A Full Evaporation Headspace Technique with Capillary GC and ITD: A Means for Quantitating Volatile Organic Compounds in Biological Samples

Jan Schuberth*

National Board of Forensic Medicine, Department of Forensic Chemistry, University Hospital, 581 85 Linköping, Sweden

Abstract

The full evaporation technique (FET), which is a variant of headspace analysis used to overcome matrix effects, was combined with capillary gas chromatography (GC) and ion-trap detection (ITD). The aim was to enable quantitative tests of volatile organic compounds (VOCs) in blood and postmortem tissue samples. FET was applied to sample sizes less than 35 mg whose VOCs were released from the matrix at an equilibration temperature of 130°C. A capillary column with a nonpolar stationary phase was used for GC, and ITD was performed with the mass spectrometer run in full-scan mode. The potential of FET–GC–ITD was studied for the analysis of blood samples spiked with low concentrations of ethanol, acetone, 2-propanol, and 2-butanone and on brain tissue that contained methyl *tert*-butyl ether (MTBE), benzene, toluene, ethylbenzene, *o*-, *m*-, and *p*-xylene, and propylbenzene. Samples were obtained from the bodies of victims who had inhaled smoke during an arson or accidental fire. There was a linear relationship between peak area and sample size, which indicates that the conditions of full evaporation were met and that the matrix effect was negated. The total analyte amount in the test sample at the limit of quantitation was in the range of 0.4–1 nmol for polar VOCs in blood and 0.03–0.1 nmol for nonpolar VOCs in brain tissue. Data on precision and accuracy of the method are reported.

Introduction

There are many problems associated with the quantitative assay of volatile organic compounds (VOCs) in a biological material. One of these problems is interaction between the analyte and the biological components. As a result of this interaction, a portion of the VOC binds to the matrix and thus becomes unavailable for determination in the gas phase. Such a matrix effect limits the use of direct headspace analysis as a simple quantitative assay for forensic postmortem samples in particular. Random environmental influences during the time period between death and the collection of the samples may cause large variations in the constituents. Different degrees of decay of the tissue or body fluid may be one cause of the changes in the concentration of organic compounds; this may lead to unforeseen matrix effects. Therefore, it can be difficult or impossible to find a matching material to be used for generating a proper calibration graph for the most common quantitation tests, notably the external standardization method (ESM) or the internal standardization method (ISM) (1).

A way of resolving such a problem is to use the standard addition method (SAM) (1). The signal response of the unknown concentration of the analyte is measured both with and without the addition of a known amount of analyte to the test sample. However, the procedure entails the dispersion in the matrix of the in vitro analyte to be the same as that of the in vivo; this assumption cannot always be made when a heterogenous biological sample is analyzed. Another way of negating matrix effects is to use the multiple headspace extraction method (2,3). Since fractions of the VOC are removed with repeated gas extractions and tested separately, one may view this technique as being the reverse of SAM and somewhat related to purge and trap analysis. Unlike the dynamic headspace method, however, only a portion of the analyte is released from the matrix by multiple headspace extraction. The total amount is calculated by extrapolation to the 0 extraction step of the straight line obtained when the logarithm of the observerd peak area is plotted versus the number of extraction steps. Multiple headspace extraction can be applied to the analysis of any heterogenous liquid (2,3) or solid (3) material, but its use is hampered by the laborious and time-consuming procedure as well as by the fact that it rests only on an empirical and not a strict mathematical basis (4). The standard graph for both multiple headspace extraction and SAM is, however, valid for any sample type and is generated simply by testing known concentrations of the VOCs dissolved in a sufficiently small volume of a solvent to enable a full evaporation of both the analyte and matrix.

Recently, Markelov and Guzowski (5) described the full evaporation technique (FET). In this method, determination is done under such conditions that the analyte in the test sample is fully or almost fully evaporated. The general approach of the method is simple: A small amount of sample is equilibrated at a high temperature. The FET should be suited for forensic work not only because it is theoretically solid and can overcome the

* Author's e-mail address is jan.schuberth@mbox300.swipnet.se.

matrix problem, but also because it requires that only a minute specimen mass be used. This may be an advantage for the forensic chemist when there is a lack of test material.

In comparison with headspace analysis, the main drawback of FET is its lower sensitivity (5). The limit of detection or quantitation depends on the amount of sample that can be tested under conditions that cause a full evaporation or that cause a partial vapor pressure below that controlling the subsequent transfer of gas from the headspace vial to the capillary. To compensate for the inherent problem of monitoring low concentrations of VOCs in a minute sample mass, the use of FET in forensic chemistry thus requires a sensitive test system.

Recently, a method applicable in forensic work to detect VOCs was reported (6). It is based on headspace analysis combined with capillary gas chromatography (GC) and ion-trap detection (ITD). This method, which is mainly aimed at searching for a VOC and identifying it, has been modified for use with the FET to make quantitative analysis possible. In the course of this work, I have studied the potential of the procedure for the analysis of small amounts of blood containing some polar VOCs that are common in forensic toxicology. In addition, a few nonpolar VOCs of smoke were determined in minute samples of brain tissue from victims who had died in an arson or accidental fire.

Experimental

Materials

Routine postmortem brain tissue samples or blood samples from blood donors were used. The makeup of the standard solutions is shown in Table I.

Instrumentation

The analyses were done on aliquots of fluid or tissue that had been weighed to within 10 pg in a closed headspace vial (MCL Research RC 201 P balance, Sartorius; Tillquist Analysis, Sweden). The headspace gas was put in a 21.4-mL vial (V.NR. 092357; Apodan, Copenhagen, Demark) and sealed with a rubber septum with a crimp cap (V.NR. 03 44 45, Apondan). The headspace vial was put in a 18906B accessory kit for constant heating and hooked up to a Hewlett-Packard Model 19395A autosampler (Sweden). GC analyses were done with an HP Model 5890 GC equipped with a DB-1 capillary column (30 m × 0.25-mm i.d. coated with 1-µm methylsiloxane) from J&W Scientific (Folsom, CA). It was inserted without a flow restrictor directly into the ion source of a Finnigan MAT Model ITD800 ion-trap detector (Sweden). The tuning of the device was done manually to resolve the *m/z* 69 and 70 peaks and the *m/z* 131 and 132 peaks. Evaluation of the raw data was carried out with Datamaster II (Version 1.3, Finnigan MAT).

Table I. Standard Solutions

Polar compounds			Nonpolar compounds	
Reference substance	Blood (mmol/L)	Water (mmol/L)	Reference substance	Methanol (mmol/L)
Ethanol	10	10	Methyl *tert.*-butyl ether	1.2
Methanol	5		Benzene	1.2
Acetone	2	2	Toluene	1.2
2-Propanol	2	2	Ethylbenzene	1.2
2-Butanone	2	2	*o*-Xylene	1.2
			m-/*p*-Xylene	1.2
			Propylbenzene	1.2

Analytical procedures

The headspace apparatus was programmed as follows: equilibration temperature, 130°C for FET or 80°C for multiple headspace extraction; equilibration time, 28 min; and auxiliary gas pressure, 210 kPa. When multiple headspace analysis was performed, the pressure in the vial had to be released to atmosphere between extractions; therefore the headspace sampler was programmed to execute an additional vent/loop fill time for 7 s after the injection. The injector temperature was 150°C. Except for the settings listed previously, the same instrumental conditions were used as described earlier for headspace GC–ITD (6). Multiple headspace extraction was done as a two-step procedure (3) on four brain tissue samples in the range of 24.84–1390 mg and on the standard solution of MTBE and aromatic hydrocarbons in methanol. To assure a complete evaporation of the standard analytes and the matrix at 80°C or 130°C, no more than 10 mg of these solutions was used.

Criteria of full evaporation

The theoretical background of the FET, which has been described in detail (5), rests on the following equation:

$$C_g = C_0 V_c (K V_r + V_g)^{-1} \qquad \text{Eq 1}$$

where C_0 is the analyte concentration in the sample, V_c is the sample volume before the equilibration, C_g is the analyte concentration in the gas phase, V_r is the condensed sample

Table II. Limit of Detection and Quantitation

Substance	*m/z*	Limit of detection*	Limit of quantitation†
Methyl *tert*-butyl ether	73	0.034	0.098
Benzene	78	0.017	0.050
Toluene	91 + 92	0.015	0.044
Ethylbenzene	91 + 106	0.013	0.038
m-/*p*-Xylene	91 + 106	0.010	0.030
o-Xylene	91 + 106	0.015	0.044
Propylbenzene	91 + 120	0.013	0.039
Ethanol	31 + 45	0.356	1.027
Acetone	43	0.123	0.356
2-Propanol	45	0.259	0.746
2-Butanone	43	0.140	0.403

* Total analyte amount (in nanomoles) in the test sample at the limit of detection. The limit of detection is equal to 3 times the standard deviation of the background noise and was calculated according to the procedure by Knoll (7).

† Total analyte amount (in nanomoles) in the test sample at the limit of quantitation. The limit of quantitation is equal to 10 times the standard deviation of the background noise and was calculated according to the procedure by Knoll (7).

volume after the equilibration, V_g is the volume of the gas (headspace vial volume), and K is the partition coefficient. When the condition of full evaporation is met, KV_r should be insignificant compared with V_g; C_g will then become independent of the temperature and linearly related to the sample size.

Estimation of the Limits of Detection and Quantitation

To assay the limits of detection and quantitation for the different VOCs, the height of the largest noise peak was measured at the appropriate mass number or combination of mass numbers (Table II) in a preselected retention time interval on chromatograms obtained from the analysis of empty headspace vials. This interval was equal to 100 multiples of the width of the calibration peak at one-half peak height. From these data, the peak height (h_{blank}), which is equal to 3 times (limit of detection) or 10 times (limit of quantitation) the standard deviation of the gross blank signal, was calculated (7). To account for variations of the h_{blank} value, the mean value of h_{blank} plus or minus the standard deviation (SD) was measured in five experiments, and the minimum peak height needed for the identification or quantitation of a VOC (Table II) was set at (h_{blank} + 3×SD). The analyte concentration that gave rise to this signal, that is, the limit of detection or quantitation, was finally calculated from the peak height of the calibration sample.

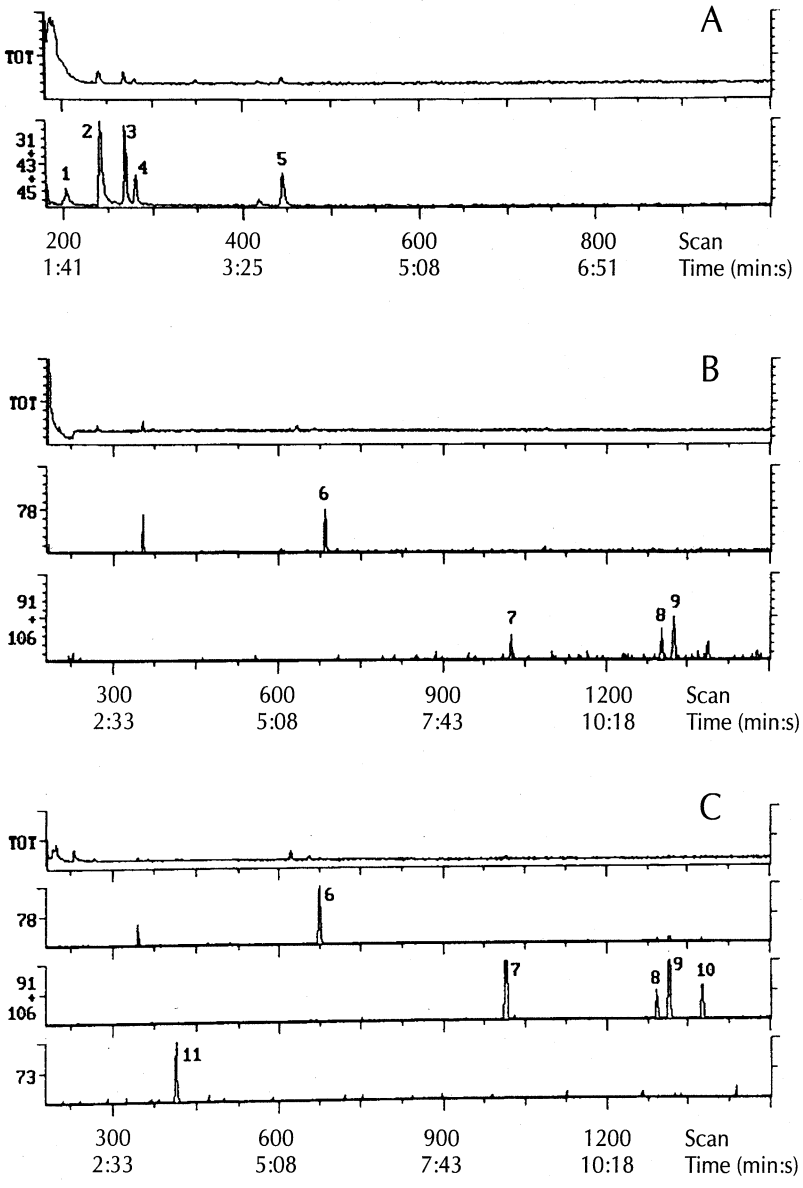

Figure 1. Total ion current and reconstituted mass chromatograms of VOCs in biological samples. The upper phase (A) shows the data from a 1.82-mg blood sample spiked with 9.1 nmol methanol, 18.2 nmol ethanol, and 3.6 nmol each of acetone, 2-propanol, and 2-butanone. The middle phase (B) shows the chromatogram from a 32.72-mg brain tissue sample from a victim who had died in an accidental fire. The bottom phase (C) shows a 4.99-mg brain tissue sample from a death by arson. Peaks: 1, methanol; 2, ethanol; 3, acetone; 4, 2-propanol; 5, 2-butanone; 6, benzene; 7, toluene; 8, ethylbenzene; 9, m-/p-xylene; 10, o-xylene; and 11, methyl *tert*-butyl ether (MTBE).

Results

Table II lists the mass numbers or combinations of mass numbers that were used for detecting or quantitating some polar and nonpolar VOCs. The Table also shows the minimum amount of the compounds needed in the headspace vial for these tests.

Figure 1 shows the total ion current and reconstituted mass chromatograms of blood or brain tissue samples run by the FET–GC–ITD. The upper phase shows 1.82 mg of blood that has 9.1 nmol methanol, 18.2 nmol ethanol, and 3.6 nmol each of acetone, 2-propanol, and 2-butanone. The peaks of the last four VOCs seemed good enough for quantitative use (see Table III), but the methanol peak was weak and distorted. Since large variations of the signal response were noted to be related to the amount of methanol, this VOC was excluded from the investigation.

The middle phase of Figure 1 depicts the analysis of a 32.72-mg brain tissue sample from a victim who died in an accidental fire that did not involve any accelerants. Among the gases inhaled during the event and deposited in the brain was benzene. The peak heights of the others were above the limit of detection but below the limit of quantitation. The lower phase of Figure 1 shows the data for a 4.99-mg brain tissue sample obtained from an arson victim (motor gasoline was used as the accelerant). In this instance, the signal responses of all of the gases associated with the fire detected on the chromatogram were above the limit of quantitation (Table IV).

To see if the criterion of full evaporation was met in accord with Equation 1, the signal response at different sample masses was studied. This was done on the same materials as used for generating chromatograms A and C in Figure 1. The data indi-

Table III. Accuracy and Precision of FET–GC–ITD of Blood Samples

Substance	Target conc. in blood (mmol/kg)	Percent mean recovery ± RSD 13.84-15.26 mg* (*n* = 7)	1.82-34.70 mg* (*n* = 9)
Ethanol	10.0	109% ± 2.4	94% ± 11.4
Acetone	2.0	115% ± 6.2	105% ± 25.9
2-Propanol	2.0	105% ± 3.2	90% ± 13.5
2-Butanone	2.0	80% ± 3.0	95% ± 13.6

* As measured using milligram amounts of sample. The number of experiments is listed in parentheses

Table IV. Accuracy and Precision of FET–GC–ITD of Brain Tissue

Substance	FET* (*n* = 7)	MHE† (*n* = 4)
MTBE	16% ± 15.2	16% ± 8.2
Benezene	56% ± 26.6	47% ± 21.1
Toluene	153% ± 25.4	161% ± 9.9
Ethylbenzene	20% ± 20.2	17% ± 51.1
m-/*p*-Xylene	34% ± 23.7	33% ± 54.3
o-Xylene	22% ± 24.0	19% ± 41.8
Propylbenzene	3% ± 21.3	3% ± 72.2

* 17.2–30.02-mg sample was equilibrated at 130°C.
† 24.84–1390-mg sample was equilibrated at 80°C in a two-step multiple headspace extraction.

cated that the peak area of the polar VOCs in the blood sample, as shown in Figure 2, and of the fire-associated gas residue in the brain tissue, as shown in Figure 3, related linearly to the sample mass up to about 35 mg.

The accuracy and precision of FET–GC–ITD is shown in Tables III and IV. When the method was used for the determination of some polar VOCs at low concentrations in blood (Table III), the precision was good at least when a fairly large and constant sample size (about 14 mg) was tested. Under these conditions, the determinations were done at analyte amounts that were about 50–100 times the limit of quantitation of the method. When FET was used to determine fire-associated gases that had been inhaled during an arson and deposited in the victim's brain, the lower precision data (Table IV) were obtained with VOC concentrations closer to the limit of quantitation of the method. The signal response of benzene in the brain of a victim of an accidental fire (Figure 1B) was 6 times the limit of quantitation. In repeated tests on seven aliquots of this material in the range of 16.51–38.17 mg, 10± 15.0% nmol/kg benzene was found (mean value plus or minus the relative standard deviation.

Discussion

VOCs cause many deaths each year. The exposure may result from swallowing or sniffing the compounds as a means of abuse or accidentally inhaling the compounds. The most common

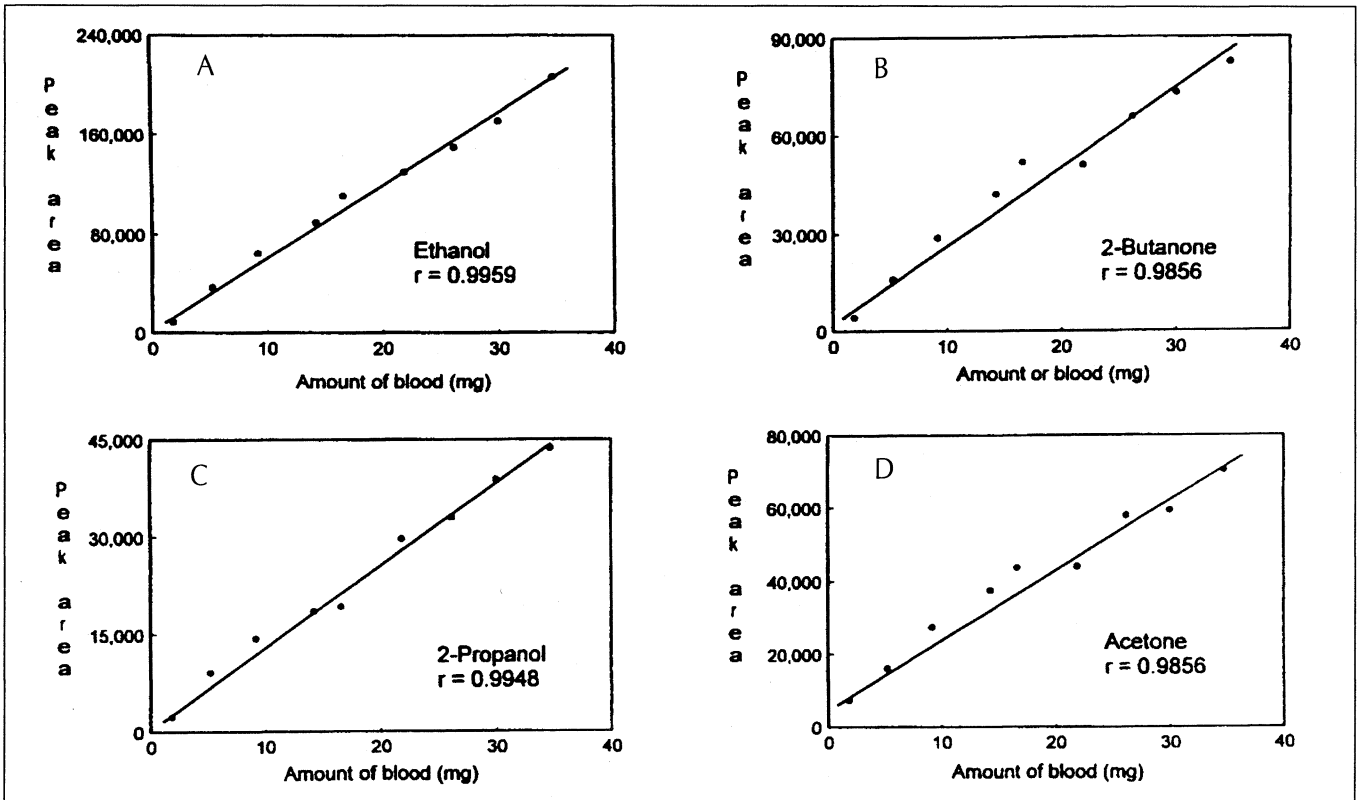

Figure 2. Peak areas of polar VOCs quantitated by FET-GC-ITD versus the amount of blood spiked with 9.1 nmol methanol, 18.2 nmol ethanol, 3.6 nmol each of acetone, 2-propanol, and 2-butanone. The mass numbers picked for measuring the peak areas of the various organic compounds are listed in Table II.

volatile intoxicants are ethanol and carbon monoxide, for which the majority of forensic laboratories have test methods. Since the biological matrices that bind carbon monoxide are well known, the analyte can be released into the gas phase by a targeted method (8). Ethanol is found in the blood at high concentrations and is, therefore, usually determined after the sample has been diluted to such an extent that any matrix effect is no longer significant (9). In forensic toxicological work, other VOCs are generally determined by ESM or ISM (10). To my knowledge, there are no reports on the use of multiple headspace analysis and only two papers dealing with with use of SAM (1,8).

For quantitating VOCs in postmortem samples, a sensitive method that is based on solid theory is needed. FET–GC–ITD seems to meet that need. If the data on the total analyte amount in the headspace vial (Table II) are recalculated for a sample size of 30 mg, the concentration at the limit of quantitation of MTBE and the aromatic hydrocarbons is in the range of 1.0–3.3 µmol/kg and the concentration at the limit of quantitation of polar VOCs is in the range of 12–34 µmol/kg. For MTBE and the aromatic hydrocarbons, these concentrations were about 10 times higher than those obtained by headspace GC–ITD with the blood samples equilibrated at 50°C (6). The sensitivity of the FET could perhaps be enhanced if more test material was used. The safety aspect, however, must then be taken into consideration. To compensate for the raised vapor pressure, the auxiliary gas pressure on the headspace vial has to be increased, and the chance of vial breakage will also increase. Moreover, the dilution of the headspace gas to be tested increases with the pressurization of the vial.

However, FET–GC–ITD is sensitive enough for measuring, with good accuracy and precision in a small sample size, ethanol, 2-propanol, acetone, and 2-butanone at low toxic concentrations. Toxic effects of ethanol are usually associated with blood concentrations of at least 20 mmol/L, and 2-propanol or acetone require about 3–10 mmol/L (11). The method may be

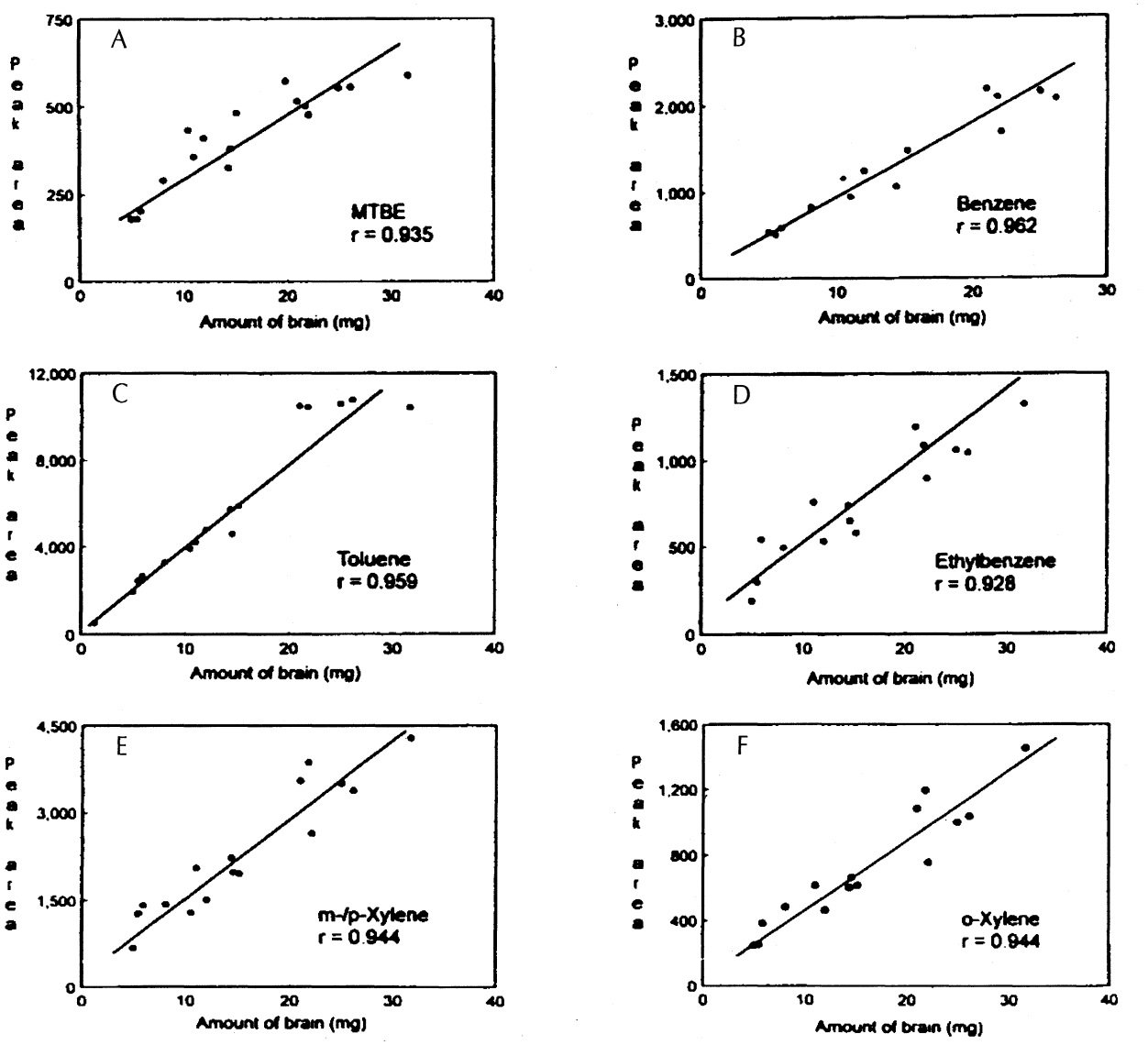

Figure 3. Peak areas of nonpolar VOCs quantitated by FET-GC-ITD versus the amount of tested brain tissue from an arson victim. The mass numbers picked for measuring the peak areas of the various organic compounds are listed in Table II.

particularly useful when no or very little blood is available. In such a situation, the chemist will be able to quantitate volatile intoxicants in any material that is at hand. An exception is methanol because it cannot be determined due to its short retention time and unfavorable mass fragmentation. A capillary with a polar stationary phase that gives a longer retention time for polar VOCs may be more useful even though methanol coelutes with 2-butanone (12).

In a fire that results in a fatality, carbon monoxide is usually judged as the direct cause of the death. Other noxious gases, formed from burning solid materials like plastics as well as from possible accelerants, may also be inhaled by the victim. In fact, such VOCs have been reported to be present in the body fluids and tissues of the victims; MTBE, which is an additive in motor gasoline, has even been used as an arson indicator in postmortem samples (13). To my knowledge, however, data on the concentrations of these fire-associated gas residues in postmortem samples do not seem to be present in the literature. As indicated in this work, FET–GC–ITD might aid in determining whether gas components other than carbon monoxide contribute to death. They may act as incapacitating agents to make the victim unable to leave the site of the disaster. Owing to their nonpolar nature, these VOCs are distributed after inhalation into body constituents that are rich in fat, and the brain is thus a proper material for such a quantitative study. As shown in this report, however, the precision of FET–GC–ITD seems to be worse and the signal responses at different sample masses appear to be more scattered when applied to brain tissue samples than when applied to blood samples. This could be caused by a low signal response of the nonpolar VOCs or an uneven in vivo distribution of these compounds in brain compartments.

Conclusion

The combination of FET with capillary GC–ITD has many advantages, such as simplicity, versatility, and low cost. A headspace sampler that can be hooked up easily to any GC is available in most laboratories. The ITD is a low-priced MS that is simple to operate and offers nearly the same sensitivity regardless of whether a wide range of ions or a single selected ion is scanned. Any volatile substance that appears on the chromatogram with a reasonable retention time and mass fragmentation should be feasible for quantitation. In some instances, the good sensitivity of the system may make it useful for both screening and quantitation during the same chromatograpic run.

References

1. P. Koupil, J. Novák, and J. Drozd. Comparison of the absolute calibration method with the method of standard addition for the determination of halothane in blood by gas chromatographic headspace analysis. *J. Chromatogr.* **425:** 99–105 (1988).
2. C. McAullife. GC determination of solutes by multiple phase equilibration. *Chem. Technol.* **1:** 46–51 (1971).
3. B. Kolb. Multiple headspace extraction—a procedure for eliminating the influence of the sample matrix in quantitative headspace gas chromatography. *Chromatographia* **15:** 587–94 (1982).
4. A. Venema. The usefulness of headspace analysis-gas chromatograpy technique for the investigation of solid samples. *J. High Resol. Chromatogr.* **13:** 537–39 (1990).
5. M. Markelov and J.P. Guzowski, Jr. Matrix independent headspace gas chromatographic analysis. The full evaporation technique. *Anal. Chim. Acta* **276:** 235–45 (1993).
6. J. Schuberth. Joint use of retention index and mass spectrum in post-mortem tests for volatile organics by headspace capillary gas chromatography with ion-trap detection. *J. Chromatogr.* **674:** 63–71 (1994).
7. J.E. Knoll. Estimation of the limit of detection in chromatography. *J. Chromatogr. Sci.* **23:** 422–25 (1985).
8. R.A. Middleberg, D.E. Easterling, S.F. Zelonis, F. Rieders, and M.F. Rieders. Estimation of perimortal percent carboxy-heme in nonstandard postmortem specimens using analysis of carbon monoxide by GC/MS and iron by flame atomic absorption spectrophotometry. *J. Anal. Toxicol.* **17:** 11–13 (1993).
9. A.W. Jones and J. Schuberth. Computer-aided headspace gas chromatograpy applied to blood-alcohol analysis: importance of online process control. *J. Forens. Sci.* **34:** 1116–27 (1989).
10. Y. Seto. Determination of volatile substances in biological samples by headspace gas chromatography. *J. Chromatogr.* **674:** 25–62 (1994).
11. A.C. Moffat and B. Widdop. *Clarke's Isolation and Identification of Drugs,* 2nd ed. The Pharmaceutical Press, London, England, 1986, pp 315, 593, 691.
12. J. Schuberth. Volatile compounds detected in blood of drunk drivers by headspace/capillary gas chromatograpy/ion trap mass spectrometry. *Biol. Mass Spectrom.* **20:** 699–702 (1991).
13. J. Schuberth. Post-mortem test for low-boiling arson residues of gasoline by gas chromatography–ion-trap mass spectrometry. *J. Chromatogr. B* **662:** 113–17 (1994).

Manuscript accepted January 22, 1996.