# Solid Phase Microextraction/Capillary GC Analysis of Drugs, Alcohols, and Organic Solvents in Biological Fluids

***Solid phase microextraction is a fast, solventless alternative to conventional sample extraction techniques. In SPME, analytes establish equilibria among the sample matrix, the headspace above the sample, and a polymer-coated fused silica fiber, then are desorbed from the fiber to a capillary GC column or HPLC column. Because analytes are concentrated on the fiber, and are rapidly delivered to the column, minimum detection limits are improved and resolution is maintained. In monitoring analytes in urine, whole blood, or blood fractions, SPME is simpler and faster, and produces cleaner extracts, than liquid-liquid or solid phase extractions. Depending on the analytes and matrix, sampling is most effective by immersing the fiber into the sample or by sampling the headspace. This bulletin summarizes a few of the procedures that have been reported in the literature.***

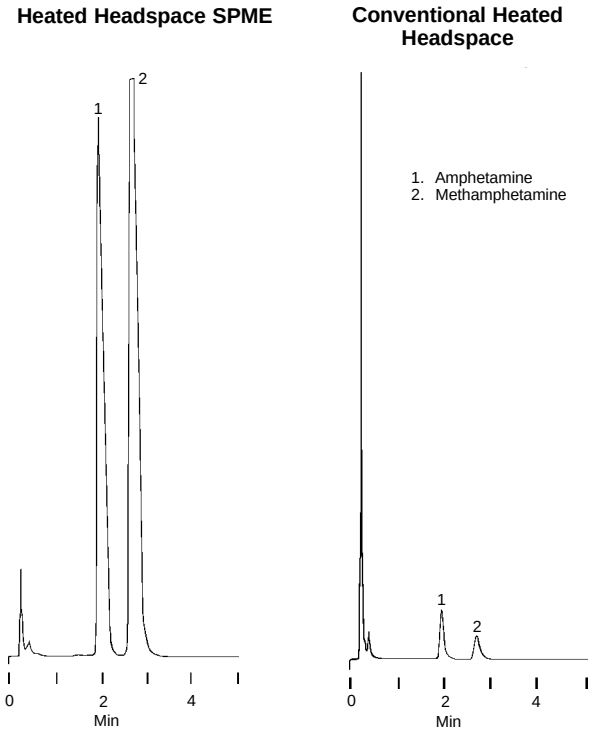
**Key Words:**
- drugs • drug abuse • amphetamines • blood alcohols
- cocaine • tricyclic antidepressants • anesthetics
- amitriptyline • chlorimipramine • imipramine
- trimipramine • lidocaine • procaine
- solid phase microextraction • SPME

In analyses of drugs in urine, blood, etc., sample preparation usually involves removing and concentrating the analytes of interest through liquid-liquid extraction, solid phase extraction, or other techniques. These methods have various drawbacks, including excessive preparation time and extravagant use of organic solvents. Solid phase microextraction (SPME)* eliminates most of these drawbacks. SPME is fast, requires no solvents or complicated apparatus, and provides linear results over wide concentrations of analytes (typically to parts per million/parts per billion levels). The technique can be used to monitor analytes in liquid samples or headspace, and can be used with any GC, GC-mass spectrometer, or HPLC system.

In SPME, equilibria are established among the concentrations of an analyte in a sample, in the headspace above the sample, and in the polymer coating on a fused silica fiber. The amount of analyte adsorbed by the fiber depends on the thickness of the stationary phase coating on the fiber and the distribution constant for the analyte, which generally increases with increasing molecular weight and boiling point of the analyte. Extraction time is determined by the time required to obtain precise extractions for the analyte with the largest distribution constant. Volatile compounds require a thick polymer coat; a thin coat is most effective for adsorbing/desorbing semivolatile analytes. Analyte recovery also is improved, or selectivity altered in favor of more volatile or less volatile compounds, by agitating or adding salt to the sample, changing the pH or temperature, or sampling the headspace rather than the sample – or *vice versa* (see *Optimizing SPME: Parameters to Control to Ensure Consistent Results* on page 6 of this bulletin).

## Figure A. Methamphetamine and Amphetamine in Urine

| | |
|---|---|
| Sample: | 1mL urine (100µg each analyte, 5µg $d_5$-methamphetamine, 0.7g $K_2CO_3$) in 12mL vial |
| SPME Fiber: | **100µm polydimethylsiloxane** |
| Cat. No.: | **57300-U** (manual sampling) |
| Extraction: | headspace, 80°C, 5 min (sample incubated 20 min) |
| Desorption: | 3 min, 250°C |
| Column: | polydimethylsiloxane, 15m x 0.53mm ID, 2.0µm film |
| Oven: | 110°C |
| Carrier: | nitrogen, 25mL/min |
| Det.: | FID, 250°C |
| Inj.: | splitless, 250°C |




795-0595, 0596

Figure provided by M. Yashiki, T. Kojima, T. Miyazaki, N. Nagasawa, and Y. Iwasaki, Dept. Legal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan and K. Hara, Dept. Legal Medicine, Fukuoka University School of Medicine, Fukuoka, Japan.

*Technology licensed exclusively to Supelco. US patent #5,691,206; European patent #0523092.

Full equilibration is not necessary for accurate and precise results by SPME, but consistent sampling time and other sampling parameters are essential. It also is important to keep consistent the vial size, the sample volume and, when using the immersion technique, the depth the fiber is immersed into the sample. Because the immersion and headspace sampling methods differ in kinetics, both approaches should be evaluated to determine which is best suited to a particular combination of analytes and matrix. Typically, for a given sampling time, immersion SPME is more sensitive than headspace SPME for analytes predominantly present in the liquid, and the reverse is true for analytes that are primarily in the headspace. Equilibrium is attained more rapidly in headspace SPME than in immersion SPME, because there is no liquid to hinder diffusion of the analytes onto the fiber. Headspace extracts from biological fluids usually exhibit lower background than extracts obtained by fiber immersion.

## Fast, Accurate Detection of Amphetamines in Urine

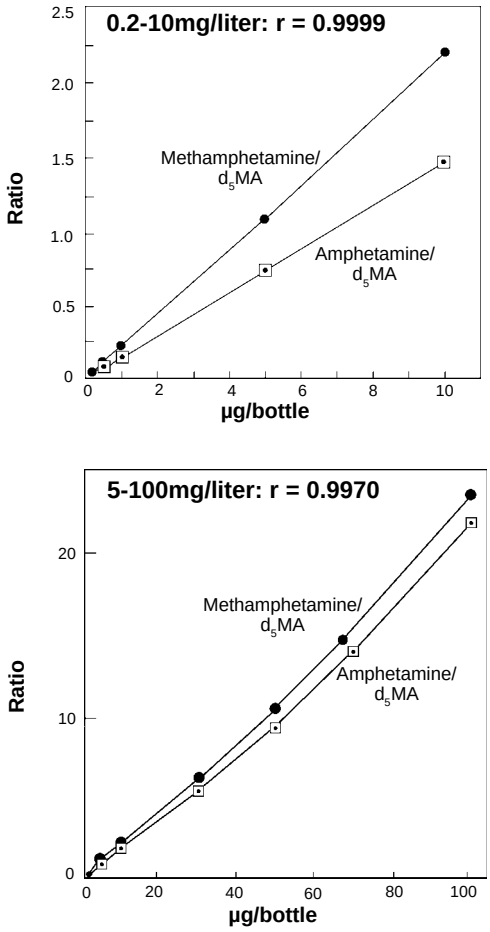
Staff members of the Department of Legal Medicine, Hiroshima University School of Medicine and the Department of Legal Medicine, Fukuoka University School of Medicine have developed an accurate, simple, and rapid method for analyzing urine for methamphetamine and its principal metabolite, amphetamine, using heated headspace SPME and capillary GC (1). The investigators seal a 1mL urine sample in a 12mL vial, add internal standard (5µg pentadeuterated methamphetamine, prepared according to reference 2) and 0.7g potassium carbonate, and heat the sample at 80°C for 20 minutes on a block heater. They then expose an SPME fiber coated with a 100µm film of polydimethylsiloxane to the headspace above the sample for 5 minutes, then introduce the fiber into the injection port of the chromatograph. In a system equipped with mass spectrometry/chemical ionization selected ion monitoring (GC-MS/CI-SIM), this analysis was 20 times as sensitive as a method incorporating conventional headspace extraction (Figure A). Correlation coefficients for methamphetamine and amphetamine, based on $d_5$-methamphetamine, were 0.9999 for concentrations of 0.2–10mg/liter and 0.9970 for concentrations of 5–100mg/liter (Figure B). Coefficients of variation for amphetamine and methamphetamine at 5mg/liter in urine were 7.0% and 5.1%, respectively.

The investigators concluded that in addition to speed, simplicity, and accuracy, the headspace SPME method could, under some circumstances, reduce the potential for interference by co-administered drugs. In an immunoassay for methamphetamine and amphetamine, chlorpromazine and its metabolites can cause false positive results (3), but these compounds did not affect analyses in which headspace SPME was used.

Subsequently, these authors developed a similar procedure for monitoring amphetamines in blood (N. Nagasawa, M. Yashiki, Y. Iwasaki, K. Hara, and T. Kojima, *Rapid Analysis of Amphetamines in Blood Using Head Space-Solid Phase Microextraction and Selected Ion Monitoring* in *Forensic Science International* 78 (2), 1996). In place of potassium carbonate, 0.5mL 1N sodium hydroxide is used to drive the analytes into the headspace (see *Optimizing SPME: Parameters to Control to Ensure Consistent Results).*

### Figure B. Linear Extraction of Methamphetamine and Amphetamine

| | |
|---|---|
| Sample: | 1mL urine (100µg each analyte, 5µg $d_5$-methamphetamine, 0.7g $K_2CO_3$)in 12mL vial |
| SPME Fiber: | **100µm polydimethylsiloxane** |
| Cat. No.: | **57300-U** (manual sampling) |
| Extraction: | headspace, 80°C, 5 min (sample incubated 20 min) |
| Desorption: | 3 min, 250°C |
| Column: | polydimethylsiloxane, 15m x 0.53mm ID, 2.0µm film |
| Oven: | 110°C |
| Carrier: | nitrogen, 25mL/min |
| Det.: | FID, 250°C |
| Inj.: | splitless, 250°C |




795-0597, 0598

Figure provided by M. Yashiki, T. Kojima, T. Miyazaki, N. Nagasawa, and Y. Iwasaki, Dept. Legal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan and K. Hara, Dept. Legal Medicine, Fukuoka University School of Medicine, Fukuoka, Japan

Investigators for the New Jersey State Police Laboratory likewise used SPME for rapid identification and quantification of amphetamines in urine. They extracted the drug from the headspace above the urine sample, exposed the drug adsorbed on the SPME fiber to the headspace above a derivatizing reagent, then introduced the derivatized drug into a capillary GC-MS system (Figure C). Derivatization enhances the sensitivity of the analysis; the headspace procedure eliminates extraneous urine components and introduces minimal residual derivatizing agent into the GC-MS system, minimizing interference with the analysis. Results are linear from 0.2µg to 100µg/mL. The method is under evaluation for detecting other drugs of abuse – cocaine and MDA/MDMA.

## Figure C. Amphetamines in Urine (Case Sample)

| | |
|---|---|
| Sample: | 1mL urine + 0.7g $K_2CO_3$ in 20mL headspace vial, equilibrated at 80°C 30 min |
| SPME Fiber: | **100µm polydimethylsiloxane** |
| Cat. No.: | **57300-U** (manual sampling) |
| Extraction: | headspace, 3-5 min, 80°C |
| Derivatization: | methyl bis-trifluoroacetamide (headspace, 0.5 min, ambient) |
| Desorption: | 270°C, 1 min |
| Column: | methylsiloxane, 12.5m x 0.2mm ID, .033µm film |
| Oven: | 60°C (1 min) to 140°C (4 min) at 30°C/min, then to 276°C at 20°C/min, 4min |
| Det.: | MS, full scan |
| Inj.: | splitless (closed 1 min), 270°C |

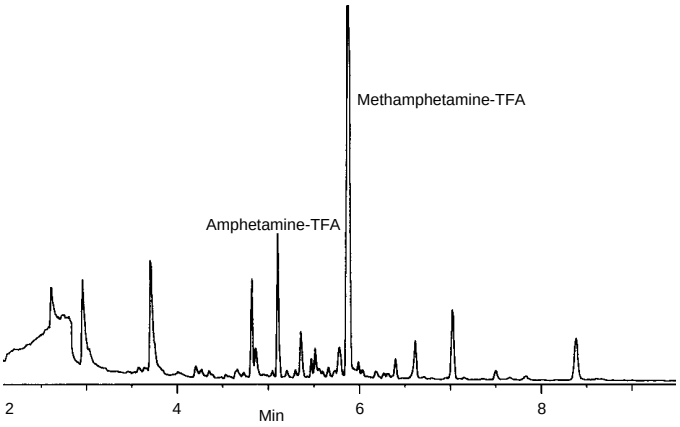
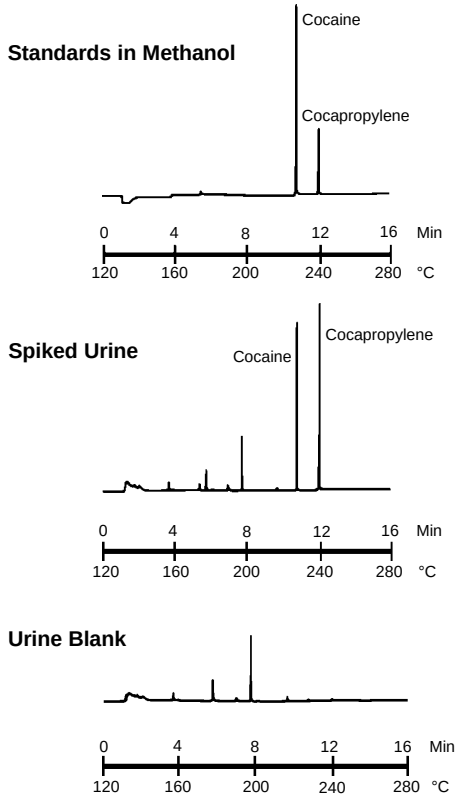



Figure provided by Thomas Brettell, New Jersey State Police Laboratory, PO Box 7068, West Trenton, NJ 08628, USA.

G000651

## Figure D. Cocaine in Urine

| | |
|---|---|
| Sample: | 0.5mL urine (250ng each analyte, 20µL 2.5% NaF) in 1mL vial |
| SPME Fiber: | **100µm polydimethylsiloxane** |
| Cat. No.: | **57300-U** (manual sampling) |
| Extraction: | immersion, 30 min |
| Desorption: | 3 min, 240°C |
| Column: | polydimethylsiloxane, 30m x 0.32mm ID, 0.25µm film |
| Oven: | 120°C to 280°C at 10°C/min |
| Carrier: | helium, 3mL/min |
| Det.: | NPD, 280°C |
| Inj.: | splitless (splitter opened after 1 min), 240°C |




796-0151, 0156, 0157

Figure provided by T. Kumazawa and K. Sato, Dept. Legal Medicine, Showa University School of Medicine, Tokyo, Japan and K. Watanabe, H. Seno, A. Ishii, and O. Suzuki, Dept. Legal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan.

Used with permission of Japanese Journal of Forensic Toxicology.

## Detecting Cocaine in Urine: An Immersion SPME Method

Investigators from the Department of Legal Medicine, Showa University School of Medicine and the Department of Legal Medicine, Hamamatsu University School of Medicine developed a method for detecting cocaine in urine, by combining SPME with capillary GC on a polydimethylsiloxane column (30m x 0.32mm ID, 0.25µm film) and a nitrogen-phosphorus detector (4). Because headspace extraction gives poor recovery values for cocaine, the analysts immerse a 100µm polydimethylsiloxane SPME fiber into the samples.

To demonstrate their technique, the analysts add 0.5mL urine plus 250ng cocaine and 250ng internal standard (cocapropylene, prepared according to reference 5) to a 1mL vial containing 20µL of 2.5% sodium fluoride solution and a small magnetic stirring bar. They insert the needle on the SPME device through the septum sealing the vial, immerse the SPME fiber in the sample for 30 minutes, then expose the fiber in the heated injection port for 3 minutes to ensure complete desorption of the extracted analyte.

Figure D shows chromatograms for cocaine and the internal standard in methanol (direct injection, 20ng each on-column), the drug and internal standard as extracted from spiked urine, and an extract from a urine blank. The extracts are very clean – urine background does not interfere with the analysis. Recovery values for cocaine and the internal standard, determined by comparing peak areas for the extracts to those for the methanol solution, were 20% and 30%, respectively. Extractions were linear from 30ng–250ng/0.5mL urine; the detection limit for cocaine was approximately 6ng/0.5mL urine.

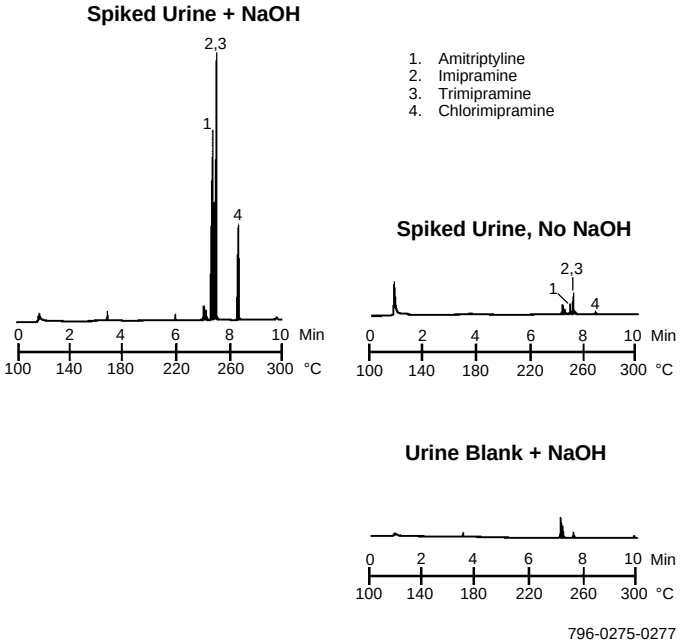
## Tricyclic Antidepressants in Urine

Staff members of the departments of legal medicine at Showa University School of Medicine and Hamamatsu University School of Medicine also have developed a method for extracting tricyclic antidepressants from urine, using headspace SPME and capillary GC (6). A 100µm polydimethylsiloxane SPME fiber is exposed to the headspace above the urine sample, and the adsorbed analytes are introduced onto a polydimethylsiloxane capillary column (30m x 0.32mm ID, 0.25µm film).

To a 7.5mL vial containing a small magnetic stirring bar, the investigators added 1mL urine plus 1µg each antidepressant (amitriptyline, chlorimipramine, imipramine, trimipramine) and 50µL of 5M sodium hydroxide. They heated the sealed vial at 100°C on a heating/stirring device. After 30 minutes, they exposed the fiber to the headspace above the sample for 15 minutes, then exposed the fiber in the heated injection port for 3 minutes.

## Figure E. Tricyclic Antidepressants in Urine

| | |
|---|---|
| Sample: | 1mL urine (1µg each analyte ± 50µL 5M NaOH) in 7.5mL vial |
| SPME Fiber: | **100µm polydimethylsiloxane** |
| Cat. No.: | **57300-U** (manual sampling) |
| Extraction: | headspace, 15 min, 100°C (sample incubated 30 min) |
| Desorption: | 3 min, 280°C |
| Column: | polydimethylsiloxane, 30m x 0.32mm ID, 0.25µm film |
| Oven: | 100°C to 300°C at 20°C/min |
| Carrier: | helium, 3mL/min |
| Det.: | FID, 280°C |
| Inj.: | splitless (splitter opened after 1 min), 280°C |




796-0275-0277

Figure provided by T. Kumazawa, X.-P. Lee, M.-C. Tsai, and K. Sato, Dept. Legal Medicine, Showa University School of Medicine, Tokyo, Japan and H. Seno and A. Ishii, Dept. Legal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan.

Used with permission of Japanese Journal of Forensic Toxicology.

Figure E shows chromatograms for the antidepressants as extracted from the headspace above spiked urine to which 50µL of 5M NaOH was added and as extracted from above spiked urine without NaOH, and an extract from above an NaOH-treated urine blank. At neutral pH, drug recovery was poor, and base was needed to increase the concentrations of analytes in the headspace. 5M NaOH did not cause other urine components to interfere with the analysis, but inclusion of 0.5g sodium chloride or potassium carbonate in addition to NaOH, in attempts to further increase drug recovery (see *Optimizing SPME: Parameters to Control to Ensure Consistent Results),* produced numerous additional peaks which coeluted with imipramine and trimipramine. Corrected values for drug recovery in the presence of NaOH were 10–20% (not as reported in reference 6). Extractions were linear from 0.05–2µg/mL urine, and the detection limit for each drug was 24–38ng/mL urine.

Relative to conventional headspace methods, the authors feel the SPME/GC analysis offers much higher sensitivity. Relative to liquid-liquid extractions and conventional solid phase extractions, SPME is simpler and faster, and produces much cleaner extracts. They concluded that their technique could be applied to monitoring other drugs and poisons of middle-sized molecular weight.

**NOTE:** In developing this technique, the analysts extracted four additional tricyclic antidepressants (carpipramine, clocapramine, desipramine, lofepramine) from urine samples, but these compounds decomposed in the GC and were eluted as multiple peaks. Such heat-sensitive analytes should be analyzed by combining the headspace SPME extraction with HPLC analysis. Our SPME/HPLC interface makes this combination possible. For information about the interface request Product Specification 496049.

## Local Anesthetics in Blood

In a third collaboration, investigators from Showa University School of Medicine and Hamamatsu University School of Medicine used headspace SPME in combination with capillary GC to extract ten local anesthetics from whole blood (7). Analytes adsorbed to a 100µm polydimethylsiloxane SPME fiber are introduced onto a polydimethylsiloxane capillary column (30m x 0.32mm ID, 0.25µm film).

The investigators added 1mL of 1M perchloric acid to a 1mL sample of whole blood containing 5µg each of 10 local anesthetics (benoxinate, bupivacaine, *p*-(butylamino)benzoic acid-2-(diethylamino)ethyl ester, dibucaine, ethyl aminobenzoate, lidocaine, mepivacaine, prilocaine, procaine, tetracaine). After 3 minutes vigorous agitation with a vortex mixer, they centrifuged the sample at 3000rpm for 5 minutes, transferred the clear supernatant to a 7.5mL vial containing a small magnetic stirring bar, then added 100µL of 10M sodium hydroxide and 0.5g of ammonium sulfate. After heating the sealed vial at 100°C for 15 minutes, they exposed the fiber to the headspace above the sample for 40 minutes. Subsequently they exposed the fiber in the heated injection port for 3 minutes.

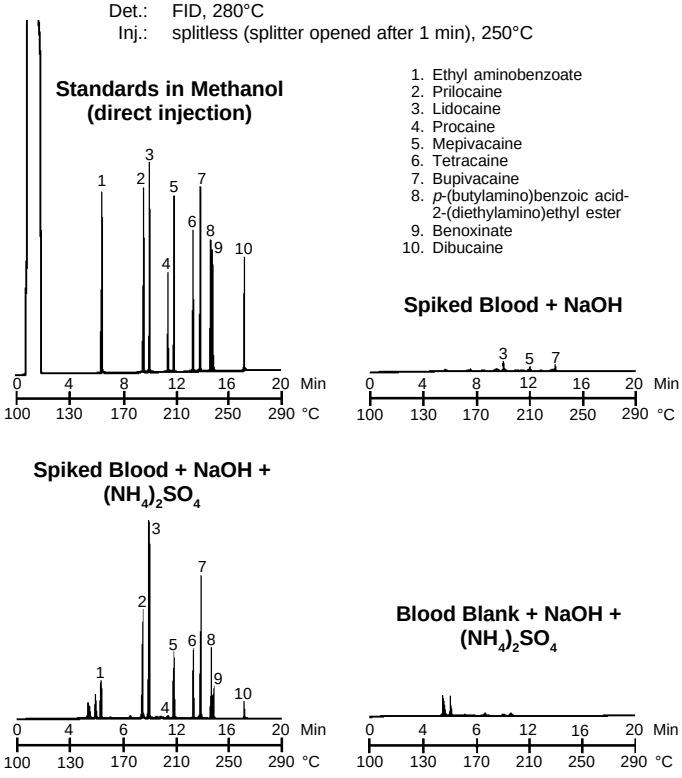
Figure F shows chromatograms for the drugs in methanol (direct injection, 50ng each on-column), as extracted from the headspace above spiked blood with 10M NaOH added, and as extracted from above spiked blood containing 10M NaOH and 0.5g $(NH_4)_2SO_4$, and a headspace extract from a blood blank. NaOH plus $(NH_4)_2SO_4$ gave the best combination of high drug recovery and low background. Various other combinations of NaOH and salt, including sodium chloride and potassium carbonate, provided lower drug recover or more interfering peaks as background.

Under the best conditions (addition of 10M NaOH plus $(NH_4)_2SO_4$), drug recovery was only 0.37% (procaine) to 11%. Nevertheless, the authors had no difficulty in quantifying their results. Extractions for these compounds, excluding procaine, were linear from 0.5–12µg/mL or 1–12µg/mL blood. Detection limits were 60–250ng/mL blood (bupivacaine, lidocaine, mepivacaine, prilocaine, tetracaine) or 250–830ng/mL blood (benoxinate, *p*-(butylamino)benzoic acid-2-(diethylamino)ethyl ester, dibucaine, ethyl aminobenzoate). With a detection limit of >2500ng/mL blood, procaine was the sole problem compound. Coefficients of variation for the ten drugs were good: 7-19% (within-day measurements) or 7-23% (day-to-day measurements). Again, relative to liquid-liquid extractions and conventional solid phase extractions, the authors stated that SPME is simpler and faster, and produces much cleaner extracts. They concluded that this technique can be used in forensic toxicology, for screening samples for local anesthetics before conducting a GC or GC/MS analysis.

Under these conditions, these investigators were able to use a single SPME fiber more than 50 times. Contaminated fibers were cleaned by thermal desorption in the GC injection port (280°C for 1-2 hours).

## Figure F. Local Anesthetics in Blood

| | |
|---|---|
| Sample: | deproteinized supernatant from (1mL blood + 1 mL 1M perchloric acid + 5µg each analyte + 100µL 10M NaOH ± 0.5g $(NH_4)_2SO_4$) in 7.5mL vial |
| SPME Fiber: | **100µm polydimethylsiloxane** |
| Cat. No.: | **57300-U** (manual sampling) |
| Extraction: | headspace, 40 min, 100°C (sample incubated 15 min) |
| Desorption: | 3 min, 250°C |
| Column: | polydimethylsiloxane, 30m x 0.32mm ID, 0.25µm film |
| Oven: | 100°C (1 min) to 290°C at 10°C/min |
| Carrier: | helium, 3mL/min |
| Det.: | FID, 280°C |
| Inj.: | splitless (splitter opened after 1 min), 250°C |




796-0278-0281

Figure provided by T. Kumazawa, X.-P. Lee, and K. Sato, Dept. Legal Medicine, Showa University School of Medicine, Tokyo, Japan and H. Seno, A. Ishii, and O. Suzuki, Dept. Legal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan.

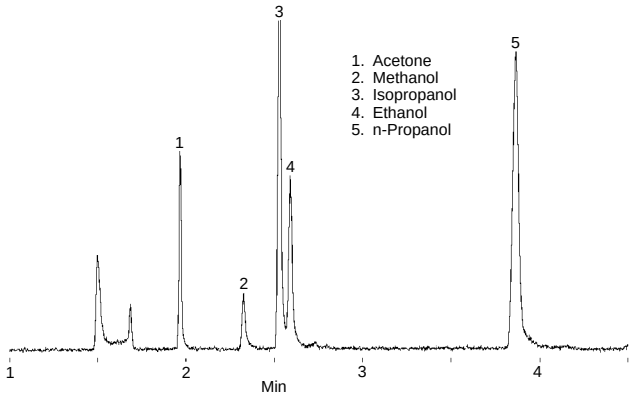
Used with permission of Japanese Journal of Forensic Toxicology.

# Alcohols in Blood

By exposing a 65µm Carbowax®/divinylbenzene SPME fiber to the headspace above whole blood, then delivering analytes adsorbed by the fiber onto a SUPELCOWAX™ 10 capillary GC column (30m x 0.20mm ID, 0.20µm film), investigators for the Office of the Chief Medical Examiner of North Carolina effectively monitor typical blood alcohols (Figure G). They quantified ethanol in the range of 50-300mg/dL with a correlation coefficient of 1.00. The coefficient of variance for 100mg/dL blood was 2.2%. Because the samples were highly concentrated, and extraction efficiency was high, carrier gas containing desorbed analytes was split to prevent column overload (8).

## Figure G. Alcohols in Blood

| | |
|---|---|
| Sample: | 100µL blood (200mg/dL each alcohol, 100mg/dL acetone) saturated with NaCl, in 0.3mL vial |
| SPME Fiber: | **65µm Carbowax/divinylbenzene** |
| Cat. No.: | **57312** (manual sampling) |
| Extraction: | headspace, 4 min, 60°C |
| Desorption: | 30 sec, 250°C |
| Column: | **SUPELCOWAX 10, 30m x 0.20mm ID, 0.20µm film** |
| Cat. No.: | **24169** |
| Oven: | 55°C |
| Carrier: | helium, 1mL/min |
| Det.: | MS (m/z = 30-70) |
| Inj.: | split (15:1), 250°C |




795-0435

Figure provided by M. Butler, Office of the Chief Medical Examiner, Chapel Hill, NC, 27555 USA.

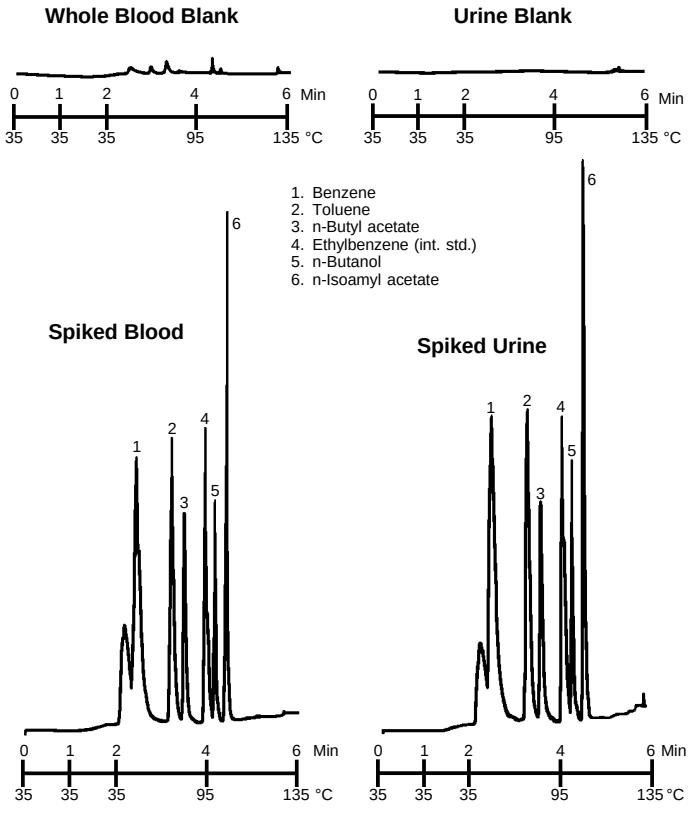
## Thinner Solvents in Body Fluids

Investigators from Showa University School of Medicine extracted five thinner compounds (benzene, toluene, n-butyl acetate, n-butanol, n-isoamyl acetate) from whole blood and urine, using headspace SPME/capillary GC (9). Analytes adsorbed by a 100µm polydimethylsiloxane SPME fiber were introduced onto a CARBOWAX-type capillary column (30m x 0.32mm ID, 0.25µm film).

Stock solutions of the five solvents (20µg/mL) and the internal standard (ethylbenzene, 2µg/mL) were prepared in methanol. To a 0.5mL sample of whole blood or urine in a 7.5mL vial containing a small magnetic stirring bar were added 5µL of each solvent stock solution (100ng solvent) and 5µL of internal standard stock solution (10ng int. std.), plus 1.5mL distilled water. The sealed vial was heated at 80°C for 15 minutes, then the SPME fiber was exposed to the headspace above the sample for 5 minutes. The fiber was exposed in the heated injection port for 3 minutes to ensure complete desorption of the analytes.

### Figure H. Solvents in Blood and Urine

| | |
|---|---|
| Sample: | 0.5mL blood or urine (100ng each analyte, 10ng int. std.) + 1.5mL water in 7.5mL vial |
| SPME Fiber: | **100µm polydimethylsiloxane** |
| Cat. No.: | **57300-U** (manual sampling) |
| Extraction: | headspace, 5 min, 100°C (sample incubated 15 min) |
| Desorption: | 3 min, 200°C |
| Column: | bonded poly(ethylene glycol), 30m x 0.32mm ID, 0.25µm film |
| Oven: | 35°C (1 min) to 230°C at 20°C/min |
| Carrier: | helium, 4mL/min |
| Det.: | FID |
| Inj.: | splitless (splitter opened after 1 min), 200°C |




796-0282-0285

Figure provided by X.-P. Lee, T. Kumazawa, and K. Sato, Dept. Legal Medicine, Showa University School of Medicine, Tokyo, Japan.

Used with permission of International Journal of Legal Medicine.

Figure H shows chromatograms for the solvents, as extracted from the headspace above spiked blood and spiked urine. Background in whole blood and urine was minor, and did not interfere with the chromatography.

Solvent recovery from blood or urine, determined by comparing peak areas for the extracts to those for the corresponding methanol solution, ranged from 50% to 70%. Extractions were linear from 2–100ng/0.5mL blood or urine. The detection limit for each compound was approximately 1.1–2.4ng/0.5mL blood or urine.

The method is simple and fast, and produces very clean extracts. Relative to conventional headspace methods, the authors feel the SPME/GC analysis offers much higher sensitivity. They concluded that the technique could be applied to monitoring other organic solvents of forensic interest. They could not extract ethanol or methanol with a 100µm polydimethylsiloxane-coated SPME fiber, but a 65µm Carbowax/divinylbenzene-coated fiber will extract these compounds (see Figure F and *Alcohols in Blood).*

## Optimizing SPME: Parameters to Control to Ensure Consistent Results

As these examples indicate, analysts using solid phase microextraction routinely obtain consistent results and reliable detection of low concentrations of analytes. The polarity and thickness of the coating on the fiber, sample agitation, the sampling method (fiber immersion or headspace sampling), the pH, salt content, and volume of the sample, and other factors affect results from SPME. It is important to remember that in SPME neither complete extraction of analytes nor full equilibrium are necessary, but consistent sampling time, temperature, fiber immersion depth, and headspace volume are crucial to reproducibility.

*Fiber Polarity* Because the SPME fiber is only 1cm long, the coating on the fiber must be either nonpolar or strongly polar in nature. In chromatography, small changes in the chemical nature of the stationary phase are useful (a 5% diphenylsiloxane/95% dimethylsiloxane phase versus a 100% dimethylsiloxane phase, for example), but such small differences will not produce appreciable selectivity differences in SPME. What *is* beneficial, however, is the incorporation of an adsorbent material in the coating. In the Carbowax/divinylbenzene fiber, the divinylbenzene polymer increases the available surface area, thus improving extractions of small polar molecules such as alcohols and amines (see Figure F). The potential value of including other adsorbents, carbons, and silica in SPME coatings is under review at Supelco.

*Fiber Coating Thickness* Diffusion of an analyte from the sample matrix or headspace into the coating on the fiber is proportional to the thickness of the coating. A thicker film retains volatile compounds and transfers them to the GC injection port without loss. For higher boiling compounds, a thin film ensures fast diffusion and release of the analyte during thermal desorption. A thick film will effectively remove high boiling compounds from the sample matrix, but the desorption rate will be prolonged, and analytes could be carried over to the next extraction.

*Sample Agitation* Sample agitation enhances extraction and reduces extraction time, especially for higher molecular weight analytes with high diffusion coefficients. Inconsistent stirring causes poor precision and is worse than no stirring. Sonication promotes analyte adsorption, but can heat the sample. This might be detrimental to some heat-sensitive analytes or, alternatively, could be useful for vaporizing analytes for headspace extraction.

## Table 1. Sampling Method and Salt Affect SPME

| | Headspace | | Immersion | |
|---|---|---|---|---|
| | Salt* | No Salt | Salt* | No Salt |
| Benzene | 5400 | 4200 | 17500 | 4300 |
| Bromodichloromethane | 2500 | 1100 | 4600 | 900 |
| Bromoform | 3600 | 1200 | 3600 | 1000 |
| Carbon tetrachloride | 2100 | 4200 | 7800 | 6200 |
| Chloroethane | 0 | 0 | 600 | 0 |
| 2-Chloroethylvinyl ether | 300 | 0 | 1100 | 0 |
| Chloroform | 1500 | 1000 | 1900 | 1000 |
| 1,3-Dichlorobenzene | 41100 | 39200 | 15300 | 15700 |
| 1,1-Dichloroethene | 200 | 500 | 2900 | 900 |
| 1,2-Dichloropropane | 1900 | 800 | 3800 | 900 |
| cis-1,3-Dichloropropene | 4200 | 1400 | 7100 | 1600 |
| Ethylbenzene | 39900 | 51500 | 34000 | 31500 |
| Methylene chloride | 600 | 200 | 1600 | 200 |
| 1,1,2,2-Tetrachloroethane | 10400 | 2300 | 8000 | 2000 |
| Tetrachloroethene | 4800 | 9800 | 6300 | 6600 |
| Toluene | 15500 | 14500 | 26800 | 13800 |
| Trichloroethene | 2200 | 2600 | 5700 | 2800 |
| Trichlorofluoromethane | 400 | 1100 | 7200 | 4300 |
| Vinyl chloride | 0 | >0 | 1200 | <100 |

*Sodium chloride, saturated solution. Analytes listed in US EPA Method 624.
Values = area counts (to nearest 100).

*Immersion versus Headspace Sampling; Effects of Salt and pH* Analytes that exhibit a vapor pressure can be extracted by immersing the fiber into the sample, or by sampling the headspace above the sample. Analytes that exhibit no vapor pressure must be extracted by immersion. Adding salt to the sample or changing the pH prior to extraction can increase the ionic strength of the solution and, in turn, reduce the solubility of some analytes. Conversely, salt can increase the solubility of other analytes – note the differing effects on the volatile compounds in Table 1. Consequently, the effects of adding salt to a sample should be evaluated for each specific combination of analytes and sample matrix. Changing the pH can minimize solubility; acidic and basic compounds are more effectively extracted at acidic and basic pH, respectively. A combination of salt and pH modification often enhances the extraction of analytes from the headspace (see *Local Anesthetics in Blood).* Equilibration is faster in headspace sampling, because in immersion sampling the analyte molecules must penetrate a static layer of water molecules surrounding the fiber. For higher sensitivity from headspace SPME, the sample headspace should be as small as is practical. A detailed theoretical discussion of headspace SPME is presented in reference 10.

*Other Factors* Less subject to control, but influencing the extraction, is analyte concentration. At low concentrations, such as 50ppb or less for the volatile compounds in Table 1, changes in sample volume do not affect response, because equilibrium is concentration-dependent. At higher concentrations, changes in sample volume become significant. With a large sample (>5mL) containing a high concentration of analyte, the amount of analyte removed from the sample is not sufficient to change the concentration. Therefore, response throughout the calibration curve is exponential, not linear, especially for compounds with high distribution constants. Responses may be linear for low concentrations. Because analyte concentration often is not known, it is best to keep sample volumes between 1mL and 5mL, and always use the same volume for samples and calibration standards. If you anticipate extracting the analytes by using an immersion sampling technique, minimize the headspace in the sample vial.

Finally, the desorption parameters – injection port temperature, depth of fiber insertion in the injection port, desorption time – also must be optimized for the analytes involved. Once established, these values should be used consistently. Desorption of an analyte from the SPME fiber depends on the boiling point of the analyte, the thickness of the coating on the fiber, and the temperature of the injection port. Some analytes can take up to 30 seconds to desorb, and cryogenic cooling might be required to focus these compounds at the inlet of a capillary GC column. Use of an inlet liner with a narrow internal diameter (e.g., 0.75-1mm) generally provides sharp peaks and can eliminate the need for cooling. As with any other extraction/concentration technique, it is best to use multiple internal standards in SPME methods, and to treat the standards and the analytes identically.

**Acknowledgments**

The procedure for detecting amphetamines in urine was developed by Mikio Yashiki, Tohru Kojima, Tetsuji Miyazaki, Nobuyuki Nagasawa, and Yasumasa Iwasaki, Department of Legal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan and Kenji Hara, Department of Legal Medicine, Fukuoka University School of Medicine, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-01, Japan.

The procedure for detecting cocaine in urine was developed by Takeshi Kumazawa and Keizo Sato, Department of Legal Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan and Kanako Watanabe, Hiroshi Seno, Akira Ishii, and Osamu Suzuki, Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan.

The procedure for detecting tricyclic antidepressants in urine was developed by Takeshi Kumazawa, Xiao-Pen Lee, Miin-Chang Tsai, and Keizo Sato, Showa University School of Medicine and Hiroshi Seno and Akira Ishii, Hamamatsu University School of Medicine.

The procedure for detecting local anesthetics in blood was developed by Takeshi Kumazawa, Xiao-Pen Lee, and Keizo Sato, Showa University School of Medicine and Hiroshi Seno, Akira Ishii, and Osamu Suzuki, Hamamatsu University School of Medicine.

The procedure for detecting thinner solvents in blood and urine was developed by Xiao-Pen Lee, Takeshi Kumazawa, and Keizo Sato, Showa University School of Medicine.

Figure F was provided by Michael Butler, Office of the Chief Medical Examiner, Chapel Hill, North Carolina, 27555 USA.

**References**

1. Yashiki, M., T. Kojima, T. Miyazaki, N. Nagasawa, Y. Iwasaki, and K. Hara, *Forensic Science International* **76:** 169-177 (1995).
2. Hara, K., T. Nagata, and K. Kimura, *Z. Rechtsmed.,* **96:** 93-104 (1986).
3. Olsen, K.M., M. Gullikssen, and A.S. Christophersen, *Clin. Chem.,* **38:** 611-612 (1992).
4. Kumazawa, T. K. Watanabe, K. Sato, H. Seno, A. Ishii, and O. Suzuki, *Japan. J. Forensic Toxicol.,* **13:** 207-210 (1995).
5. Roy, I.M., T.M. Jefferies, M.D. Threadgill, and G.H. Dewar, *J. Pharm. Biomed. Anal.,* **10:** 943-948 (1992).
6. Kumazawa, T., X.-P. Lee, M.-C. Tsai, H. Seno, A. Ishii, and K. Sato, *Japan. J. Forensic Toxicol.,* **13:** 25-30 (1995).
7. Kumazawa, T., X.-P. Lee, K. Sato, H. Seno, A. Ishii, and O. Suzuki, *Japan. J. Forensic Toxicol.,* **13:** 182-188 (1995).
8. Shirey, R., V. Mani, and M. Butler, *The Reporter* **14 (5):** 4-5, Supelco, Inc., Bellefonte, PA (1995).
9. Lee, X.-P., T. Kumazawa, and K. Sato, *Int. J. Legal Med.,* **107:** 310-313 (1995).
10. Zhang, Z. and J. Pawliszyn, *Anal. Chem.,* **65:** 1843-1852 (1993).

References 1-7, 9,10 not available from Supelco.

**For a listing of journal references on using SPME for toxicology determinations, request SPME Application Guide T199925.**

## Ordering Information:

| Description | Cat. No. |
|---|---|
| **SPME Holder*** | **26358,25** |
| For manual sampling | **57330-U** |
| For Varian 8100/8200 AutoSampler | **57331** |
| For Leap/CTC Combi-PAL Autosampler | **57347-U** |
| For HPLC | **57331** |
| **SPME/HPLC Interface** | |
| With Valco® Valve | **57350-U** |
| With Rheodyne® Valve | **57353** |
| **SPME Fiber Assemblies** (pk. of 3) | |
| For manual sampling | |
| 100µm polydimethylsiloxane | **57300-U** |
| 65µm Carbowax/divinylbenzene | **57312** |
| 70µm Carbowax/divinylbenzene, StableFlex◆ | **57336-U** |
| For HPLC or for GC autosamplers shown above | |
| 100µm polydimethylsiloxane | **57301** |
| 65µm Carbowax/divinylbenzene | **57313** |
| 70µm Carbowax/divinylbenzene, StableFlex◆ | **57338-U** |
| **SPME Sampling Stands** | |
| For consistent fiber immersion. | |
| Stand for 4mL vials (8 slots) | **57333-U** |
| Stand for 15mL vials (6 slots) | **57357-U** |
| Vial Puck for 15mL vials | **57358-U** |
| **SPME Inlet Guide** | |
| Supports manual holder during injection. | **57356-U** |
| **Heat/Stir Plates** | |
| 120VAC | **Z262129-1EA** |
| 230VAC | **Z262137** |
| **Magnetic Stirring Bars** | |
| 10 x 3mm, pk. of 3 | **Z118877-3EA** |
| **SPB™-1 Capillary Column** | |
| 30m x 0.32mm ID, 0.25µm film | **24044** |
| **SUPELCOWAX 10 Capillary Columns** | |
| 30m x 0.20mm ID, 0.20µm film | **24169** |
| 30m x 0.32mm ID, 0.25µm film | **24080-U** |

* Initially you must order both holder and fiber assembly. Holder is reusable indefinitely. Use with AutoSampler requires Varian SPME upgrade kit (available from Varian).

◆ StableFlex fiber is more flexible than original fibers, and is less likely to break during use.

**Trademarks**

ChromFax, SPB, StableFlex, SUPELCOWAX – Sigma-Aldrich Co.
Carbowax – Union Carbide Corp.
Hewlett-Packard – Hewlett-Packard Corp.
Perkin-Elmer – Perkin-Elmer Corp.
Rheodyne – Rheodyne, Inc.
Shimadzu – Shimadzu Corp.
Valco – Valco Instruments Co., Inc.

| Description | Cat. No. |
|---|---|
| **Inlet Liners for SPME** | |
| Increase sensitivity and sharpen peaks by reducing inlet volume. Replace a standard 2mm ID liner with this specially designed 0.75mm ID liner. | |
| **Hewlett-Packard®** (5880, 5890 series, 6890) | |
| Each | **26375,01** |
| pk. of 5 | **26375,05** |
| pk. of 25 | **26375,25** |
| **Varian 1075/1077 Injectors** | |
| Each | **26358,01** |
| pk. of 5 | **26358,05** |
| pk. of 25 | **26358,25** |
| **Varian 1078/1079 Splitless** | |
| Each | **26378,01** |
| pk. of 5 | **26378,05** |
| pk. of 25 | **26378,25** |
| **Varian 1093/1094 SPI Injectors** | |
| Each | **26364,01** |
| pk. of 5 | **26364,05** |
| pk. of 25 | **26364,25** |
| **Perkin-Elmer®** | |
| (Auto System Split/Splitless Injector) | |
| Each | **26312,01** |
| pk. of 5 | **26312,05** |
| pk. of 25 | **26312,25** |
| **Shimadzu® GC Models 9A/15A/16** | |
| (SPL-G9/15 Injector) | |
| Each | **26329,01** |
| pk. of 5 | **26329,05** |
| pk. of 25 | **26329,25** |
| **Shimadzu GC Models 14/15A/16** (SPL-14 Injector) | |
| Each | **26335,01** |
| pk. of 5 | **26335,05** |
| pk. of 25 | **26335,25** |
| **Shimadzu GC Models 17A** (SPL-17 Injector) | |
| Each | **26339,01** |
| pk. of 5 | **26339,05** |
| pk. of 25 | **26339,25** |
| **SPME Inserts** | |
| Flash On-Column, Varian SPME Injector | |
| pk. of 5 | **26364,05** |

Fused silica columns manufactured under HP US Pat. No. 4,293,415.



For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

**ARGENTINA** · Sigma-Aldrich de Argentina, S.A. · Buenos Aires 1119 **AUSTRALIA** · Sigma-Aldrich Pty. Ltd. · Castle Hill NSW 2154 **AUSTRIA** · Sigma-Aldrich Handels GmbH · A-1110 Wien
**BELGIUM** · Sigma-Aldrich N.V./S.A. · B-2880 Bornem **BRAZIL** · Sigma-Aldrich Quimica Brasil Ltda. · 01239-010 São Paulo, SP **CANADA** · Sigma-Aldrich Canada, Ltd. · 2149 Winston Park Dr., Oakville, ON L6H 6J8
**CZECH REPUBLIC** · Sigma-Aldrich s.r.o.· 186 00 Praha 8 **DENMARK** · Sigma-Aldrich Denmark A/S · DK-2665 Vallensbaek Strand **FINLAND** · Sigma-Aldrich Finland/YA-Kemia Oy · FIN-00700 Helsinki
**FRANCE** · Sigma-Aldrich Chimie · 38297 Saint-Quentin-Fallavier Cedex **GERMANY** · Sigma-Aldrich Chemie GmbH · D-82041 Deisenhofen **GREECE** · Sigma-Aldrich (o.m.) Ltd. · Ilioupoli 16346, Athens
**HUNGARY** · Sigma-Aldrich Kft. · H-1067 Budapest **INDIA** · Sigma-Aldrich Co. · Bangalore 560 048 **IRELAND** · Sigma-Aldrich Ireland Ltd. · Dublin 24 **ISRAEL** · Sigma Israel Chemicals Ltd. · Rehovot 76100
**ITALY** · Sigma-Aldrich s.r.l. · 20151 Milano **JAPAN** · Sigma-Aldrich Japan K.K. · Chuo-ku, Tokyo 103 **KOREA** · Sigma-Aldrich Korea · Seoul **MALAYSIA** · Sigma-Aldrich (M) Sdn. Bhd. · Selangor
**MEXICO** · Sigma-Aldrich Química S.A. de C.V. · 50200 Toluca **NETHERLANDS** · Sigma-Aldrich Chemie BV · 3330 AA Zwijndrecht **NORWAY** · Sigma-Aldrich Norway · Torshov · N-0401 Oslo
**POLAND** · Sigma-Aldrich Sp. z o.o. · 61-663 Poznañ **PORTUGAL**· Sigma-Aldrich Quimica, S.A. · Sintra 2710 **RUSSIA** · Sigma-Aldrich Russia · Moscow 103062 **SINGAPORE** · Sigma-Aldrich Pte. Ltd.
**SOUTH AFRICA** · Sigma-Aldrich (pty) Ltd. · Jet Park 1459 **SPAIN** · Sigma-Aldrich Quimica, S.A. · 28100 Alcobendas, Madrid **SWEDEN** · Sigma-Aldrich Sweden AB · 135 70 Stockholm
**SWITZERLAND** · Supelco · CH-9471 Buchs **UNITED KINGDOM** · Sigma-Aldrich Company Ltd. · Poole, Dorset BH12 4QH
**UNITED STATES** · Supelco · Supelco Park · Bellefonte, PA 16823-0048 · Phone 800-247-6628 or 814-359-3441 · Fax 800-447-3044 or 814-359-3044 · email:supelco@sial.com **H**