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Determination of cocaine and cocaethylene in plasma by solid-phase microextraction and gas chromatography–mass spectrometry

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Abstract

The present paper describes a method for the simultaneous determination of cocaine and cocaethylene in plasma. It was based in the extraction of the analytes by solid-phase microextraction (SPME), and gas chromatography–mass spectrometry (GC–MS) was used to identify and quantify the analytes in selected ion monitoring (SIM) mode. The method showed to be very simple, rapid and sensitive. The method was validated for the two compounds, including linearity (range 25–1000 ng/mL) and the main precision parameters. It was applied to ten plasma samples from cocaine and alcohol users, obtaining positive results in all cases.

Keywords: Cocaine; Cocaethylene; Solid-phase microextraction; Gas chromatography–mass spectrometry

1. Introduction

Cocaine (COC) has become one of the most prominently abused drugs, and its illicit uses have prompted considerable interest in the development of methods for the detection of users and abusers of the drug. The concurrent use of COC and ethanol results in a biologically active molecule, cocaethylene (CE), which is nearly as psychoactive as COC but produces a more long lasting high [1–3]. Moreover, CE is even more toxic than COC, and its potency results in an increased risk of death due to overdose [4].

Currently, one of the most common biological specimens used for drug testing is plasma, in which the concentration of toxic drug is directly proportional to the individual's clinical state. This explains the interest in the development and optimisation of an analytical technique to detect COC and CE in plasma. Methods for the analyses of COC, CE and several of their metabolites and/or products are reported in the literature. These methods range from thin-layer chromatography (TLC) to high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) [5–17]. However, these methods involve multi-step extractions and/or derivatizations. Solid phase microextraction (SPME), introduced by Pawliszyn's group in 1990, offers some advantages against conventional techniques of extraction, such as simplicity, rapidity, less sample manipulation and solvent-free extraction. In fact, SPME has proven to be an important sample preparation technique when it is applied to forensic specimens [18–19]. SPME was chosen because it allows the sampling of small amounts of samples from aqueous medium and direct GC–MS analysis. Methodologies that make it possible to analyze cocaine as well as its major metabolites in plasma are highly important, and their development can provide the necessary elements for many studies, such as the distribution of cocaine and its derivatives in many organic tissues, in order to establish their interrelationship and influence on the diagnosis of acute intoxication.

So, the objective of this work was to propose a method to detect cocaine and cocaethylene (trans-esterification product of the coingestion of COC with ethanol) in plasma samples using solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS).

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2. Experimental

2.1. Material and methods

2.1.1. Reagents and standards

Methanol and acetonitrile from Merck® (Barcelona, Spain) were gradient grade solvents. Cocaine, cocaethylene and their respective deuterated-labelled analogues, cocaine-d3 and cocaethylene-d3, were purchased from Cerilliant® (Round Rock, TX, USA). Sodium hydroxide, sodium chloride, hydrochloric acid and boric acid were obtained from Merck® (Barcelona, Spain). Distilled water was processed through a Milli-Q water system (Millipore, Bedford, MA, USA).

2.1.2. Preparation of solutions

The borax buffer, pH 9, was prepared by mixing 12.37 g of H_3BO_3 with 100 mL of 1 N NaOH and making up to 1 L by mixing with distilled water. Then, 83.5 mL of this solution was mixed with 16.5 mL of 0.1 N HCl.

Working solutions of cocaine, cocaine-d3, cocaethylene and cocaethylene-d3 at a concentration of 0.01 mg/mL were prepared with acetonitrile in volumetric glassware. Stock solutions were stored at 4 °C when they were not in use.

2.1.3. Instrumentation

Solid-phase microextraction devices were obtained from Supelco® (Bellefonte, PA, USA) and equipped with 100 μm polydimethylsiloxane (PDMS) coating fiber.

Chromatographic analyses for COC and CE were performed using a gas chromatograph model 6890 interfaced to a mass selective detector (MSD) model 5973 from Hewlett-Packard® (Little Falls, DF, USA).

2.1.4. Analytical conditions

Chromatographic elution was done with a 12 m × 200 μm i.d. capillary column internally coated with 0.33 μm thick film of 5% phenylmethylsiloxane, purchased from Agilent Technologies® (Las Rozas, Spain). The injector temperature was 250 °C, and a purging time of 2 min was used. The temperature program was started at 90 °C (2 min), followed by a 30 °C/min ramp to 215 °C (5 min) and a second 30 °C/min ramp to 260 °C. Two ramps in the temperature program were chosen to accelerate the separation process. The mass selective detector was kept at 320 °C, the ion source at 250 °C and the quadrupole at 100 °C. Initially, neat standards of cocaine and cocaethylene (2 μL of a 0.01 mg/mL solution) were injected into a mixture and analyzed using the full scan mode of the GC/MS, which scanned from 50 to 550 amu. Quantifier and qualifier ions used for each analyte were selected on the basis of their abundance and mass-to-charge ratio (*m*/*z*). Because of their reproducibility and lack of interference, high mass ions were selected when possible. The ions selected for each compound studied were: *m*/*z* 182, 272, 303 (COC); *m*/*z* 196, 272, 317 (CE); *m*/*z* 185, 306 (COC-d3) and *m*/*z* 199, 320 (CE-d3). The underline ions were used for quantitation. Upon selection of ions, the MS was run in selected ion monitoring (SIM) mode.

2.1.5. Sample preparation

To carry out the calibration curves, drug-free plasma obtained from a local blood bank was used. At the laboratory, the blood was centrifuged for 10 min at 4000 rpm to separate the plasma from the other blood components. Forty microlitres of each deuterated internal standard (0.01 mg/mL) was added to 1 mL of plasma. Because they are dissolved in acetonitrile and it causes the precipitation of plasmatic proteins that interfere in the SPME process, plasma was centrifuged for 5 min to 12,000 rpm. Four hundred microlitres from this solution was taken, and 200 μL of borax buffer, pH 9, was added. To improve the extraction efficiency, 50 mg of sodium chloride was added and the mixture was shaken.

Furthermore, the fiber was introduced in this solution, and it was dipped for 25 min. The fiber was then placed in the injection port of the chromatograph for 5 min.

2.2. Validation of the method

The analytical validation of the method was performed by establishing selectivity, linearity, intra and inter-day precision, accuracy and limits of detection (LOD) as follows.

The selectivity of the method was demonstrated by analyzing ten blank plasma samples. Standard calibration curves were obtained in triple runs with the described method using drug-free control plasma spiked with standard solutions to obtain the concentrations of 25, 50, 100, 200, 400, 600, 800, and 1000 ng/mL for each compound. Quantitation was based on target peak area ratios of cocaine (*m*/*z* 182) and cocaethylene (*m*/*z* 196) to their internal standards (*m*/*z* 185 and *m*/*z* 199, respectively).

Precision and accuracy were determined by inter- and intra-day precision. Inter-day precision and accuracy were performed by analyzing negative human plasma samples spiked with cocaine, cocaethylene, cocaine-d3 and cocaethylene-d3 at three concentrations; the lower limit of quantitation (LLOQ), the upper limit of quantitation (ULOQ) and an intermediate level were assessed by analyzing five replicates each day for 5 days for each level of concentration.

Intra-day precision and accuracy were determined at three concentrations, 25, 200 and 800 ng/mL, by preparing and analyzing five replicates for each level on the same day.

Precision, expressed as the coefficient of variation (CV) of the measured values, was expected to be less than 15% at all concentrations, except for the LLOQ for which 20% was acceptable. In the same way, accuracy was evaluated using the mean relative error (MRE), which had to be less than 15% of the theoretical values at each concentration level except for the LLOQ, for which 20% was acceptable.

The sensitivity of the method was determined by calculation of the limit of detection (LOD) and the lower limit of quantitation (LLOQ). LOD was determined by an empirical method that consists of analyzing a series of plasma samples containing decreasing amounts of the analytes. LOD was the lowest concentration that presented a signal-to-noise ratio higher than 3 for at least three diagnostic ions for each substance. The LLOQ was the lowest concentration of analytes in a sample that can be determined quantitatively with appropiate precision and accuracy.

3. Results and discussion

Liquid–liquid extraction is a widely used and generally accepted sample preparation method for a large variety of applications [20,21]. Recently, SPME gained considerable interest in a broad field of analysis including drug-abuse area. The majority of examples of the use of microextraction for drug analysis reported till date, employ SPME fiber with GC analysis. The method was originally thought to be applicable only to forensic samples where concentrations of analytes are quite high. More recently, however, methods have appeared for the analysis of drugs at therapeutic concentrations. As techniques and coatings continue to improve, it is likely that more trace analysis at successively lower levels will be possible. Cocaine, which is a naturally occurring alkaloid and stimulant, has been analyzed from spiked urine samples by Kumazawa et al. [17,25] using direct immersion SPME with a PDMS fiber and GC-NPD. Strano-Rossi and Chiarotti described the application of solid-phase microextraction to cannabis testing in hair, and they propose that their method is also suitable for the analysis of methadone, cocaine and cocaethylene [22].

Drugs bound to plasma proteins in varying degrees depending on their individual physicochemical properties. In general, acidic and neutral drugs bound primarily to albumin, and basic drugs primarily to α-acid glycoprotein. The nature of the bond between the protein and the drug molecule is a determining factor in the molecule metabolism. The bond can be an ester or an amide. In the case of cocaine and cocaethylene, the bond is an amino ester. Only free drugs are available for extravascular distribution and elimination, are able to cross cellular membranes and interact with drug receptors. Although it is a general practice to report total drug concentration (free and protein-bound) in serum, SPME is ideally suited for measuring free drug (therapeutically relevant) concentration, as the protein binding equilibrium is not disturbed when an insignificant amount of free drug is removed in a microextraction [18]. The proposed method did not test benzoyl ecgonine (main metabolite of cocaine) because it is not a pharmacologically active metabolite. The possibility of "in vitro" production of this compound was avoided by storing plasma in adequate conditions (NaF, 4 °C).

SPME as an extraction method was chosen because it presents some advantages such as simplicity, rapidity, less sample manipulation and solvent-free extraction which produces less residues.

In this method, a little amount of sample (400 μL) was needed, which permits to make use of the remaining amount for other analysis, using other or the same extraction procedure.

According to Pawliszyn [23], PDMS fibers were employed for the extraction of analytes of medium and low polarity, and the coating thickness of 100 μm was selected against 30 μm because it provides shorter equilibrium times for unvolatile analytes, such as cocaine and cocaethylene. In our case, pH of the extraction mixture was controlled with a buffer, and then more drug could be extracted by an absorptive fiber coating than that extracted from an extraction mixture where the pH is not buffered. An alkaline pH was necessary to extract both substances, and borax buffer at pH 8, 8.5, 9 and 9.5 was checked. pH 9 showed the best performance in terms of recovery and fiber life. The addition of a salt can often improve the response in SPME [18]. Several kinds of salts, such as sodium sulphate, potassium carbonate and sodium chloride were used by other authors [15,16,24,25]. In our case, three salts were tried (KCl, $NaSO_4$ and NaCl), and sodium chloride proved to be the most suitable salt for detection of cocaine and cocaethylene because it allows to obtain higher abundances because of salting-out effects. Adsorption times of 15, 20, 25 and 30 min were checked. Twenty-five minutes was set as the most suitable adsorption time. This choice was made on the relative abundance of the drug and its deuterated analogue. Absorption times less than 25 min provided minor abundances. The increase of time did not cause a significant increase of the abundance, and on the contrary, made the extraction process longer.

In the consulted literature, it had not been possible to find any recent paper in which cocaine and cocaethylene were extracted from plasma by SPME. However, a comparison was made between this method and other similar cases that use different extraction techniques. Wang et al. [16] analyze cocaine, heroin and metabolites in several biological matrix including plasma. In this paper, the extraction step is a SPE of about 30 min (which is more laborious than the extraction described in this paper) and the eluate is evaporated. The same problem can be found in the analysis of Caufield et al. [26] which uses a complex SPE procedure.

With respect to LLE, SPME offers some advantages. One is that if we compare the chromatograms obtained using SPME or LLE, we can see the superiority of SPME. Normally in LLE chromatogram, several matrix peaks elute in the retention time range of the analytes. With SPME, we obtained slight matrix interference.

In other consulted publications using SPME, such as the one from Pereira de Toledo et al. [19], the authors have used SPME to extract cocaine benzoyl ecgonine and cocaethylene; the results show an absortion time of 20 min, but this paper showed a desorbtion time 15 min higher than the one that we have used.

Figs. 1 and 2 show typical chromatograms obtained for a blank and a spiked sample with 25 mg/mL for cocaine and cocaethylene, respectively. Fig. 1 shows no significant interferences of any peak appearing at the expected retention times for the analytes (cocaine: 8.5 min and cocaethylene: 9.1 min), demonstrating a good selectivity of the proposed method. Fig. 2

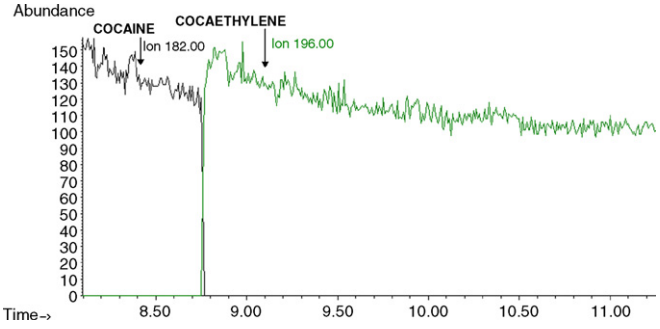

Fig. 1. Chromatogram for a blank plasma sample.

Table 1
Limits of detection, limits of quantitation and calibration results of cocaine and cocaethylene

Compound	LOD	LLOQ	Linearity	Slope standard error	Intercept standard error	R
Cocaine	19	25	$y = -0.066 + 2.934\,x$	0.055	0.028	0.992
Cocaethylene	11	25	$y = 0.075 + 2.757\,x$	0.050	0.026	0.996

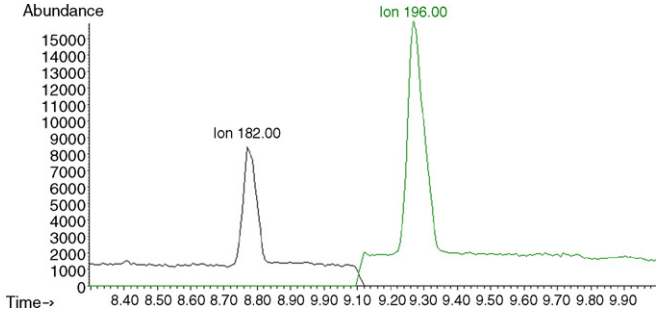

Fig. 2. Chromatogram for a plasma sample spiked with cocaine and cocaethylene (25 ng/mL).

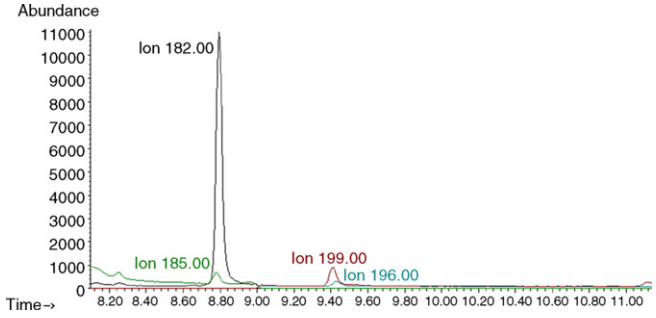

Fig. 3. Chromatogram of a real sample (cocaine: 136.92 ng/mL and cocaethylene: 41.89 ng/mL).

Table 2
Inter-day parameters ($n = 5$)

Compound	Concentration (ng/mL)	CV (%)	Mean relative error (%)
Cocaine	25	17.93	8.91
	400	0.24	12.86
	1000	0.12	0.62
Cocaethylene	25	19.17	17.40
	400	12.23	6.60
	1000	0.77	2.60

shows a correct separation of cocaine and cocaethylene during the chromatographic process.

The calibration plots were linear for the considered drugs over the specific range (25–1000 ng/mL). A simple linear regression analysis was performed. The LODs, LOQs and calibration results are detailed in Table 1. The limits of detection were 19 ng/mL for cocaine and 11 ng/mL for cocaethylene, and the lower limit of quantitation was 25 ng/mL for cocaine and cocaethylene. The confidence parameters of the validated method (inter- and intra-day precision and accuracy) for the determination of the studied drugs are shown in Tables 2 and 3.

The analytical procedure proposed for the determination of cocaine and cocaethylene in plasma showed to be highly precise with the use of the respective deuterated internal standards. Good sensitivity and linearity were also obtained for both analytes.

Finally, the developed method was used to analyze 10 real plasma samples that were collected from drug abusers. All ten human plasma samples that were tested contained cocaine and cocaethylene in various concentrations. The mean concentrations found were 166.8 ng/mL (range 50.17–635.4 ng/mL) for cocaine and 96.53 ng/mL (range 25.39–596.1 ng/mL) for cocaethylene. A representative chromatogram of a real sample is presented in Fig. 3.

Table 3
Intra-day parameters ($n = 5$)

Compound	Concentration (ng/mL)	CV (%)	Mean relative error (%)
Cocaine	25	14.4	7.35
	200	2.68	0.15
	800	1.49	1.17
Cocaethylene	25	16.3	9.23
	200	3.63	3.34
	800	1.15	0.97

4. Conclusion

The application of SPME followed by GC–MS for the determination of cocaine and cocaethylene in plasma was tested and successfully applied to the analysis of plasma samples from drug abusers. The use of the SPME turned out to be a substantially simpler and faster procedure than the conventional sample processing, and GC–MS was found to be specific, sensitive and selective enough for determining the low drug concentrations to be expected in plasma. So, the method has the sensitivity and the selectivity requirements of clinical and forensic toxicology.

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