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## Evaluation of Internal Standards for the Analysis of Ignitable Liquids in Fire Debris

**ABSTRACT:** An evaluation of eight compounds for use as an internal standard in fire debris analysis was conducted. Tests were conducted on tetrachloroethylene, chlorobenzene, n-octylbenzene, 3-phenyltoluene, and deuterated compounds toluene-d8, styrene-d8, naphthalene-d8, and diphenyl-d10 to measure the extraction efficiency of each compound in the presence of an interfering volatile compound (carbon disulfide). Other tests were conducted to evaluate whether or not the presence of an ignitable liquid or pyrolysis/combustion products from fire debris would interfere with the identification of these compounds when used as an internal standard. The results showed that while any of the eight compounds could be used as an internal standard in fire debris analysis, the more volatile compounds (toluene-d8, tetrachloroethylene, chlorobenzene, and styrene-d8) showed better extraction efficiencies at room temperature than when heated to 60°C. Each of the less volatile compounds (naphthalene-d8, diphenyl-d10, n-octylbenzene, and 3-phenyltoluene) performed well during extraction at 60°C, while naphthalene-d8 showed better extraction efficiency in the presence of competing volatiles when extracted at room temperature.

**KEYWORDS:** forensic science, fire debris, ignitable liquid, internal standard, gas chromatography-mass spectrometry

The use of an internal standard to monitor the extraction and analysis of target analytes from a matrix is a standard component of laboratory quality control. The field of forensic toxicology has routinely employed a variety of internal standards to quantitate the recovery of volatile hydrocarbons in body fluids. Deuterated toluene (toluene-d8) and indane have been used as internal standards for the quantitative analysis of volatile hydrocarbons in blood (1,2). Similarly, n-octylbenzene has been used in the toxicological analysis of gasoline and nitrobenzene in biological specimens (3,4). Toluene-d8 has also been used as an internal standard for the environmental analysis of light volatile organic compounds found in indoor and outdoor air (5). Halogenated compounds, such as 1,1,2-trichlorotrifluoroethane, have been used as an internal standard for the analysis of lighter fluid in blood (6), and chlorobenzene-d5 was used as an internal standard in the chemical analysis of food exposed to smoke (7). Finally, an internal standard solution of n-pentane and 1-pentanol was added to the blood of fire victims during a headspace extraction for volatile hydrocarbons (8).

There are few reports in the scientific literature that describe the use of an internal standard to qualitatively monitor the extraction process used in fire debris analysis. The ASTM method E1412 has suggested the use of either 3-phenyltoluene or diphenylmethane as an internal standard added to the eluting solvent (9). Tan and coworkers reported the addition of naphthalene-d8 as an internal standard to the solvent extract obtained from accelerant-spiked solid matrices (10). Cavanagh et al. used 2-hexanone in the eluting solvent as an internal standard for the study of gasoline from motor vehicle carpets (11,12). Similarly, Almirall and Furton have reported the addition of tetrachloro-m-xylene (TCMX) to the solvent used to elute activated charcoal strips used to collect pyrolysis

products from fire debris (13,14). However, in these cases the addition of an internal standard to the eluting solvent does not demonstrate the efficiency of the headspace extraction. Touron reported the addition of the internal standard n-tetradecane directly to fire debris received in routine casework (15). Chasteen described the addition of 3-phenyltoluene directly to fire debris (16), while Stauffer reported the use of the internal standard tetrachloroethylene in the eluting solvent in combination with the addition of 3-phenyltoluene directly to the fire debris prior to adsorption of the volatile hydrocarbons onto activated charcoal (17). To our knowledge, no evaluation of the suitability of any of the above compounds as internal standards for fire debris analysis has been published.

Ideally, an internal standard for fire debris analysis should have four characteristics: it should be a compound that is not commonly found in pyrolysis, combustion, or distillation products normally present in fire debris or in an ignitable liquid; it should elute within the typical boiling range of the compounds normally present in fire debris and ignitable liquids; it should have a sufficiently unique mass spectrum that it can be extracted from a total ion chromatogram obtained from the analysis of fire debris; and, it should be cost effective. The goal of this study was to evaluate eight different compounds for their use as an internal standard in the analysis of fire debris.

### Materials and Methods

#### *Standards and Reagents*

Eight compounds were selected as internal standard candidates (ISCs) for this study. Two compounds were chlorinated (tetrachloroethylene and chlorobenzene), two were hydrocarbons (n-octylbenzene and 3-phenyltoluene), and four were deuterated (toluene-d8, styrene-d8 stabilized with 4-t-butylcatechol, naphthalene-d8, and diphenyl-d10). All compounds were obtained from Sigma-Aldrich (Oakville, Canada), except chlorobenzene which was purchased from Fisher Scientific (Nepean, Canada). A comparison of the cost of each internal standard is given in Table 1. Some of the physical

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TABLE 1—Relative cost of each ISC.

Compound	Unit Cost (US\$)*	Relative Cost
Toluene-d8	6.73	84
Tetrachloroethylene	0.45	6
Chlorobenzene	0.08	1
Styrene-d8	110.00	1375
Naphthalene-d8	40.80	510
Diphenyl-d10	58.70	734
n-Octylbenzene	3.96	50
3-Phenyltoluene	39.30	491

\*Unit cost expressed as the cost per 1 mL or 1 g based on 2007 prices.

and chemical properties for each compound are listed in Table 2. n-Undecane and n-dodecane were purchased from ChemService (West Chester, PA) and used as internal standards in the eluting solvent carbon disulfide (CS<sub>2</sub>) during the evaluation of extraction efficiency of each ISC. Low benzene CS<sub>2</sub> and dichloromethane solvents were purchased from Fisher Scientific and used as received. New, unused metal paint cans and glass canning jars were obtained locally and each had a volume of c. 1 L. Activated charcoal strips were purchased from Albrayco Laboratories (Cromwell, CT). A charcoal strip was suspended inside a container with a safety pin. Each safety pin was washed before use with dichloromethane, and the head bent perpendicular to the pin shaft so that a rare-earth magnet placed on the outside of the lid could firmly hold the safety pin to the inside of the lid.

#### Gas Chromatography-Mass Spectrometry Conditions

All samples were analyzed on a Hewlett-Packard (HP) 6890 series gas chromatograph (GC) interfaced with an HP 5973 mass selective detector (MS) using electron impact ionization. A 30 m × 0.25 mm HP-1MS capillary column (0.25-μm film thickness) with hydrogen carrier gas at a constant flow rate of 1.1 mL/min was used. The inlet temperature was set to 250°C and a split ratio of 20:1 with a split flow rate of 22.0 mL/min was used. Electron energy was 70 eV and full scans from 30 to 300 amu were collected. Except for the determination of the extraction efficiencies, the column was initially held at 40°C for 3 min,

ramped at 8°C/min to 250°C, and held for 0.75 min. For the analysis of the extraction efficiency of a mixture of all eight ISCs, the column was initially held at 40°C for 3 min, ramped at 12°C/min to 250°C, and then held for 0.75 min. The higher ramp rate was required to separate n-octylbenzene and 3-phenyltoluene. For both temperature programs, the post-run temperature was maintained at 300°C for 5 min.

#### Extraction Efficiency

A passive headspace extraction method based on ASTM E1412 was used to determine the extraction efficiency for each ISC. An ISC stock solution containing 10 mg each of naphthalene-d8 and diphenyl-d10 dissolved in 10 μL each of toluene-d8, tetrachloroethylene, chlorobenzene, styrene-d8, n-octylbenzene, and 3-phenyltoluene was prepared. This stock solution was used to prepare all other solutions. A Kimwipe™ (Kimberly-Clark, Mississauga, ON, Canada) was placed into a 1-L container and spiked with either 25, 50, 100, 500, 1000, or 2000 μL of CS<sub>2</sub> containing 1 μL of the original ISC stock solution. This procedure was also repeated in the absence of CS<sub>2</sub> where a Kimwipe™ was placed into a 1-L container and spiked with 1 μL of the original ISC stock solution. Passive headspace extractions were performed for 16 h at room temperature and at 60°C. For the heated passive headspace extraction, the container containing 2000 μL of CS<sub>2</sub> was omitted due to concerns of excessive solvent vapor pressure being built up inside the container during heating. All extractions were performed in triplicate. The charcoal strips were eluted with 600 μL of CS<sub>2</sub> spiked with an n-alkane internal standard (either n-undecane or n-dodecane) at a concentration of 1:2000 (v/v). The results of the passive headspace extractions were compared with the GC-MS results obtained (in triplicate) when 1 μL of stock solution was pipetted onto an activated charcoal strip followed by elution of the strip with 600 μL of CS<sub>2</sub> spiked with n-alkane internal standard.

The peak area ratio of each ISC to n-alkane internal standard was calculated for each run. The extraction efficiency of each compound was calculated from the ratio of each ISC compared with the mean peak area ratio of the corresponding compound when the stock solution was deposited directly onto the charcoal strip and eluted.

TABLE 2—Selected properties for the ISCs.

Internal Standard Candidates	Formula	Formula Weight	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure (kPa)
Toluene-d8 (perdeuterotoluene)					
C <sub>6</sub> D <sub>5</sub> CD <sub>3</sub> (99.6 atom % deuterated)	C <sub>7</sub> D <sub>8</sub>	100.19	-93*	110	38.8* <sup>†</sup>
Tetrachloroethylene (perchloroethylene)					
CCl <sub>2</sub> = CCl <sub>2</sub>	C <sub>2</sub> Cl <sub>4</sub>	165.83	-22	121	27.6 <sup>†</sup>
Chlorobenzene (phenyl chloride)					
C <sub>6</sub> H <sub>5</sub> Cl	C <sub>6</sub> H <sub>5</sub> Cl	112.56	-45	132	19.7 <sup>†</sup>
Styrene-d8 (perdeuterostyrene)					
C <sub>6</sub> D <sub>5</sub> CD = CD <sub>2</sub> (98 atom % deuterated)	C <sub>8</sub> D <sub>8</sub>	112.20	-31*	145–146*	12.2* <sup>†</sup>
Naphthalene-d8 (perdeuteronaphthalene)					
C <sub>10</sub> D <sub>8</sub>	C <sub>10</sub> D <sub>8</sub>	136.22	80–82	218	0.99* <sup>†</sup>
Diphenyl-d10 (1,1'-biphenyl-d10)					
C <sub>12</sub> D <sub>10</sub> (99 atom % deuterated)	C <sub>12</sub> D <sub>10</sub>	164.27	70–72	255*	0.20 <sup>†,§</sup>
n-Octylbenzene (1-phenyloctane)					
C <sub>6</sub> H <sub>5</sub> (CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub>	C <sub>14</sub> H <sub>22</sub>	190.32	-36	261–263	0.32 <sup>†,‡</sup>
3-Phenyltoluene (3-methyl-1,1'-biphenyl)					
C <sub>6</sub> H <sub>5</sub> C <sub>6</sub> H <sub>4</sub> CH <sub>3</sub>	C <sub>13</sub> H <sub>12</sub>	168.23	4–5	272	0.20 <sup>†,§</sup>

\*Value calculated for the non-deuterated compound.

<sup>†</sup>Vapor pressures calculated from NIST data using the Antoine equation at 80°C. Frenkel, M (Director), "Thermodynamics Source Database" in NIST Chemistry WebBook, NIST Standard Reference Database Number 69, P.J. Linstrom and W.G. Mallard, Editors. June 2005, National Institute of Standards and Technology, Gaithersburg, MD 20899 (<http://webbook.nist.gov>).

<sup>‡</sup>Vapor pressure estimated using data for n-heptylbenzene.

<sup>§</sup>Vapor pressure estimated using data for diphenyl (C<sub>12</sub>H<sub>10</sub>).

### Evaluation of ISCs in the Presence of Different Ignitable Liquids

A passive heated headspace extraction method based on ASTM E1412 was used to evaluate each ISC in the presence of different types of ignitable liquids. An ISC stock solution containing 50 mg each of naphthalene-d8 and diphenyl-d10 was dissolved in 50  $\mu\text{L}$  each of toluene-d8, tetrachloroethylene, chlorobenzene, styrene-d8, n-octylbenzene, and 3-phenyltoluene. A 100  $\mu\text{L}$  aliquot of this stock solution was diluted in  $\text{CS}_2$  to a final volume of 500  $\mu\text{L}$ . A 25- $\mu\text{L}$  aliquot of the  $\text{CS}_2$ :ISC solution was spotted onto a Kimwipe™ in a container fitted with a charcoal strip, 100  $\mu\text{L}$  of ignitable liquid added, and the container sealed. Samples without any ISCs were prepared by spotting 100  $\mu\text{L}$  of an ignitable liquid onto a Kimwipe™ in a 1-L container fitted with a charcoal strip and the container sealed. Each container was heated for 16 h at 60°C, after which time each charcoal strip was removed from its container and eluted with 600  $\mu\text{L}$   $\text{CS}_2$ . Ignitable liquids used in this part of the study were two different gasolines (Esso™ regular and Esso™ premium) at three different levels of evaporation (0%, 50%, and 90% evaporated), two light petroleum distillates (Coleman™ camp fuel and Zippo™ lighter fluid), two medium petroleum distillates (Varsol™ paint thinner and Mastercraft™ odorless paint thinner) and two heavy petroleum distillates (STP™ fuel injector cleaner and Esso™ diesel fuel).

The ISCs were also evaluated in the presence of a standard accelerant mixture (SAM) consisting of a 1:1 (v/v) mixture of 50% evaporated gasoline and unevaporated diesel fuel. Fifty microliters of SAM was spotted onto a Kimwipe™ in a 1-L metal can. An ISC stock solution containing 10 mg each of naphthalene-d8 and diphenyl-d10 dissolved in 10  $\mu\text{L}$  each of toluene-d8, tetrachloroethylene, chlorobenzene, styrene-d8, n-octylbenzene, and 3-phenyltoluene was prepared. One microliter of the stock solution was spotted onto a piece of filter paper (Whatman), the paper immediately placed inside the can containing the SAM, and the can fitted with a charcoal strip. Separate extractions were conducted at room temperature and in an oven set at 60°C. After 16 h, each charcoal strip was removed from its container and eluted with 600  $\mu\text{L}$   $\text{CS}_2$ . The extracts were analyzed by GC-MS.

### Evaluation of ISCs in the Presence of Fire Debris

Pyrolysis and combustion products were generated by burning a variety of materials commonly encountered at fire scenes. Pieces of dressed spruce lumber (9 × 3.5 × 4 cm), nylon carpet and polyurethane foam underlay (8 × 5 cm), vinyl flooring (15 × 8 cm), high density polyethylene plastic container lids (12 cm diameter), polyurethane chair foam (8 × 8 × 4 cm), newsprint (10 pages), file folder (cut up), and corrugated cardboard (36 × 5 cm) were each placed in a ceramic dish lined with aluminum foil and charred in a muffle furnace set to 300°C. Each material was heated in the furnace until significant charring of the surface was observed. The heating time in the furnace was determined by trial and error, and was dependent on the type of material being charred. Heating times for each material were as follows: dressed spruce lumber (10 min); nylon carpet and polyurethane foam underlay (7 min); vinyl flooring (6 min); high density polyethylene plastic container lids (18 min); polyurethane chair foam (5 min); newsprint (12 min); file folder (10 min); and corrugated cardboard (5 min). Immediately after the material was removed from the furnace, it was extinguished with water, placed into a container, and sealed. An ISC stock solution containing 10 mg of naphthalene-d8 dissolved in

10  $\mu\text{L}$  each of toluene-d8, tetrachloroethylene, chlorobenzene, styrene-d8, n-octylbenzene, and 3-phenyltoluene was prepared (diphenyl-d10 was not included in this stock solution). The stock solution was diluted with 200  $\mu\text{L}$  of  $\text{CS}_2$  and a 25- $\mu\text{L}$  aliquot of the solution was added directly to the cooled debris using a microliter syringe. The extracts were analyzed by GC-MS.

## Results and Discussion

### Selection of ISCs

Ideally, an internal standard should not be a compound commonly found in pyrolysis, combustion, or distillation products from fire debris, or in an ignitable liquid. For this reason, a large number of halogenated compounds could serve as internal standards because halogenated compounds are not commonly encountered at fire scenes as ignitable liquids or as pyrolysis or combustion products from the debris. Chlorinated compounds such as tetrachloroethylene and n-chlorobenzene were considered over brominated and fluorinated compounds because they tended to be more readily available and significantly less expensive. For a similar reason, isotopically labeled compounds are commonly employed as internal standards in toxicology and drug analysis (18). A common isotope used is deuterium in place of hydrogen. Three *perduetero* compounds were selected based on the fact that one compound is common to fire debris (styrene-d8), and two are common to both fire debris and some ignitable liquids such as gasoline (toluene-d8 and naphthalene-d8) (17). The compound n-chlorobenzene was used instead of the deuterated analog reported in the literature (7) because it was felt that the nondeuterated compound would have a sufficiently unique mass spectrum that it could be readily extracted from a total ion chromatogram obtained from the analysis of fire debris. Tetrachloroethylene and 3-phenyltoluene have been used as internal standards in the analysis of fire debris and so these were also included in this study (16,17). It was important to test some other higher boiling compounds aside from 3-phenyltoluene and so n-octylbenzene, an internal standard reported in the toxicology literature (3,4), was selected for this study. Diphenylmethane (boiling point = 264°C) and 3-phenyltoluene are chemical isomers ( $\text{C}_{12}\text{H}_{13}$ ) with similar boiling points and mass spectra; therefore, there seemed to be little value in testing both compounds. Furthermore, even though diphenylmethane is suggested as a possible internal standard by ASTM E1412 (9), to our knowledge the use of this compound as an internal standard in fire debris has never been reported in the literature. A fourth *perdeuterio* compound, diphenyl-d10, was chosen as a substitute for diphenylmethane because it has a similar chemical structure to, but a different mass spectrum from, diphenylmethane and 3-phenyltoluene. It is important to note that one drawback to using 3-phenyltoluene as an internal standard is that, unlike the other compounds used in this study, it was only commercially available at the relatively low purity of 95%.

Another internal standard reportedly used in fire debris analysis, TCMX (or 1,2,3,5-tetrachloro-4,6-dimethylbenzene) (13,14) was not used in this study because at the time this study was conducted, a suitable standard of TCMX was not readily available from the major chemical suppliers in North America. Furthermore, the melting point of this compound is between 220 and 222°C and so this compound was expected to have a relatively low vapor pressure and, therefore, poor extraction efficiency. In the field of toxicology, two other internal standards, indane and n-C14 (1,2), have been used; however, these were not selected for this study because these two compounds can be found in some classes of ignitable liquids.

### Extraction Efficiency

An internal standard added to fire debris must have relatively high extraction efficiency. The extraction efficiency of an internal standard added to fire debris will depend not only on the physical properties of the compound itself, but also on the way it is introduced into the debris and the concentration of interfering volatiles present in the debris. It is difficult to introduce a small volume of internal standard (e.g., 1  $\mu\text{L}$ ) to a solid substrate (e.g., fire debris) and have confidence that the internal standard was actually deposited. One of the simplest methods of overcoming this problem is to make a solution by dissolving the internal standard in the eluting solvent and introduce a small quantity of internal standard to the debris as part of a larger, more visible, volume of liquid. However, the presence of eluting solvent as well as other volatiles in the fire debris may have an effect on the amount of internal standard that is recovered from the debris.

The extraction efficiencies for each ISC extracted at room temperature are given in Table 3. In the absence of  $\text{CS}_2$ , all of the ISCs tested had good extraction efficiencies at room temperature (78–94%). The addition of a small volume of  $\text{CS}_2$  (25  $\mu\text{L}$ ) tended to reduce the room temperature extraction efficiency somewhat, while the addition of slightly more  $\text{CS}_2$  (50 and 100  $\mu\text{L}$ ) led to an overall improvement in the room temperature extraction efficiencies. When 500  $\mu\text{L}$  of  $\text{CS}_2$ , or more, was added to the container, the room temperature extraction efficiency would decrease with increasing volume of  $\text{CS}_2$ . In the presence of 2000  $\mu\text{L}$   $\text{CS}_2$ , the four lower boiling ISCs had poor extraction efficiencies, while the four higher boiling ISCs did not extract at all.

The extraction efficiencies for each ISC extracted at 60°C are given in Table 4. In the absence of  $\text{CS}_2$ , all of the ISCs tested had fair to good extraction efficiencies when heated (48–88%) with the more volatile compounds exhibiting lower extraction efficiencies than the less volatile compounds. As with the room temperature extractions, the addition of a small amount of  $\text{CS}_2$  (25–100  $\mu\text{L}$ ) tended to increase the overall extraction efficiency (except for styrene-d8), and the extraction efficiency would begin to decrease for each compound in the presence of 500 or 1000  $\mu\text{L}$  of  $\text{CS}_2$ . Overall, the most volatile compounds exhibited the poorest extraction efficiencies when extracted at 60°C.

$\text{CS}_2$  is very volatile (boiling point = 46°C) and is normally used as an eluting solvent because it efficiently desorbs trapped volatiles from activated charcoal. Therefore, it was expected that the  $\text{CS}_2$  would compete strongly with each ISC for adsorption sites on the activated charcoal and that this would allow a relative assessment to be made of how well each ISC would adsorb onto activated charcoal in the presence of competing volatiles such as an ignitable liquid or pyrolysis, combustion, or distillation products from the debris. The recovery of the analytes generally improved for extractions at both room temperature and when heated as the volume of

$\text{CS}_2$  was increased from 25 to 100  $\mu\text{L}$ . One reason this improvement in extraction was observed may be that the  $\text{CS}_2$  wetted the surface of the charcoal strip resulting in the creation of an additional adsorptive layer of  $\text{CS}_2$  molecules. The results showed that when 500  $\mu\text{L}$  or more of  $\text{CS}_2$  was in the container the extraction efficiency for each of the ISCs began to decrease. This data also showed that an ISC containing chlorine or deuterium atoms (e.g., toluene-d8, styrene-d8, diphenyl-d10, tetrachloroethylene, and chlorobenzene) did not hinder their adsorption onto activated charcoal. In the presence of a relatively high concentration of volatile vapors (i.e., 1000 or 2000  $\mu\text{L}$   $\text{CS}_2$ ), the higher boiling compounds (e.g., naphthalene-d8, diphenyl-d10, n-octylbenzene, and 3-phenyltoluene) may not be recovered at room temperature, whereas the lower boiling compounds (toluene-d8, tetrachloroethylene, chlorobenzene, and styrene-d8) may exhibit poor recovery at 60°C.

### Evaluation of ISCs in the Presence of an Ignitable Liquid

The six most abundant ion mass fragments ( $m/z$ +) for each ISC, together with their relative abundance, were determined directly from their mass spectra and are given in Table 5.

With the exception of n-octylbenzene, all ISCs have mass spectra that are relatively unique compared with the mass spectra typical of alkanes, cycloalkanes and alkenes, aromatics, naphthalenes, and indanes normally found in common, petroleum-derived ignitable liquids (Table 6). The *perdeutero* compounds (toluene-d8, styrene-d8, naphthalene-d8, and diphenyl-d10) were chosen as ISC because they would each possess a unique mass spectrum whilst having similar chemical properties relative to their nonisotopically labeled analogs.

The elution order, retention time, and relative retention time for each ISC is summarized in Table 7 and in Fig. 1. The ISCs were evaluated against a broad range of ignitable liquids to determine whether or not coelution with any of the compounds present in the ignitable liquids would interfere with either the identification of the internal standard or the ignitable liquid. The chromatogram of SAM spiked with the ISCs extracted at room temperature is shown in Fig. 2. Expanded views of the extracted ion profile for each ISC from the room temperature extraction is given in Fig. 3. The chromatogram of SAM spiked with the ISCs extracted at 60°C is shown in Fig. 4.

Ideally, in addition to having good extraction efficiency, an internal standard for fire debris will not be obscured by the presence of pyrolysis and combustion products from the debris and it should not interfere with the identification of an ignitable liquid. As can be seen in Fig. 1, each internal standard will elute with more than one ignitable liquid and it is expected that, depending on the temperature program and column used, each ISC will at least partially coelute with a compound from one of the ignitable liquids listed here. For example, using the analytical conditions

TABLE 3—Extraction efficiency for each of the ISCs at room temperature.

Volume $\text{CS}_2$ Added ( $\mu\text{L}$ )	Percent Extraction Efficiency (% Relative Standard Deviation in Parentheses)							
	Toluene-d8	Tetrachloroethylene	Chlorobenzene	Styrene-d8	Naphthalene-d8	Diphenyl-d10	n-Octylbenzene	3-Phenyltoluene
0	84.3 (5.9)	86.2 (6.7)	85.0 (8.4)	84.1 (9.6)	97.4 (9.4)	94.7 (7.9)	77.7 (7.6)	83.9 (6.4)
25	63.1 (4.0)	62.8 (4.8)	60.9 (6.1)	57.0 (7.2)	67.1 (8.9)	64.5 (10.4)	53.4 (9.5)	58.2 (8.5)
50	88.0 (16.4)	87.6 (17.0)	85.8 (17.8)	79.1 (18.3)	94.2 (17.0)	92.8 (18.1)	74.0 (18.8)	80.1 (18.3)
100	96.8 (1.0)	97.3 (1.3)	95.9 (2.5)	81.9 (3.7)	106.4 (3.4)	102.0 (3.0)	80.8 (3.9)	82.9 (6.5)
500	55.3 (2.4)	58.1 (2.8)	68.0 (4.6)	59.2 (5.5)	72.4 (9.4)	55.8 (29.5)	53.9 (23.5)	39.0 (41.4)
1000	42.1 (1.1)	47.8 (1.7)	64.3 (1.4)	70.2 (3.2)	66.2 (10.2)	17.1 (21.1)	22.7 (11.6)	10.0 (21.5)
2000	13.3 (7.8)	13.0 (11.1)	20.4 (10.4)	25.6 (12.5)	0.0	0.0	0.0	0.0



TABLE 4—Extraction efficiency for each of the ISCs at 60°C.

Volume CS <sub>2</sub> added (μL)	Percent Extraction Efficiency (% Relative Standard Deviation in Parentheses)							
	Toluene-d8	Tetrachloroethylene	Chlorobenzene	Styrene-d8	Naphthalene-d8	Diphenyl-d10	n-Octylbenzene	3-Phenyltoluene
0	47.9 (10.6)	55.5 (11.3)	63.7 (10.9)	65.5 (13.4)	84.1 (22.8)	87.5 (26.0)	80.5 (32.2)	85.4 (26.6)
25	63.7 (11.5)	71.9 (9.7)	81.1 (9.1)	77.4 (9.3)	106.6 (23.7)	107.1 (18.7)	96.2 (5.9)	103.5 (15.8)
50	69.8 (4.8)	78.1 (1.3)	86.7 (3.5)	45.3 (7.8)	93.0 (30.1)	96.7 (28.2)	100.4 (15.6)	92.4 (30.2)
100	65.8 (26.1)	75.3 (17.6)	84.5 (11.6)	21.5 (14.5)	108.2 (2.8)	111.8 (0.3)	101.7 (0.7)	109.1 (0.4)
500	41.7 (26.0)	48.6 (20.2)	60.9 (14.8)	33.9 (18.8)	78.5 (8.5)	88.2 (8.2)	89.7 (5.9)	86.8 (5.5)
1000	24.5 (20.8)	32.0 (17.0)	64.3 (1.4)	26.5 (21.7)	78.9 (18.4)	80.0 (26.0)	85.2 (17.2)	71.5 (15.0)

TABLE 5—Six most abundant ion mass fragments (*m/z*+) for each ISC.

Compound	Mass Fragment ( <i>m/z</i> +)					
Toluene-d8	98 (100)	100 (66)	42 (12)	70 (12)	99 (11)	54 (8)
Tetrachloroethylene	166 (100)	164 (78)	129 (68)	131 (66)	168 (48)	94 (39)
Chlorobenzene	112 (100)	77 (61)	114 (33)	51 (22)	50 (21)	74 (10)
Styrene-d8	112 (100)	84 (43)	110 (37)	54 (24)	82 (18)	111 (13)
Naphthalene-d8	136 (100)	137 (11)	108 (9)	134 (9)	54 (7)	68 (6)
Diphenyl-d10	164 (100)	162 (37)	160 (21)	163 (14)	165 (13)	80 (13)
n-Octylbenzene	92 (100)	91 (94)	190 (29)	41 (13)	57 (12)	105 (11)
3-Phenyltoluene	168 (100)	167 (56)	165 (24)	152 (22)	153 (15)	169 (14)

TABLE 6—Compound classes and ions common to each class for compounds typically found in ignitable liquids.

Compound Class	Common Ions ( <i>m/z</i> +)
Alkanes	43, 57, 71, 85
Cycloalkanes/alkenes	55, 69, 83
C0- to C4-Alkylbenzenes	78, 91, 92, 105, 106, 119, 120, 134
C0- to C3-Naphthalenes	128, 141, 142, 155, 156, 170
C0- to C2-Alkylindanes	117, 118, 131, 132, 146

TABLE 7—Retention time (RT) and relative retention time (RTT) for ISCs.

Compound	8°C/min		12°C/min	
	RT	RRT	RT	RRT
Toluene-d8	2.89	740	2.93	742
Tetrachloroethylene	3.83	795	3.82	794
Chlorobenzene	4.47	824	4.38	824
Styrene-d8	5.45	867	5.20	868
Naphthalene-d8	11.48	1149	9.57	1152
Diphenyl-d10	14.93	1345	11.87	1345
n-Octylbenzene	16.62	1450	13.02	1451
3-Phenyltoluene	16.64	1451	13.06	1455

described in this study (see Fig. 1), toluene-d8 coeluted with 2,3,4-trimethylpentane found in premium gasoline. However, the ISCs can still be identified in a chromatogram using an extracted ion profile (Figs. 2–4) in the same way that extracted ion profiles can be generated from a total ion chromatogram and used to identify the compound classes in an ignitable liquid (see Table 6). Although the less volatile ISCs did not adsorb onto charcoal very well at room temperature in the presence of relatively large volumes of CS<sub>2</sub>, Fig. 2 clearly shows that they can extract well in the presence of the hydrocarbons present in the SAM. Clearly the adsorption of any of the ISCs is a complicated process that is dictated by what other compounds, and their concentration, are present in the headspace.

### Evaluation of ISCs in the Presence of Fire Debris

As with the ignitable liquids, it is expected that each ISC will at least partially coelute with a compound arising from the pyrolysis and combustion products from the debris. In this study, we had little difficulty in identifying any of the ISCs in either the total ion chromatogram or in the extracted ion profiles in the presence of pyrolysis, combustion, and/or distillation products arising from burned debris.

### Conclusions

Eight compounds were tested for their suitability as an internal standard that could be added directly to fire debris prior to extraction and analysis. We found that each of the compounds tested would be a suitable internal standard. However, based on our results we would recommend that the internal standard be delivered to the fire debris either in solution, where the internal standard is diluted in the same solvent used to elute the charcoal strip, or by spotting the internal standard directly on a piece of filter paper that is then placed into the evidence container. For ISCs that are solids at room temperature (e.g., naphthalene-d8), the compound must either be dissolved in a suitable solvent such as CS<sub>2</sub>, or dissolved with another (liquid) internal standard compound. There was no data to support the selection of one ISC over another based on the likelihood of it being resolved in the total ion chromatogram because this result will be dependent on not only the instrument conditions used in the laboratory, but also the volatiles present in each extract. It is clear, however, that extracted ion profiles must be used in combination with retention time data in order to identify an internal standard in a chromatogram. Overall, we favor the use of two internal standards in combination, one with a low boiling point and one with a higher boiling point. Tetrachloroethylene is a good choice as the low boiling internal standard because it is readily available, inexpensive, showed good extraction efficiency, and has a unique mass spectrum. Furthermore, when analyzed with our instrument conditions, tetrachloroethylene did not coelute with compounds present in pyrolysis, combustion, and/or distillation products in our simulated fire debris, nor did it coelute with the

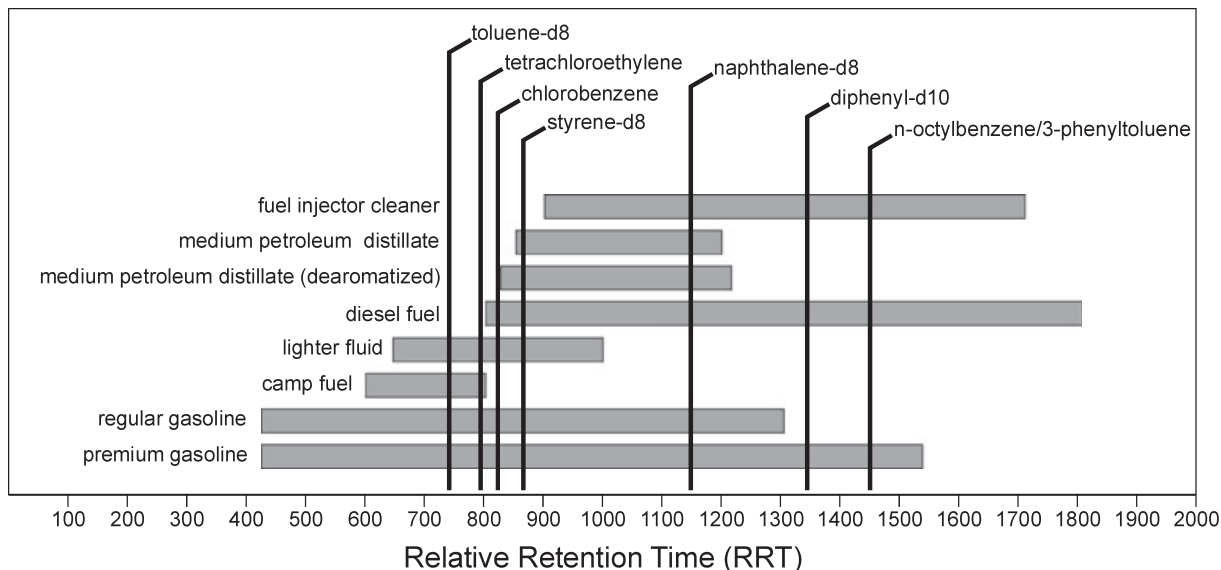


FIG. 1—Relative retention times for the internal standard candidates (ISCs) and the relative retention time ranges for selected ignitable liquids (GC temperature program: 40°C for 3 min, ramped at 8°C/min to 250°C).

compounds present in some common ignitable liquids. For the higher boiling internal standard, we would recommend naphthalene-d8 because this compound had room temperature extraction efficiencies that, in the presence of an interfering volatile

compound (CS<sub>2</sub>), were significantly better than those for diphenyl-d10, n-octylbenzene, or 3-phenyltoluene. Also, naphthalene-d8 is an attractive internal standard because, as deuterated isotope, it will not be present in fire debris or any ignitable liquids.

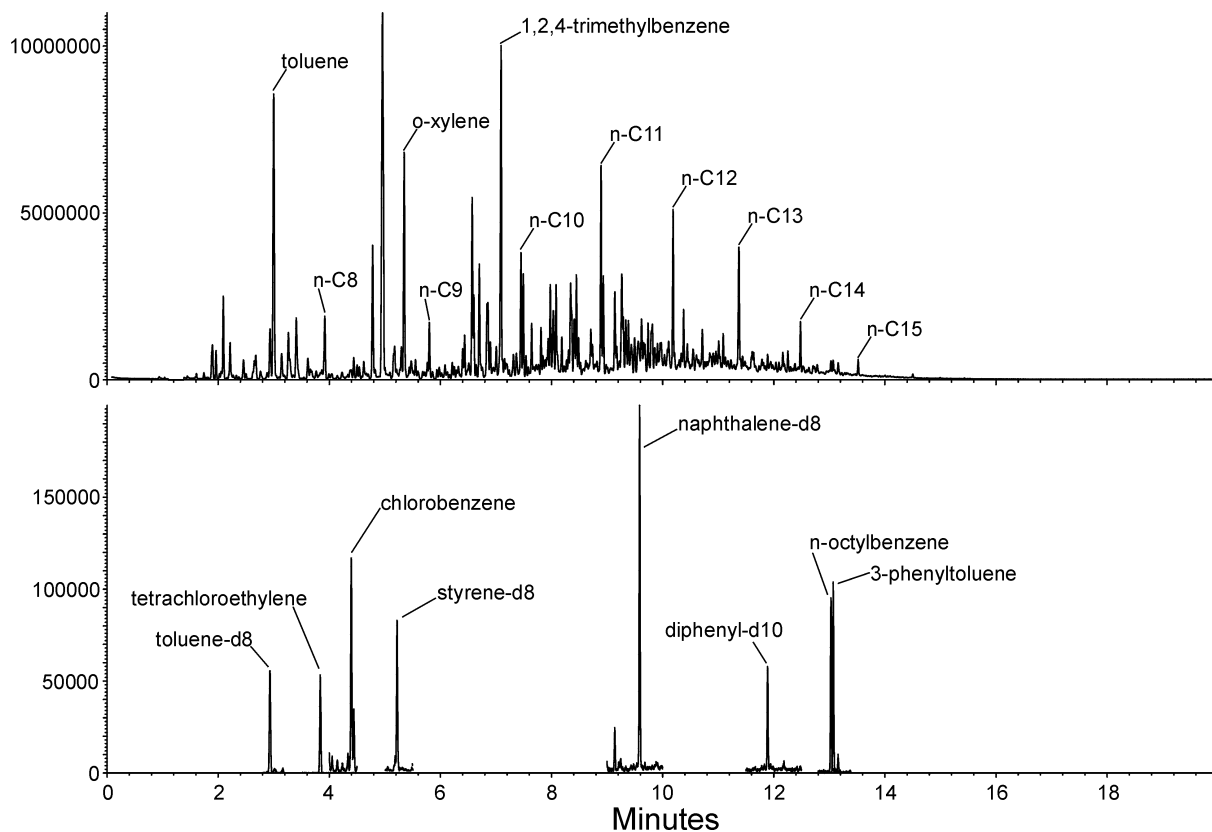


FIG. 2—Total ion chromatogram obtained from 50 µL standard accelerant mixture (SAM) combined with 1 µL of internal standard stock solution in a 1-L can and extracted onto charcoal via passive headspace adsorption at room temperature (top); and extracted ion profile of each ISC from the above TIC (bottom) (GC temperature program: 40°C for 3 min, ramped at 12°C/min to 250°C).

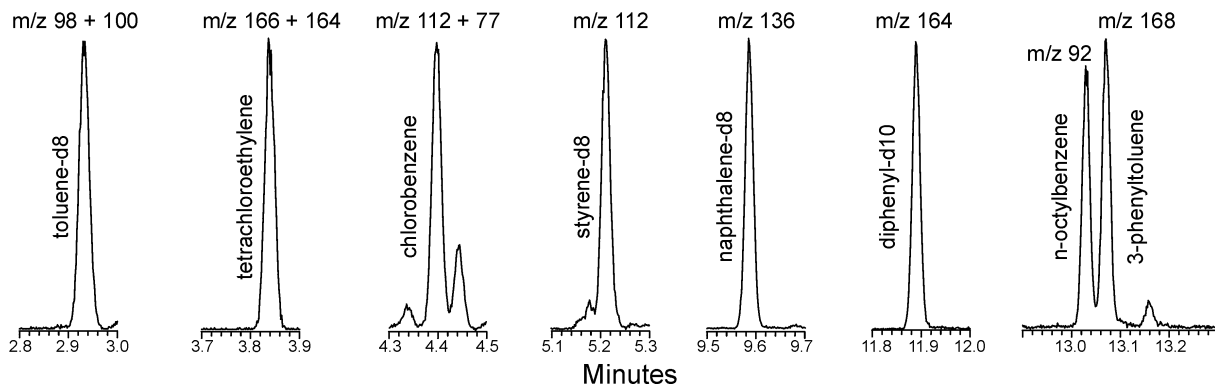


FIG. 3—Detailed view of the extracted ion profile for each ISC shown in Fig. 2.

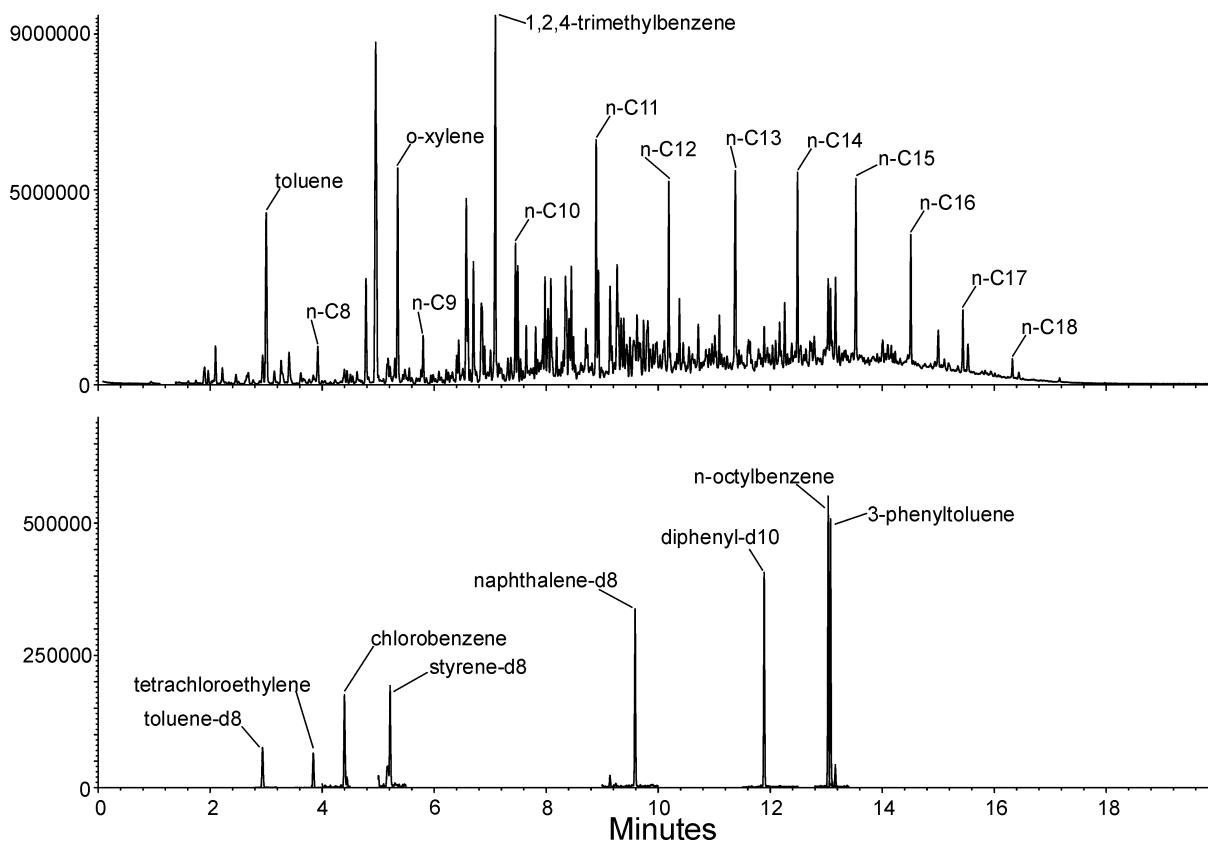


FIG. 4—Total ion chromatogram obtained from 50  $\mu$ L SAM combined with 1  $\mu$ L of internal standard stock solution in a 1-L can and extracted onto charcoal via passive headspace adsorption at 60°C (top); and extracted ion profile of each ISC from the above TIC (bottom) (GC temperature program: 40°C for 3 min, ramped at 12°C/min to 250°C).

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