

Characterization of Volatiles Using Solid-Phase Microextraction / Gas Chromatography-Mass Spectrometry (SPME/GC-MS)

Two Customs Applications

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INTRODUCTION

The relatively new technique of SPME/GC-MS has been applied to a wide variety of analytical problems, including extraction and quantitation of drugs in biological matrices,¹ extraction of volatile organic compounds (VOCs) from water² and extraction of explosive residues from post blast soil samples.³ The SPME fiber can be thought of as a very short GC column turned inside out. An outer polymer coating absorbs volatiles, which are then desorbed in the hot GC inlet and chromatographed in the usual manner. These volatiles are subsequently identified using a mass spectrometer.

The attraction of SPME is that the extraction is fast and simple and can be done without solvents, and detection limits can reach parts per trillion (ppt) levels for certain compounds.⁴ SPME also has great potential field application: onsite sampling can be done even by nonscientists without the need to have a GC-MS at each location. When properly stored, samples can be analyzed days later in the lab without significant loss of volatiles.

This article serves as a brief introduction into the SPME/GC-MS technique. The emphasis will be on characterization of volatiles associated with cocaine and heroin materials used in the detector dog training program, but the technique can be just as easily used to answer other Customs-related questions such as quantitation of citronella oil in wax samples or identification of counterfeit perfumes. Examples of the latter will be given.

MATERIALS AND METHOD

Cocaine and Heroin Analysis

The following fibers, all available from Supelco (Bellefonte, PA), were tested for their ability to extract efficiently volatiles associated with cocaine and heroin training aid materials:

- 85 μm polyacrylate (PA)
- 75 μm Carboxen/PDMS (CAR)
- 65 μm PDMS/divinylbenzene (DVB)
- 65 μm Carbowax/DVB (CWDVB)
- 50/30 μm divinylbenzene/Carboxen/PDMS (CBXDVB)

Before it is used the first time, each fiber is conditioned until a clean chromatogram is obtained under normal run conditions. In addition, to minimize background signals, the fibers are heated in the GC inlet for 2 to 5 minutes before each headspace sampling. To eliminate carryover, the fibers are left in the inlet for the full length of a run, typically 20 to 30 minutes.

To avoid sampling particulate matters, powder samples are separated from the fiber by two layers of stainless steel fine mesh (20 and 25 μm) sieves (Fig. 1a). Experiments were done with either a standard 10-gram sample or the full stock bottle (Fig. 1b-c). The pan/sieve

assembly is wrapped in foil to minimize loss of volatiles during incubation.¹ The foil also helps minimize accidental environmental contamination. Sampling is done by puncturing the foil through one of the holes and exposing the fiber. Stainless steel was used instead of brass because the finish on brass caused unacceptable background signals. All pans and sieves were solvent cleaned, using sequentially, methanol, acetone, and chloroform, and dried in a 150°C oven for at least 12 hours before use.

Sampling and incubation times varied. Injections were done manually, and data were acquired on an HP 5890/5970 GC/MSD. After testing several temperature programs and columns, the following conditions were chosen:

Column: XTI-5 (Restek #12223), 30-m x 0.25 mm ID x 0.25µm df

Constant column head pressure: 10 psi, equivalent to 1.2 mL/min at 50°C

Inlet liner: 0.75 mm ID SPME Injection Sleeve for Hewlett Packard (Supelco #2,6375)

Inlet temperature: 260°C

MSD transfer line temperature: 300°C

Cocaine oven temperature program:

50°C for 3 min, ramp 5°C/min to 90°C, ramp 15°C/min to 150°C, ramp 30°C/min to 270°C

Heroin oven temperature program:

50°C for 1 min, ramp 5°C/min to 150°C, ramp 25°C/min to 275°C and hold 3.8 min

Injection mode: splitless with purge activation at 0.75 min

Solvent delay: 1 min

m/z Scan range: 40-440

MSD settings were at autotune values.

Counterfeit Perfume Analysis

Authentic and counterfeit Eau de Toilette samples of Giorgio and Chloé were sprayed into individual 10-mL Erlenmeyer flasks, which are then sealed with a Teflon-lined rubber cap. Sampling was done by puncturing the rubber seal and exposing the fiber to the headspace for 15 minutes at room temperature. Because of the high concentration of volatiles in these samples, no incubation was necessary. GC-MS conditions are the same as those used for heroin headspace analysis.

RESULTS AND DISCUSSION

Of the fibers that were tested, the one that worked best and that was used in all subsequent analyses is the dual-layer CBXDVB (Fig. 2). The outer DVB coating captures large molecules, while smaller, lighter volatiles diffuse through the DVB layer to be trapped by the inner Carboxen/PDMS layer. Thus, the dual-layer fiber can efficiently extract a greater range of analytes than other fibers, which is an important factor when analyzing unknowns such as the headspace components of different narcotic materials. Figure 3 compares the extraction efficiency of the CBXDVB (red curve) and PA (blue curve) fibers for a cocaine hydrochloride stock sample. The other three fibers that were used gave extraction efficiencies roughly between those of the CBXDVB and PA fibers. Similar results were also obtained for heroin.

Figure 4 compares the effect of incubation versus sampling time for a cocaine hydrochloride stock material (COC285). Two 10-g portions of COC285 were incubated and sampled using the setup in Fig. 1b. The total time from start to finish is the same for both samples, and that is 48 hours. However, the red curve came from a 24-h sampling, whereas the blue curve

¹ Incubation simply means to allow volatiles to build up and equilibrate in the headspace for a certain amount of time before sampling with the SPME fiber.

came from a 4-h sampling. The first thing to notice is that longer sampling time leads to a significant increase in the signals of later eluting materials such as higher alkyl benzoates (1a,b) and the cinnamates (2a,b). The inset shows, for example, that ethyl benzoate appears only as a shoulder in the 4-h sample, whereas it is a distinct signal that was automatically integrated and identified in the 24-h sample. This is not because there are more volatiles present in the headspace of one sample than the other since the total time is the same in both cases. Rather, it is because these larger molecules take longer to absorb into the polymer coating of the fiber, and a longer sampling time allows more interaction between the vapor phase and the polymer phase. While a long sampling time might be necessary to obtain a more complete headspace profile of cocaine, problems might be encountered as some signals get so large that they obscure smaller signals. The inset also reveals this problem: the menthol signal around 8.7 minutes is so large in the 24-hour sample that it obscured the smaller methyl phenyl acetate signal eluting immediately after it.

To determine whether incubation time could be reduced by using a larger quantity of drug, the following experiment was done (Fig. 5). Two chromatograms were generated from 4-hr headspace samplings of the same cocaine stock material (COC284). The top chromatogram came from a 1-kg sample that was incubated for only one hour prior to sampling. Only 10-g were used for the bottom chromatogram, but it was incubated for 44 hours prior to sampling. The integrated areas are about the same for benzaldehyde in both cases, possibly indicating fiber saturation for this one component. However, the percent of total integrated area for benzaldehyde is significantly less in the 10-g sample because longer incubation allowed greater build up in the headspace of less volatile compounds. These are subsequently sampled and contribute to the total area. It is not surprising, then, that a long incubation time gave a more complex headspace profile – the integrated area count is higher for almost all components in the 10-g sample.

In addition to characterizing stock materials used to prepare training materials, onsite headspace sampling of seizure samples can provide a better picture of the odor matrix that a drug-sniffing dog must face. Samples taken out in the field will generally not be analyzed until we get back to the lab, and so we have to be sure that the headspace profiles do not change in transit. The experiment in Figure 6 shows that samples can be stored sealed in a septum for at least 4 days without significant change in the headspace profile. A small amount of the smaller, more volatile compounds like acetic acid and propyl acetate is lost in storage, but key components like methyl and propyl benzoates remain dissolved in the fiber coating and come off only in the heated GC inlet. It is also important to make sure that the sealing septum does not add to the profile. In this example, even the plasticizer (DMP) signal remains the same between the sample analyzed immediately and those done 4 days later. A blow up of the smaller signals also show agreement among these three samples.

We have, in fact, used SPME to sample volatiles associated with a “Colombian” heroin seizure at Houston Intercontinental Airport. All four fibers were analyzed the next day at the Research Laboratory and gave reproducible detection of acetic acid, the key odor component of heroin (Fig. 7).

The importance of characterizing volatiles associated with narcotic materials comes from the Research Lab’s involvement with the detector dog training program. We prepare and maintain inventory control of all hard narcotic training aids and have worked with the Canine Enforcement Training Center to develop pseudococaine and pseudoheroin formulations for initial scent association training as well as proficiency training. SPME enables us to screen stock materials to minimize the risk of sending out training aids that contain some unusual odors such that a whole class of dogs get trained on the wrong scent and fail to alert when they encounter “typical” real samples. As more stock samples are analyzed, we will also be

able to determine odor components that are common for each type of drug, and this will enable us to refine the pseudo formulations to mimic more accurately real-world samples.

The last examples focus on samples that a field laboratory is more likely to encounter – identification of counterfeit perfumes. Figures 8 and 9 compares counterfeit and authentic samples of Giorgio and Chloé, respectively. The odor profile of the counterfeit Giorgio closely resembles that of the authentic sample, the difference occurring mainly in a lower concentration of the high-boiling components that would correspond to the “base note” of the perfume. The counterfeit Chloé is not only missing some base note components as compared to the authentic sample, but it also has some additional components that would give it a different wear characteristic from the authentic sample.

CONCLUSION

The examples given above illustrate some of the factors that need to be considered when doing SPME/GC-MS. Although most of our work to date has emphasized characterization of odors associated with detector dog training materials, the technique can just as easily be applied to any analysis involving volatiles (or semivolatiles). The references cited herein also show that SPME can be used to extract materials from a liquid medium, be it water or blood.² With appropriate use of an internal standard, SPME/GC-MS can give quantitative results as well.

REFERENCES

1. Namera, A., Watanabe, T., Yashiki, M., Iwasaki, Y., and Kojima, T., *J. Anal. Toxicology*, **1998**, 22, 396-400; a good survey of SPME toxicology applications can be also found in Supelco Technical Bulletin No. 901, *Solid Phase Microextraction/Capillary GC Analysis of Drugs, Alcohols, and Organic Solvents in Biological Fluids*.
2. Llompert, M.; Li, K. and Fingas, M., *Anal. Chem.* **1998**, 70, 2510-2515.
3. Pukkila, J., and Jäntti, S., *Curr. Top. Forensic Sci., Proc. Meet. Int. Assoc. Forensic Sci.*, 14th (1997), Meeting Date 1996, Volume 4, 214-217. (IAFS, Tokyo, Japan, August 1996.).
4. Shirey, R.E., Mani, V., and Mindrup, R.F., Supelco Technical Presentation No. 98-0070 (T498041).

² Care is needed when extracting from an organic solvent, as some solvents, such as chloroform, may dissolve the glue holding the fiber to the needle and/or may cause swelling of the polymer coating. We have done extraction of drug standards from a methanol solution without deleterious effects, and the retention times are the same as when doing a direct injection.

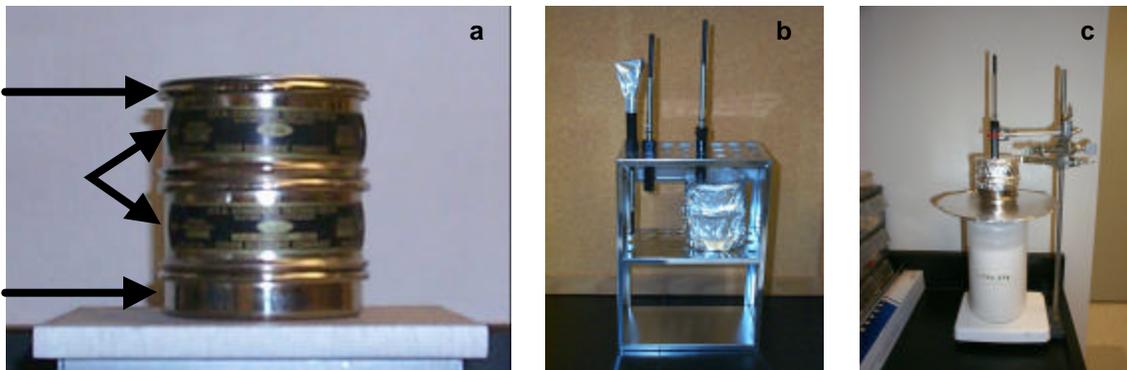


Figure 1. Setup for incubation and sampling of volatiles associated with cocaine and heroin stock materials: (a) pan/sieve assembly (W.S. Tyler, Mentor, OH) (b) 10-g sample (c) 1-kg stock sample.

Cross Sectional View of Dual-Coated SPME Fiber

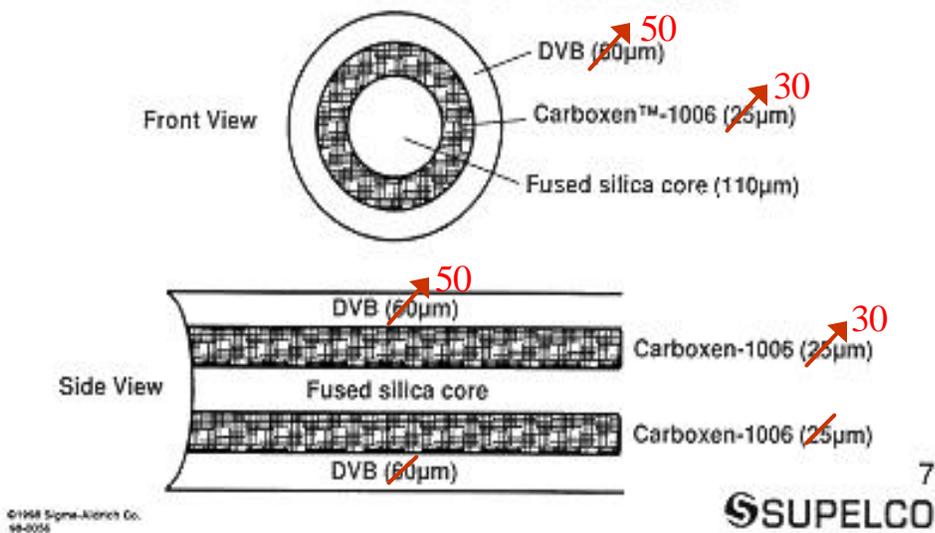


Figure 2. Cross-sectional view of the dual-layer DVB/Carboxen/PDMS fiber. Reprinted with permission from Supelco.

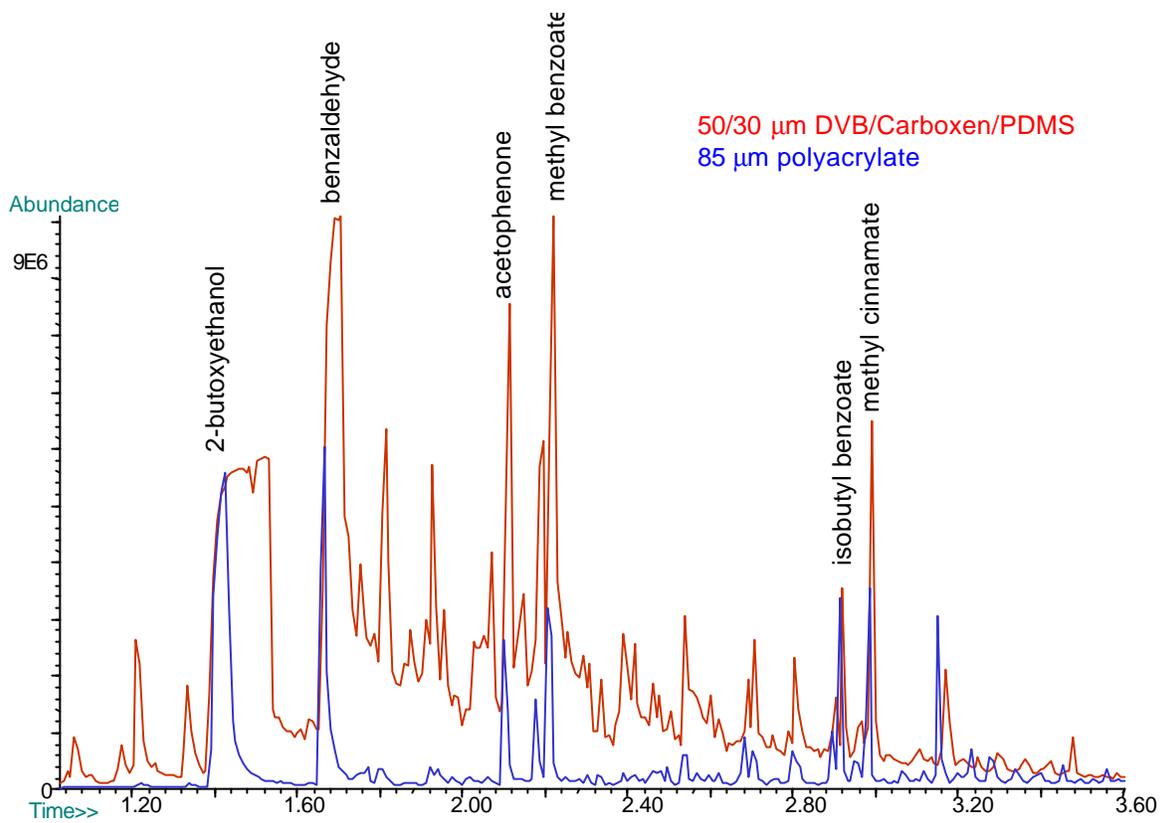


Figure 3. Comparing extraction efficiency of two different SPME fibers for a cocaine hydrochloride stock sample (90-min incubation/90-min sampling of 50-g COC219).

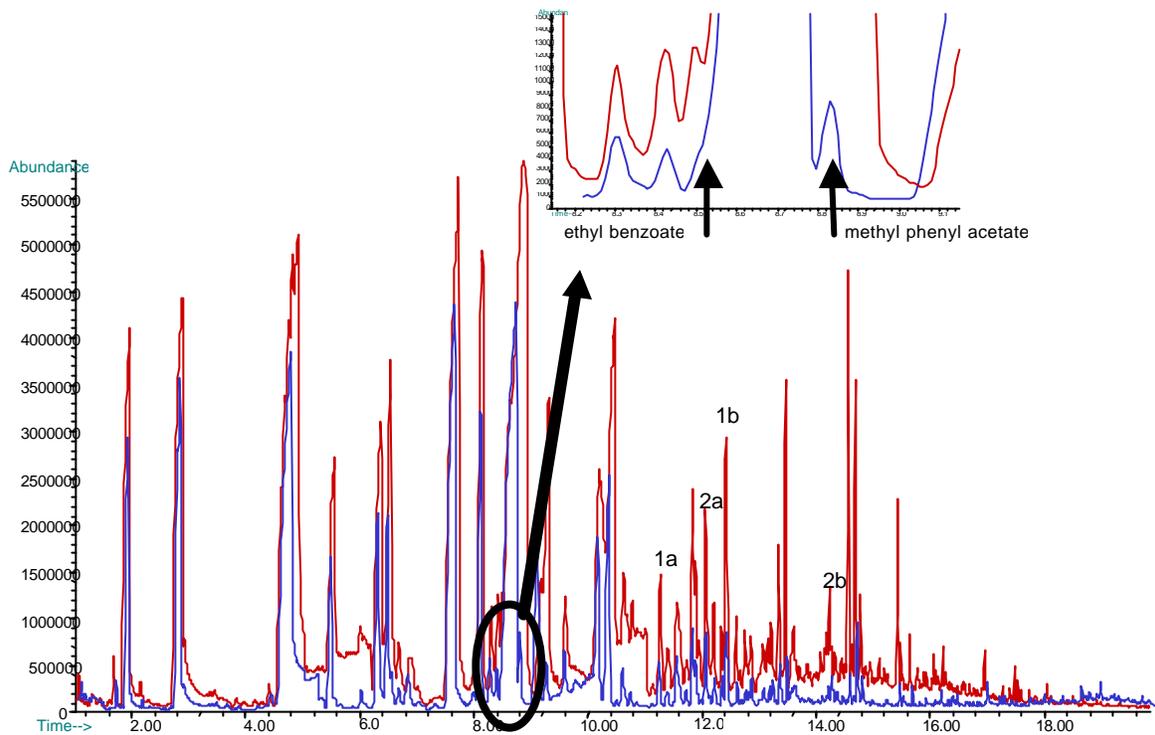


Figure 4. Comparing the effect of incubation versus sampling time for a cocaine hydrochloride stock material (COC285). See text for discussion.

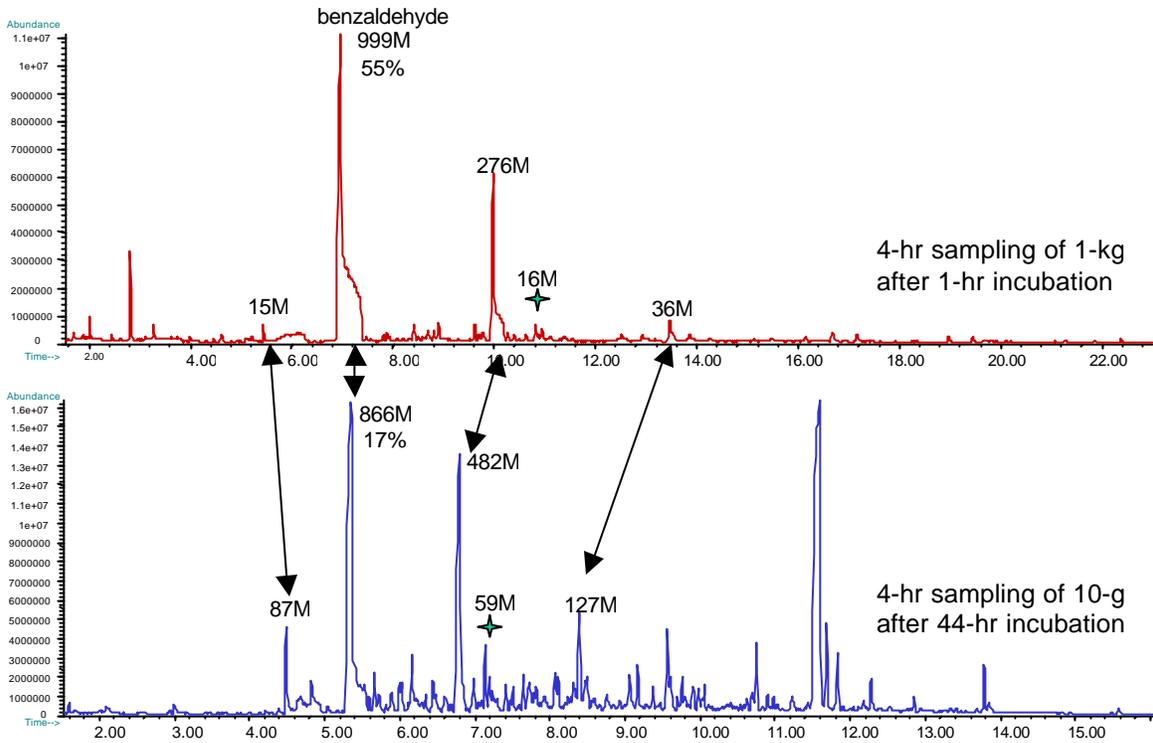


Figure 5. Determining whether incubation time could be reduced by using a larger quantity of drug (COC284). The chromatograms cannot be overlaid because different temperature programs and columns were used.

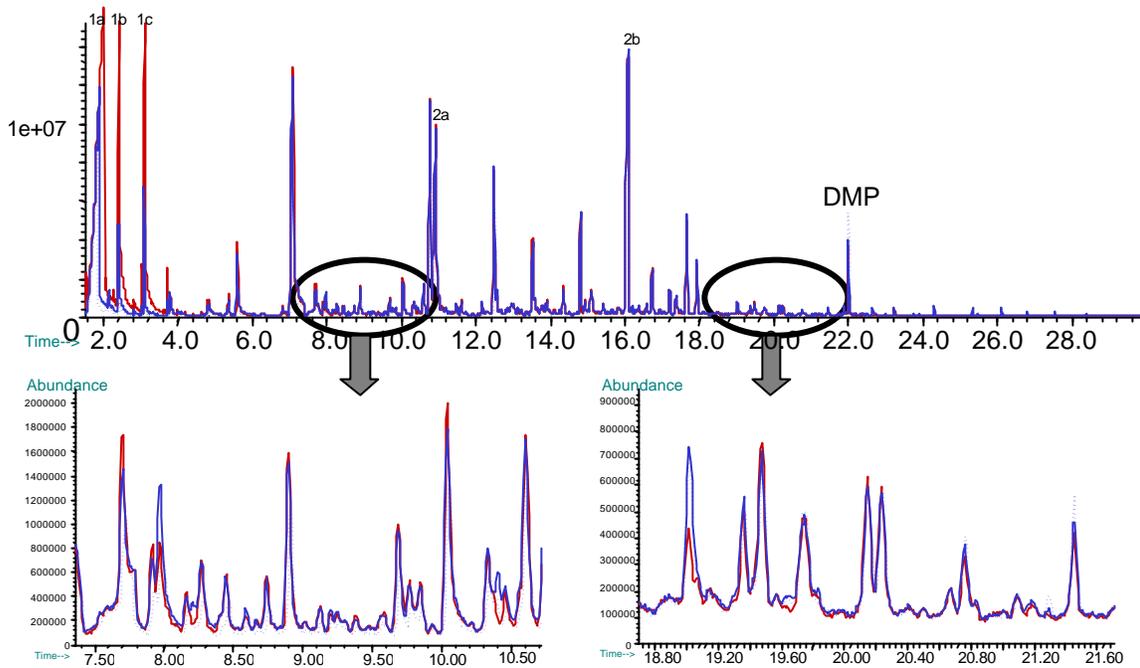


Figure 6. 1-hr samplings of COC276 analyzed immediately (red curve) and 4 days later (blue curves). 1a-c are, respectively, acetic acid, propyl acetate and isobutyl acetate. 2a-b are, respectively, methyl and propyl benzoate.

Abundance

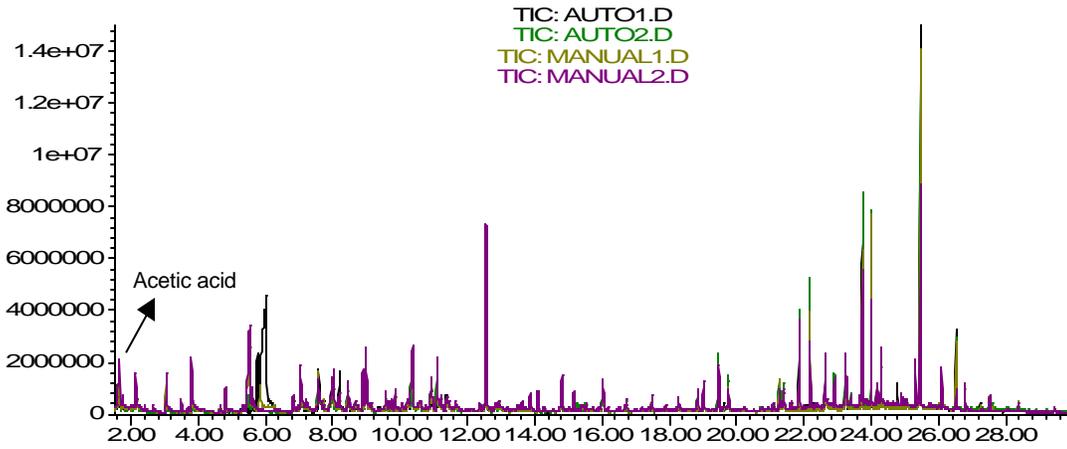


Figure 7. Total ion chromatograms comparing the four headspace samples that were taken onsite at Houston Intercontinental Airport, December 9, 1998. The SPME fibers were exposed and placed in an open cardboard box, which was then placed inside the hand-carry bag containing the concealed heroin. The bag was zipped up and left undisturbed during the 100-min sampling period. Samples were analyzed the next day in the Research Lab. Note the reproducible detection of acetic acid in all four samples.

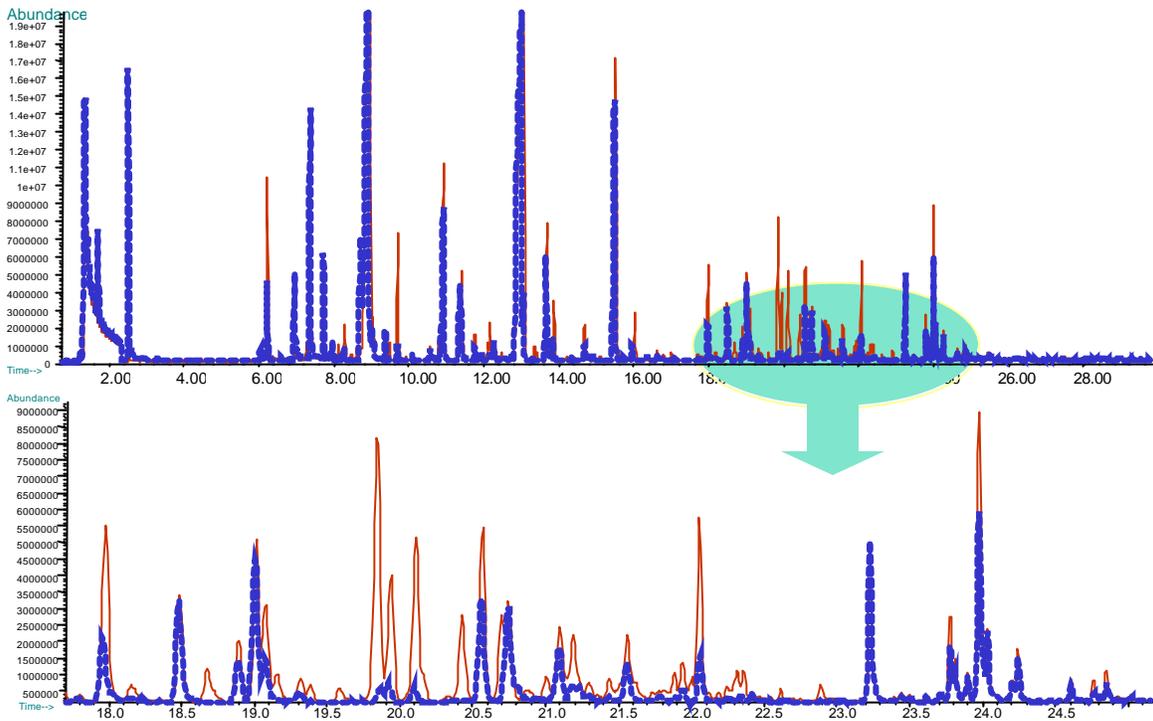


Figure 8. 15-min SPME sampling of the headspace of authentic (red curve) and counterfeit (blue curve) Giorgio Eau de Toilette.

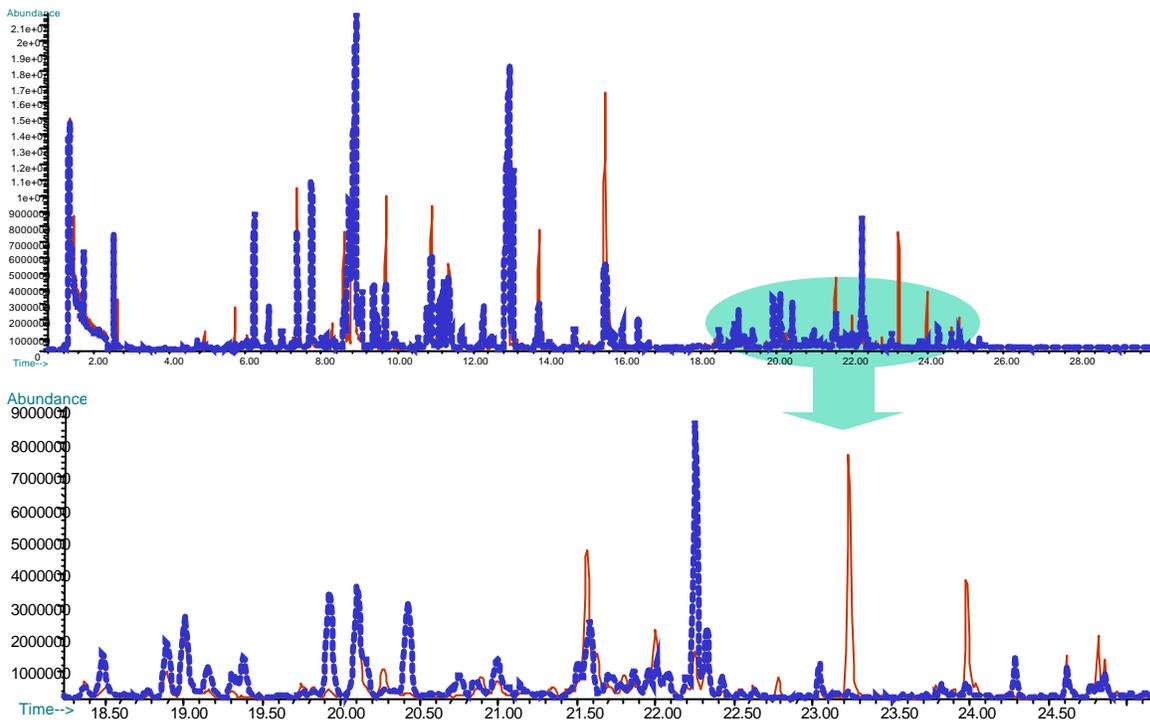


Figure 9. 15-min SPME sampling of the headspace of authentic (red curve) and counterfeit (blue curve) Chloé Eau de Toilette.