

Solventless determination of caffeine in beverages using solid-phase microextraction with fused-silica fibers

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ABSTRACT

Caffeine concentrations in beverages were determined using a simple and rapid method based on microextraction of caffeine onto the surface of a fused-silica fiber. The uncoated fiber was dipped into the beverage sample for 5 min after the addition of isotopically labeled (trimethyl ^{13}C)caffeine. The adsorbed caffeine was then thermally desorbed in a conventional split/splitless injection port, and the concentration of caffeine was determined using gas chromatography with mass spectrometric detection. Quantitative reproducibilities were *ca.* 5% (relative standard deviation) and the entire scheme including sample preparation and gas chromatographic analysis was completed in *ca.* 15 min per sample. The potential of the microextraction technique for the analysis of flavor and fragrance compounds in non-caffeinated beverages is also demonstrated. Since no solvents or class-fractionation steps are required, the method has good potential for automation.

INTRODUCTION

Recent concerns about the use of liquid solvents for the extraction of dissolved analytes from water-based samples had led to the development of several solid-phase extraction methods in an effort to greatly reduce or eliminate the need for liquid solvents in routine laboratories [1,2]. The majority of such methods require that the sample is passed through a relatively large (several grams) sorbent packing followed by elution of the trapped analytes with a few milliliters of solvent. Present methods for the determination of caffeine in coffee, tea, and other liquids generally require pH adjustment and either liquid solvent extraction or solid-phase adsorption followed by elution of the caffeine with liquid solvents.

Analysis of the extracts is performed using gas chromatography (GC) or high-performance liquid chromatography (HPLC) [3–8]. However, methods that are solvent-free and simpler to automate for the analysis of caffeine in beverages are desirable, particularly for quality control applications and to satisfy regulatory requirements for products such as decaffeinated coffee [3,7].

A microextraction technique in which analytes are adsorbed directly onto a fused-silica fiber from water samples has recently been reported that completely eliminates the need for liquid solvents and solid-phase adsorbents [9,10]. This method utilizes a short length of fused-silica optical fiber which is mounted in a conventional GC syringe. Following exposure of the uncoated silica surface to the sample, adsorbed analytes are recovered from the fiber by thermal desorption in a conventional GC injection port. In the present study, isotopically labeled (trimethyl ^{13}C) caffeine was added as an internal

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standard so that the native caffeine concentration could be directly determined without the need to obtain 100% extraction of the native caffeine onto the fiber. This approach has been developed in this study for the quantitation of caffeine in coffee, tea and carbonated beverages based on isotopic dilution analysis.

EXPERIMENTAL

Preparation of the fiber

The solid-phase microextraction (SPME) devices were constructed from polyimide-coated fused-silica optical fibers having outer diameters of *ca.* 170 μm (Polymicro Technologies, Phoenix, AZ, USA) and a Hamilton series 7000 syringe. A 22-cm-long fiber was mounted in the syringe by placing a small

drop of epoxy on one end of the fiber, allowing the epoxy to dry, and inserting the fiber through the hollow syringe plunger and the removable syringe needle. Thus, the fiber can be withdrawn into the syringe needle, or when the plunger is depressed, *ca.* 2 cm of the fiber extends out of the end of the syringe needle (Fig. 1). After the fiber was mounted in the syringe, the plunger was depressed to expose the end of the fiber, and the polyimide coating was carefully burned away from the last *ca.* 1.5 cm of the exposed end of the fiber with an oxygen-acetylene torch as shown in Fig. 1. The exposed end of the fiber was then briefly rinsed in methanol and cleaned for *ca.* 5 min by inserting into a heated (300°C) GC injection port. Mounting the fiber in the syringe in this manner provides a convenient method of protecting the uncoated portion of the fiber during storage and for inserting the fiber into the beverage samples and into the GC injection port. Construction and cleaning of a new SPME device requires *ca.* 15 min.

Sample preparation and analysis

Regular coffee, decaffeinated coffee and tea were brewed as for normal consumption. All other beverages were used directly from the beverage container, except that they were allowed to warm to room temperature prior to analysis. No sample preparation steps were used except that, for the caffeine quantitations, 20 μl of a methanol solution containing 150 μg of isotopically labeled [$^{13}\text{C}_3$]caffeine (trimethyl- $^{13}\text{C}_3$ purchased from Cambridge Isotope Laboratories, Woburn, MA, USA) was added to a 1-ml sample of each beverage which had been placed into a 1.5-ml autosampler vial. After brief mixing, *ca.* 1 cm of the end of the uncoated portion of the fiber was inserted into the sample to allow adsorption of the caffeine. (Mounting the syringe in a ring stand clamp prior to depressing the syringe plunger to insert the end of the fiber into the sample provides a simple way to reproducibly expose the same length of adsorbent surface for each analysis.) After the 5-min exposure period, the syringe plunger was withdrawn to pull the uncoated end of the fiber out of the sample and into the syringe needle. (Note that no water adheres to the fiber because of water's high surface tension, as described in ref. 9.) The syringe needle was then inserted into a heated (300°C) split/splitless injection

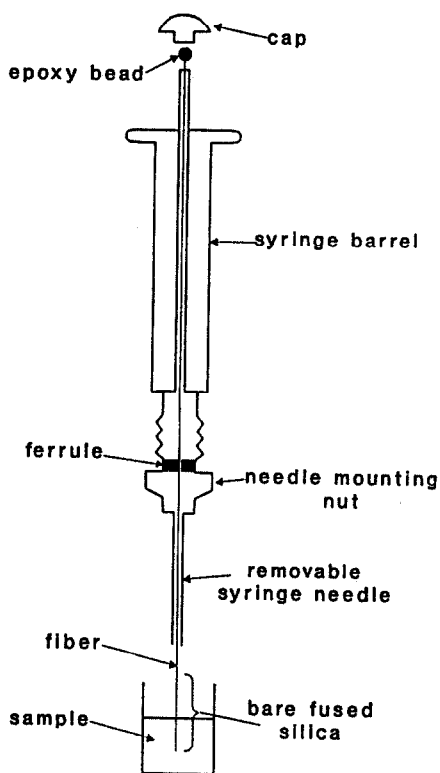


Fig. 1. Schematic of the SPME device assembled from a removable needle syringe. All components come with the syringe except for the fused-silica fiber and the epoxy bead on the end of the fiber. Construction of the device is described in the Experimental section.

port to thermally desorb the adsorbed analytes into the GC–mass spectrometry (MS) system.

The sequential steps required to perform the method are:

(1) Pipette 1 ml of the sample beverage into a 1.5-ml autosampler vial, add the [$^{13}\text{C}_3$]caffeine internal standard, and mix briefly.

(2) Depress the syringe plunger to insert *ca.* 1 cm of the uncoated end of the fused-silica fiber into the sample for 5 min (no mixing is required during the adsorption step). Then withdraw the uncoated end of the fiber out of the sample into the syringe needle.

(3) Insert the syringe needle into the GC injection port, close the split vent, and depress the syringe plunger to place the sample end of the fiber into the injection port liner.

(4) Let the adsorbed analytes thermally desorb for 1 min, withdraw the syringe plunger to pull the uncoated end of the fiber into the syringe needle, and remove the syringe from the injection port.

(5) Open the split vent, and perform a conventional GC separation.

GC–MS analysis conditions

All analyses were performed using a Hewlett-Packard Model 5985 GC–MS system equipped with a 25 m \times 0.32 mm I.D. HP-5 column (Hewlett-Packard, 0.17 μm film thickness) and a conventional unpacked injection port liner. No modification of the split/splitless injection port was required. During the splitless desorption step, the syringe was inserted through the injection port septum so that when the syringe plunger was depressed the sample end of the fiber was inside of the injection port liner *ca.* 1 cm above the inlet of the analytical column. (Sample desorption can also be performed in the split mode, and is preferred for samples with very high concentrations of analytes to avoid overloading the GC column stationary phase.) For the caffeine analyses, the GC oven was maintained at 70°C during the 1 min desorption step so that the desorbed caffeine was efficiently focused at the head of the analytical column. After the desorption step was completed and the fiber was removed from the injection port, the split vent was opened, and the GC oven was heated at 30°C/min to 190°C, followed by a temperature ramp of 10°C/min to 280°C. Analyses of the non-caffeinated beverages were per-

formed in the same manner, except that the GC oven temperature was held at lower temperatures during the thermal desorption step to efficiently focus analytes that were more volatile than caffeine. All GC–MS analyses were performed using electron

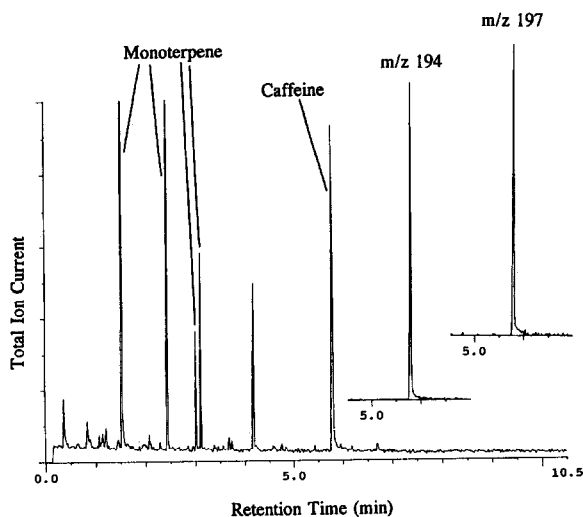
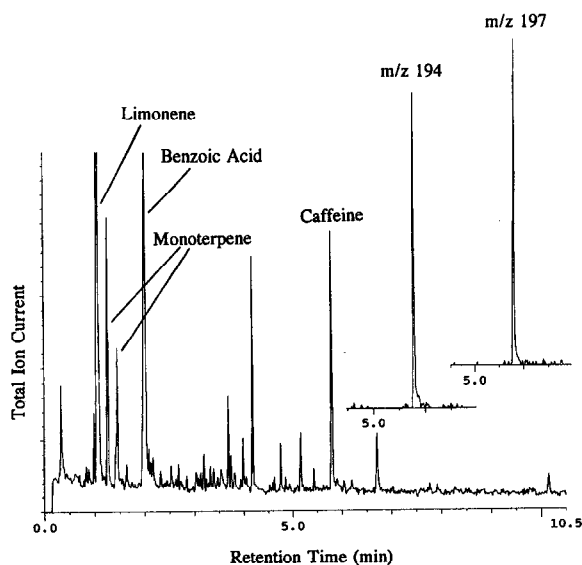


Fig. 2. GC–MS analysis of caffeine from a carbonated cola soft drink (top) and from brewed tea (bottom) using microextraction onto a fused-silica rod. The mass chromatograms for m/z 194 (caffeine) and m/z 197 ($^{13}\text{C}_3$ caffeine) were generated from the reconstructed total ion current chromatograms (TIC). Microextraction and GC–MS conditions are given in the text.

impact ionization (70 eV) with a mass range of 50 to 300 a.m.u. The integrated areas from the mass chromatograms of the molecular ions (194 and 197 for caffeine and $[^{13}\text{C}_3]$ caffeine, respectively) were used for all quantitations.

RESULTS AND DISCUSSION

Typical GC–MS chromatograms resulting from the SPME analysis of tea and a carbonated cola drink (Coca-Cola) are shown in Fig. 2. Both samples showed caffeine as a major peak along with several monoterpenes. The cola beverage also showed significant amounts of benzoic acid. Benzoic acid and the monoterpene, limonene, were also predominant species in many of the non-cola soft drinks tested. When the mass chromatograms were generated for the molecular ions of the native caffeine (m/z 194) and the $[^{13}\text{C}_3]$ caffeine (m/z 197), only the caffeine species showed significant chromatographic peaks for any of the caffeinated beverages tested (Fig. 2).

During the initial investigations, there was concern that adsorbed sample species, including caffeine or $[^{13}\text{C}_3]$ caffeine, might not be efficiently removed from the uncoated fiber during the one-minute thermal desorption step, and thus contaminate the fiber for subsequent samples. To determine whether analyte carryover could be a significant source of error, the SPME fiber was desorbed into the GC–MS a second time after the analysis of each beverage sample was completed. No carryover of analyte species could be observed in the second GC–MS analyses of any of the samples tested, demonstrating that one minute was sufficient for the thermal desorption step.

While carryover from sample to sample was not observed, two significant experimental problems were encountered during this study. First, there is a temptation for the analyst to touch the syringe needle to help guide the needle into the sample vial, or into the GC injection port. This procedure results in significant contamination, as shown by the SPME analyses of a coffee sample without touching the needle, and when the analyst guided the syringe needle into the injection port with his fingers. As shown in Fig. 3, touching the syringe needle causes significant peaks in the GC–MS chromatogram resulting from biological acids.

The second experimental problem occurs when care is not taken to protect the uncoated portion of the fused-silica fiber. Since the polyimide coating has been removed, this part of the fiber is somewhat brittle, and should be drawn into the syringe needle for physical protection unless the adsorption or desorption steps are being performed. However, with reasonable care, the fibers have good lifetimes, and all analyses in this study (> 100 microextractions) were performed with only two fibers. It is also reasonable to expect that the fiber could be deactivated from some sample matrices and, therefore, become

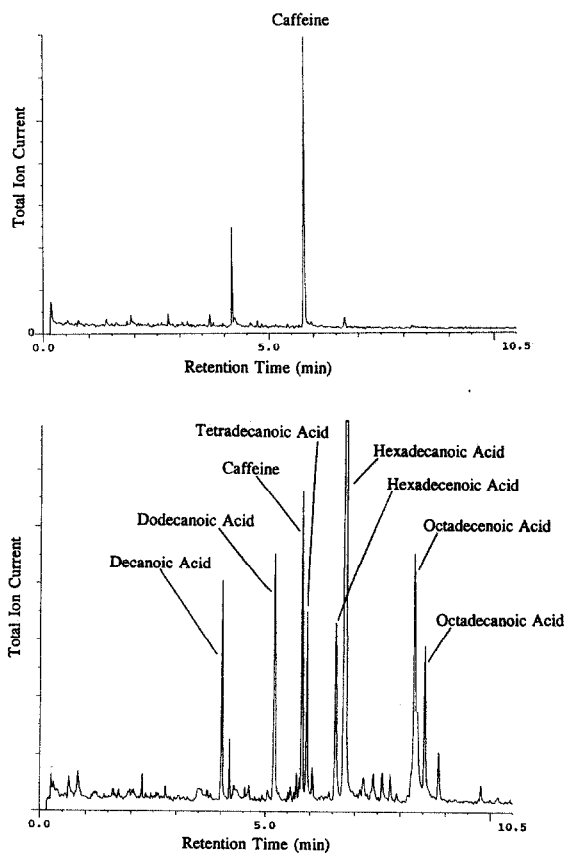


Fig. 3. Effect of touching the syringe needle on the analysis of coffee extracted using fused-silica rod microextraction followed by GC–MS. The top part of the figure shows a typical reconstructed total ion current chromatogram (TIC) of coffee. The bottom part of the figure shows the TIC chromatogram resulting from the analysis of an identical coffee sample when the analyst touched the syringe needle while inserting the needle into the GC injection port. Microextraction and GC–MS conditions are given in the text.

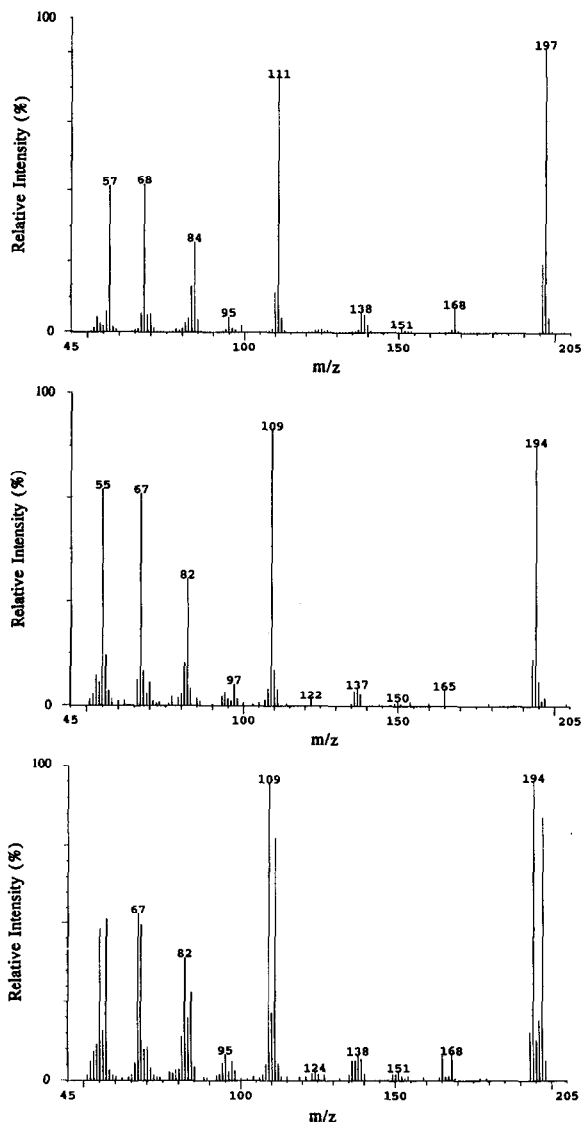


Fig. 4. Mass spectra of $[^{13}\text{C}_3]$ caffeine (top), native caffeine from tea (middle), and the mixed caffeine- $[^{13}\text{C}_3]$ caffeine spectra from the analysis of a carbonated cola soft drink (bottom).

useless. However, no such loss of activity was observed for the samples used in this study.

Mass spectra for the native caffeine (from coffee) and the $[^{13}\text{C}_3]$ caffeine standard are shown in Fig. 4 along with the mixed mass spectra of the caffeine chromatographic peak obtained from the analysis of the cola beverage. As shown in Fig. 4, the minimum overlap in the mass spectra occurred in the

molecular ion region. Since the molecular ions (m/z 194 and 197) were also the most intense mass spectral peaks and showed no interfering chromatographic peaks in the analysis of the beverage samples, the ratio of the peak areas of m/z 194 to m/z 197 was used for all quantitations.

Quantitative standard curves were generated using two caffeine-free matrices, a cola drink (caffeine-free Coca-Cola), and HPLC-grade water. Standards were prepared by spiking 1-ml aliquots of the cola or water sample with 37.5, 75, 150, 375 and 750 μg of caffeine in methanol. Each standard was then spiked with the internal standard (150 μg) and analyzed in a manner identical to that used for the samples. Prior to spiking with the caffeine standards, the caffeine-free cola was first analyzed using the SPME method, and no caffeine was detected.

Both the spiked caffeine-free cola and the water yielded similar standard curves, so subsequent calibration was performed using the caffeine-free cola. A typical standard curve based on the ratio of 194/197 peak areas is shown in Fig. 5, and displays acceptable linearity ($r^2 = 0.998$) for quantitative calibration. It should be noted that the standard curve does not have an exact zero intercept whether the calibration matrix is pure water or caffeine-free cola. With the zero caffeine standard for both matrices, the 194/197 ratio was slightly above zero (ca. 0.01). This non-zero intercept was found to occur because of the presence of a small peak at m/z 194 in

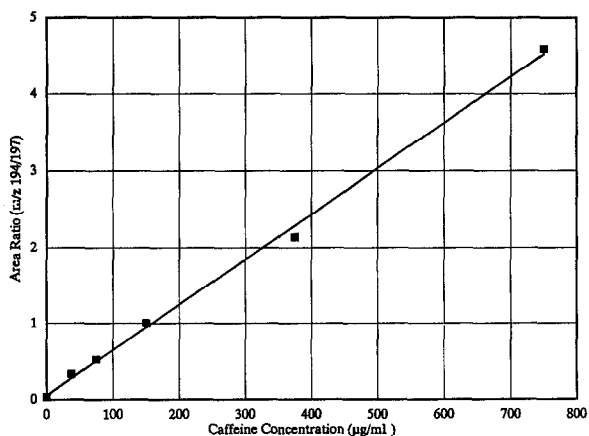


Fig. 5. Quantitative calibration curve based on the addition of caffeine to a caffeine-free cola drink containing 150 $\mu\text{g/ml}$ of $[^{13}\text{C}_3]$ caffeine as an internal standard.

TABLE I
QUANTITATION OF CAFFEINE IN BEVERAGES USING THE MICROEXTRACTION TECHNIQUE

Sample	Caffeine concentration ($\mu\text{g}/\text{ml}$)				$\bar{x} \pm \text{S.D.}^a$	Relative S.D. (%)
	Trial					
	1	2	3	4		
Coffee	295	290	291	292	292 ± 2	0.7
Decaffeinated coffee	33	29	31	30	31 ± 1.7	5.5
Tea	398	366	405	368	384 ± 20	5.1
Diet cola	140	126	124	132	131 ± 7.2	5.5

^a Mean concentration \pm one standard deviation.

the mass spectra of the [$^{13}\text{C}_3$]caffeine internal standard. Therefore, with the addition of 150 μg internal standard, the practical detection limit was *ca.* 2 $\mu\text{g}/\text{ml}$ to have a 194/197 ratio twice the level of the zero calibration standard. The [$^{13}\text{C}_3$]caffeine spike concentration of 150 $\mu\text{g}/\text{ml}$ was used in this study because it resulted in a concentration approximately in the range of the native caffeine in the samples studied. However, if lower detection limits are need-

ed, the sensitivity of the technique can be improved by adding less of the internal standard (and, therefore, adding less of the interfering m/z 194 peak which is contributed by the [$^{13}\text{C}_3$]caffeine). The analyses in this study were also performed in the full scan mode, since we were interested in identifying any species in addition to caffeine that may have been transferred to the GC by the SPME technique. However, additional sensitivity could also be

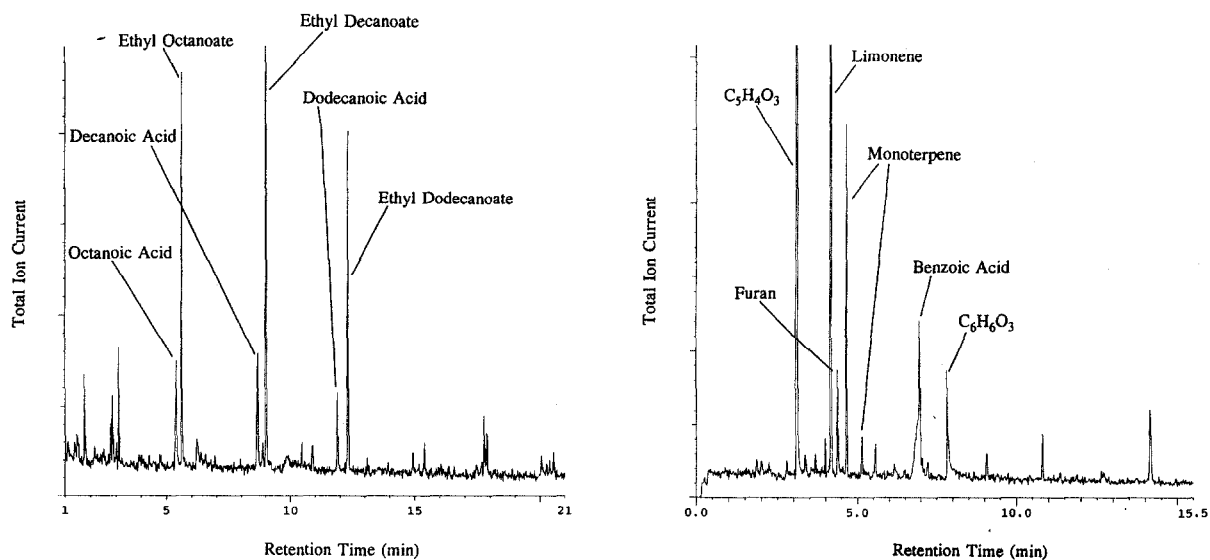


Fig. 6. SPME GC-MS analysis of flavor and fragrance compounds from lemon juice (left) and brandy (right). Both samples were extracted and analyzed as described in the Experimental section. GC oven temperature during the desorption of the fiber for lemon juice was 5°C followed by a temperature ramp of 30°C/min to 65°C, then a temperature ramp at 8°C/min. GC oven temperature during the desorption of the brandy sample was 20°C followed by a temperature ramp of 30°C/min to 80°C, then a temperature ramp at 8°C/minute. All peak identifications were based on the GC-MS analysis. The species labeled as $\text{C}_5\text{H}_4\text{O}_3$ and $\text{C}_6\text{H}_6\text{O}_3$ appear to be oxygenated furans.

gained by analyzing the samples using selected ion monitoring (e.g., monitoring only the molecular ion of caffeine), and, with selected ion monitoring analysis, detection limits in the low ng/ml range have been achieved for caffeine in spiked water samples.

The ability of the SPME technique to yield reproducible results was investigated by performing replicate analyses of four samples including brewed coffee and decaffeinated coffee, tea, and a carbonated cola soft drink. Quadruplicate 1-ml aliquots of each sample were spiked with the internal standard and analyzed using the SPME technique. The results of these replicate analyses are shown in Table I. In general, the relative standard deviations were ca. 5%, which compare favorably with those reported for coffee of 2–7% using liquid solvent extraction [7] and 1–8% using solid-phase adsorption [3].

While quantitative investigations were performed only caffeine, the SPME technique may also be useful for analyzing beverages for a variety of flavor and fragrance compounds. SPME followed by GC–MS analysis of different types of samples including several carbonated soft drinks, fruit juices, and alcoholic beverages have shown acceptable chromatographic peaks for major flavor and fragrance compounds. As shown by the analysis of lemon juice and brandy in Fig. 6, SPME followed by GC analysis is a very simple and potentially useful qualitative analysis approach that requires no liquid solvents and virtually no sample preparation. However, quantitation of the individual species is not possible without the addition of an appropriate internal standard or a knowledge of the distribution

coefficient of each analyte species between the beverage sample and the fiber [9].

CONCLUSIONS

Microextraction onto a fused-silica fiber followed by thermal desorption and capillary GC–MS isotope dilution analysis is a simple and rapid method for the quantitation of caffeine in beverages. Sample preparation consists solely of adding an internal standard, and, since all subsequent sample preparation and analysis steps are performed in a GC autosampler vial and require only the manipulation of a GC syringe, the method has good potential for automation.

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