TECHNICAL NOTE

David J. Tranthim-Fryer,[1] *B.App.Sc., Grad.Dip.Chem.; Robert C. Hansson,*[1] *B.App.Sc., Grad.Dip.Chem.; and Keith W. Norman,*[1] *B.App.Sc., MSc.*

Headspace/Solid-Phase Microextraction/Gas Chromatography-Mass Spectrometry: A Screening Technique for the Recovery and Identification of Volatile Organic Compounds (VOC's) in Postmortem Blood and Viscera Samples*

REFERENCE: Tranthim-Fryer DJ, Hansson RC, Norman KW. Headspace/solid-phase microextraction/gas chromatography-mass spectrometry: a screening technique for the recovery and identification of volatile organic compounds (VOC's) in postmortem blood and viscera samples. J Forensic Sci 2001;46(4):934–946.

ABSTRACT: This paper describes the application of Headspace/Solid-Phase Microextraction/Gas Chromatography-Mass Spectrometry (HS/SPME/GC-MS) to the recovery and identification of volatile organic compounds in blood and viscera samples from deceased persons. The technique is used as a screening procedure to rapidly obtain information relating to toxicological investigations. The technique is suitable for the detection of volatiles (of wide boiling range) including butane, halothane, toluene, xylenes, and petrol residues in blood and viscera (lung, brain, and body fat).

KEYWORDS: forensic science, forensic toxicology, headspace analysis, solid-phase microextraction, human postmortem fluids and viscera, volatile organic compounds, solvent abuse, gas chromatography/mass spectrometry

Solvent abuse is a particular problem in Western Australia among sections of our youth population. The necessity to quickly determine circumstances surrounding a sudden death has brought about the application of Solid-Phase Microextraction (SPME) technology to toxicological analysis. Often the toxicologist is required to determine if a victim had been inhaling volatile organic compounds. Solid-Phase Microextraction, a technique developed by Pawliszyn and co-workers (1,2), is a solvent-free sample preparation technique that uses polymer coated glass fibers for the recovery [by direct immersion (2) or headspace sampling (3)] of organic compounds from aqueous solutions. Polymers used to coat the SPME glass fiber can be either homogeneous or mixtures (4). Homogeneous polymer coatings containing a liquid phase only (PDMS-polydimethylsiloxane or polyacrylate), extract analytes by absorption (5). Nonhomogeneous coatings, containing porous particles suspended in a liquid phase (e.g., PDMS/divinylbenzene, PDMS/carboxen, carbowax/divinylbenzene, carbowax/templated resin), extract analytes primarily by adsorption (5). Thermal desorption, via the GC injection port into a gas chromatograph is used to remove analytes from the polymer coated glass fiber.

Over the past five years an increasing number of papers have appeared in the literature reporting forensic applications of SPME, including the analysis of flammable liquid residues (6,7), explosive residues (8), impurities in illicit methoxyamphetamine (9) and drugs of toxicological interest (10,11). Traditional headspace techniques (static headspace, using a gas syringe or automated headspace sampler) have been used for the recovery of volatile organic compounds from body fluids (12) and viscera (13). These methods are now being replaced by Headspace Solid-Phase Microextraction (HS/SPME). Over recent years a number of papers have appeared in the literature reporting the use of HS/SPME for the recovery of VOC's from body fluid samples. Polyacrylate coated fibers have been used for the recovery of alcohols and acetone from blood serum (14). PDMS coated fibers have proven useful for the recovery of thinners (15) and charcoal lighter fluid (16), from blood, urine, and gastric fluid. Carboxen/PDMS coated fibers have been employed for bio-monitoring of benzene and toluene in human blood (17). A paper reporting the comparison of HS/SPME and automatic headspace sampling of toluene in blood and urine samples was recently published (18).

From 1996 to 2000, headspace SPME, using a PDMS coated fiber (100 μm film thickness) has been used with success in our laboratory for the qualitative analysis of VOC's in human blood and viscera samples. To date, the method has been employed in the examination of 34 sudden death cases where solvent abuse was suspected. Some data from this work has been presented as poster

[1] Senior chemist and research officer, principal chemist, and chemist and research officer, respectively, Chemistry Centre (WA), Forensic Science Laboratory, East Perth, WA, Australia.

* This paper was presented by David Tranthim-Fryer at the 52nd Annual Meeting, American Academy of Forensic Sciences, Reno, NV, February 2000.

Received 5 July 2000; and in revised form 1 Sept. 2000; accepted 5 Sept. 2000.

papers at conferences (19,20). The data corroborate well with similar work, reporting the detection and quantitative analysis of halogenated organic compounds in blood, urine, and tissue samples (21).

This paper reports the recovery and identification of volatile organic compounds (butane, halothane, toluene, and petrol residues), from human blood and viscera samples. Sample preparation, manual holder/fiber assembly manipulation and instrument conditions are given.

Materials and Methods

Materials

Solid-phase microextraction was performed by manual technique according to the manufacturer's instructions (22). A Supelco, SPME manual fiber assembly, with polydimethylsiloxane (100 μm film thickness) coated fiber was used for all analysis. 50 mL glass bottles were used as headspace vessels. Butyl rubber stoppers and crimp seals (Alltech Associates, Baulkham Hills, Australia) were used to seal the glass bottles after the addition of sample and glass distilled water. Methanol (HiperSolv, BDH laboratory Supplies, Poole, England), halothane (Fluothane, ICI, Melbourne, Australia) and isoflurane (Forthane, Abbott Laboratories, Kurnell, Australia) were used for quantitations.

Headspace-SPME Sampling Procedure

Prior to each analysis, the SPME fiber was "blanked" by thermal desorption in the injection port of the gas chromatograph (5 min at 220°C, purge on). Blood (2 to 5 g), lung, brain, or fat tissue samples (5 to 10 g, finely chopped) were placed into a 50 mL glass bottle, to which 25 mL of distilled water was added, giving a headspace volume of between 15 and 23 mL. The glass bottle was then crimp sealed and placed into an oven at 80°C for 1.5 h. The bottle was then removed from the oven, the SPME syringe inserted into the bottle and the fiber exposed to headspace for 30 min. The SPME syringe was removed from the bottle and inserted into the injection port of the gas chromatograph/mass spectrometer. The plunger of the SPME holder was depressed and the fiber exposed in the injection port, (1 min. at 220°C) to ensure desorption of volatiles. A blank (water) was run with each group of samples.

Instrument Conditions

A Hewlett-Packard 5890 series II, GC coupled to a HP 5971 series mass selective detector (MSD) with HP Chemstation control and Windows 95 software, was used for analysis of all samples. A 30 m × 0.25 mm DB1701 (J and W Scientific) fused silica capillary column with a film thickness of 0.25 μm was used for capillary GC separation of analytes. Helium carrier gas was used at a head pressure of 10 psi and a flow of 1 mL/min. The GC oven temperature was held at 35°C for 1 min, then ramped at 8°C per minute to a final temperature of 270°C (hold time 1 min). The injection port and transfer line temperatures were set at 220°C and 270°C, respectively. Volatiles were desorbed from the fiber under splitless GC inlet conditions (purge on at 0.41 min.). The MS was scanned from 28 to 400 amu at 1.9 scans per second. The electron multiplier and threshold were set to 2400 V and 500 amu respectively. A non HS/SPME quantitative analysis of halothane was accomplished by selected ion monitoring of ions between 1.0 and 2.5 min at m/z 198, 196 (halothane) and m/z 149 and 117 (isoflurane internal standard) for methanol extracts of blood. The dwell times were set at 10 ms per ion.

Results and Discussion

Examples of typical cases where HS/SPME/GC-MS has been used for the qualitative analysis of VOC's from blood, lung, brain, or fat samples from deceased persons, are given. Recovered volatile organic compounds were identified by comparing analyte spectra with those from mass spectral libraries (Wiley and NBS) or spectra generated from "in house" reference compounds. Retention times of detected VOC's were in agreement with those of "in house" reference compounds.

Case 1—A male (aged 17 years) died after allegedly inhaling butane from a pressure pack can. Toxicological analysis revealed no common drugs or poisons. A subsequent headspace/SPME screen revealed butane in postmortem blood, lung, and brain samples. Figures 1*a*–1*c* show the summed, extracted ion profiles (ions 41,43,57, and 58-indicative of butane) and a mass spectrum of butane, recovered from blood, lung, and brain samples. Figure 1*d* shows the total ion chromatogram (TIC) and mass spectrum of butane (standard). An evaluation of the ratio of detector response (m/z 43)/sample weight, showed the highest concentration of butane to be in the brain sample with lesser concentrations in blood and lung samples respectively. Brain tissue, due to its high fat content, is a reliable accumulator for lipophilic substances such as VOC's. Unless there is severe head trauma, the brain is usually protected and may be more resistant to postmortem decomposition and is therefore a good sample for analysis. We have instructed the Forensic Pathologist to provide brain tissue for analysis from all alleged substance abuse victims.

Case 2—A female (aged 13 years) died due to hanging. It was believed that the deceased had been sniffing solvents immediately prior to death. Toxicological analysis for common drugs and poisons showed the presence of alcohol only. A subsequent headspace/SPME screen revealed toluene in postmortem blood, lung, brain, and fat tissue. Figures 2*a*–2*d* show TIC's of VOC's (including background volatiles from the butyl rubber septum) and a mass spectrum of toluene from blood, lung, brain, and fat samples.

We are currently developing the technique further for the quantitative analysis of toluene and petrol using cyclohexanone as an internal standard. Results (unreported) to date allow for a cut of level of 200 ng for a 2.0 g blood sample (i.e, 0.1 mg/kg). This was readily achievable and suitable for toluene quantitation while using the GC/MS in scan mode. These preliminary findings were in agreement with those of Kim and Park who reported toluene levels of 0.1 to 17.6 mg/L for blood taken from deceased persons and live patients who were suspected of glue sniffing (18).

An evaluation of the ratio of detector response (toluene)/sample weight, showed the highest concentration of toluene to be in the brain and blood samples, with lesser concentrations in the fat and lung samples respectively. This trend correlates well with previously published data (23). The analysis of body fat may prove valuable in some circumstances where it is the only sample available at autopsy. It was our first request to the Forensic Pathologist for such a sample.

Case 3—A male (aged 22 years) died during a high speed chase by police after the vehicle that he was driving collided with a power pole. It was believed that the deceased had been sniffing petrol prior to the crash. Toxicological analysis for common drugs and poisons showed the presence of alcohol only. A subsequent

```
File       : C:\HPCHEM\1\DATA\DTF1105\BLOOD2.D
Operator   : DTF
Acquired   :  5 Nov 98    1:23 pm using AcqMethod HSSPME
Instrument :    5971 - In
Sample Name: HS/SPME,  98F1312, MORT ADMIN BLOOD 2 (2.5G)
Misc Info  : 30M DB1701(15)/8PSI
Vial Number: 1
```

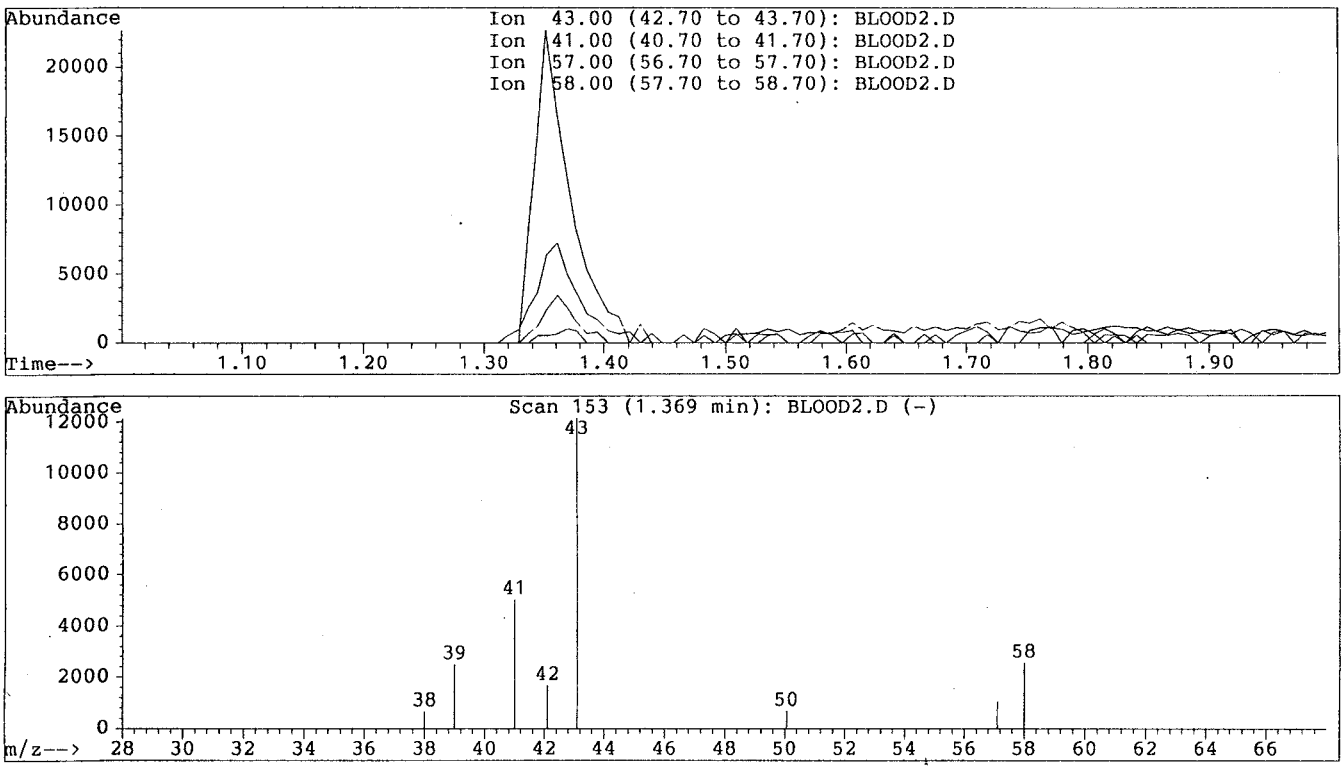

FIG. 1a—*Summed extracted ion profile and mass spectrum of butane (blood sample).*

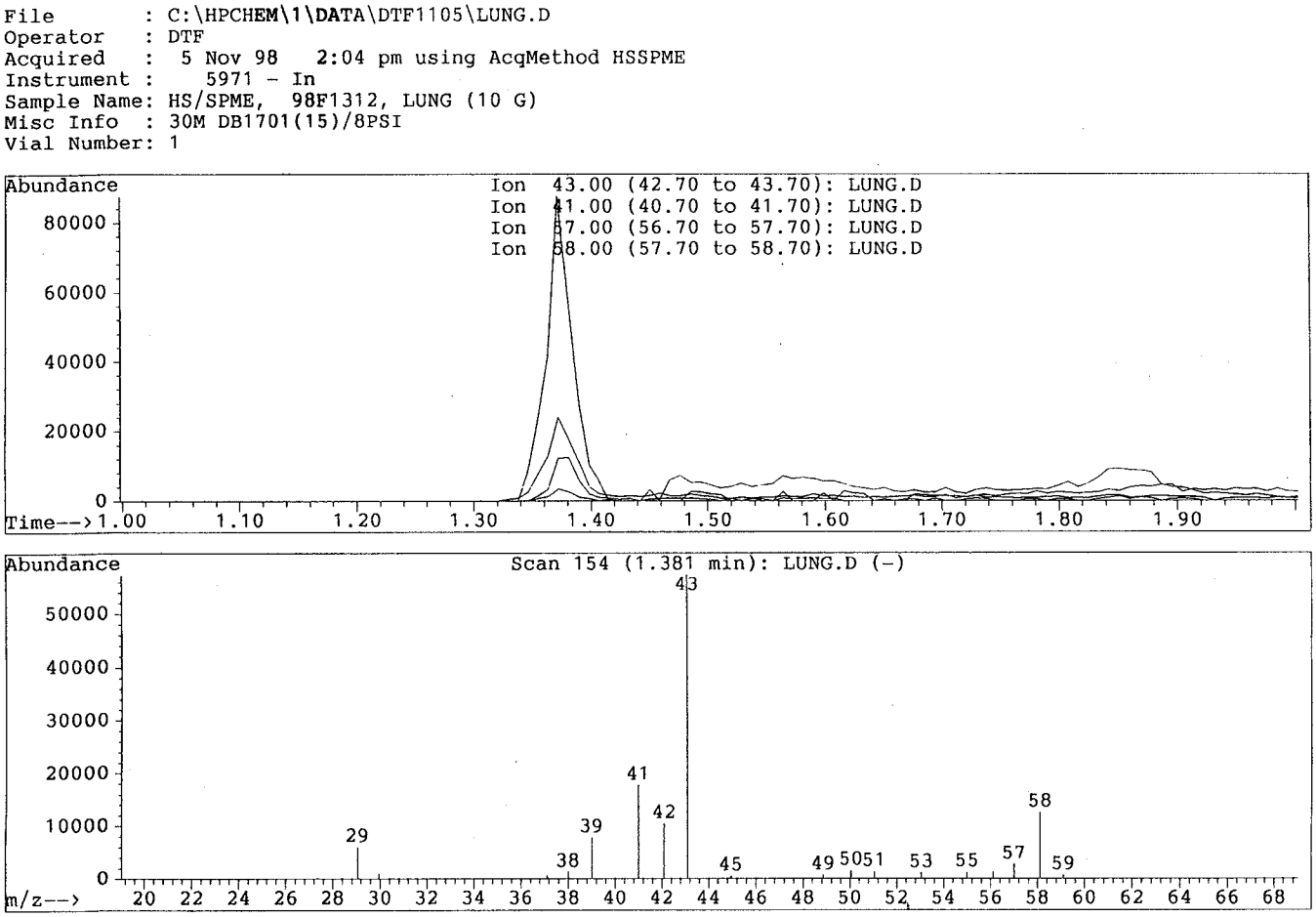

FIG. 1b—*Summed extracted ion profile and mass spectrum of butane (lung sample).*

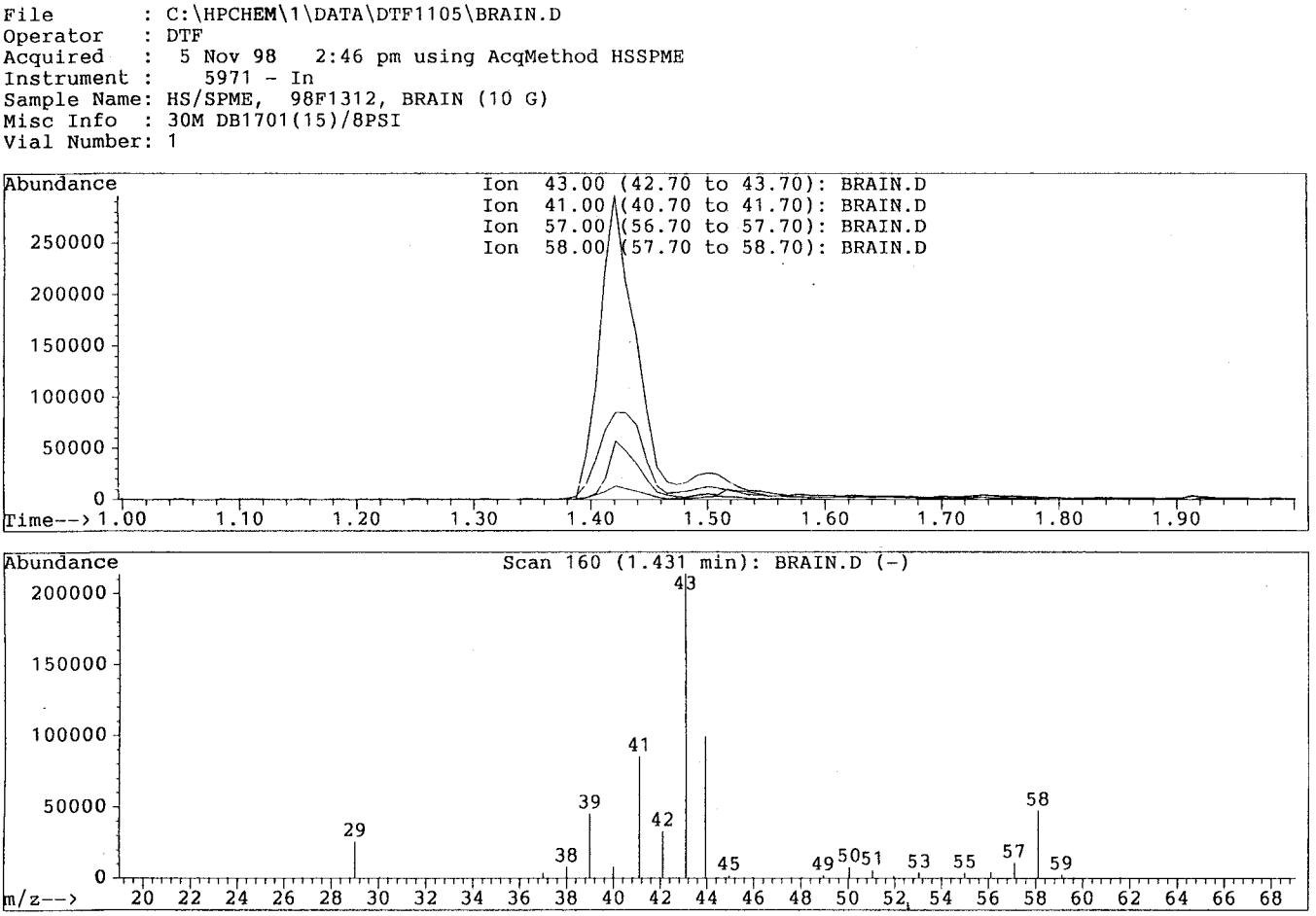

FIG. 1c—*Summed extracted ion profile and mass spectrum of butane (brain sample).*

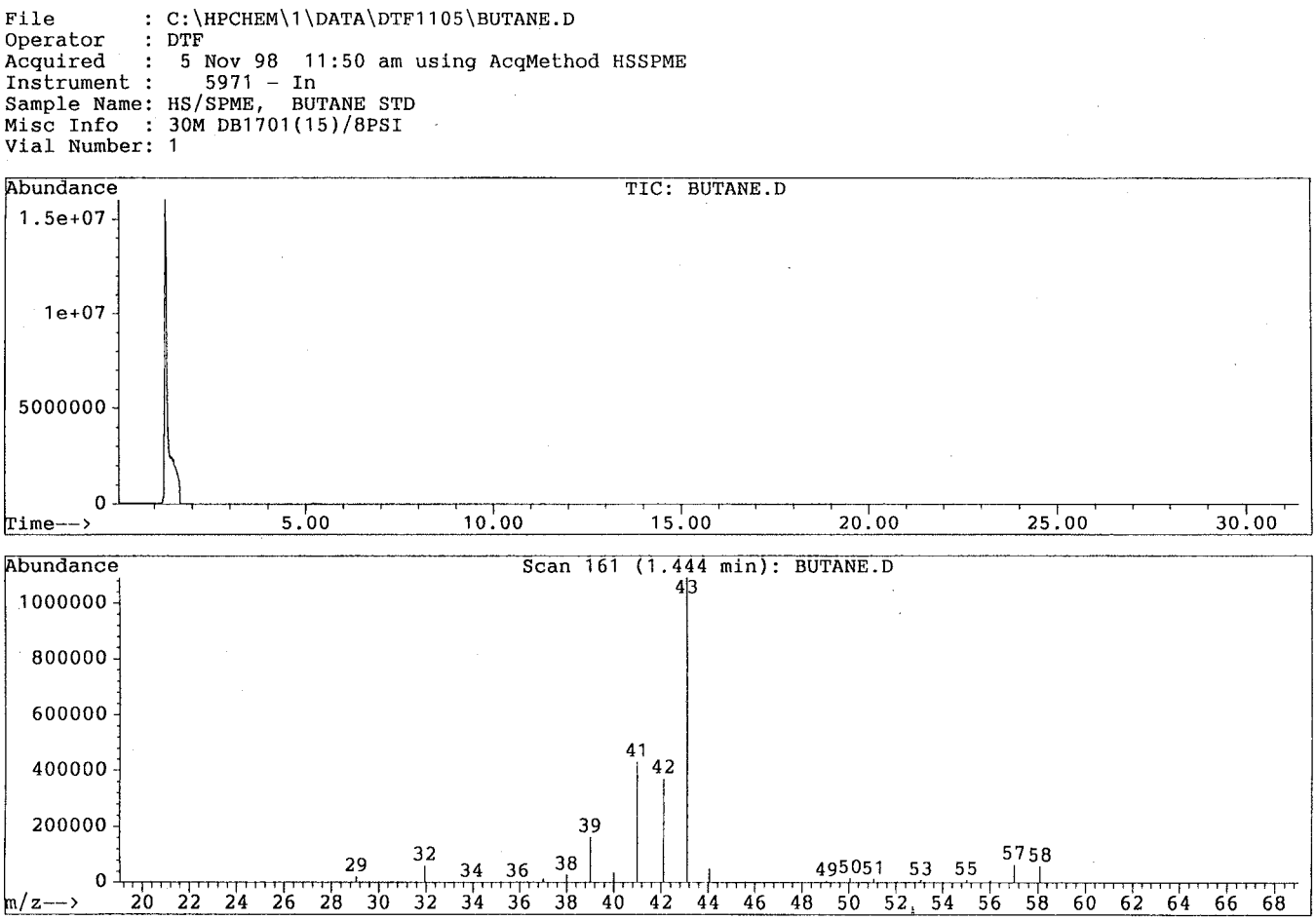

FIG. 1d—*Total ion chromatogram and mass spectrum of butane (standard).*

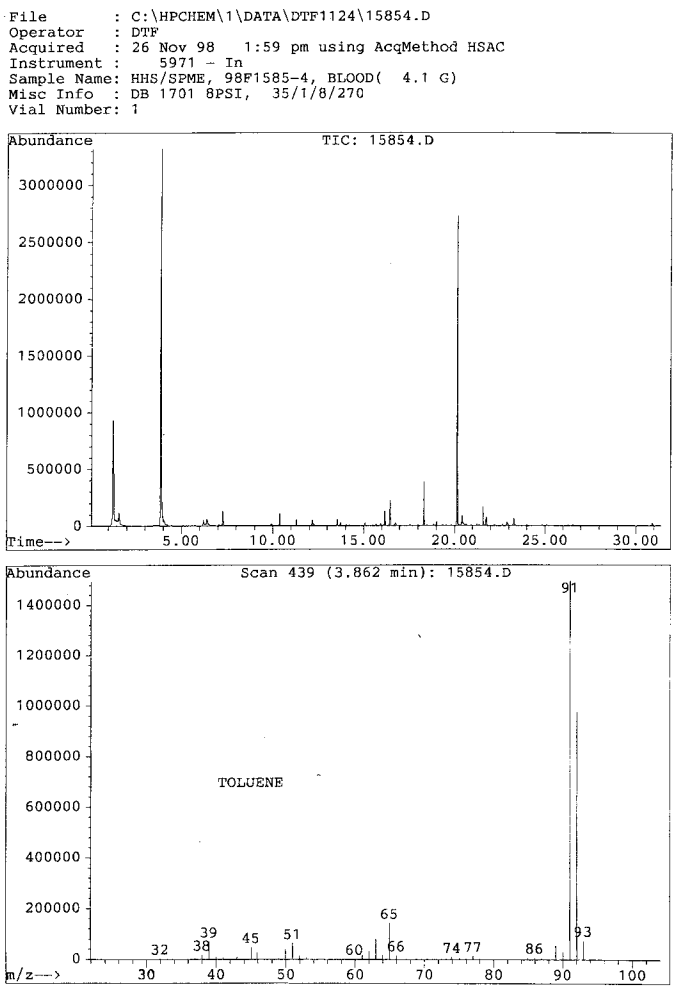

FIG. 2a—*Total ion chromatogram of VOC's and mass spectrum of toluene (blood sample).*

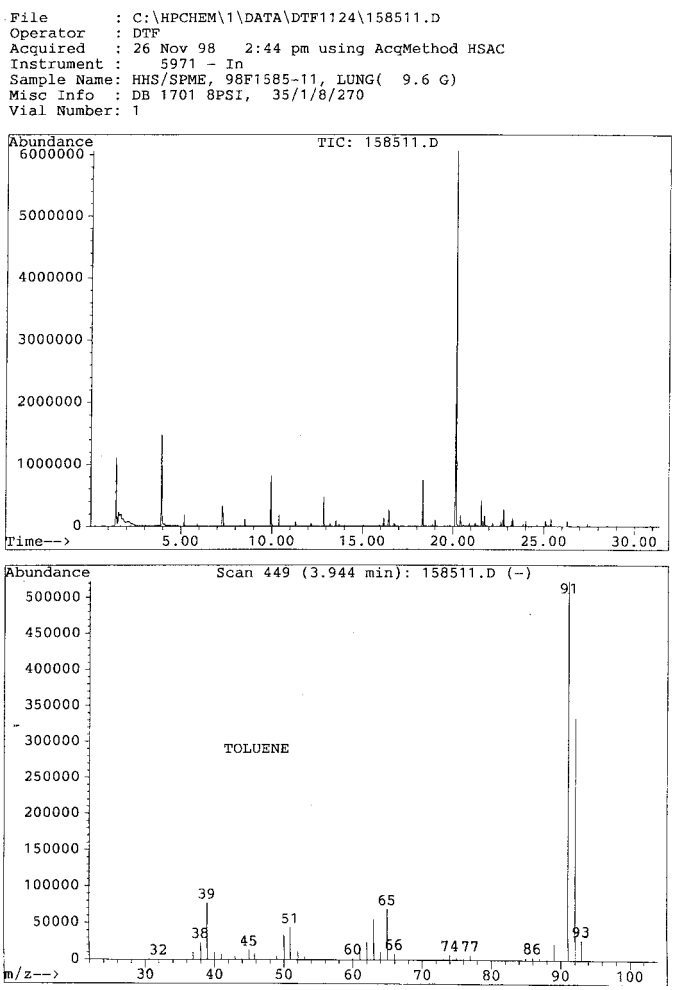

FIG. 2b—*Total ion chromatogram of VOC's and mass spectrum of toluene (lung sample).*

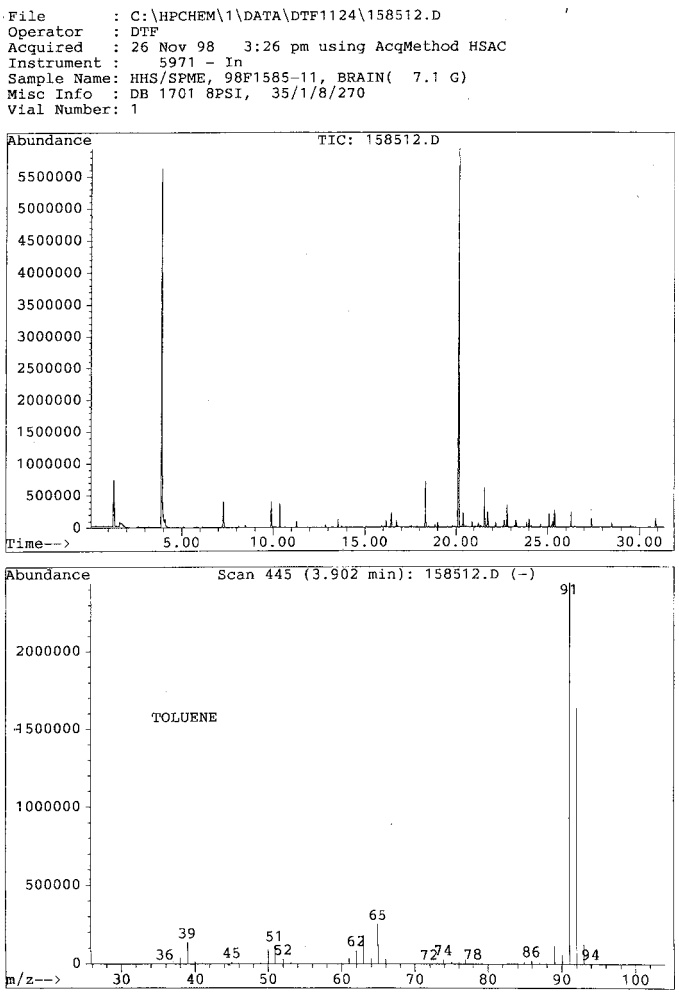

FIG. 2c—*Total ion chromatogram of VOC's and mass spectrum of toluene (brain sample).*

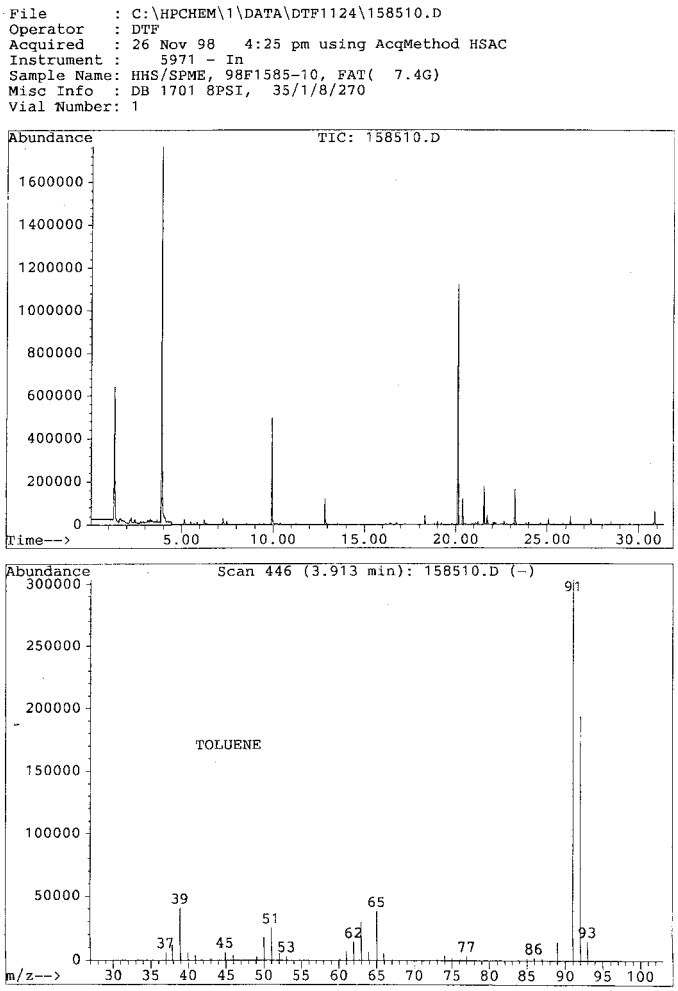

FIG. 2d—*Total ion chromatogram of VOC's and mass spectrum of toluene (fat sample).*

headspace/SPME screen revealed petrol residues in four post-mortem blood samples (no lung or brain samples were submitted for analysis).

Figure 3*a* shows the TIC and mass spectra (up to scan 1399) for VOC's (including background volatiles from butyl rubber) from one blood sample. Figure 3*b* shows the summed, extracted ion profile (ions 91, 105, 117, 119-indicative of petrol) from the sample. The technique has proved most valuable for the discrimination between toluene and petrol exposure of alleged solvent abuse victims. Petrol and toluene are the most commonly used solvents for abuse, by sections of the youth population in Western Australia.

Case 4—A male (aged 17 years), known drug user, died suddenly under suspicious circumstances. Toxicological analysis for common drugs and poisons showed the presence of ketamine, di-

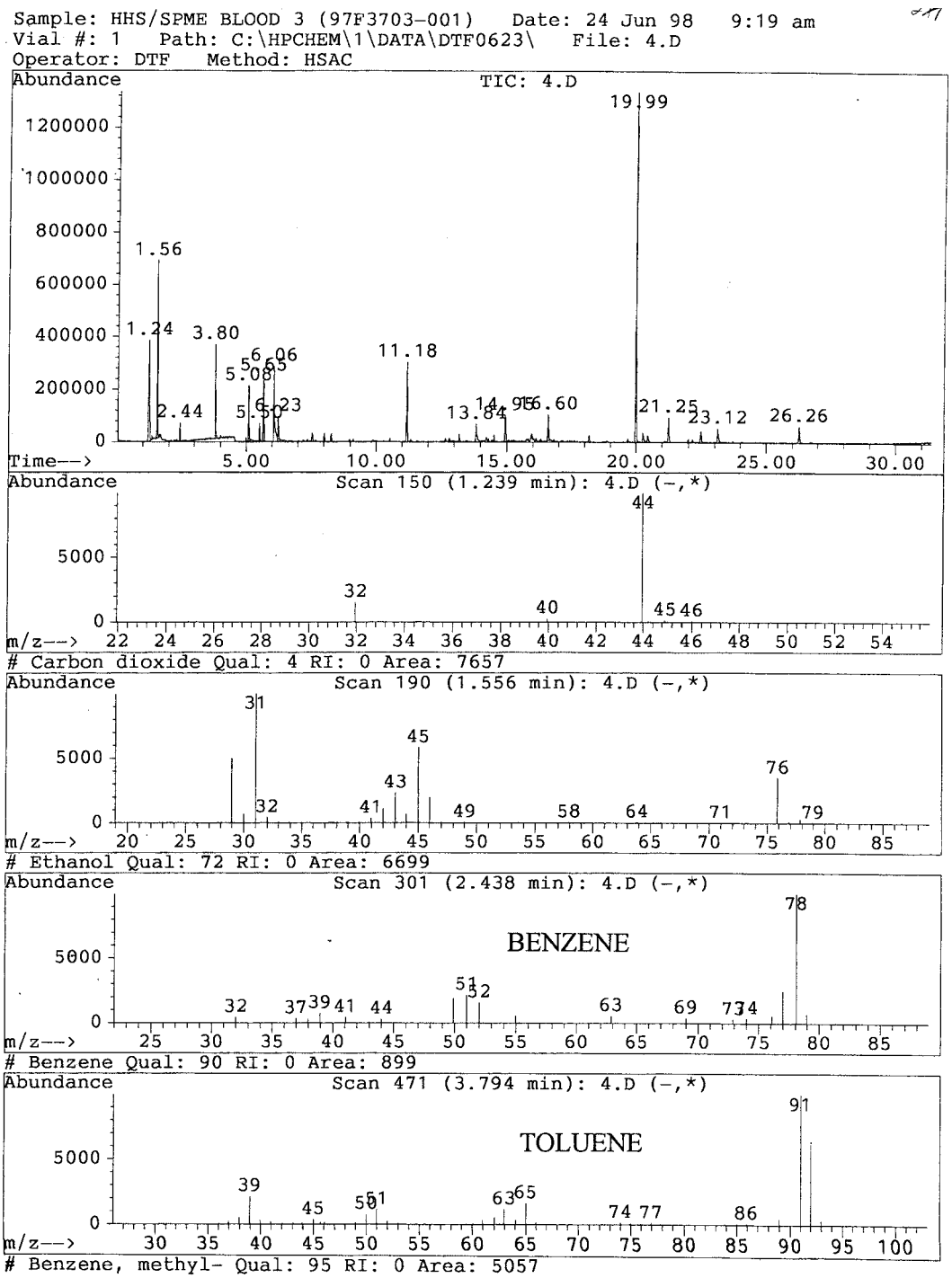

FIG. 3a—*Total ion chromatogram of VOC's and mass spectra, up to scan 1399 (blood sample).*

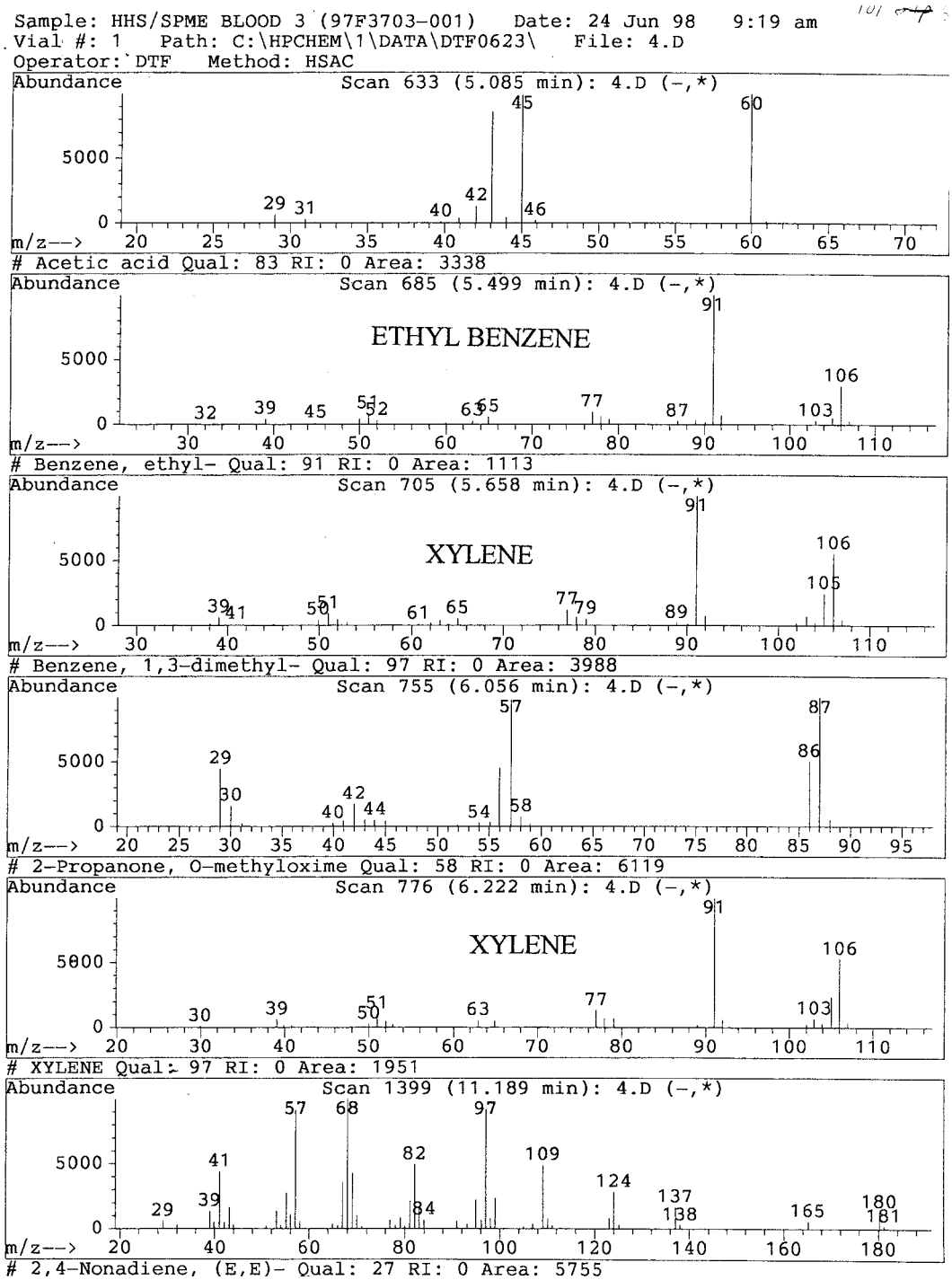

FIG. 3a—*Continued.*

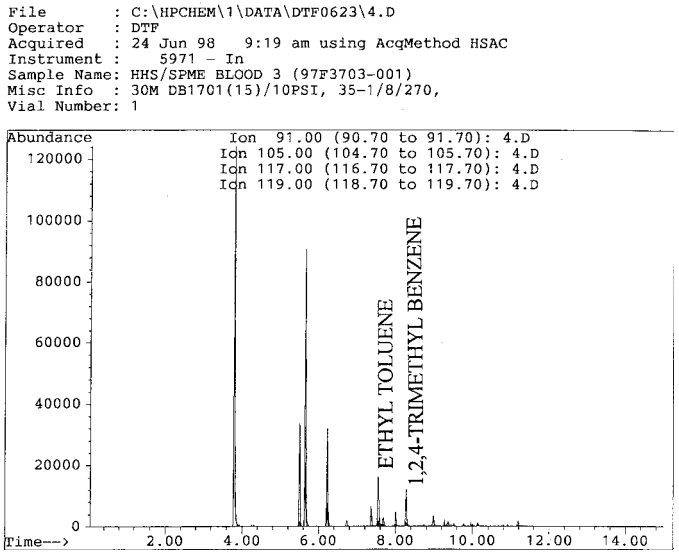

FIG. 3b—*Summed extracted ion profile, indicative of petrol (blood sample).*

azepam, and THC. The appearance of tissue damage to the decreased's mouth (possible chemical burns) allowed for consideration of solvent abuse. A subsequent headspace/SPME sceen revealed halothane in postmortem blood, lung, and brain samples.

Figures 4*a*–4*c* show TICs for VOC's (including background volatiles from butyl rubber) and a mass spectrum of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane). An evaluation of the ratio of detector response (halothane)/sample weight, showed the highest concentration of halothane to be in the brain sample, with lesser concentrations in the lung and blood samples respectively. This trend correlates well with previously published data (24).

The halothane level in a mortuary admission blood sample (methanol extract with isoflurane [2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane] internal standard, 290 ng/uL) was determined at 51 mg/L. This level was greater than that of a previously reported fatality (25). Although HS/SPME was not used for the quantitation of halothane, it has been reported that halogenated organic compounds (tetrachlorethylene and trichlorethylene) can be quantified in human body fluids and tissue using HS/SPME with GC-ECD (21).

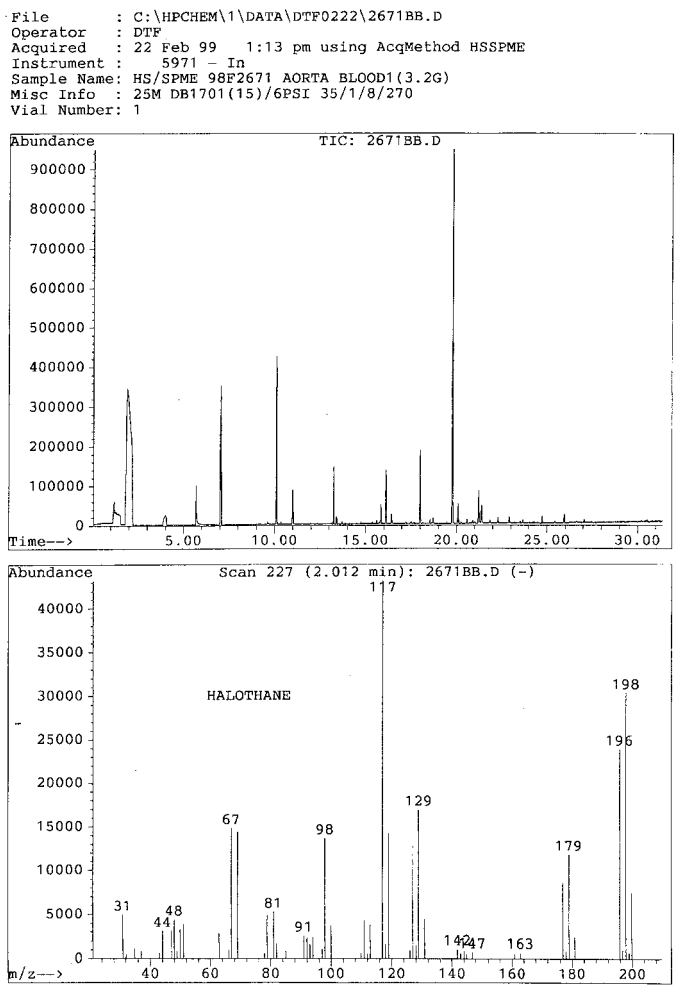

FIG. 4a—*Total ion chromatogram of VOC's and mass spectrum of halothane (blood sample).*

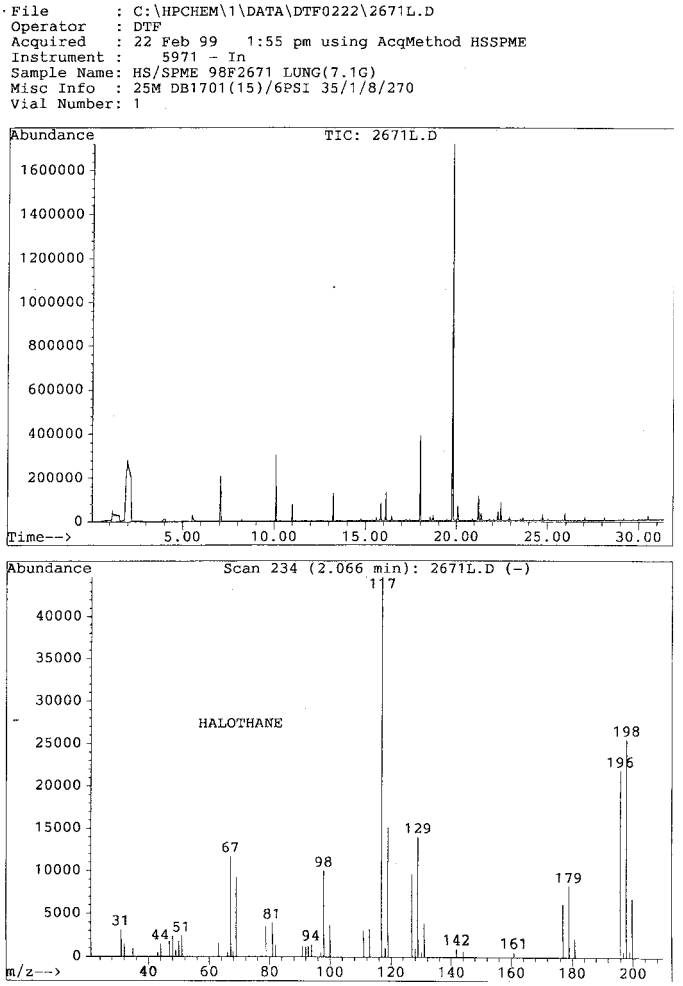

FIG. 4b—*Total ion chromatogram of VOC's and mass spectrum of halothane (lung sample).*

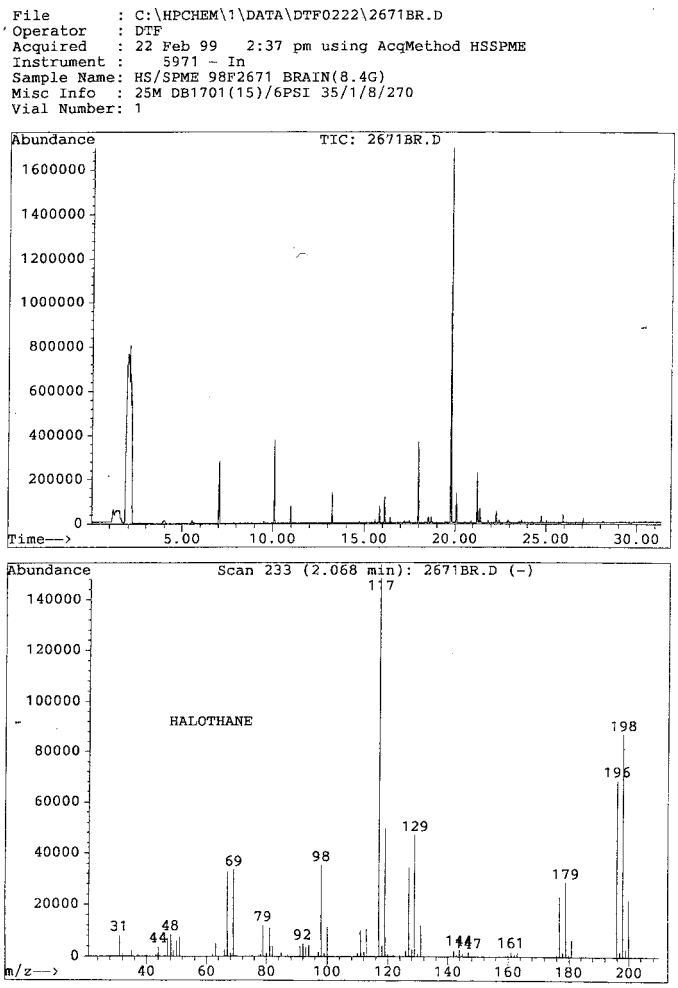

FIG. 4c—*Total ion chromatogram of VOC's and mass spectrum of halothane (brain sample).*

Conclusions

The headspace/SPME technique described provides for the qualitative analysis of volatile organic compounds from blood, lung, brain, or fat tissue of decreased persons. Several advantages of the technique, over the more traditional static headspace technique (manual syringe or autosampler) include:

(1) Simplicity of sample preparation and manipulation of the SPME injection needle and fiber.
(2) Sensitivity and selectivity for a wide range of volatiles because of the pre-concentration and selectivity effects of the fiber coating.
(3) Provides for rapid screening of samples for volatiles (especially non-routine volatiles such as halothane). This is essential for some toxicological investigations.
(4) The avoidance of mass spectral interferences from air, due to the absorption selectivity of the polydimethylsiloxane coating.
(5) The fiber assembly and manual holder is an inexpensive piece of equipment, which is particularly suitable for those laboratories that do not possess expensive automated headspace sampling equipment.

The analysis of blood in combination with viscera samples (lung, brain, and fat) allows for a more complete evaluation of a person's exposure to VOC's prior to death. This information has proved most valuable in assisting the Coroner.

Acknowledgments

We express our appreciation to the Western Australian State Coroner (Mr. Alistair Hope), Director Chemistry Centre WA (Dr. John Hosking), and the Chief, Forensic Science Laboratory (Mr. Neil Campbell) for their support and permission to publish case results.

References

1. Belardi R, Pawliszyn J. The application of chemically modified fused silica fibres in the extraction of organics from water matrix samples and their transfer to capillary columns. Water Pollut Res J Can 1989;24:179–82.
2. Arthur CL, Pawliszyn J. Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal Chem 1990;62:2145–8.
3. Zhang Z, Pawliszyn J. Headspace solid-phase microextraction. Anal Chem 1993;65:1843–52.
4. Mani V. Properties of commercial SPME coatings. In: Pawlysyn J, editor. Applications of solid phase microextraction. Cambridge UK: Royal Society of Chemistry Monograph, 1999;60–67.
5. Gorecki T. Solid versus liquid coatings. In: Pawlysyn J, editor. Applications of solid phase microextraction. Cambridge UK: Royal Society of Chemistry Monograph, 1999;92.
6. Ren Q, Bertsch W. A comprehensive sample preparation scheme for accelerants in suspect arson cases. J Forensic Sci 1999;44(3):504–15.
7. Almirall JR, Wang J, Lothridge K, Furton KG. The detection and analysis of ignitable liquid residues extracted from skin using SPME/GC. J Forensic Sci 2000;45(2):453–61.
8. Kirkbride KP, Klass G, Pigou PE. Application of solid-phase microextraction to the recovery of organic explosives. J Forensic Sci 1998;43(1):76–81.
9. Coumbaros JC, Kirkbride KP, Klass G. Application of sold-phase microextraction to the profiling of an illicit drug: manufacturing impurities in illicit 4-methoxyamphetamine. J Forensic Sci 1999;44(6):1237–42.
10. Jurado C, Gimenez MP, Soriano T, Mendenez M, Repetto M. Rapid analysis of amphetamine, methamphetamine, MDA and MDMA in urine using solid-phase microextraction, direct on-fiber derivatisation and analysis by GC-MS. J Anal Toxicol 2000;24(1):11–6.
11. Bermejo AM, Seara R, dos Santos Lucas AC, Tabernero MJ, Fernandez P, Marsili R. Use of solid-phase microextraction (SPME) for the determination of methadone and its main metabolite, EDDP in plasma by gas chromatography-mass spectrometry. J Anal Toxicol 2000;24(1):66–9.
12. Park SW, Kim NY, Yang YG, Seo B, Paeng KJ. Toluene distribution of glue sniffers' biological fluid samples in Korea. J Forensic Sci 1998;43 (4):888–90.
13. Schuberth J. Gas residues of engine starting fluid in postmortem sample from an arsonist. J Forensic Sci 1997;42(1):144–7.
14. Degel F. Comparison of new solid-phase extraction methods for chromatographic identification of drugs in clinical toxicological analysis. Clin Biochem 1996;29(6):529–40.
15. Lee X-P, Kumazawa T, Sato K. A simple analysis of 5 thinner components in human body fluids by headspace solid-phase microextraction. Int J Legal Med 1995;(107), 310–13.
16. Brewer WE, Galipo RC, Morgan SL, Habben K. The confirmation of volatiles by solid-phase microextractio and GC/MS in the investigation of two traffic fatalities. J Anal Toxicol 1997;21(1):286–90.
17. Schimming E, Levsen K, Kohme C, Schurmann W. Biomonitoring of benzene and toluene in human blood by headspace-solid-phase microextraction. Fresenius' J Anal Chem 1999;363(1):88–91.
18. Kim NY, Park SW. The comparison of toluene determination between headspace-solid phase microextraction and headspace methods in glue-sniffer's blood and urine samples. J Forensic Sci 2000;45(3):702–7.
19. Tranthim-Fryer DJ, Hansson RC, Norman KW. Recovery and identification of volatile organic compounds in ante and postmortem samples by headspace/solid-phase microextraction/gas chromatography-mass spectrometry. Melbourne, Australia: Australian International Symposium on Analytical Science 1999; poster paper 2.40
20. Tranthim-Fryer DJ, Hansson RC, Norman KW. Headspace/solid-phase microextraction/gas chromatography-mass spectrometry: a screening technique for the recovery and identification of volatile organic com-

pounds (VOC's) in postmortem blood and viscera samples. Proc Amer Acad For Sci, Reno USA 2000;(paper K12):271–2.
21. Dehon B, Humbert L, Devisme L, Stievenart M, Mathieu D, Houdret N. et al. Tetrachlorethylene and trichlorethylene fatality: case report and simple headspace SPME-capillary gas chromatographic determination in tissues. J Anal Toxicol 2000;24(1):22–6.
22. SUPELCO Bulletin 923. Solid phase microextraction: theory and optimization conditions 1998, Sigma-Aldrich Co.
23. Baselt RC, Cravey RH. Disposition of toxic drugs and chemicals in man, 5th edition. Chemical Toxicology Institute, Foster City, CA: 2000;842.
24. Baselt RC, Cravey RH. Disposition of toxic drugs and chemicals in man, 5th edition. Chemical Toxicology Institute, Foster City, CA: 2000;404.
25. Lofti H, Clement S, Debord J, Brosset A, Dumont D, Lachatre G. A fatal case involving halothane intoxication. TIAFT Bulletin Case Notes 1994;24(3):34–7.

Additional information and reprint requests:
David J. Tranthim-Fryer
Chemistry Centre (WA)
Forensic Science Laboratory
125 Hay Street
East Perth WA 6004
Australia
E-mail: *dfryer@ccwa.wa.gov.au*