrate as necessary using $N_{2(g)}$.
6. Analyze the extract solutions using relevant instrumentation (e.g., GC/MS). Clean instrumentation appropriately in-between samples.

B. Liquids:

1. Alternate instrumental techniques can be employed, if necessary and approved (e.g., infrared, headspace-GC/MS).
2. Dilute liquid samples by adding an appropriate amount of pentane (or other solvent). The amount is dependent on the concentration of the items to be analyzed. Each item will be evaluated by the analyst before an appropriate amount is determined.
3. Filter and concentrate, if necessary. Analyze extracts using relevant instrumentation (e.g., GC/MS). Clean instrumentation appropriately in-between samples.

C. Residues:

1. Consider using adsorption/elution
2. Rinse submitted evidence using appropriate amount of solvent (e.g., pentane).
3. Filter and concentrate, if necessary.
4. Analyze extracts using relevant instrumentation (e.g., GC/MS). Clean instrumentation appropriately in-between samples.

c. Record appropriate information within either case notes or worksheets if not captured within procedure (e.g., techniques used, solvents, volumes, concentrating/dilution volumes).

  i. Reagents and steps not explicitly stated in this procedure will be recorded within examination documents (e.g., dilutions, alternate extraction solvents, appropriate standards)

  ii. Lot numbers of solvents and other appropriate materials will be recorded within examination documents.

d. Sample Preservation

After each sample is analyzed solvent is added to the sample vial and it is placed into the submitted can. The sample vial should be placed under proper seal prior to being returned with the can. Evidence may be sub-itemized, if necessary. All evidence will be properly secured and accounted for during transfers and return.

9 Instrumental Parameters

The following are the typical operating parameters for the instrument used in this procedure. With approval from the Lead Examiner (or higher) these instrument conditions may be modified to adjust or improve the procedure. Documentation of these parameters should be included with sequence data so that any instrumental parameter changes may be recorded in case files.

GC Oven:

Initial Temp	40 °C
Initial Time	2.0 min.
Ramp	10 °C/min.
Final Temp	300°C
Hold Time	12.5 min.
Equil. Time	0.1 min.

GC Inlet/Injector:

Inj. Vol.	1 µL
Mode	Splitless
Inlet Temp.	250 °C
Total Flow	55 mL/min
Septum Purge	4 mL/min

GC Column:

Type	HP-5MS (or equivalent		Mode	Constant Flow
Length	30 m		Init. Flow	1 mL/min
Diameter	0.25 mm		Ave. Linear Velocity	36 cm/sec
Film Thickness	0.25 µm		Carrier Gas	Helium (He)

Mass Spectrometer:

Transfer Line	300 °C
Source	230 °C
Quadrupole	150 °C
Low Mass	40
High Mass	400

10 Decision Criteria

a. Evaluation of Data

i. The total ion chromatogram (TIC) and associated spectra for each sample are reviewed using appropriate GC/MS software. Each significant chromatographic peak should be reviewed to determine if it is an analyte of interest.

ii. Preliminary identification of an analyte or mixture can be achieved by operator recognition of particular chromatographic retention times/profiles, by instrumental library providing matching mass spectral information, use of reference standard controls for comparison, using other accepted methods of evaluation (e.g., ASTM guidelines), or a combination.

iii. A full identification occurs when the sample analyte (or mixture of analytes) is compared to a positive control (e.g., an in-house validated standard) and the following criteria are met:

A. Chromatography

Retention Times: Analyte peak retention times (or relative retention times (RRT)) are within +/- 5% between sample and positive control.

Profile: The overall chromatographic pattern profile of a mixture should compare favorably to the chromatographic profile pattern of the known. While pattern matching of extracted ion profiles rarely gives perfect correlation with reference ignitable liquids, some sort of correlation should be present.

Ratios: Based on accepted/published criteria (e.g., ASTM), certain analyte ratios within mixtures should be present.

Note-03: Exceptions may be made based on the analyte/mixture in question. For example, some weathered gasoline samples may not exactly compare in all aspects to pure gasoline and analyst experience/knowledge may factor in to decision making.

B. Mass Spectrometry

1. Mass spectra will compare favorably to mass spectra from positive controls (e.g., reference standards). Comparison of mass spectral fragment pattern ion ratios should also compare favorably but may differ based on certain factors (e.g., large concentration differences between controls and samples). Spectra may also compare favorably to known library spectra, although library spectra may have been produced under different mass spectral parameters.
2. Mass spectral reference libraries (e.g., Wiley, NCFS, in-house) will be used during the evaluation process. An internal library may be made of substances analyzed in-house and may not be in published libraries. Entries within an internal in-house library should be compared to published spectra from reliable sources prior to use in casework.

iv. To accept GC/MS data the following should be followed:

A. Blanks

1. The blank prior to the injection of the sample must not contain significant unexplainable peaks. However, due the nature of samples analyzed within the

Unit (i.e., burnt debris) certain substances may remain on a GC column even after the method has completed and thereby elute after multiple injections have been performed. If retention times significantly differ (i.e., minutes) then column carryover is likely the issue (as opposed to injection carryover) and data may be acceptable. Consultation of an FSE2 or higher should occur in these situations if considering acceptance of data.

Note-04: Significant Peaks: To justify the existence of a significant chromatographic peak the peak's baseline signal-to-noise ratio (SNR), based on height, should equal or exceed three (3).

2. If the analyte(s) being identified in a sample is present in the blank just prior to its injection then that sample peak(s) should be ten-times (10x) greater in height than that of the analyte peak(s) from the previous blank.
3. When it is known that there is a carry-over issue with a sample then multiple blanks can be injected to minimize the carryover and only the blanks after a sample and just prior to the next sample need to be evaluated.
4. Blanks injected after a sample injection should be free of any analytes of interest. Any analytes found in the blanks may indicate that the method's temperature program was not long enough to elute all the peaks from the GC column.

B. Controls

1. Negative controls will be free of analytes of interest and no significant unexpected peaks should be present. Explainable peaks will be acceptable.
2. Positive control analytes must have acceptable chromatographic and mass spectral data.
3. Unexpected results within positive or negative control data should be reported to the Lead Examiner or higher for evaluation.

v. In cases where identifications cannot be made due to the lack of obtaining a positive control then indications can be made, if appropriate (e.g., based on mass spectral library data). Consultation with other analysts/technical reviewers or the Lead Examiner (or higher) may be appropriate in such situations.

vi. Positive controls should be analyzed contemporaneously when identifying analytes in evidentiary samples or when specifically looking for analytes in samples which result in negative results.

Note-05: If reference standards/positive controls have been analyzed previously (i.e., not contemporaneously) then such data may be used for comparison purposes if no significant instrument/method changes have occurred (e.g., column clipping, column change, temperature program change).

vii. Documentation: Appropriate instrumental printouts, including reagent blanks and controls, will be included in the case file such that an independent reviewer would be able to readily interpret analyst conclusions.

- A. All appropriate chromatograms will be retained in case files. Label the chromatographic peaks for all analytes being identified.
- B. All identified analytes will have documented spectra and appropriate comparative reference spectra (e.g., from positive controls and/or spectral libraries) within the case files.
- C. In samples where no analytes are identified the analyst should include the TIC along with any appropriate extracted ion chromatograms (EICs). If there are significant peaks that cannot be identified, or attributed to common artifacts, then such spectra should be included in the case file.

Note-05: Mass spectral data is considered significant if, by not including such data in a case file, it would diminish be detrimental to the quality of the examination/results.

11 Calibration

Not applicable.

12 Uncertainty

Not applicable – this is a qualitative procedure only.

13 Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment should be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use, especially. When casework samples are being processed/analyzed brown paper (or other similar barrier) should be placed in between the surface and the specimens.

14 References

a. General Laboratory (GL) Manual and Procedures

b. ASTM Standard Methods

c. GC-MS Guide to Ignitable Liquids

d. Bertsch, W., Holzer, G., and Sellers, C. S., *Chemical Analysis for the Arson Investigator and Attorney***,** Huthig Buch Verlag: Heidelberg, 1993

e. Hendrikse, J., Grutters, M., Schäfer, F. *Identifying Ignitable Liquids in Fire Debris. A Guideline for Forensic Experts*. Academic Press, 2016

f. Kelly, R. L., and Martz, R. M., *Accelerant Identification in Fire Debris by Gas Chromatography/Mass Spectrometry Techniques,* Journal of Forensic Sciences, Vol. 29, No. 3, July 1984, pp. 714-722

g. Stauffer, E., Dolan, J.A, Newman, R., *Fire Debris Analysis*, Academic Press, Burlington, MA, 2008.

h. Kelly, R. L., and Martz, R. M., *Accelerant Identification in Fire Debris by Gas Chromatography/Mass Spectrometry Techniques,* Journal of Forensic Sciences, Vol. 29, No. 3, July 1984, pp. 714-722

i. Stauffer, E., Dolan, J.A, Newman, R., *Fire Debris Analysis*, Academic Press, Burlington, MA, 2008.

Rev. #	History
2	Slight format change. Replaced section with unit. Modified the 'Materials' section. Added a 'Standards and Controls' section specifically requiring controls to be analyzed. Re-worded the 'smell' portion of the procedure. Decreased injection volume in 'Heated Headspace' section from 1 mL to 0.5 mL. Fixed type from 180 °C to 80 °C within 'Absorption/Elution' section. Decreased amount of pentane used during extraction within 'Absorption/Elution' section from 0.5 mL to approximately 0.3 mL. Added requirement to record lot numbers and solvents within examination documents. Added a 'Revised History' section to the document.
3	Revised sections 4c and 5b to reflect that if samples are accelerant-free, the analysis of a blank after that particular sample is not required.
4	General format change throughout document ; Added sections: 'Introduction,' 'Scope,' 'Principle,' 'Specimens' ; Updated the 'Materials' section ; Updated 'Samples and Controls' section ; added 'Sampling' section ; updated 'Procedure' section to be in-line with current practices ; specified documentation needs within casefiles ; added steps to address potential cross-contamination ; Added 'Instrumental Parameters' section ; Added 'Decision Criteria' section ; updated references. Updated how to detect odors within Section 8.