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Article

Development of a Powdered Activated Carbon in Bar Adsorptive Micro-Extraction for the Analysis of Morphine and Codeine in Human Urine

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In the present work, bar adsorptive microextraction using an activated carbon (AC) adsorbent phase followed by liquid desorption and high-performance liquid chromatography with diode array detection was developed to monitor morphine (MOR) and codeine (COD) in human urine. Under optimized experimental conditions, assays performed in aqueous media spiked at the 30.0 μg/L level yielded recoveries of 41.3 ± 1.3% for MOR and 38.4 ± 1.7% for COD, respectively. The textural and surface chemistry properties of the AC phase were also correlated with the analytical data for a better understanding of the overall enrichment process. The analytical performance showed good precision (relative standard deviation < 8.0%), suitable detection limits (0.90 and 0.06 μg/L for MOR and COD, respectively) and convenient linear dynamic ranges ($r^2 > 0.991$) from 10.0 to 330.0 μg/L. By using the standard addition methodology, the applications of this analytical approach to water and urine matrices allowed remarkable performance to monitor MOR and COD at the trace level. This new confirmatory method proved to be a suitable alternative to other sorptive micro-extraction methodologies in monitoring trace levels of opiate-related compounds, because it was easy to implement, reliable, sensitive and required a low sample volume.

Introduction

The consumption of drugs of abuse, such as heroin (3,6-diacetylmorphine), remains a major social issue. Opiate-dependent individuals are often depressed, and suicide is 14 times as frequent in this group as in the general population. In addition to the serious toxicological and social risks for users, there is also a great risk for non-users when present in certain circumstances. In traffic or in the workplace, people under the influence of drugs of abuse can cause severe damage (1). Heroin intoxication has a well-recognized toxicity syndrome involving depression of the central nervous and respiratory systems and pupil constriction. The use of heroin can be proved by detecting the presence of 6-monoacetylmorphine (6-MAM), the heroin-specific metabolite, on several biological fluids, namely urine. However, 6-MAM can only be detected in urine by sensitive techniques for approximately 2–8 h after the heroin consumption. Many laboratories involved in screening drugs of abuse lack the ability to identify the presence of 6-MAM in urine specimens. Therefore, to confirm heroin abuse, the metabolites of 6-MAM and acetylcodeine (a by-product of illicit heroin manufacture), i.e., morphine (MOR) and codeine (COD), respectively, are usually screened (2). The increasing availability of drugs of abuse has resulted in a growing demand for rapid and universal screening methods, such as antibody-based assays for their analysis in biological fluids, combined with fast and easily accessible data interpretation procedures (3). However, in recent years, numerous confirmatory methods have been developed for the analysis of the opiate related compounds, including liquid–liquid extraction (4), solid-phase extraction (5–7) and more recently, solid-phase microextraction (SPME) (8, 9) with derivatization before chromatographic analysis. Currently, gas chromatography and high-performance liquid chromatography (HPLC) or hyphenated to mass spectrometry, are the most common techniques for the determination of heroin metabolites in different biological matrices, including blood, human hair, saliva and urine (10–14). Nevertheless, almost all the enrichment techniques usually employed are time-consuming and require considerable amounts of toxic organic solvents, which make them neither high throughput nor environmental friendly. In recent years, stir bar sorptive extraction (SBSE) has been successfully employed as a novel sample preparation technique based on the same principles as those of SPME (15). For enrichment and sensitive determination of priority organic compounds in water or in other matrices, SBSE is particularly useful (16–18). In SBSE, the commercial stir bars are just coated with 24–126 μL of polydimethylsiloxane (PDMS), a nonpolar polymeric phase that makes this enrichment technique unique in particular for compounds having nonpolar characteristics (log $K_{O/W} > 3$). Nevertheless, when polar compounds (log $K_{O/W} \leq 3$) such as MOR and COD are envisaged, a derivatization step has to be performed before the enrichment step to enhance selectivity and sensitivity (19). To overcome this limitation, our group has recently proposed novel polymeric phases based on polyurethane foams for stir bar coating (20–23), as well as novel analytical approaches, such as adsorptive microextraction techniques (AμE) (24), using convenient sorbent materials such as activated carbons (ACs) for enrichment purposes of the more polar metabolites. For the latter methodology in particular, and because ACs are amorphous solids, composed essentially by carbon and presenting high adsorption capacities, they can be prepared from carbon-rich raw materials. Depending on the preparation methodology used and the precursor, it is possible to tailor the pore size distribution and surface chemistry to

meet the requirements of a given application (25). The acidic/basic nature of the AC surface depends on the content of heteroatoms as, for instance, oxygen, hydrogen and nitrogen, correspond usually to a small percentage of the total AC composition. These heteroatoms form surface species that strongly influence the material adsorptive properties in liquid phase (25), which is often ideal to retain organic compounds with higher polar characteristics, as recently proved in water analysis (26, 27). Nevertheless, the potentialities of these analytical approaches for the enrichment of target analytes at trace levels from biological matrices prior to chemical analysis have not yet been explored. To our knowledge, only in very few studies is a correlation made between the analytical results and the AC characteristics (28).

The present contribution aims at the development, optimization and application of bar adsorptive microextraction using commercial AC as adsorbent phase followed by liquid desorption and high-performance liquid chromatography with diode array detection (BAμE(AC)-LD/HPLC–DAD) for the analysis of MOR and COD in aqueous media. The performance of the proposed methodology was evaluated in terms of accuracy, precision, linearity and detection limits, correlated with textural and surface chemistry properties of the solid materials. The application to urine matrices and the comparison of the data obtained by BAμE(AC) with SBSE(PDMS) is also addressed.

Materials and Methods

Chemicals and standards

All reagents and solvents were of analytical grade and used with no further purification. HPLC-grade methanol (MeOH, 99.9%) and acetonitrile (ACN, 99.9%) were purchased from Sigma-Aldrich (Germany). Sodium chloride (NaCl, 99.9%) and sodium hydroxide (NaOH, 98.0%) were obtained from AnalaR (BDH Chemicals, Poole, UK). *o*-Phosphoric acid (85.0%) was purchased from Aldrich (Germany). Sodium hydrogen phosphate (99.0%) and sodium hydrogen carbonate were purchased from Panreac (Spain). Ultra-pure water was obtained from Milli-Q water purification systems (Millipore, Bedford, MA). The AC (lot 01930), hydrochloric acid (37.0%) and sodium carbonate (99.5–100%) were purchased from Riedel-de Haën (Germany). MOR and COD in methanolic solutions with concentrations of 1,000 mg/L were supplied by Instituto de Desporto de Portugal (Portugal).

Preparation of solutions and samples

The 30 mM phosphate buffer (pH 6.25) was prepared weekly by dissolution of sodium hydrophosphate in ultra-pure water at the desired concentrations. The pH was adjusted with *o*-phosphoric acid and checked periodically using a Metrohm 744 pH Meter (Switzerland). Urine samples were collected in the morning from a healthy 15-year-old male and were filtered before use (Whatman No 1 filters, UK) followed by ultrasonic treatment (Branson, ultrasonic cleaner, model 3510 E-DTH) for 15 min. The stock solutions, working standards and samples were wrapped in aluminium foil and stored at −4°C.

Experimental setup

AC characterization

The ash content of the AC was estimated by the mass residue left after the combustion of the samples in air, according to the procedure described by Mestre *et al.* (28). Briefly, approximately 1 g of dried AC was heated in a horizontal furnace equipped with a Eurotherm 2416 controller, from ambient to 500°C in 10 min, kept for 30 min and then raised to 815°C in 15 min and kept for 2 h 30 min. The ash content (mean of three assays) is the amount of the sample that remains after this treatment, expressed by dried mass of AC. Mass weights were determined in a Mettler AE 240 analytical balance. The characterization on the surface chemistry was made by measuring the pH at the point of zero charge (pH_{PZC}) of the sample through reverse mass titration, following the method proposed by Noh *et al.* (29). Slurries of 1, 2, 6 and 10% were prepared by mixing the AC with ultra-pure water in a glass bottle, bubbled and sealed under nitrogen (to eliminate carbon dioxide). The pH of the slurry was measured after shaking for at least 24 h at room temperature. The pH_{PZC} value corresponds to the plateau of the curve of equilibrium pH versus solid weight fraction. The textural characterization was made by the nitrogen adsorption isotherm at −196°C and determined in a conventional volumetric apparatus equipped with an MKS-Baratron (310BHS-1000) pressure transducer (0-133 kPa). Before the isotherm acquisition, the sample (≈50 mg) was outgassed for 2 h at 300°C, under a vacuum better than 10^{-2} Pa.

Recovery assays and method validation

The μ-extraction bars were prepared in house by coating a polyethylene bar using a adhesive film (15 mm length and 0.5 mm thickness), which was covered with AC powder (approximately 1.5 mg). The μ-extraction bars were previously cleaned by treatment with MeOH followed by ultra-pure water before use. The detailed description of the manufacturing devices has previously been published (24).

In a typical assay, 30 mL of ultra-pure water (pH 7, 25°C) spiked with MOR and COD working standards at different concentrations (10.0–330.0 μg/L), a μ-extraction bar device and a conventional Teflon magnetic bar were introduced into glass sampling flasks (30 mL, Macherey-Nagel, Germany). For the optimization of the BAμE(AC) efficiency, assays were performed in a fifteen agitation point plate (Variomag H + P Labortechnik AG Multipoint 15, Germany) at room temperature (25°C). Parameters such as agitation speed (750, 1,000 and 1,250 rpm), equilibrium time (1.5, 2.5, 3.5 and 16.5 h), pH (3, 5, 7 and 10), organic modifier (MeOH; 5, 10 and 15%, v/v) and ionic strength (NaCl; 5, 10 and 15%, w/v) were systematically studied in triplicate. To evaluate the best LD conditions, several assays using MeOH, ACN and equal volumes (1:1) of both as back-extraction solvents and different desorption times (15, 30 and 60 min) were performed in triplicate. For back-extraction, the μ-extraction bars were removed from the samples with clean tweezes, dried with a lint-free tissue, placed into a 2-mL amber vial (Chromacol, UK) containing 1.5 mL of the stripping solvent, ensuring their total immersion prior to ultrasonic treatment at a constant temperature (25°C). After back-extraction, the

μ-extraction bars were removed with clean tweezes, the stripping solvent was evaporated to dryness under a gentle stream of nitrogen (>99.5 %) followed by reconstitution with 200 μL of HPLC mobile phase. The vials were then sealed and placed on the auto-sampler for HPLC–DAD analysis. For validation experiments, 30 mL of ultra-pure water were spiked with 200 μL of both working standards at desired concentrations, and the extraction and back-extraction experiments were performed as described previously under optimized conditions. For comparison purposes, assays with commercial stir bars (Twister, Gerstel, Germany) coated with PDMS (20 mm length and 0.5 mm film thickness, 47 μL) were performed under optimized experimental conditions. Before use, the stir bars were cleaned by treatment with ACN. For real sample assays, 1 mL of urine was diluted to 30 mL with ultra-pure water, spiked with working standards at the desired concentrations (10.0–330.0 μg/L) and performed by using the standard addition methodology (SAM) to suppress matrix effects. Blank assays were also performed using the procedure described previously without spiking.

HPLC-DAD settings

Analyses were carried out on an Agilent 1100 Series LC system (Agilent Technologies, Germany), constituted by the following modules: vacuum degasser (G1322A), quaternary pump (G1311A), autosampler (G1313A), thermostatted column compartment (G1316A) and DAD (G1315B). The data acquisition and instrumental control were performed by the software LC3D ChemStation [version Rev.A.10.02 (1757), Agilent Technologies]. Analyses were performed on a Tracer excel 120 ODS-A column, 150 × 4.0 mm, 5 μm particle size (Teknokroma, Spain). The mobile phase consists of a mixture of ACN and 30 mM phosphate buffer (pH 6.52) with ratio of 20/80% (25°C) and a flow rate of 1.0 mL/min. The injection volume was 20 μL with a draw speed of 200 μL/min. The detector was set at 220 nm. For identification purposes, standard addition was used by spiking the samples with pure standards, as well as by comparing the retention parameters and peak purity with the ultraviolet-visible (UV-vis) spectral reference data. For recovery calculations, peak areas obtained from each assay were compared with the peak areas of standard controls used for spiking. For quantification purposes on real matrices, calibration plots using the SAM were also performed, using peak height whenever lack of resolution was observed.

Table I
Ash Content, pH_{PZC}, Specific Surface Area, Micro, Meso, and Total Porous Volumes and Mean Micropore Width Observed in the AC Used in the Present Study

Parameter	Value
Ash (%)	0.7
pH_{PZC}	6.50
Specific surface area (m^2/g)	937
Total porous volume* (cm^3/g)	0.65
Mesoporous volume† (cm^3/g)	0.25
Microporous volume (cm^3/g)	0.40
Mean micropore width (nm)	1.38

*Volume adsorbed at $p/p^\circ = 0.95$.
†Difference between total porous volume and microporous volume.

Results and Discussion

Textural and surface chemistry characterization of the AC

The results of ash content and textural and surface chemistry characterization assays carried out on the commercial AC are presented in Table I. The AC has very low ash content and its pH_{PZC} value indicates that it has a slightly acidic surface. In fact, at pH_{PZC} the number of positive and negative surface groups is equal and thus total surface charge becomes zero, so the net surface charge of the studied AC is positive when the solution pH is below 6.50 and negative for higher pH values. The analysis of the nitrogen adsorption isotherm (data not shown) reveals the microporous (pore width < 2 nm) nature of the AC, associated with a mesoporous (2 nm < pore width < 50 nm) structure. The specific surface area obtained applying the BET equation (30), for values between $0.05 < p/p^\circ < 0.15$, is in the range of the data reported for this type of adsorbents (25). For a quantitative assessment of the sample microporosity, the results were analyzed by the Dubinin-Radushkevich equation (30). The corresponding volume of the total porosity was taken from the volume of nitrogen adsorbed at $p/p^\circ = 0.95$ and the mesoporous volume obtained by the difference between the total and microporous volumes. The data in Table I confirm the conclusions of the isotherm shape analysis because estimated microporous volume corresponds to approximately 60% of the total volume. The mean micropore width, 1.38 nm, estimated using the empirical equation proposed by Dubinin and Stoeckli (31) allow us to admit that the target molecules would not have steric restrictions to access to the porous structure of this AC (Figure 1). Moreover, the mesopore size distribution (data not shown) is very broad, revealing that the solid has pores with openings in the entire range of the mesopores, which favors the diffusivity of MOR and COD to the inner porosity. Considering the molecular dimensions of the target compounds, estimated from crystallographic data (32), presented in Figure 1, and the mean micropore width, 1.38 nm, estimated using the empirical equation proposed by Dubinin and Stoeckli (31), one can admit that MOR and COD would not have steric restrictions to access to the porous structure of the carbon.

Instrumental conditions

In a first approach, the HPLC–DAD conditions were evaluated, including the UV-vis spectral data for the detection of MOR and COD, as well as suitable retention times. In agreement with literature (7), the wavelength (λ_{max}) of 220 nm was selected because it maximizes the response of both heroin metabolites for DAD. By combining a conventional reversed-phase column with a mobile phase constituted of ACN and 30 mM phosphate buffer (20/80 %, pH 6.52, 25°C), a good response was obtained by HPLC–DAD, showing suitable resolution within convenient analytical time (< 6 min). The instrumental sensitivity was checked through the limits of detection (LODs) and quantification (LOQs) for both heroin metabolites, obtained by the injection of diluted calibration standards and calculated with a signal-to-noise ratio (S/N) of 3/1 and 10/1, respectively. Values of 15.0 and 24.0 μg/L for LODs, an 50.0 and 81.0 μg/L for LOQs were measured for MOR

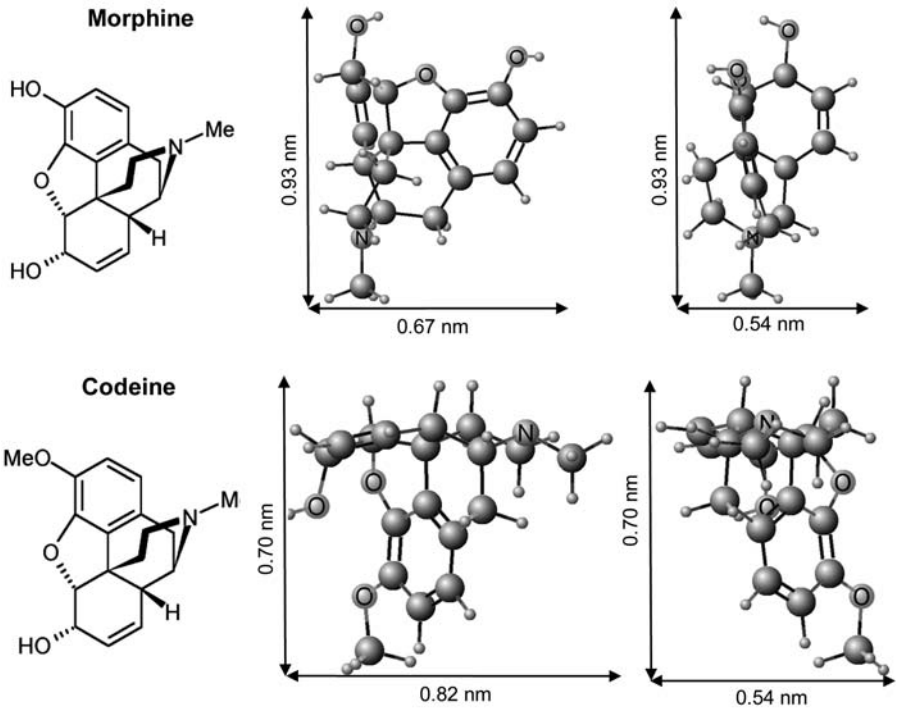

Figure 1. Molecular structures of morphine (MOR) and codeine (COD): general structures and arrangements of atoms in space presenting molecular dimensions estimated from crystallographic data (32).

and COD, respectively. Subsequently, instrumental calibration was performed with six standard solutions with concentrations ranging from 1.0 to 10.0 mg/L. From these data, excellent linear dynamic responses were observed for MOR and COD metabolites with correlation coefficients higher than 0.9995. To evaluate the instrumental precision, repeated injections for each calibration level were carried out, resulting in relative standard deviations (RSD) below 1.2 %. Finally, no carry-over was observed by series of replicate injections because the background was always below the LODs achieved.

Optimization of the BAμE(AC)-LD assays

Throughout the present work, several parameters affecting the extraction and back-extraction efficiency yields were evaluated. Therefore, systematic assays were performed in a univariate way to optimize parameters that are known to influence this analytical process, such as, equilibrium time, agitation speed, matrix characteristics (pH, polarity and ionic strength) and LD conditions (15–19).

In a first approach, the LD conditions that ensure that complete back-extraction for both heroin metabolites from the μ-extraction bars were optimized. Solvents such as MeOH, ACN and a mixture with equal volumes of both were assayed to survey the desorption performance, followed by solvent switch, more suitable for HPLC–DAD analysis. Figure 2A depicts the data obtained using standard conditions (equilibrium time: 2.5 h (1,000 rpm) and desorption time: 0.5 h), in which the mixture of MeOH/ACN (1:1) was selected as back-extraction solvent, because it presents the highest ability to desorb both analytes from the μ-extraction bars. After the selection of the most effective solvent for back-extraction, desorption times of 15, 30 and 45 min were assayed. A slight increment on the back-extraction efficiency was observed for 0.5 h and no advantages were obtained for longer periods. Because the evaporation step is essential for solvent switch, it is necessary to carefully check for possible analyte losses during this process. As expected, the data showed that negligible losses of MOR and COD occur during the evaporation step once MOR and COD are non-volatile compounds. Furthermore, no carry-over was observed by a series of replicate desorptions for which the background was always below the LODs achieved.

In analogy to SBSE theory (15), equilibrium time and agitation speed are very important parameters to be optimized to achieve better steady state conditions by BAμE(AC). The stirring rate can have a great influence on the mass transfer process of both heroin metabolites towards the AC phase during the equilibrium process. Therefore, three stirring levels (750, 1,000 and 1,250 rpm) were assayed, and according to the data obtained (Figure 2B), 1,000 rpm of agitation speed leads the higher recovery yields for both analytes under the experimental conditions. Furthermore, it seems that COD is less sensitive to variations of experimental parameters, unlike MOR. Subsequently, the extraction time was evaluated by carrying out experiments within 1.5 and 16.5 h. As illustrated in Figure 2C, the recovery yields for MOR reach the maximum equilibrium after 2.5 h and then decrease, whereas for COD, after 2.5 h the extraction efficiency does not change with the time, showing that this system was basically at a steady state and no advantages were observed after this period of time. Therefore, the extraction time was set at 2.5 h for further experiments. According to previous works (15–19), the matrix characteristics, i.e., pH, ionic strength and polarity, are also important parameters that significantly affect the extraction

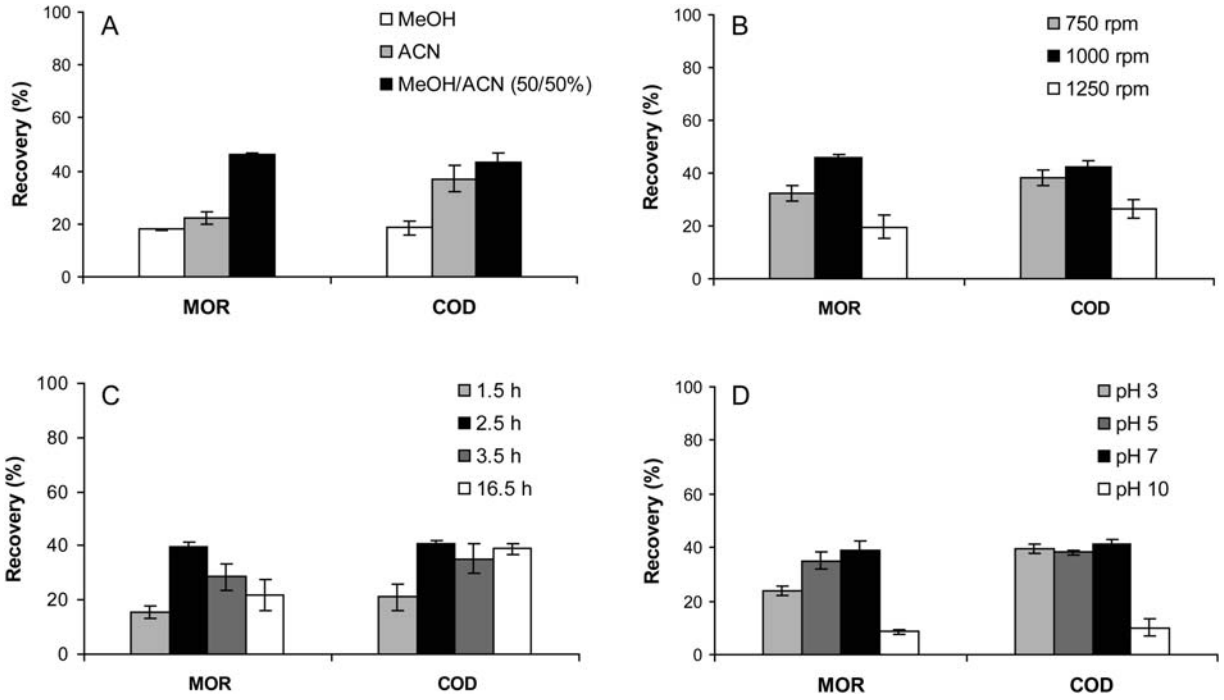

Figure 2. Effect of LD solvent (A), agitation speed (B), equilibrium time (C) and pH (D) on the recovery yields of MOR and COD by BAμE(AC)-LD/HPLC-DAD.

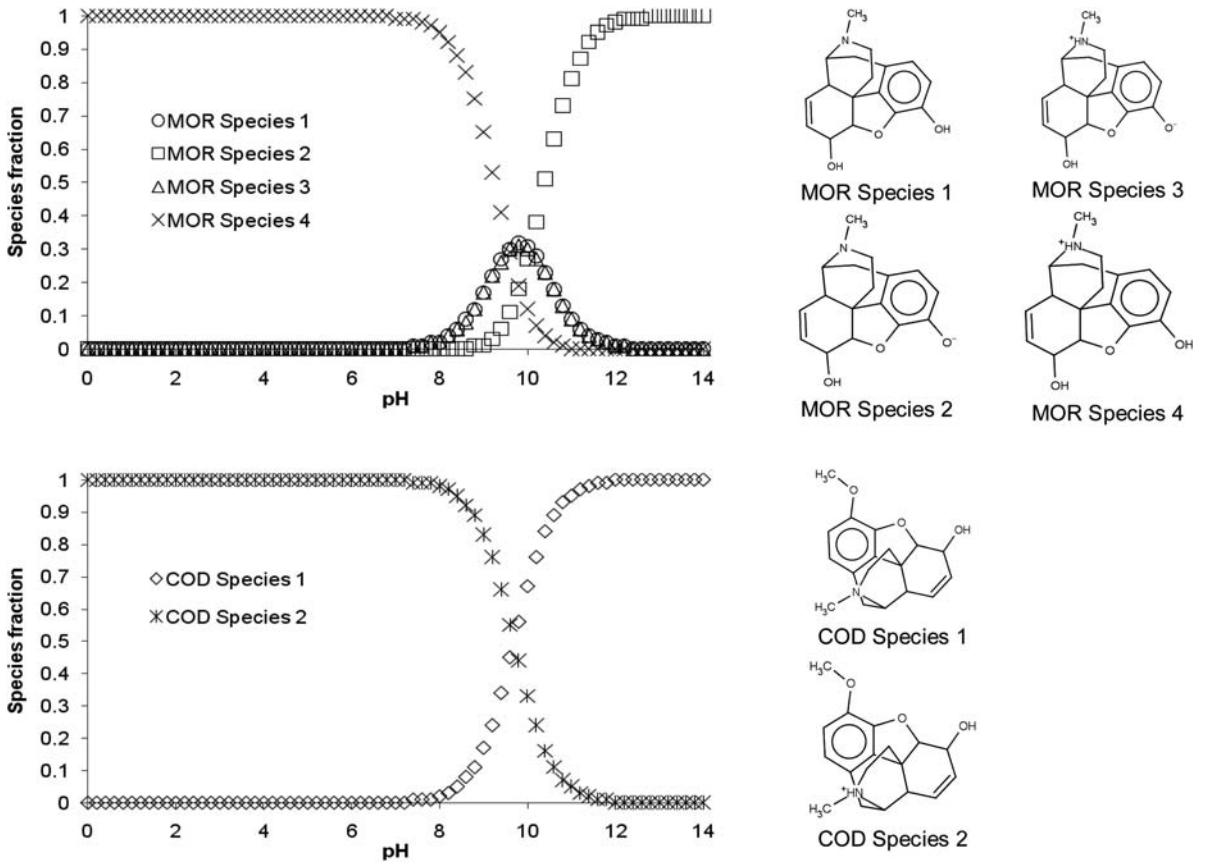

Figure 3. Speciation plots of MOR and COD against the solution pH (33).

efficiency. For the particular case of MOR and COD, the pH plays an important role because these metabolites are ionizable compounds. Figure 3 depicts the speciation plots for MOR and COD against solution pH using the SPARC (Sparc performs automated reasoning in chemistry) approach (33), showing that the aqueous media of the former is a much more complex system than the latter. Actually, in the pH range at which more than one species are present in the solution ($8 < pH < 12$), MOR presents four species with different net charges, while COD presents only two, a neutral and a positively charged. In addition, it is also well known that pH plays an important role in the adsorption process of AC because the net surface charge depends on the pH solution. In Figure 2D the results of pH effect on the recovery yields of both analytes are displayed for four different pHs (3, 5, 7 and 10) tested at room temperature. The pH effect profile shows that for COD, the recoveries are almost the same for pHs ranging from 3 to 7. Nevertheless, MOR has a different profile, because an increment of the recovery is observed until pH 7. Also, both compounds present identical recovery that yields at pH 7 and a considerable decrease is observed for the assayed alkaline pH. To justify this behavior, we can admit that at pH 10 the studied AC has a negative net surface charge and a particular interaction with positively charged species will be strong enough to enable the desorption during the back-extraction process. The ionic strength and polarity were modified through the addition of NaCl and MeOH (5, 10 and 15%) onto matrix media, respectively. A progressive addition of NaCl or MeOH significantly reduces the recovery yield of both MOR and COD (data not shown). According to the matrix modification assays performed, it was demonstrated that the addition of NaCl or MeOH is disadvantageous.

Validation of the BAμE(AC)-LD/HPLC–DAD method

After optimizing the primary parameters that affect the recovery efficiency, we proceed to method validation. The linear dynamic ranges of the present methodology, under the optimized experimental conditions [equilibrium time: 2.5 h (1,000 rpm; pH_{PZC}); LD: MeOH/ACN (1:1), 0.5 h], were evaluated on 30 mL of water samples spiked with analytical standards. The concentration range (10.0 to 330.0 μg/L) was chosen because the minimal concentration that defines a positive-opiate analysis in triage exams is usually 300 μg/L and the minimal concentration requested in toxicological confirmation assays is 80 μg/L (3). According to the validation parameters, an excellent linearity, with remarkable correlation coefficients ($r^2 > 0.991$) was observed. Additionally, the precision achieved for the present methodology, using within-day and between-day repeatability assays calculated as RSD on three replicates, gave rise to variations lower than 8.0%. Furthermore, the sensitivity of the methodology was also verified through the LOD and LOQ achieved for both compounds and measured with S/N of 3/1 and 10/1, respectively. The values attained for MOR and COD were 0.90 and 0.06 μg/L and 2.90 and 0.20 μg/L for LODs and LOQs, respectively. No carryover was observed by series of replicates, for which the background was always below the achieved LODs. Table II summarizes the experimental recoveries, linear dynamic ranges and determination coefficients (r^2), LOD, LOQ and repeatability data for MOR and COD in aqueous media under optimized experimental conditions attained by the present methodology. To demonstrate the advantages of the proposed analytical approach by comparing with other sorptive microextraction techniques, several assays at the 30.0 μg/L level were performed by BAμE(AC) and SBSE(PDMS) under the optimized experimental conditions. The data obtained by the latter seems to be consistent with the theoretical predictions (15), in which no detection (n.d.) was observed for both heroin metabolites in aqueous media (Table II), due the high polarity they exhibited ($\log K_{O/W} = 0.70$), estimated according to a fragment constant estimation methodology (34). This observation reinforced the unsuitability of PDMS polymeric phase for compounds with these polarity characteristics. On the other hand, the proposed methodology shows the ability to recover both analytes, in which average recoveries yielded $41.3 \pm 1.3\%$ and $38.4 \pm 1.7\%$ for MOR and COD, respectively (Table II).

Table II
Estimated log $K_{O/W}$, LODs, LOQs, Correlation Coefficients (r^2), Average Recoveries Obtained for Both Heroin Metabolites in Aqueous Media by BAμE(AC) and SBSE(PDMS) Followed by LD/HPLC–DAD Analysis Under Optimized Experimental Conditions

Heroin Metabolites	Linear range* (μg/L)	LOD (μg/L)†	LOQ (μg/L)‡	r^2	Average recovery§ (% ± RSD; n = 3)	
					BAμE(AC)	SBSE(PDMS)
MOR ($\log K_{O/W} = 0.70$)**	10.0–330.0	0.90	2.90	0.996	41.3 ± 1.3	n.d.
COD ($\log K_{O/W} = 0.70$)**	10.0–330.0	0.06	0.20	0.991	38.4 ± 1.7	n.d.

*Five levels of concentration: conditions as described in the "Experimental" section.
†S/N >10.
§Assays at the 30.0 μg/L level.
**According to Meylan (34).

Application to real matrices

To evaluate the applicability of the proposed methodology to real matrices, assays on biological fluids, i.e., human urine, were performed. To account for intrinsic contamination and particular pronounced matrix effects, SAM is always recommended. In a first approach, the matrix was fortified with four working standards to produce the corresponding spiking levels (10.0–330.0 μg/L) for both analytes under study. Blank assays (zero-point) were also performed without spiking to assure maximum control of the analytical methodology. Preliminary blank assays on urine matrices showed MOR and COD contents below the LODs achieved for the present methodology, under optimized experimental conditions. The results obtained from the assays performed by the SAM using the proposed methodology present a convenient linear dynamic range ($r^2 > 0.991$). Figure 4 shows chromatogram profiles obtained from the application of the proposed methodology using the SAM under optimized experimental conditions. The proposed methodology presents remarkable performance in water media (Figure 4A), although some fluctuation and complexity is noticed when urine matrices are involved (Figure 4B). Therefore, although the present methodology has proven to be a suitable analytical tool to monitor heroin metabolites at trace level, the

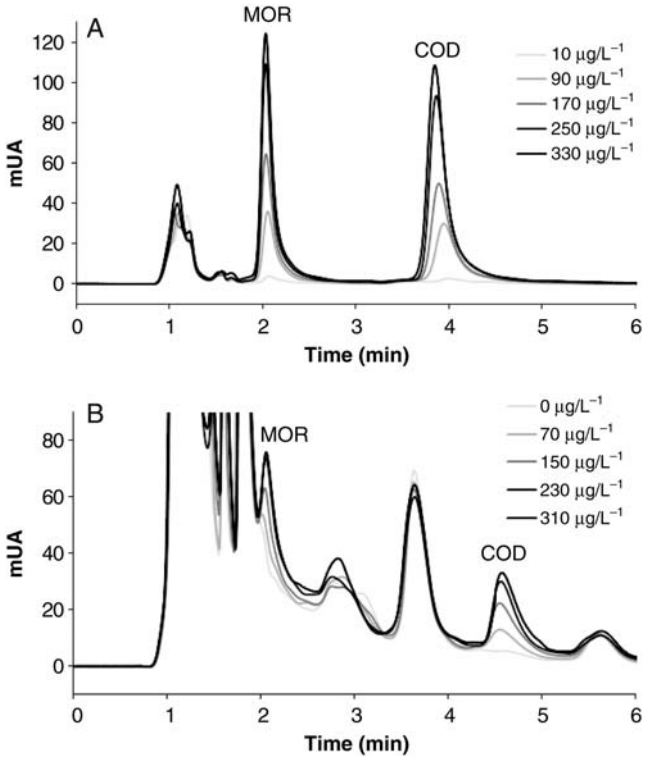

Figure 4. Chromatograms profile from spiked water (A) and urine (B) matrices using the SAM for different levels, obtained by BAμE(AC)-LD/HPLC-DAD, under optimized experimental conditions.

performance can be further improved by using HPLC coupled to mass spectrometry or tandem systems (LC–MS or LC–MS/MS) to achieve better analytical selectivity and sensitivity. Even so, low traces of MOR and COD could be selectivity detected, which make this analytical approach a novel confirmatory method for the analysis of opiate-related metabolites.

Conclusions

The methodology proposed in the present work (BAμE(AC)-LD/HPLC–DAD) was successfully applied to determine traces of MOR and COD in aqueous media. By using a commercial AC as sorbent phase under optimized experimental conditions, good accuracy, suitable precision, convenient linear dynamic ranges and low detection limits were achieved. The obtained analytical data were correlated with the textural and surface properties of the AC to gain a better understanding of the overall enrichment process. The application of the present methodology to monitor traces of heroin metabolites in water and urine matrices provided very good performance through the standard addition methodology.

The proposed method also demonstrated to be easy to use, selective and sensitive, and to require little sample requirement. This new confirmatory method has proved to be a convenient alternative to monitor trace levels of opiate-related metabolites in comparison with other dedicated sorptive microextraction techniques.

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