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# Application of solid-phase micro-extraction technology to drug screening and identification

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SUMMARY. Benzodiazepines, tricyclic antidepressants and local anaesthetics are frequently involved in poisoning episodes and fatalities. A specific, sensitive and rapid procedure for identifying and quantifying such drugs in postmortem matrices has been developed using solid-phase micro-extraction (SPME) and gas chromatography–mass spectrometry. Very clean extracts were obtained in one step using SPME. The most commonly used fibre coatings were tested to select the best coating for SPME of the drugs. The appropriate fibre coating for most drugs was polyacrylate, followed by Carbowax–divinylbenzene. A Hewlett-Packard 5890 gas chromatograph in combination with a Trio 2000 mass spectrometer was used to analyse the samples. Temperature, time, pH and addition of sodium chloride were optimized to obtain consistent extraction. The between-day and within-day coefficients of variation were less than 16% and less than 6%, respectively.

## INTRODUCTION

Liquid–liquid and solid-phase extraction (SPE) methods are widely used for separating drugs from biological samples. However, liquid–liquid extraction can produce emulsions and a large amount of organic solvent is often needed to extract the drugs. SPE methods also require large amounts of expensive organic solvents and excessive preparation time, although they can be automated. Such methods can be cumbersome[1] and can cause harm to the human body and the environment.

Solid-phase micro-extraction (SPME), introduced by Belardi and Pawliszyn in 1989,[2] is an excellent alternative to the classical methods and is a solvent-free extraction technique that incorporates sample extraction, concentration and sample introduction in a single step.[3]

SPME initially used chemically modified fused-silica optical fibres to extract organic volatiles for analysis by gas chromatography (GC).[2] The technique has since been developed so that, using commercially available fibres, many compounds can be analysed, not only by GC, but also by high-performance liquid chromatography (HPLC).[3]

SPME is now routinely applied in several different areas, including environmental analysis (water, pesticides, solids and air), analysis of drugs and other products during chemical manufacture, toxicology, forensic science and analysis of food and drink.[3]

This new extraction technique is based on partitioning the analyte between a phase immobilized on a fused-silica fibre and the matrix (air, water, plasma, etc.). After equilibration, the absorbed analytes are thermally desorbed by exposing the fibre in the injection port of a gas chromatograph or redissolving them in an organic solvent using HPLC. The technique was made practical by mounting the fibre in a syringe-like device.[4]

Benzodiazepines are an important class of sedative, hypnotic and anticonvulsant drugs that are widely prescribed throughout the world. They are often involved in drug intoxication, are liable to abuse and are capable of affecting

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human skilled performance.[5] They are also frequently involved in poisoning episodes and traffic accidents. In 1978 about 200 deaths were reportedly associated with benzodiazepines in England and Wales. Detection and quantification of these drugs in postmortem matrices are therefore often necessary for medical and legal purposes.[5,6]

Antidepressant drugs are used in psychiatry for the treatment of endogenous depression and are commonly involved in self-poisoning because of the widespread prescribing of large amounts to vulnerable patients.[7]

A number of publications have reported the usefulness of SPME for the analysis of drugs in biological matrices. These include the analysis of amphetamines and related compounds,[8,9] benzodiazepines,[10,11] local anaesthetics,[12,13] meperidine,[14] methadone,[15] tricyclic antidepressants[16,17] and cocaine.[18]

The aim of the present work was to test SPME as a sample preparation method in the qualitative and quantitative analysis, by gas chromatography–mass spectrometry (GC–MS), of drugs in postmortem matrices and to make this procedure as simple and as fast as possible.

## MATERIALS AND METHODS

### Reagents

Methanol, ethyl alcohol and n-butyl acetate were all HPLC grade. All other chemicals were of analytical grade.

Solutions of glycine 0·1 mol/L, NaCl 0·1 mol/L, citric acid 0·1 mol/L and disodium phosphate 0·2 mol/L were prepared in de-ionized water. Glycine–NaOH buffers (pH 8–12) were prepared by adding appropriate amounts of NaOH 0·1 mol/L in glycine 0·1 mol/L. Citric acid–phosphate buffers (pH 4–7) were prepared by adding appropriate amounts of citric acid 0·2 mol/L in disodium phosphate 0·2 mol/L.

Pure samples of drugs were purchased from manufacturers. The stock solutions of individual drugs were prepared by dissolving the appropriate amount of each drug in methanol to make solutions of 1 g/L for each drug. Working standard solutions (50, 100, 200, 300, 400 and 600 mg/L) of the drugs were prepared in methanol. During the study, identical amounts of working solutions were added to drug-free plasma in order to keep the amount of methanol in the samples constant.

### Equipment

An SPME fibre holder and the following SPME fibres were purchased from Supelco (Poole, UK): 100-$\mu$m polydimethylsiloxane (PDMS), 85-$\mu$m polyacrylate (PA), 65-$\mu$m polydimethylsiloxane–divinylbenzene (PDMS–DVB), Carboxen™–polydimethylsiloxane (CAR–PDMS), 65-$\mu$m Carbowax®–divinylbenzene (CW–DVB).

Samples were stirred using a magnetic stirrer with a Teflon-coated stir bar. A Hewlett-Packard 5890 gas chromatograph was used in combination with a Trio 2000 quadrupole mass spectrometer. Screw-top glass sample vials (5 mL) were purchased from Thames Restek (Windsor, UK).

GC separation was achieved on a Hewlett-Packard HP-5 fused-silica capillary column (30 m × 0·32 mm i.d., 0·25-$\mu$m film thickness). The samples were injected in the splitless mode and the splitter was opened after 1 min. The injector port temperature was 250°C. The oven temperature was held at 45°C for 2 min, then programmed to 290°C at 6°C/min and held at this temperature for 5 min. The GC–MS interface temperature was 300°C. Ultra-pure helium CP grade (B.O.C. Gases, Guildford, UK) was used as a carrier gas with a constant on-column pressure of 8 p.s.i. The mass spectrometer was operated in electron impact mode at 70 eV and tuned to heptacosafluorotributylamine (a reference calibration compound) to achieve the best sensitivity. The mass range scanned was from 45 to 600 a.m.u. A computer equipped with Masslab software, which was also devoted to data acquisition and processing, controlled the whole procedure. Searching the related spectrum in commercial libraries NIST, LIBTX and Wiley identified the drugs. The concentration was achieved by calculating the peak area ratios of the drugs to internal standard (IS) and comparing with a calibrator.

### Structure and use of the SPME fibre and holder

The fibre holder was constructed and operated in a similar way to a GC syringe. The fibre sheath had a needle point for penetrating a rubber septum when the fibre was in the withdrawn position. Once the sheath had penetrated the septum, pushing the plunger downwards exposed the fibre.

In use, the prepared sample was contained in a 5-mL glass vial with small stirrer magnet and a septum cap. The vial was heated in a beaker of water at 60°C for 45 min (Fig. 1). With the clean fibre retracted, the sheath was passed through

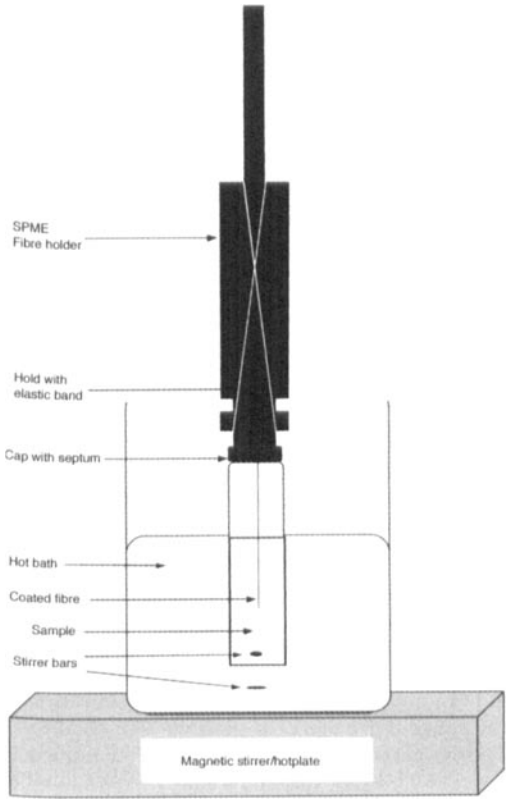


FIGURE 1. *Schematic illustration of the solid-phase micro-extraction (SPME) method.*

the septum and the fibre was exposed. At the end of the equilibration time, the fibre was retracted and the sheath withdrawn. The GC injection port septum was then penetrated by the sheath and the fibre was exposed to allow desorption.

**Urine sample preparation**

Ten microlitres of working internal standard solution (100 mg of prazepam per litre of methanol) were added to 1 mL of urine. Centrifugation at 3000 r.p.m. was performed for 5 min and the supernatant was transferred to a 10-mL beaker. The solution was buffered to the desired pH (4, 7, 10) with 3 mL of the appropriate buffer and the pH was adjusted with either 1 mol/L NaOH or 1 mol/L perchloric acid. The solution was transferred to a 5-mL glass vial with a magnetic stir bar. The fibre was placed in the sample solution, where extraction was allowed to take place for 45 min.

**Plasma and blood sample preparation**

Ten microlitres of working internal standard solution (100 mg of prazepam per litre of methanol) was added to 1 mL of plasma followed by 1·5 mL of 1 mol/L perchloric acid. The mixture was vortex-mixed for 3 min.

The sample was sonicated for 5 min to complete deproteinization and then centrifuged at 3000 r.p.m. for 10 min. The clear supernatant (2 mL) was transferred into a 10-mL beaker containing 2 mL of the appropriate buffer; the pH was then adjusted and extraction achieved as for urine samples.

**Maintenance of fibres**

Before use, fibres were cleaned by immersion for 5 min in de-ionized water at 60°C; the water was stirred constantly using a magnetic stirrer. The fibres were then reconditioned in the GC injector for 3 min as instructed by the manufacturer. A blank analysis was performed daily to check the baseline and fibre cleanliness.

**Experimental**

The effects of changes in salt (NaCl) concentrations, pH and temperature were studied.

For preparation of the standard curves, 1 mL of drug-free plasma was spiked to obtain drug concentrations ranging from 0·5 to 6 mg/L of plasma. The matrices were then sampled using the same procedure as for sampling of plasma.

Addition of NaCl, extraction temperature, sample pH and fibre type were included as variables in the screening design. The optimal values found for each factor were then adopted for the extraction and analysis of the postmortem samples.

Addition of a saturated solution of NaCl facilitated extraction of the drugs by reducing their solubility and so permitting greater partitioning to the SPME fibre. However, the saturated salt solution caused the formation of salt crystals in the hollow barrel of the SPME syringe causing blockage and damage to the needle. To overcome this problem the concentration of NaCl was reduced to 5 mol/L and the fibre was cleaned by inserting it into a vial containing de-ionized water after every five injections to prevent build up of NaCl around the fibre.

Reproducibility was evaluated by analysing plasma samples containing different concentrations of selected drugs of interest on the same

day (within-day reproducibility) and over 5 days (between-day reproducibility).

## RESULTS AND DISCUSSION

The choice of an appropriate fibre coating is essential for the development of an SPME method. The sensitivity of the fibre towards an analyte depends on the molecular weight and polarity of the analytes to be extracted.[3] Retention times (in minutes), using the selected fibres at different pH values for the targeted drugs, are shown in Table 1. In all cases we observed that PA, followed by CW–DVB, was the best fibre for the extraction of the drugs. Therefore, PA and CW–DVB were chosen for the extraction of postmortem specimens for SPME.

### Addition of NaCl

The addition of salt, such as NaCl, into the sample matrix decreases the solubility of the targeted analytes. This causes an increase in the amount of analyte extracted by the fibre coating. As a result, the sensitivity can be significantly increased for polar compounds. It was observed that addition of NaCl (5 mol/L) solution enhanced the extraction of methadone, imipramine, bupivacaine, diazepam and prazepam. The enhancement achieved by addition of NaCl ranged from 1·4-fold for bupivacaine to 7-fold for diazepam. The only drug that was less well extracted after the addition of NaCl was trifluoperazine. Therefore a sodium chloride concentration of 5 mol/L was used for all further extractions.

### Temperature effects

The extraction rate was found to increase over a temperature range from room temperature up to approximately 75°C (Table 2). However, it proved difficult to stabilize the temperature at 75°C, so 60°C was used for the SPME extraction of the postmortem samples.

### pH effects

The targeted drugs have different $pK_a$ values and therefore the partitioning of the drug between the sample and the fibre was strongly affected by pH. The effect of pH on the extraction was tested over the pH range 4–11. The optimum pH for the extraction of diazepam and prazepam was pH 7. Trifluoperazine and bupivacaine were optimally extracted at pH 9 and methadone and imipramine at pH 11. There was no measurable difference for the extraction of prazepam and diazepam at acidic pH (Table 3). We therefore selected pH values of 4, 7 and 10 for the extraction of the samples.

### Precision

The precision was calculated after the analysis of five replicates on the same day (within-day precision) and after repeated analysis over 5 days (between-day precision). The between-day coefficients of variation (%CV) for five targeted drugs (1 mg/L) in human plasma were 15% for bupivacaine, 13% for diazepam, 12% for imipramine, and 13% for prazepam and trifluoperazine. The within-day %CV for all drugs was less than 6%. The drugs were extracted using polyacrylate fibre at pH 7 and 60°C.

### Linearity

Calibration curves of amitriptyline and diazepam were generated by least-squares linear regression. They were constructed by plotting the peak area ratios ($A_{analyte}/A_{IS}$) at six concentrations of each drug (between 0·5 and 6 mg/L), which were extracted from plasma using polyacrylate fibre at pH 7 and 60°C. Both drugs showed linearity in the range 0·5–6 mg/L of plasma. The equations for the curves were: $y = 0{\cdot}11x + 0{\cdot}2$ (confidence interval 95%) for diazepam and $y = 0{\cdot}89x - 0{\cdot}06$ (confidence interval 95%) for amitriptyline. The $R^2$ (squared correlation coefficient) of the calibration curve was 0·98 for diazepam and 0·99 for amitriptyline. The detection limits (at a signal to noise ratio of 3) of amitriptyline, bupivacaine, diazepam, prazepam, imipramine, trifluoperazine and haloperidol were less than 0·02, 0·01, 0·02, 0·01, 0·01, 0·02 and 0·1 mg/L, respectively.

### Application to postmortem samples

To evaluate the reliability of the method, some postmortem samples were analysed (Table 4). The samples were found to contain dothiepin, medazepam, dextropropoxyphene and metabolites, amitriptyline, thioridazine, mesoridazine, diphenhydramine, paracetamol, pethidine, diazepam, nordiazepam, chlordiazepoxide, procyclidine, codeine, chlorpromazine, carbamazepine, chlorazepate, dicyclomine, trifluoperazine, nefopam, pseudoephedrine, phenelzine and dihydrocodeine.

## CONCLUSION

To our knowledge, the analytical procedure presented here is the first to describe the detection

TABLE 1. *Retention time (in minutes) of some benzodiazepines, analgesics, local anaesthetics and antidepressants (1 μg/mL) detected using various fibres and pH levels*

| Analyte | pH | Fibre | | |
|---|---|---|---|---|
| | | PA | CW–DVB | PDMS |
| Diazepam | 7 | 34·44 | 0 | NA |
| Nitrazepam | 4 | NA | 38·93 | NA |
| Nitrazepam | 7 | NR | NR | NR |
| Nitrazepam | 10 | NA | NR | NA |
| Prazepam (IS) | 7 | 37·35 | NA | NA |
| Oxazepam | 4 | NR | NR | NR |
| Oxazepam | 7 | NR | NR | NR |
| Oxazepam | 10 | NR | NR | NR |
| Chlordiazepoxide | 4 | NA | 35·59, 37·34 | NA |
| Chlordiazepoxide | 7 | 35·59, 37·34 | 35·59, 37·34 | NA |
| Flunitrazepam | 4 | NR | 36·83 | NA |
| Flunitrazepam | 7 | NR | NA | NR |
| Medazepam | 4 | 31·59 | NA | NA |
| Medazepam | 7 | 31·59 | NA | 31·59 |
| Ketazolam | 7 | 34·44 | NA | NR |
| Midazolam | 4 | NA | 34·98 | NA |
| Clobazam | 4 | NA | 34·33 | NA |
| Clonazepam | 4 | NR | NR | NR |
| Clonazepam | 7 | NR | NR | NR |
| Clonazepam | 10 | NR | NR | NR |
| Codeine | 7 | NR | NA | NR |
| Codeine | 10 | NR | 33·61 | NR |
| Cocaine | 7 | NR | 31 | NR |
| Cocaine | 10 | NR | NR | NR |
| Heroin | 7 | NR | NR | NR |
| Heroin | 10 | NR | NR | NR |
| Meperidine | 7 | 22·74 | NA | NA |
| Meperidine | 10 | 22·74 | NA | NA |
| Dihydrocodeine | 7 | NR | NA | NA |
| Dihydrocodeine | 10 | NR | 32·93 | NA |
| Methadone | 7 | 30·04 | NA | NA |
| D-Propoxyphen | 7 | 30·77 | NA | NA |
| Bupivacaine | 7 | 32·2 | NA | NA |
| Amitriptyline | 7 | 30·87 | NA | 30·87 |
| Clomipramine | 10 | 33·96 | NA | NA |
| Mianserin | 7 | 30·88 | NA | NA |
| Dothiepin | 7 | 33·65 | NA | NA |
| Butriptyline | 7 | 30·78 | NA | NA |
| Imipramine | 7 | 31·3 | NA | NA |
| Trimipramine | 7 | 31·08 | NA | NA |
| Nortriptyline | 7 | NR | NA | NA |
| Protriptyline | 7 | 31·67 | NA | NA |
| Doxepine | 7 | 31·1 | NA | NA |
| Loxapine | 10 | 34·62 | NA | NA |
| Haloperidol | 7 | 40·85 | NA | NR |
| Trimeprazine | 7 | 32·36 | NA | 32·36 |
| Chlorpromazine | 7 | 35·13 | NA | NA |
| Trifluoperazine | 7 | 36·6 | NA | NA |

PA = polyacrylate; CW–DVB = Carbowax–divinylbenzene; PDMS = polydimethylsiloxane; IS = internal standard; NR = no response; NA = not analysed.

of such a number of drugs of different groups. Factors found to be significant for detection were the type of fibre, pH, extraction temperature and the addition of sodium chloride. The SPME procedure has been shown to be useful for the detection of therapeutic and toxic concentrations of the drugs in clinical and postmortem samples.

TABLE 2. *Effect of temperature on peak area responses of various drugs (polyacrylate fibre)*

| Temperature (°C) | Methadone | Imipramine | Bupivacaine | Diazepam | Prazepam | Trifluoperazine |
|---|---|---|---|---|---|---|
| Room temperature | 260 | 746 | 48 | 622 | 1895 | 1981 |
| 35 | 234 | 626 | 198 | 1655 | 4229 | 3788 |
| 50 | 368 | 1527 | 643 | 3030 | 7592 | 6943 |
| 60 | 1138 | 4253 | 2002 | 4703 | 10351 | 7321 |
| 75 | 1660 | 6810 | 3627 | 5831 | 13187 | 9305 |

TABLE 3. *Effect of pH on peak area response of various drugs (polyacrylate fibre, 60°C)*

| pH | Methadone | Imipramine | Bupivacaine | Diazepam | Prazepam | Trifluoperazine |
|---|---|---|---|---|---|---|
| 4 | – | 35 | – | 711 | 2305 | – |
| 5 | 49 | 107 | 14 | 843 | 2532 | 543 |
| 6 | 62 | 97 | 11 | 922 | 2843 | 871 |
| 7 | 142 | 370 | 54 | 990 | 2868 | 2531 |
| 8 | 854 | 4170 | 350 | 664 | 1922 | 3824 |
| 9 | 1358 | 7307 | 400 | 801 | 2305 | 6014 |
| 10 | 2854 | 8172 | 358 | 702 | 1982 | 4674 |
| 11 | 3387 | 9216 | 293 | 656 | 1621 | 5847 |

TABLE 4. *Comparison of results between SPME/GC–MS as a screening procedure and HPLC*

| Case no. | SPME/GC–MS | HPLC |
|---|---|---|
| 1 | Dextropropoxyphene, amitriptyline, dothiepin, thioridazine, mesoridazine | Thioridazine, mesoridazine |
| 2 | Dextropropoxyphene, naproxyphene, desmethyl-propoxyphene, paracetamol, diphenhydramine | Propoxyphene, naproxyphene, paracetamol, diphenhydramine |
| 3 | Pethidine, dothiepin | Pethidine, norpethidine |
| 4 | Codeine, dothiepin, paracetamol | Codeine, dothiepin, paracetamol |
| 5 | Amitriptyline, procyclidine, diazepam, nordiazepam | Amitriptyline, procyclidine, diazepam, nordiazepam, nortriptyline |
| 6 | Carbamazepine, chlorpromazine | Chlorpromazine, zopiclone |
| 7 | Diphenhydramine, nefopam | Diphenhydramine, nefopam |
| 8 | Diazepam, chlordiazepoxide, procyclidine, nefopam | Diazepam, morphine, benzoylecgonine |
| 9 | Dothiepin, medazepam | Dothiepin, desmethyldothiepin |
| 10 | Chlordiazepoxide, dihydrocodeine, diazepam, nordiazepam, propoxyphene, phenelzine | Dihydrocodeine, venlafaxine |
| 11 | Procyclidine, chlorpromazine, trifluoperazine | Procyclidine, chlorpromazine, nefazodone |
| 12 | Codeine, dihydrocodeine | Codeine, dihydrocodeine, morphine |
| 13 | Dihydrocodeine, dicyclomine, chlorazepate | Dihydrocodeine |
| 14 | Chlorpromazine, dihydrocodeine | Chlorpromazine, dihydrocodeine |
| 15 | Pseudoephedrine, diphenhydramine | Pseudoephedrine |
| 16 | Diazepam, nordiazepam, dothiepin | Diazepam, nordiazepam, dothiepin |

SPME = solid-phase micro-extraction; GC = gas chromatography; MS = mass spectrometry; HPLC = high-performance liquid chromatography.

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