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A combination of single-drop microextraction and open tubular capillary electrochromatography with carbon nanotubes as stationary phase for the determination of low concentration of illicit drugs in horse urine

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ABSTRACT

In this study we developed an interesting alternative to HPLC–mass spectrometry for the quantification of seven important drugs of abuse in racehorses. The procedure proposed in this work is a combination of single-drop microextraction (SDME) and an open tubular capillary electrochromatography (OT-CEC) using multi-wall carbon nanotubes (MWCTs) immobilized into a fused-silica capillary as a stationary phase. The SDME showed to be a powerful tool for extraction/preconcentration of the seven drugs analyzed in the study, showing an enrichment factor between 38- and 102-fold depending on the drug. We have investigated the electrophoretic features of MWCTs immobilized fused-silica capillary by covalent modification of the inner surface of the capillary. The results show a good run-to-run, day-to-day and capillary-to-capillary reproducibility of the method. Compared with the capillary zone electrophoresis (CZE), the coating of the capillary allowed the separation of the analytes with high resolution, with less band-broadening and without distortion of the baseline. The interactions between the analytes and the MWCTs resulted in an increased migration time and probably this was the reason of the front tailing effect. The results showed a good capillary efficiencies and an improved of the electrophoretic separation.

1. Introduction

The definition of doping is the administration of illegal drugs to animals in competition with the intention of altering their physical performance, in either positive or negative sense [1]. For horse races is a requirement that the horse should not be declared as a participant if in their tissues, body fluids or excretions there is any prohibited substances or its metabolite, even if any substance that although has an endogenous origin it is in higher levels than the normal. The prohibit drugs in racehorses are classifying in five different groups related to their effect in the animal by the Association of Racing Commissioners International (ARCI). Alkaloids are a group of complex nitrogen-containing compounds derived from a variety of sources, including microbes, marine organisms and plants. Strychnine, morphine, cocaine and ephedrine are classified as “Class 1” substance. This class of drugs has a highest potential to affect racing performance and they are not used as treatment in any common suffering. Moreover, caffeine and theophylline are classifying as “Class 2”. Into this group are including psychotropic compounds, stimulants and depressants of the nervous and cardiovascular system, local anesthetic and neuromuscular blockers. Finally, piroxicam is a non-steroidal anti-inflammatory drug (NSAID) which has potent anti-inflammatory, analgesic and antipyretic properties. This drug is part of the “Class 4” substance; this group includes diuretics, steroids, anabolic steroids, corticosteroids, expectorants, mucolytic, hemostatic, cardiac glycoside antiarrhythmics, topical anesthetics, and non-steroidal anti-inflammatory.

Several chromatographic and electrophoretic techniques have been used for the determination of illicit drugs. GC, which is often used in many forensic applications, presents some limitations due to the analysis of highly polar compounds such as amphetamines and morphine require a derivatization process before its determination [2,3]. For this reason, HPLC and, especially, HPLC–mass spectrometry (MS) are the current separation techniques mostly used in this area, which can analyze polar, thermally labile and non-volatile compounds [4–8]. The most important disadvantage of this methods is the amount of reagent and sample that it needs for each analysis. Capillary electrochromatography (CEC) is a relatively new miniaturized separation technique that combines the advantages of the high speed and efficiency of CE with the high selectivity and high sample loading [9]. Open tubular capillary electrochromatography

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(OT-CEC) have important advantages over packed capillary column. Among them we can name easy preparation without the requirement of a frit, variety and availability of surface for modification and immobilization procedures, no bubble formation and the possibility to work with small internal diameters capillaries in the range 2–25 μm to facilitate an efficient solute diffusion to the stationary phase surface. Tsuda et al. [10] develop for the first time an OT-CEC method and since then, novel materials have been used as a stationary phase. The rapid progress in nanotechnology has opened a wide range of horizons for its applications in capillary electrophoresis, due to their surface-to-volume ratio as well as their applicability in miniaturization. A stationary phase with large surface area in combination with an electro-osmotic flow-driven system has great potential in a highly efficient separation system [11]. Carbon nanotubes have been the object of intense research due to their physical, mechanical and chemical properties and their applications have been proposed in several analytical chemistry fields. Their high thermal and mechanical stability as well as high surface area available for chemical interactions confers to carbon nanotubes a great analytical potential [12], particularly in term of retention [13]. In more recent developments, the application of carbon nanotubes was evaluated in separation techniques because they present similar properties to porous graphitic carbon (PGC). The use of nanoparticles as stationary phase in CEC has recently been proposed [14,15]. The incorporation of carboxylic multi-walled carbon nanotubes (MWCTs) as stationary phase resulted in the addition of nanoscale interactions that enhanced sensitivity and resolution of the complex mixture.

The biological fluids samples are especially contaminated samples, these contaminants may be absorbed into the capillary electrochromatography (CEC) column and interfered with the analysis. That is the reason why most of this type of sample should be treated before they are injected into the OT-CEC. Several techniques that have been published previously [16–19] for biofluids sample preparation for the detection of drugs by CEC method. During the past decade, an important improvement in the development of sample preparation techniques has been the miniaturization of the liquid–liquid extraction method [20,21]. This single-drop microextraction (SDME) method provides an excellent clean-up of the samples [22,23]. Two main approaches can be used to perform single-drop microextractions. In direct single-drop microextraction, a drop of a water-immiscible solvent is suspended directly from the tip of a microsyringe needle immersed in the aqueous sample. The second approach a drop of extracting phase is suspended directly from the tip of a microsyringe needle in the headspaces of the vial, which contain the sample. This extraction approach, combined with GC or HPLC, has been demonstrating to be quite effective for extraction and analysis of various organic compounds [24].

The aim of the present study was to demonstrate the facility of OT-CEC method to separate seven illicit drugs. Special attention was paid to the selection of the stationary phase due to the well-known difficulties in the separation of basic compounds by CEC [25]. We also demonstrate that the combination of a SDME and OT-CEC not only result as an excellent combination to obtain a low limits of detection using a little amount of sample and solvents but it is also a cheap alternative to HPLC–MS and GC–MS. The combination of the extraction and separation techniques proposed in this paper was applied to horse urine to demonstrate its simplicity and sensitivity.

2. Materials and methods

2.1. Instrumentation

A Beckman P/ACE MDQ instrument (Beckman Instruments, Fullerton, CA, USA) equipped with a diode array detector and a data handling system comprising an IBM PC and P/ACE System MDQ Software (ESANCO) was used. The fused silica capillaries were obtained from MicroSolv Technology Corporation and had the following dimensions: 57 cm total length, 50 cm effective length, 50 mm ID, and 375 mm OD. The pH of the electrolyte was measured by an Orion 940 pH meter equipped with a glass combined electrode. All the glass instruments used were previously washed with a 10% (v/v) HNO_3/water solution and then with ultrapure water.

2.2. Chemicals and reagents

MWCTs with diameters between 110 and 170 nm and length in the range 5–9 μm, 1,3-dicyclohexylcarbodiimide, 3-aminopropyl triethoxy silane (APTS), and all drugs (ephedrine, cocaine, strychnine, morphine, caffeine, theophylline, and piroxicam) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Anhydrous acetone, sulphuric acid (98%), nitric acid (60%), boric acid and dimethylformamide (DMF) were supplied by Merck (Buenos Aires, Argentina).

2.3. Standards preparation

The seven drugs, ephedrine (EP), cocaine (CO), strychnine (ST), morphine (MO), caffeine (CF), theophylline (TP), piroxicam (PI), were dissolved in water to prepare stock solutions (1 mg mL^{-1}, 100 mg/L) and stored at 4 °C. All stock solutions were mixed and diluted to give suitable concentrations for CEC analysis.

2.4. Single-drop microextraction

The urine for five different horses was collected before a horse race. One of these samples, which not contain any drug, was used as a control. The samples were placed immediately at −20 °C. The SDME was carried out as follows: 10 mL of sample solution were placed in a sample vial and its pH was adjusted. Then, 5 μL of extracting solution (chloroform/methanol, 90:10) was then inserted into the headspace of the sample solution with needle of the syringe. The syringe plunger was depressed and a microdrop of extracting phase was suspended from the needle tip. After the stirring optimized time, the plunger was withdrawn and the microdrop was retracted back into the syringe. The needle was removed from the headspace and its content was introduced into a CE vial, the content of the vial was evaporated to dryness and the residue was reconstituted with 5 μL of running buffer for subsequent analysis.

2.5. Preparation of carboxylic multi-wall nanotubes

To attach the MWCTs to the wall of the capillary they must be activated, which means that they have to be oxidized. Carboxylic MWCTs were prepared by adding 20 mg of MWCTs to 80 mL of H_2SO_4:HNO_3 (3:1). This mixture was ultrasonicated (50 W, 60 Hz) during 5 h, diluted with water (60 mL) and filtered through a 0.45 μm cellulose acetate filter. Finally, carboxylic MWCTs were washed and left to dry at room temperature [26].

The immobilization of the carboxylic MWCTs in the wall of the capillary was carried out according to Sombra et al. [27] with modifications. The most important steps during the immobilization are: (i) modification of the surface of the capillary by a covalent interaction between silanized bare fused silica and APTS in order to get amino groups in the wall of the capillary, and (ii) oxidation of the MWCTs to obtain carboxylic groups on the surface, capable to react with APTS functional groups. The complete procedure was performed on line as following; the capillary was rinsed with 1 M of NaOH for 30 min, followed by Milli-Q water (5 min). Then, it was flushing with a solution of 2% (v/v) APTS prepared in anhydrous acetone during 15 min, followed by anhydrous acetone

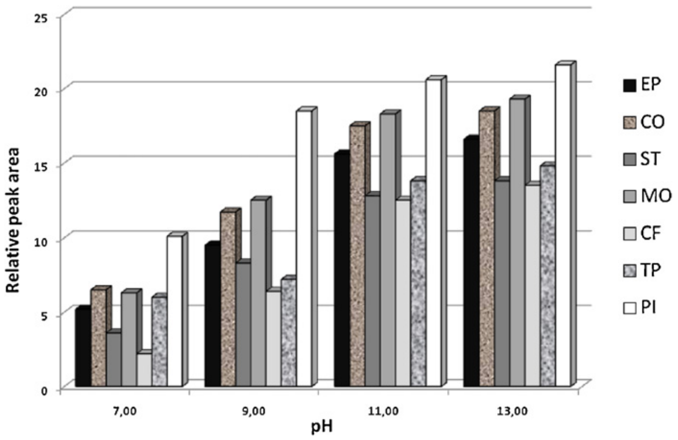

Fig. 1. Effect of sample pH on the extraction efficiency. Extraction conditions: time 10 min, temperature 25 °C, extracting phase chloroform/methanol, 90:10. Running conditions: 20 mmol L^{-1} sodium tetraborate; applied voltage, 15 kV; temperature, 25 °C; injection 0.5 psi during 10 s, detection at 214 nm.

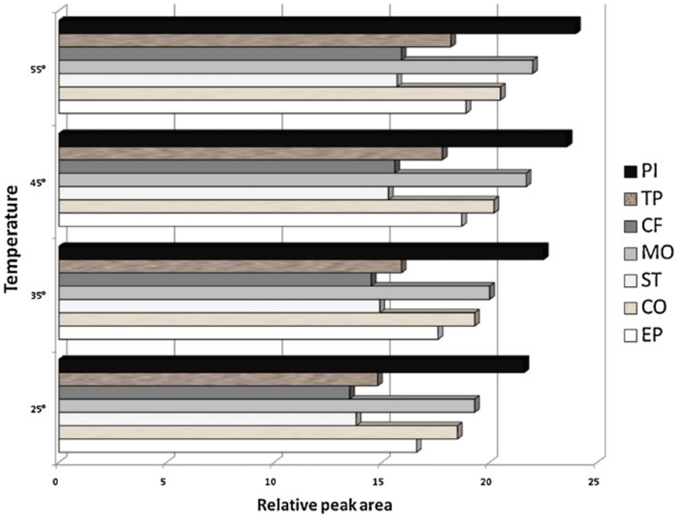

Fig. 2. Effect of sample temperature on the extraction efficiency. Extraction conditions: time 10 min, extracting phase chloroform/methanol, 90:10. Running conditions: 20 mmol L^{-1} sodium tetraborate; applied voltage, 15 kV; temperature, 25 °C; injection 0.5 psi during 10 s, detection at 214 nm.

(5 min) to eliminate the excess of APTS. Finally, a carboxylic MWCTs solution (5 mg mL^{-1}) dissolved in a DMF containing 0.5 mg of 1,3-dicyclohexylcarbodiimide was passed through the capillary for 1 h and rinsed with water to remove the unimmobilized carboxylic MWCTs.

2.6. LC/MS–MS

LC/MS–MS was used as a validation method. The LC was equipped with an ODS column (100 mm length, 2.1 mm i.d., 3.5 μm particle size), and the separation was carried out at constant temperature (40 °C). The mobile phase was composed by phosphate buffer 20 mmol L^{-1} (pH = 300)/acetonitrile (20:80, v/v). The column was flushed for 4 min with acetonitrile and finally re-equilibrated mobile phase for 4 min. The flow rate was set at 0.8 mL min^{-1}. Multiple reactions monitoring (MRM) mode was used to detect the seven analytes in positive ionization mode. Product ion scan experiments were performed employing collision-induced dissociation (CID) with nitrogen as the collision gas at 5.33e−3 Pa (obtained from a CMC nitrogen generator, Eschborn, Germany). The spray voltage was set at 4.0 kV and the ion source was operated at 330 °C. Nitrogen was also used as nebulizing and drying gas, and the pressure was set at 35 psi.

3. Results and discussion

3.1. Optimization of SDME

All the SDME experiments were performed with 10 mL of sample solution and 5 μL of extracting phase. The basic drugs studied in this paper were: EP ($pK_a = 9.36$), CO ($pK_a = 8.5$), ST ($pK_a = 9.7$), MO ($pK_a = 7.9$), CF ($pK_a = 10.4$), TP ($pK_a = 8.8$), and PI ($pK_a = 6.3$). The extracting phase was developed following the procedure using by group of Tanaka et al. [28]. The better extraction was observed with chloroform/methanol, 90:10 (data not showed). The pH of the sample solution is a very important parameter in headspace SDME and its effect was examined in a range between 7.0 and 13.0 at room temperature for 10 min. Standard solutions of all the studied drugs were added in horse urine which not contains any drug. Three independent experiments for each pH value were carried out. The results obtained for this studied are show in Fig. 1. The pH range with the higher extraction was 11–13, so the further experiments were performed at pH = 11. The reason why the better extraction of this group of drugs was at high pHs was that they were basic drugs, but another important factor which affected the extraction was the different solubility of the drugs in water and organic solvents. Even piroxicam which had the lowest pK_a the extraction was better in acidic pHs. This phenomenon was mostly due to its high solubility in organic solvents.

The second factor considered was the effect of the temperature and the results are show in Fig. 2. As can be seen, the extraction capacity gradually increases with temperature due to the increasing distribution constant of analyte between the sample and extracting phase. However, the evaporation of the organic phase was observed when the extraction temperature was set higher than about 30–35 °C. Furthermore, when extraction temperature was set at 35 °C or higher, the microdrop became unstable and tended to detach from the needle tip. Although the maximum peak areas were obtained at 45 and 55 °C, room temperature was adopted to ensure a stable, more reproducible and simple operation without decreasing significantly the sensitivity of the method.

SDME is an equilibrium-based technique and there is a direct relationship between the amount extracted and the microdrop exposure time in the sample headspace. Extraction time effect was studied in the range of 5–30 min at 25 °C. A plot of relative peak area versus extraction time (Fig. 3) shows that drugs amount extracted increases with sampling time and reaches the equilibrium after 20 min, and then stays constant until 30 min. Therefore, the incubation time was setting at 20 min for further studies. It is well known that the agitation enhance the mass transfer of the compounds from the sample solution to the extracting solution, this eventually leads to a shorter equilibrium time and consequently a lower extraction time. Thus, the last factor optimized was the velocity of the stirring; this feature was studied in the range of 200–800 rpm. The best extraction was obtained in the rage of 500–800 rpm, but the study was performed at 500 as the drop became unstable above 600 rpm.

3.2. Studies of the capillary surface

The electrophoretic behavior of analytes in CEC depends on the EOF, the chemical nature of the analytes and the chromatographic retention due to partitioning of the analytes between the electrolyte and the stationary phase. To evaluate all the steps of the modifications of the capillary wall, the behavior of the EOF were studied and the different stages were compared. The electrophoretic behavior was evaluated as a function of the pH

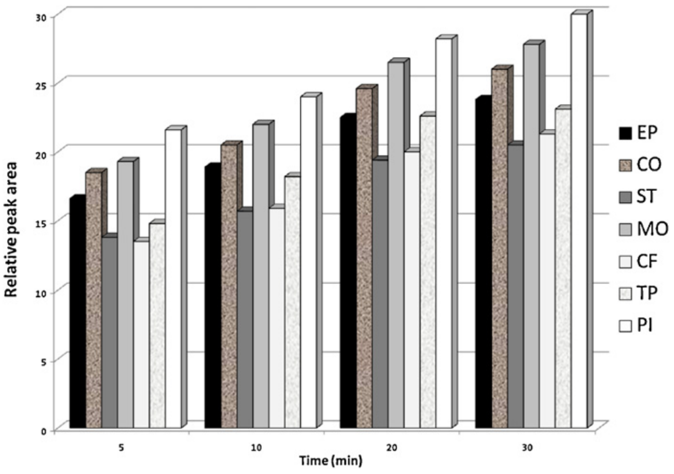

Fig. 3. Effect of time of stirring on the extraction efficiency. Extraction conditions: temperature 25 °C, extracting phase chloroform/methanol, 90:10. Running conditions: 20 mmol L^{-1} sodium tetraborate; applied voltage, 15 kV; temperature, 25 °C; injection 0.5 psi during 10 s, detection at 214 nm.

within the interval 6–10 and Fig. 4 shows the variation of the electro-osmotic mobility, μ_{eo} (10^{-4} cm^2 s^{-1} V^{-1}) for the bare capillary, the amino-silylated and the MWCTs immobilized capillary. The EOF increased with increasing pH values for the bare capillary as well as for the immobilized capillary, though the mobility is higher on the modified capillary, this increasing in the μ_{eo} can be explained due to the large area of the MWCTs in the surface of the capillary as well as the high amount of negative charges, product of the carboxylic groups of the MWCTs on the inner wall of the capillary. In contrast, a reversed EOF was obtained with the amino-silylated capillary; this is the result of positives charges in the wall of the capillary.

3.3. Development of the separation conditions

The optimization of the experimental conditions has been accomplished by the traditional method of one-at-a-time. The peak areas and migration times were used to evaluate the extraction efficiency, and separation/quantification efficacy under different experimental conditions. Sodium tetraborate was chosen as the BGE solution considering selectivity, reproducibility, baseline and current performance of the method. The concentration and the pH of the BGE were also evaluated considering the resolution of the studied drugs. The concentration of the BGE was studied between 10 and 30 mmol L^{-1} and a good resolution was archived from 20 to 30 mmol L^{-1} of sodium tetraborate (Fig. 5a). However, we did not observe appreciable improvements of buffer concentrations above 20 mmol L^{-1}. The effect of the pH on the resolution is shown in Fig. 5b and 9.0 was the optimum pH to carry out the following studies with high resolution. Finally, the background electrophoretic buffer (BGE) used was 20 mmol L^{-1} sodium tetraborate, pH 9. The samples were injected hydrodynamically at 0.5 psi for 10 s and the temperature of the capillary was set up at 25 °C. The separation was performed applying 15 kV and the wavelength was fixed at 214 nm. Between runs and after the immobilization of the MWCTs, the capillary was washed sequentially at 20 psi with Milli-Q water (2 min) and the electrophoretic buffer (2 min). Under these conditions all the analytes studied were baseline resolved within 25 min.

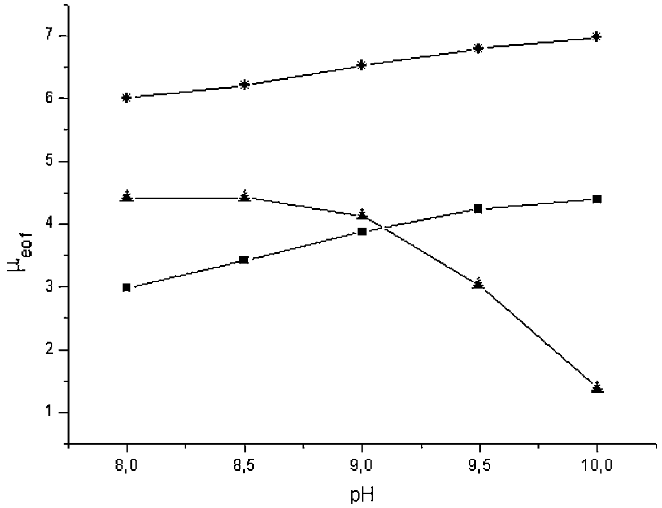

Fig. 4. Variation of electro-osmotic flow mobility, μ_{eof} (10^{-4} cm^2 s^{-1} V^{-1}) measured using DMSO as a marker with pH. (*) Bare capillary, (■) c-MWNT immobilized capillary and (▲) amino-silylated fused silica capillary. Running conditions: 20 mmol L^{-1} sodium tetraborate; applied voltage, 15 kV; temperature, 25 °C; injection 0.5 psi during 10 s, detection at 254 nm.

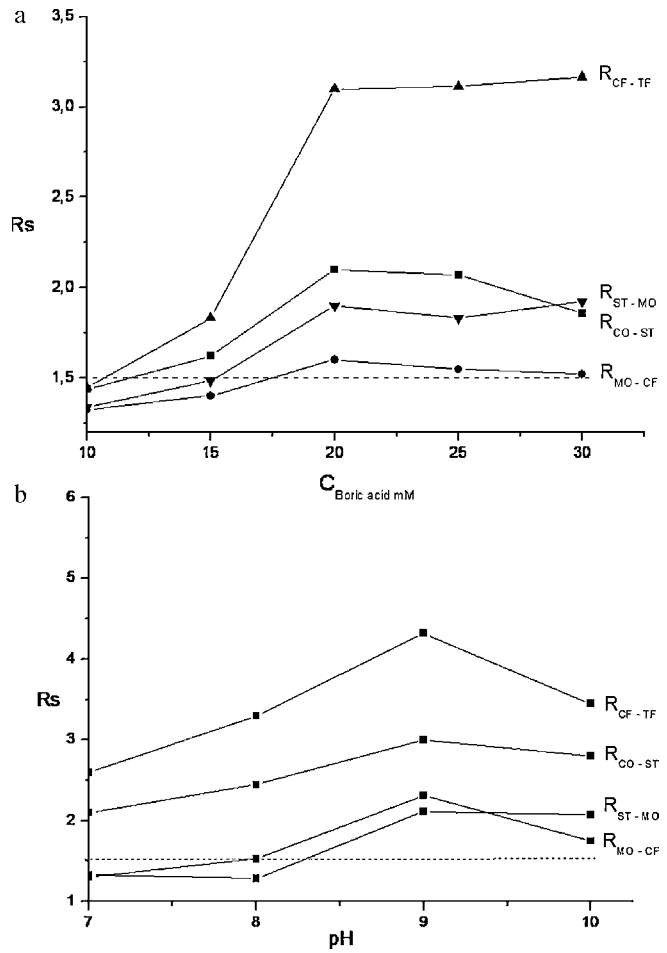

Fig. 5. Resolution values (*Rs*) obtained for caffeine and theophylline (RCF-TF), cocaine and strychnine (RCO-ST), strychnine and morphine (RST-MO) and morphine and caffeine (RMO-CF) as function of the (a) boric acid concentration and (b) pH in the BGE. The concentration of the illicit drugs was 10 mg/L. The dotted line indicated the threshold value for the baseline separation.

The separation obtained with the OT-CEC was compared with the one obtained with CZE under the same electrophoretic conditions. Fig. 6 shows the electropherograms of a standard solution contains the seven drugs analyzed by OT-CEC (A) and by CZE (B). The coating of the capillary with MWCTs allowed the separation of the analytes with high resolution, with less band-broadening and without distortion of the baseline. The interactions between

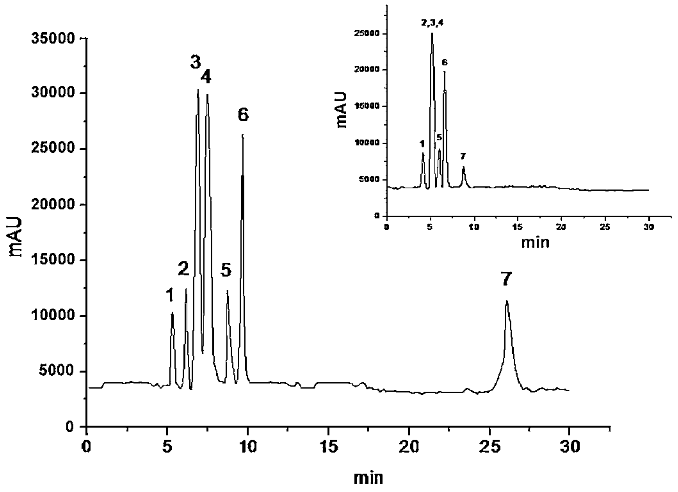

Figure 6. Comparison of the electrophoretic behavior of a mixture of 1: ephedrine (EP), t_r 5.3 min; 2: cocaine (CO), t_r 6.1 min; 3: strychnine (ST), t_r 6.9 min; 4: morphine (MO), t_r 7.5 min; 5: caffeine (CF), t_r 8.9 min; 6: theophylline (TP), t_r 9.6 min; 7: piroxicam (PI), t_r 26.1 min using a capillary with the MWNT immobilized in the wall (a) and bare capillary (b). Running conditions: 20 mmol L^{-1} sodium tetraborate; applied voltage, 15 kV; temperature, 25 °C; injection 0.5 psi during 10 s, detection at 214 nm.

the analytes and the MWCTs resulted in an increase of the migration time. Regardless of carboxylic groups on the surface of MWCTs acted as electrons withdrawing groups localizing electron from π-system of MWCTs, which might be expected to interfere with and weaken π–π dispersion forces between the aromatic ring of the studied drugs and the graphitic structure of MWCTs. Moreover, the oxidation of the nanotubes generates a disruption in the electronic cloud. Previous studies on the adsorption of aromatics on the activated carbon suggested that surface oxygen complexes increased the affinity of water to activated carbon, which reduced the accessibility and affinity for aromatic adsorbates, and thus led to the lower adsorption capacity [29,30]. However, according to our results, it seems that some of the π–π interactions are still active since the molecules with smaller aromatic systems are less retained than those with more extensive systems. The best example in this study is the piroxicam, which was the most retained and has the highest π-system between the molecules that considered for this investigation. Moreover and as expected, we could not achieve a good separation of the analytes with a fused silica capillary.

Under the optimum conditions determined in the current work, seven abuse drugs could be separated in less than 25 min giving separation efficiencies of up to 46,972 (TP) average experimental electrophoretic plates (N).

3.4. Evaluation of the methodology

For the evaluation of the methodology all the assays were based on the use of horse urine spiked with the drugs. The limits of detection (LDs) and limits of quantification (LQs) were calculated as the analyte concentrations that give rise to peak heights with a signal-to-noise ratio of 3 and 10, respectively. Linear range, correlation coefficient, detection limits and quantification limits, under the optimum analysis conditions for CEC methodology, are list in Table 1. With the aim of validate the proposed method, Table 1 also shows the LQs obtained with LC/MS–MS method typically used to determinate the seven illicit drugs.

Repeatability studies were carried out at three assay concentrations (20, 40, 80 ng mL^{-1}) of all drugs added to a not doping horse urine. In order to determine the repeatability within-day precision of the method, replicate injections were carried out in the same day ($n = 6$). In all cases, the precision, expressed as RSD was better than 0.75% for the migration time and 2.83% for the corrected peak area (peak area/migration time). Repeatability between-day precision was also evaluated over 3 days by performing six injections each day. Reproducibility (RSD values) on the basis of migration time and corrected peak area were better than 1.25 and 4.32%, respectively. The MWCTs immobilized capillaries are stable at least for 50 injections of samples with negligible variation in the quantitative/qualitative parameters. The capillary-to-capillary was also evaluated and the RSD obtained for the migration times were between 1.93 and 2.35%. The enrichment factors for the SDME were calculated as the ratio of the concentration determinate in the extraction phase to the total concentration added before the extraction procedure and the results for a 10 mL of sample are shown in Fig. 7. These results show clearly that the extraction of the drugs which has more affinity for water (hydro soluble) was lower than those which have more affinity for non-polar solvents. Although, respect to the extracting phase methanol play an essential role related to the hydro soluble drugs.

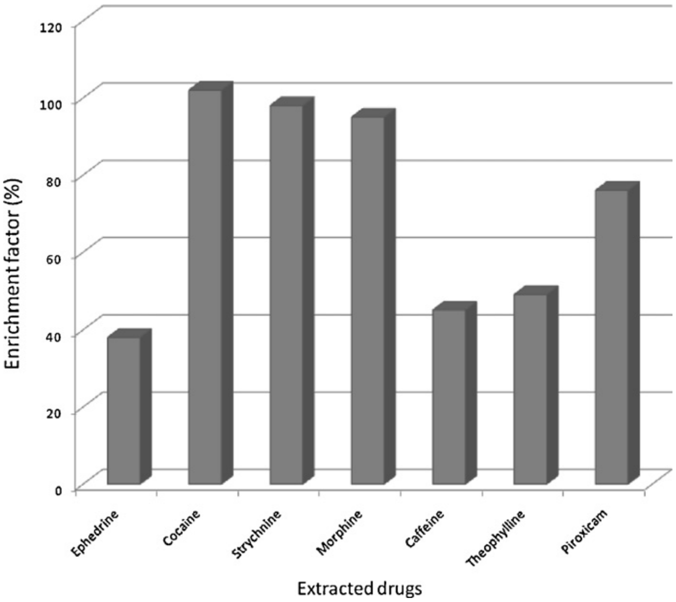

Fig. 7. Enrichment factor for the seven studied drugs obtained using SDME as a preconcentration/extraction technique.

3.5. Method accuracy

As a certified value for EP, CO, ST, MO, CF, TP and PI does not exist for any certified reference material of horse urine, the method of standard addition was considered as a validation method. Thus, the method was applied to the determination of horse urine spiked samples in order to validate it. About 100 mL of sample was collected and divided into ten portions of 10 mL each. Increasing quantities of the analyte were added to the aliquots of sample, and the content of each drug was determined. The results are summarized in Table 2.

3.6. Urine sample analysis

This simple OT-CEC method developed for rapid screening of abused drugs was applied to real samples of horse urine to demonstrate the potential of the combination of SDME and OT-CEC with MWCTs as a stationary phase. The concentrations measured were shown in Table 3. The measures were in good agreement with those of obtained by HPLC–MS.

Table 1
Validation of the electrochromatography method for the seven illicit drugs.

Studied drugs	Calibration range ($ng\,mL^{-1}$)	Regression coefficient of the calibration (r^2)	Proposed method LDs ($ng\,mL^{-1}$)	Proposed method LQs ($ng\,mL^{-1}$)	HPLC–MS method LQs ($ng\,mL^{-1}$)
Ephedrine	9.2–85	0.994	7.71	9.12	9.87
Cocaine	10.5–215	0.997	8.27	9.78	10.04
Strychnine	1.5–175	0.996	0.94	1.19	2.00
Morphine	6.5–95	0.995	5.12	5.98	6.05
Caffeine	11–350	0.996	9.07	10.73	9.83
Theophylline	2–105	0.999	1.32	2.02	1.85
Piroxicam	20–645	0.996	17.64	19.04	25.64

Table 2
Recovery study for the seven studied drugs.

Studied drugs	Recovery (%)[a]	±SD
EP	80.3	1.25
CO	103.1	2.14
ST	98.8	1.54
MO	108.3	2.03
CF	83.5	1.22
TO	87.5	1.3
PI	94.8	1.46

Concentration of the added drug. EP, CO and CF: 15, 30, 60 $ng\,mL^{-1}$. ST and TO: 5, 10, 20 $ng\,mL^{-1}$. MO: 10, 20, 40 $ng\,mL^{-1}$. PI: 25, 50, 100 $ng\,mL^{-1}$.

[a] [(Found-base)/added] × 100.

Table 3
Concentration of illicit drugs found it in four urine samples.

	Samples			
	M1	M2	M3	M4
EP ($ng\,mL^{-1}$)	–	17.6	–	84.6
CF ($ng\,mL^{-1}$)	113.2	–	–	–
MO ($ng\,mL^{-1}$)	–	–	26.2	–
ST ($ng\,mL^{-1}$)	–	–	–	62.4

4. Conclusions

The OT-CEC procedure developed here for the simultaneous analysis of seven doping racehorse drugs was precise, reproducible, and sensitive. The advantages of this method include simplicity with high selectivity, identification and quantification in the separation of the drugs besides to low cost and efficiency by using MWCTs as a stationary phase. The potential of OT-CEC method was shown performing the analysis of urine sample with the studied illicit drugs after a SDME procedure. This extraction method used as a sample pre-treatment enabled a good yield in the extraction with satisfactory precision and suitable purification from interferences. The main success of the approach is that the MWCTs stationary phase introduced new nanoscale interactions that resulted in an excellent electrochromatographic characteristics and high resolutions. Good run-to-run, day-to-day and capillary-to-capillary reproducibility have been obtained. In addition, the regeneration of the functionalized capillary allows its reusability for at least 6 months. The positive results obtained demonstrate that the coupling of these methods have a great potential to be applied in the toxicology field. It can be used as a comparative method to HPLC, owing to several advantages such as lower costs, minimal consumption of solvents and samples and short analysis time.

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