

# Evaluation of Solid-Phase Microextraction Desorption Parameters for Fast GC Analysis of Cocaine in Coca Leaves

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## Abstract

By its simplicity and rapidity, solid-phase microextraction (SPME) appears as an interesting alternative for sample introduction in fast gas chromatography (fast GC). This combination depends on numerous parameters affecting the desorption step (i.e., the release of compounds from the SPME fiber coating to the GC column). In this study, different liner diameters, injection temperatures, and gas flow rates are evaluated to accelerate the thermal desorption process in the injection port. This process is followed with real-time direct coupling a split/splitless injector to a mass spectrometer by means of a short capillary. It is shown that an effective, quantitative, and rapid transfer of cocaine (COC) and cocaethylene (CE) is performed with a 0.75-mm i.d. liner, at 280°C and 4 mL/min gas flow rate. The 7- $\mu$ m polydimethylsiloxane (PDMS) coating is selected for combination with fast GC because the 100- $\mu$ m PDMS fiber presents some limitations caused by fiber bleeding. Finally, the developed SPME-fast GC method is applied to perform in less than 5 min, the quantitation of COC extracted from coca leaves by focused microwave-assisted extraction. An amount of  $7.6 \pm 0.5$  mg of COC per gram of dry mass is found, which is in good agreement with previously published results.

## Introduction

Speeding up the entire analytical process has become a priority in several laboratories. Faster analyses are attractive not only for reducing the time-response delivery but also the costs of operation in numerous domains. The global approach to decrease the analysis time is to curtail both the sample preparation step as well as the separation and detection procedures. Because of its high efficiency and easy coupling with selective and sensitive detectors, gas chromatography (GC) remains a frequently selected separation method. Although the theoretical bases of fast GC were already established in the 1960s (1), the recent disposal of commercially available instrumentation has encouraged the interest

for this technique in the last decade only (2). Depending on the sample complexity and required separation, there are different methods for achieving fast GC analysis. Korytar et al. (3) proposed some guidelines for accelerating GC runs and also outlined the practical implications. Nowadays, the fast GC instruments offer good performance, such as rapid oven temperature programming, rapid data acquisition, as well as different possibilities to enhance the injection rate. Whatever the method, injection remains a critical step in fast GC because injection plugs must be narrow enough to avoid any loss of chromatographic efficiency. Beside classical injection techniques [split/splitless (4,5), on-column (6,7), and programmed vaporization temperature], other sample introduction approaches were also evaluated but found to be of limited use because of the sophisticated instrumentation requirements (8,9). An interesting approach is the application of a pressure pulse during splitless injection, which permits the increase in flow rate in the injector and, therefore, accelerates the sample transfer rate. This technique, called pressure pulsed splitless (PPS) (10), also allows the introduction of higher solvent amounts and minimizes degradation problems during conventional GC analysis (11–13).

Solid-phase microextraction (SPME), introduced in 1990 by Arthur and Pawliszyn (14), appears also as an alternative for sample introduction in fast GC. It is a solvent-free sample preparation technique. Indeed, analytes are extracted on a coated silica fiber, which is then directly inserted into the injection port and desorbed at a high temperature (15). Although SPME is a straightforward extraction technique in comparison with other classical methods, several parameters have to be optimized for ensuring a quantitative, as well as rapid, transfer of the analytes of interest from the fiber coating to the GC column.

The purpose of the present study was to evaluate the simple combination of SPME with fast GC (i.e., without sophisticated instrumental modifications). Thus, the SPME desorption step appeared as the most important parameter to evaluate. This desorption optimization was realized for cocaine (COC) and the internal standard cocaethylene (CE) by directly coupling a split/splitless injection port to a mass spectrometer (MS), which

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allowed for real-time monitoring (16). A simple SPME–fast GC method was then applied to the quantitative analysis of COC obtained from coca leaves by focused microwave-assisted extraction (FMAE). Indeed, previous works made in this laboratory and dedicated to the extraction of COC in plant material by means of different techniques (17–19) demonstrated that FMAE was the most efficient technique in terms of solvent consumption, analysis time, and reproducibility (20). Furthermore, a successful FMAE–GC–MS method was previously reported (17). In addition, to improve selectivity and reduce analysis time, SPME was integrated into the entire analytical process, as discussed by Bieri et al. (21).

## Experimental

### Plant material and chemicals

Leaves of *E. coca* Lam. var. *coca* were harvested in Tingo Maria (Peru), authenticated by T. Plowman, and kindly provided by Dr. L. Rivier (Lausanne, Switzerland). Coca leaves were air-dried in the shade at a temperature below 40°C. Plant material was powdered with a ball-mill (MM Retsch, Switzerland) and finally sieved to an average particle size between 90 and 125 µm. A voucher specimen is deposited in the laboratory.

Analytical-grade solvents were purchased from Panreac Quimica SA (Barcelona, Spain). COC hydrochloride was obtained from Siegfried Handel (Zofingen, Switzerland) and CE from Lipomed AG (Arlesheim, Switzerland). All other chemicals were purchased from Fluka (Buchs, Switzerland). Ultrapure water was provided by a Milli-Q unit from Millipore (Bedford, MA).

### Fiber desorption profiles

For these preliminary experiments, SPME was performed with 1.5 mL of standard solutions containing COC and CE, at 15 and 25 ppm, respectively, in a 50mM phosphate buffer solution at pH 8.1. Samples were extracted for 5 min at 25°C under sonication with 100- and 7-µm polydimethylsiloxane (PDMS) fibers pur-

chased from Supelco (Bellefonte, PA) and conditioned before use according to the supplier instructions.

Desorption profiles of the fibers were studied by coupling the split/splitless injector of an HP 5890 series II GC (Agilent Technologies, Waldbronn, Germany) to an HP 5972 MS by means of a fused-silica capillary (1 m × 100-µm i.d. × 375-µm o.d.) deactivated with OV-1701-OH (BGB Analytik, Anwil, Switzerland). The GC oven and GC–MS transfer line were set at 280°C. Helium was used as carrier gas. The influence of liner diameter, injector temperature, and gas flow rate was studied as described in Table I. To obtain a stable baseline, the MS was turned on for 1.5 min before inserting the SPME fiber. After this stabilization period, injections were performed in the splitless mode with an overall 6.5-min splitless time. The MS was operated in the selected ion monitoring (SIM) mode with *m/z* 182 and 196 for COC and CE (internal standard), respectively.

Furthermore, additional experiments were performed at different gas flow rates to verify that the desorption of COC and CE in the improved conditions (injection temperature, 280°C; liner diameter, 0.75 mm) was quantitative. For this purpose, the fused-silica capillary was replaced by a classical HP-5MS capillary column (30 m × 0.25-mm i.d., 0.25-µm film thickness). The injection was performed at 280°C in the splitless mode with a 2-min splitless time, and a pressure program was applied to provide the desired flow rate during the injection step. The analyses were performed in the constant flow mode at 1 mL/min with helium as the carrier gas. The oven temperature program was: 70°C (2 min) to 280°C (2 min) at 30°/min. The MS was operated in electron impact (EI) ionization mode at 70 eV in the scan range *m/z* 30–400.

### Sample extraction by FMAE and SPME

FMAEs of plant material were performed in the previously published optimized conditions (17). Dry powdered coca leaves (100 mg) were placed into a 20-mL quartz extraction vessel with 5 mL MeOH. The sample was stirred during the extraction process and a condenser was placed above the sample flask. Extractions were realized under atmospheric pressure at a frequency of 2450 MHz using a 3.6 FMAE apparatus (Prolabo, Fontenay-sous-Bois, France). A heating power of 125 W was applied for 30 s. After cooling, the extracts were filtered (0.45-µm polytetrafluorethylene filter). To 1 mL of organic solution, an internal standard (100 µL of a methanolic CE solution at 1000 ppm) and aqueous buffer were added up to 5 mL. The buffer was a 50mM phosphate solution adjusted to pH 8.1. An aliquot of 1.5 mL was placed in a 2-mL vial for SPME liquid sampling at 25°C. A 7-µm PDMS fiber was immersed in the solution for precisely 2 min with sonication. The fiber was withdrawn and further inserted in the injection port of the fast GC for thermal desorption.

### Fast GC analysis

Fast GC analyses were carried out on an HP 6850 GC (Agilent Technologies, Waldbronn, Germany) coupled to a flame ionization detector (FID) and equipped with a DB-5 capillary column (3 m × 100-µm i.d., 0.1-µm film thickness) (J&W Scientific, Folsom, CA). A split/splitless injector with an SPME liner (0.75-mm i.d.) was used. Injection was performed at 280°C in the pres-

**Table I. Experimental Conditions Used to Test the Direct Desorption Profiles of COC and CE with the 100- and 7-µm PDMS Fibers**

Liner diameter (mm)	Injector temperature (°C)	Flow rate (mL/min)	
4	230	0.4	
2			
0.75			
0.75	200	0.4	
	230		
	260		
0.75	280	0.4	
	280		1
			2
4			

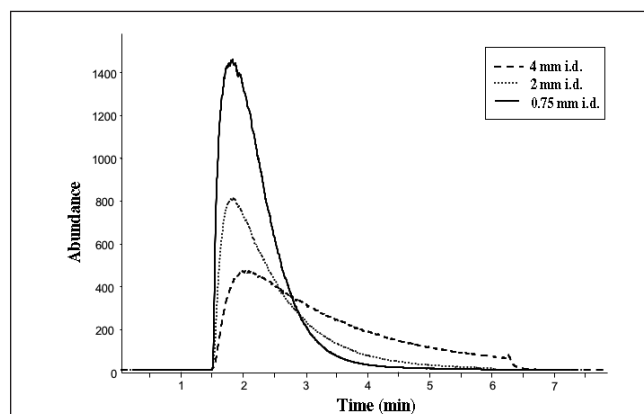
sure-pulsed splitless mode for 12 s with a column head pressure of 58.5 psi corresponding to an initial flow rate of 4 mL/min. The pressure was then reduced to 12.4 psi at 99 psi/min. After that, the electronic pressure control operated in the constant flow mode at 0.4 mL/min. Hydrogen was employed as the carrier gas. The oven temperature program was: 130°C (0.2 min) to 285°C (1 min) at 120°/min, resulting in a total run time of 2.50 min. The detector temperature was maintained at 280°C.

## Results and Discussion

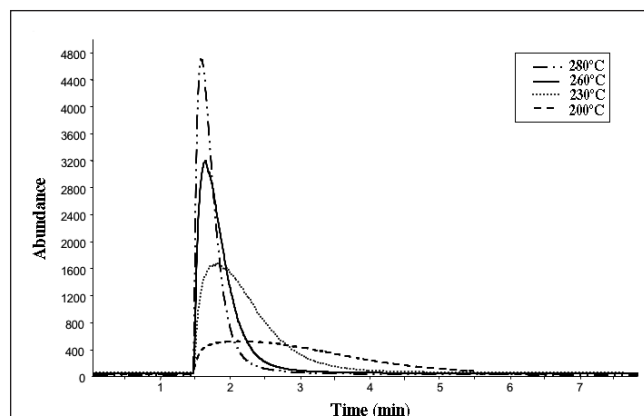
### Fiber desorption profiles

The influence of the different parameters was studied with the 7- and 100- $\mu$ m PDMS fibers. Although results were similar with both fibers, for the sake of clarity, only those obtained with the thinner polymer are presented. In addition, as discussed later, the 100- $\mu$ m fiber was not appropriate for combination with fast GC because instability features were observed.

As shown in Figure 1, the liner diameter had a strong effect on the desorption rate. Indeed, this process was much shorter with



**Figure 1.** Real-time desorption profiles obtained with the 7- $\mu$ m PDMS fiber with different liner diameters. Measures were performed by MS in the selected ion monitoring mode ( $m/z$  182 and 196 for COC and CE, respectively).



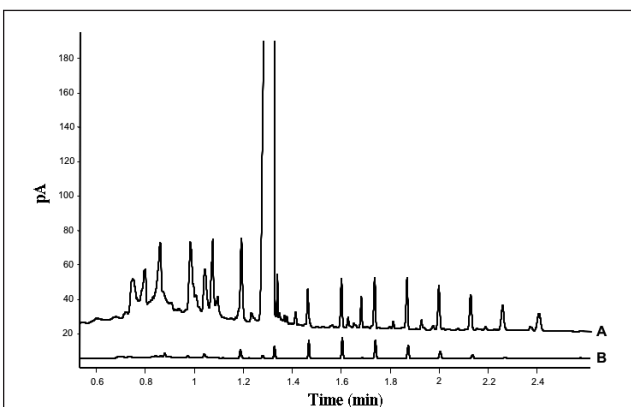
**Figure 2.** Real-time desorption profiles obtained with the 7- $\mu$ m PDMS fiber at different injection temperatures. Measurements were performed by MS in the SIM mode ( $m/z$  182 and 196 for COC and CE, respectively).

thinner liners. This can be explained by the generation of higher linear flow rates by the carrier gas around the fiber coating, induced by the reduction of the insert diameter (22). The thinnest liner was thus selected for the following tests.

The injection temperature is another parameter that affects analyte desorption. It is noteworthy that the diffusion coefficient of compounds in the polymeric phase increases and the gas/polymer distribution constant ( $K_R$ ) rapidly decreases with temperature increase (22). Furthermore, in the case of a quantitative analysis, a total transfer of the extracted compounds is required. For these reasons, it is usually recommended to work at the highest temperature supported by the polymer, which corresponds to 280°C for PDMS. In the case of COC and CE, which are semivolatile molecules, an elevated temperature showed a significant effect on the desorption rate, which was largely increased at 280°C in comparison with 260°C, 230°C, and 200°C (Figure 2). Consequently, the highest injection temperature was chosen for further experiments.

An important increase of the desorption rate was thus obtained with a thin liner and at an elevated injection temperature, as already reported by Langenfeld et al. (16). For these experiments, the gas flow rate was fixed at 0.4 mL/min, corresponding to the value applied in fast GC analysis and given by the method translation software (MXLATOR, version 2.0a, Agilent Technologies). However, when combining SPME with fast GC, the gas flow rate can affect the desorption rate and, therefore, was studied at higher values (Table I). As expected, the desorption times decreased (0.5, 0.4, 0.3, and 0.2 min) when the gas flow rate increased (0.4, 1, 2, and 4 mL/min, respectively).

From these results, it was observed that a significant enhancement of the SPME desorption rate of COC and CE was obtained simply by varying the liner diameter, injection temperature, and gas flow rate. The real-time measurement of the desorption suggested that COC and CE were released from the 7- $\mu$ m PDMS fiber in a very short time (i.e., 0.2 min) with a liner of 0.75-mm i.d., at 280°C and 4 mL/min. In these improved conditions, the quantitative desorption of the target analytes was verified. Experiments were conducted by classical SPME-GC-MS analyses with a pressure program during the injection step, ensuring different gas flow rates. Results provided identical chromatograms (same peak



**Figure 3.** Comparison of fiber bleeding observed on fast GC-FID chromatograms. Fibers were inserted into the injection port without any immersion (100- $\mu$ m PDMS, A; 7- $\mu$ m PDMS, B).

areas) in all cases, indicating that the same amount of COC and CE was desorbed independently on the applied gas flow rate during the injection step.

It is important to note that fast GC analysis of standard solutions of COC and CE extracted by SPME in the previously mentioned conditions showed a great number of additional peaks when the 100- $\mu\text{m}$  fiber was used. Further investigations on fibers inserted into the injection port without any immersion demonstrated that these peaks originated from fiber bleeding as a consequence of the non-bonded stationary phase. Moreover, in classical SPME–GC–MS procedures, chromatograms also showed a fiber bleeding (data not shown), though to a lesser extent. On the other hand, the 7- $\mu\text{m}$  fiber, with a bonded polymer, was more stable and demonstrated a cleaner chromatogram by fast GC, as shown by the comparison of fast GC analyses of the two fibers presented in Figure 3.

#### Extraction time with the 7- $\mu\text{m}$ PDMS fiber

In SPME, extraction times are determined both by the distribution constant of the compounds and the coating thickness. As COC and CE demonstrated a good affinity for PDMS, the extraction time needed with the 100- $\mu\text{m}$  fiber was 15 min, and 5 min was sufficient with the 7- $\mu\text{m}$  coating (shown in Figure 4). The choice of the extraction time is usually guided by sensitivity and

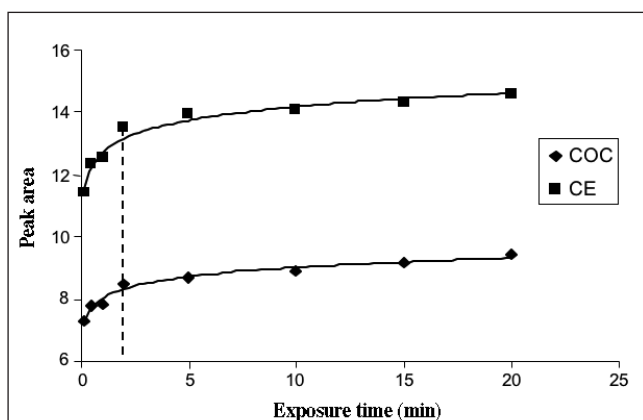


Figure 4. SPME extraction time for COC and CE obtained with the 7- $\mu\text{m}$  PDMS fiber.

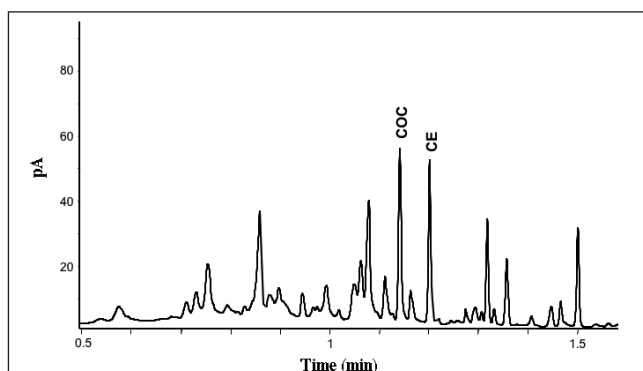


Figure 5. Fast GC–FID chromatogram of coca leaves after FMAE and 2-min SPME with the 7- $\mu\text{m}$  PDMS fiber.

repeatability, as well as total analysis time. As a compromise, a 2-min SPME time was selected.

#### Quantitative plant analysis

SPME combined with fast GC was finally applied to the extraction and quantitation of cocaine in FMAE extracts of coca leaves. A chromatogram is given in Figure 5. In this case, because the coating volume and, therefore, the quantity of extracted compounds was low, it was necessary to work with concentrated solutions. For this reason, the initial methanolic FMAE extract was diluted five times instead of 50 times, as reported previously (17). External calibration was performed on standard solutions containing COC at different concentrations with CE as internal standard at 20 ppm. The relative standard deviation (RSD) (2%) on the peak area ratios COC/CE was calculated for five consecutive analyses of the solution containing COC and CE both at 20 ppm. The linear regression equation (peak area ratios COC/CE plotted in function of the corresponding concentration ratios) was:

$$y = 0.5458x + 0.103 \quad \text{Eq. 1}$$

and the coefficient of determination was 0.9974. Finally, COC was quantitated in real plant material by SPME–fast GC. Based on three independent extractions analyzed in triplicate, the content in cocaine was determined as  $7.6 \pm 0.5$  mg/g of dry mass, with an RSD of 2.6%. Thus, the results showed a satisfactory precision, and the percentage was in agreement with those obtained previously (17,21).

#### Conclusion

In this study, SPME was evaluated as an attractive and interesting alternative injection technique compatible with fast GC. A real-time monitoring of the desorption process by directly coupling a split/splitless injector to an MS allowed the ability to select conditions that fit with fast GC requirements. The desorption rate of COC and CE was significantly enhanced, which finally ensured a very short desorption time of 12 s for the compounds of interest. Moreover, because of the stationary phase stability, it was shown that the thinnest PDMS fiber (7  $\mu\text{m}$ ) was more appropriate for such applications because less undesirable compounds were released from the fiber during the injection process. The use of a thin fiber coating also allowed for a decrease in extraction time from 15 to 2 min. Finally, the developed SPME–fast GC method permitted a quantitative determination of COC in FMAE extract in less than 5 min.

#### Acknowledgments

The authors are grateful to Dr. L. Rivier (Lausanne, Switzerland) for kindly providing the coca samples. We would also like to thank Dr. O. Panero and Dr. I. Tonutti (Tradall SA, Meyrin, Switzerland) for their valuable assistance, which was greatly appreciated, and the loan of the fast GC apparatus.

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Manuscript received September 14, 2005;  
revision received December 16, 2005.