In-line Solid-phase Extraction-Capillary Zone Electrophoresis for the Determination of Barbiturate Drugs in Human Urine

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The abuse of barbiturate drugs is widespread, and the development of methods for their efficient separation and quantification is needed. Three barbiturate drugs were preconcentrated and determined by in-line solid-phase extraction (SPE) capillary electrophoresis (CE) in urine samples. Different parameters affecting preconcentration were evaluated, such as the sample pH, the volume of the elution plug and the sample injection time. This strategy enhanced the detection sensitivity in the range of 170- to 1840-fold, compared with normal hydrodynamic injection. The method provides limits of detection (LODs) for standard samples in the range of 0.5 to 5 ng/mL with good repeatability and reproducibility values. The LODs obtained for urine samples were in the range of 5 to 60 ng/mL. The validation with human urine samples spiked with the studied compounds demonstrated the applicability of the optimized method. This method provides a reproducible and sensitive analysis of urine samples in the determination of barbiturates drugs.

Keywords Barbiturates, drugs of abuse, capillary electrophoresis, in-line solid phase extraction, urine samples

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Introduction

Although growing interest has occurred in capillary electrophoresis (CE) as an analytical technique in recent years, its major drawback is related to the inherent low concentration sensitivity. In order to overcome this issue, there has been increasing interest in the development of several on-line preconcentration techniques in CE. Among them, solid-phase extraction (SPE) coupled to CE is a very promising technique because low limits of detection (LODs) can be achieved due to the high sample volume that can be injected. SPE can mainly be performed in four different setups: off-line, on-line, at-line and in-line. The latter three of these setups have been of particular interest due to an increasing trend towards fully automated analytical methods; among them, in-line coupling on SPE and CE is the most widely reported at present. In this setup, the preconcentration column is an integrated part of the CE system, and presents several advantages, such as a low volume of the organic solvent needed, easy automation, low requirement of sorbent material for constructing the SPE device, and its capability to analyze the complete eluate from SPE by CE.

Barbiturate drugs are typical sedative-hypnotic drugs and, depending on the substituting groups, exhibit a wide variety of responses in the body. In high doses, these kinds of drug depress the respiratory system, which accounts for their toxicity. Barbiturates are abused for recreational purposes and, in relatively low doses, they cause relaxation and sleepiness. Moreover, barbiturates may result in drug tolerance and dependence, and may display additive effects with other central nervous system depressants. Barbiturate drugs, like other drugs of abuse, are mainly excreted in urine in their original form. Therefore, this sample is highly suitable for the determination of these drugs in medical and forensic science.

The occurrence of these drugs in urine samples is variable with respect to the time after administration. In the analysis of biological samples in clinical cases of patients, the concentrations of these drugs have been reported in the range of 0.2 to 21 μg/mL. However, these values depend on the individual metabolism, and could be lower in combination with alcohol and/or amphetamines. These compounds have been determined in biological fluids by LC and GC. For example, Martin-Biosca et al. used micellar liquid chromatography for the determination of barbiturates in urine with direct injection of the sample with LODs ranging between 130 and 270 ng/mL. The potential of CE for the determination of barbiturates in biological samples has also been demonstrated by other authors. For example, Jiang et al. reported on the determination of barbiturates in urine by CE using a dynamically coated capillary with polycationic polymers. The LODs for standard samples were in the range of 870 to 3500 ng/mL. Wang et al. used a moving reaction boundary (MRB)-induced stacking procedure in combination with capillary zone electrophoresis (CZE) for the determination of barbital and phenobarbital in urine samples. In that case, the LODs were 270 ng/mL for barbital and 260 ng/mL for phenobarbital. More recently, lower LODs were achieved by different authors, who showed the potential of different preconcentration techniques in combination with CE for the determination of barbiturate compounds; in this sense Kadi et al. reported LODs of 16.8 ng/mL in rat urine samples using a method involving a sample stacking preconcentration induced by a reverse migrating pseudostationary phase (SRMP)

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technique. On the other hand, our research group showed the potential of an electrokinetic stacking procedure, in particular electrokinetic supercharging (EKS); the obtained LODs ranged from 8 to 15 ng/mL for human urine samples.

The principal aim of this work was the determination, for the first time, of a group of barbiturate drugs in urine samples by in-line SPE-CE-UV. The optimization of the in-line SPE-CE system in the determination of the studied barbiturates was performed by evaluating different parameters affecting the preconcentration factors. Thus, studies of the sample pH, the volume of the elution plug and the sample loading time were carried out. Urine samples from a healthy volunteer have been analysed with the proposed method.

Experimental

Standards and reagents

All reagents used were of analytical-reagent grade. Secobarbital, phenobarbital and barbital, sodium hydroxide (NaOH) and disodium tetraborate anhydrous were purchased from Sigma Aldrich (St. Louis, MO). Ultrapure reagent water were prepared by dissolving each compound in MeOH at a concentration of 1000 mg/L, and stored at 4°C. Working standard solutions of the mixture of all the compounds at a concentration of 10 μg/mL were prepared weekly by diluting the standard solutions in ultrapure water. Working solutions were prepared daily by diluting these solutions with an appropriate volume of MilliQ water. The separation buffer (background electrolyte (BGE)), which consisted on 20 mM sodium tetraborate (pH 9.2), was prepared by dissolving the appropriate amount of disodium tetraborate anhydrous in MilliQ water.

Instrumentation

The instrumentation used for electrophoretic separation was an Agilent 3D CE (Agilent Technologies, Waldbronn, Germany) equipped with UV diode-array detection (DAD). Detection of the barbiturates was performed at 214 nm. A fused-silica capillary having a length of 100 cm (91.5 cm effective length) and an internal diameter (ID) of 50 μm was used.

CZE

New capillaries were conditioned with 1 M NaOH for 40 min prior to use and daily with 1 M NaOH for 10 min and water for 10 min. Between separations, the capillary was conditioned with NaOH for 4 min, water for 4 min, and BGE for 4 min. Injections were performed by placing the sample in the inlet vial and applying 50 mbar for 10 s. A normal polarity of 30 kV was used for separating the drugs with UV detection.

In-line SPE-CE-UV procedure

The construction of the SPE device was performed in a similar way to a method described in a previous paper by our group. This consisted on cutting 2 mm of a bare fused-silica capillary of 150 μm ID (the anolyte concentrator (AC) device). Then, it was introduced 1 mm into a 0.5-cm piece of PTFE tubing (Grupo Taper S.A., Madrid, Spain) with an ID of 0.250 mm. Then, a 7.5-cm piece of the separation capillary was introduced at the other end of the PTFE tubing until connecting with the AC (inlet); the free end of this capillary of 7.5 cm was connected to a vacuum pump using a syringe. Subsequently the AC was introduced into a vial that contained the Oasis HLB sorbent (particle size higher than 50 μm); this was loaded into the AC device. Then, the 7.5-cm capillary and the AC were moved until the preconcentrator was located in the half-way position of the PTFE tubing. Finally, a CE separation capillary (92.5 cm) was introduced into the other part of the PTFE tubing until joining the other side of the AC (outlet). The entire process of fabricating the concentrator was monitored under a microscope. Finally, the assembly was installed in a CE cartridge, which was checked for abnormal flow by filling the whole capillary with water and applying a pressure of 930 mbar to a vial containing MeOH. For the capillary dimensions chosen, the time needed for MeOH to reach the detector was measured by monitoring the signal at 200 nm. This time was about 70 s in a properly constructed capillary (in-line SPE-CE), which is the same as that in a capillary without an in-line SPE device. Capillaries with this AC were conditioned daily, and between separations at 930 mbar with MeOH for 3 min and BGE for 4 min. The in-line SPE-CE-UV procedure shown in Fig. 1 was as follows: firstly, the sample (adjusted to pH 6 with a solution of 1 M of HCl) was loaded at 930 mbar for 60 min. The next step was a clean-up with the BGE solution at 930 mbar for 3 min in order to remove any unretained molecules, and to equilibrate the capillary prior to elution and separation. Then, for the elution step, a plug of MeOH was injected at 50 mbar for 40 s; this was subsequently pushed by the BGE solution at 50 mbar for 220 s. The normal polarity of 30 kV was used for separating the three analytes.

Human urine sample pretreatment

Urine samples were obtained from a healthy volunteer. The extraction procedure used was based on the existing literature with some modifications. Briefly, the procedure was as follows: before extracting the drugs from the samples, the pH value was adjusted to 4.0 with HCl 1 M. Spiked urine samples were prepared by adding a standard solution containing the analytes at different concentrations into 500 μL of blank urine in an Eppendorf tube. Sample pretreatment was carried out by adding 500 μL of ethyl acetate/n-hexane (40/60 v/v) to the spiked urine samples for extracting the barbiturates. After vortex mixing, the sample was centrifuged for 10 min at 9000 rpm. The organic phase containing the barbiturates was then transferred to another Eppendorf tube and a second extraction of the residual was performed by adding 500 μL of ethyl acetate/n-hexane (40/60 v/v) and repeating the procedure previously described. Finally, the two organic phases were combined and then evaporated to dryness under a gentle stream of dry nitrogen (UHP grade). The final residue was then dissolved with 500 μL 10⁻⁴ M HCl (final pH of 6). This solution was transferred to a micro-vial for in-line SPE-CE analysis.

Results and Discussion

Separation of barbiturates

The pKₐ values of the barbiturates studied are between 7 and 8. Since the barbiturates are negatively charged at basic pH values, separation of these compounds at positive voltages was performed under counter-EOF conditions. Different BGEs have been reported in the literature for the separation of barbiturate drugs in CE, with borate and Tris based buffers being the most commonly used. Hence, in order to optimize the separation, three different BGEs were tested. The electrolytes
studied were 20 mM Na₂B₄O₇ (pH 9.2), 150 mM Tris buffer (adjusted to pH 7.8 with 5 M HNO₃) and 50 mM ammonium acetate (adjusted to pH 9.7 with NH₄OH 32%). The latter of these electrolytes was considered due to the possibility of coupling CE with MS detection as, in this case, the use of volatile buffer is highly recommended. Even though baseline separations were obtained with all of the BGEs studied, the best results in terms of the peak height and a short analysis time were obtained using 20 mM Na₂B₄O₇ (pH 9.2) as the BGE. Under these conditions, the run time was around 13 min.

**Drug analysis by in-line SPE-CE-UV**

In this work Oasis HLB was chosen based on previous literature, in which good recoveries were reported for barbiturates by using this sorbent in an off-line mode. Moreover, clean extracts were obtained by using this sorbent when preliminary experiments have been performed to test its suitability. Oasis HLB is a polymeric sorbent with a polar group, with a hydrophilic-lipophilic-balance and reversed-phase interactions. As a result, it is a universal sorbent for acidic, basic, and neutral compounds. Different parameters affecting the in-line SPE-CE-UV procedure were studied in order to obtain the optimal response in terms of the peak height and an electropherogram free of interferences.

**Effect of sample pH**

One of the main factors that influence the extraction efficiency of analytes is the sample pH, which plays a critical role in the SPE procedure because its value determines in which state the analytes are in. Depending on the sample pH, the analytes can be in either a charged or neutral form; this determines its retention in the SPE sorbent. Based on this fact, different pH values in the range between 2 and 10 were tested. The study was performed using a standard mixture containing the analytes at a concentration of 0.2 mg/L for secobarbital, 0.1 mg/L for phenobarbital and 0.5 mg/L for barbital (prepared by an appropriate dilution of the working standards in MilliQ water and adjusted with dilutes HCl or diluted NaOH according to the desired pH). This mixture was then loaded into the capillary at 930 mbar for 5 min. A washing step with the BGE solution was then carried out at 930 mbar for 5 min. Finally, for the elution of the compounds from the SPE sorbent, a plug of methanol was introduced into the capillary at 50 mbar for 30 s; and this plug was then pushed through the SPE sorbent by introducing the BGE solution at 50 mbar for 220 s. Based on previous work by our group,7 with respect to the capillary dimensions used, the selected pushing conditions with BGE were found to be sufficient to move the elution solvent out of the SPE sorbent. From the obtained results we can conclude that the signal response in terms of the peak area was higher when the pH value of the sample was below 7 (data not shown). At higher pH values, a lower retention of the compounds was observed, which can be explained by the negative charge of the barbiturates at these pH values. For secobarbital and barbital, the differences in the retention behaviour were not significant for pH values under 7; in the case of phenobarbital, however, because higher retention was observed at pH 6, this was the value chosen for further studies.

**Study of the elution step**

Different organic solvents were tested for the elution of three barbiturates from the Oasis HLB sorbent. For this study, MeOH, basic MeOH (pH 9), MeOH/Isopropanol (80:20, v/v), MeOH/Isopropanol (50:50, v/v) and MeOH/ACN (80:20, v/v) were evaluated. This selection was based on the existing literature regarding the off-line SPE process involving barbiturates and Oasis HLB. Based on the obtained results, we can conclude that the signal response in terms of the peak area was higher when the pH value of the sample was below 7 (data not shown). At higher pH values, a lower retention of the compounds was observed, which can be explained by the negative charge of the barbiturates at these pH values. For secobarbital and barbital, the differences in the retention behaviour were not significant for pH values under 7; in the case of phenobarbital, however, because higher retention was observed at pH 6, this was the value chosen for further studies.

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Fig. 1 Diagram of the in-line SPE-CE-UV procedure developed for the preconcentration of secobarbital, phenobarbital and barbital. The different steps followed to preconcentrate and separate the analytes are shown from (A) to (E): (A) sample loading; (B) washing step; (C) introduction of the elution plug; (D) elution of the analytes from the SPE sorbent by means of the pushing step, and (E) separation process. For a detailed description of the method, please see the Experimental section.
solution at 50 mbar for 220 s. The results demonstrated that the differences in the organic solvent and mixtures tested, in terms of peak area, were not significant. Thus, MeOH was chosen as the elution solvent for further experiments.

Subsequently, a study of the MeOH plug time was performed in order to obtain higher sensitivity in terms of the peak area. To this end, different plugs of methanol were introduced into the capillary at 50 mbar for different periods of time ranging from 20 to 60 s; then, the organic solvent was pushed through the SPE sorbent by the BGE solution (at 50 mbar for 220 s). The results showed that the peak areas for the compounds studied increased with the increment of the MeOH plug time. However, at over 40 s, there was an increase in the time required to reach the current stabilization, probably caused by the presence of this very low conductivity plug inside the separation capillary. Therefore, 40 s was selected as the elution time for further studies.

**Study of sample loading time**

In order to be able to detect low concentrations of the compounds, a high sample volume should be introduced to the analyte concentrator and thus avoiding losses of the compounds. With this in mind, the sample loading time at 930 mbar was tested using a standard mixture containing the analytes at a concentration of 0.2 mg/L for secobarbital, 0.1 mg/L for phenobarbital and 0.5 mg/L for barbital (pH 6). Within the range of loading times tested (from 10 to 60 min), the peak height increased with the loading time, as can be observed in Fig. 2. For secobarbital and phenobarbital, the peak height increased with the sample loading time; this indicated that the breakthrough volume of the SPE sorbent was not exceeded. However, in the case of barbital, for sample loading, above 40 min, the responses obtained remained constant. This could be explained because barbital is less hydrophobic than the other two barbiturates, and hence it is most probably less retained on the SPE sorbent. Taking into account all of these considerations, a sample loading time of 60 min was selected as an optimal value because this time period provided maximum signal.

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**Table 1** Regression equations, values for reproducibility, repeatability and LODs for the studied drugs obtained for standard and urine samples by in-line SPE-CE

|                      | Secobarbital | Phenobarbital | Barbital  |
|----------------------|--------------|---------------|-----------|
| **Standard samples** |              |               |           |
| Linearity/ng mL⁻¹    | 2 – 500      | 2 – 500       | 10 – 500  |
| Calibration equation | $y = 26.3 + 3621x$ | $y = 6.9 + 6809x$ | $y = 23.3 + 438x$ |
| $r^2$                | 0.9999       | 0.9998        | 0.9989    |
| Reproducibility (RSD, %) | 5.8          | 7.1           | 7.5       |
| 100 ng mL⁻¹          | 5.8          | 7.1           | 7.5       |
| 300 ng mL⁻¹          | 7.9          | 8.7           | 8.9       |
| Repeatability (RSD, %) | 3.7          | 2.4           | 4.1       |
| 10 ng mL⁻¹           | 3.7          | 2.4           | 4.1       |
| 100 ng mL⁻¹          | 4.1          | 3.9           | 4.4       |
| LOD/ng mL⁻¹          | 1.0          | 0.5           | 5.0       |
| LOQ/ng mL⁻¹          | 2.0          | 2.0           | 10.0      |
| SEF                  | 1410         | 1840          | 173       |

| **Urine Samples** |          |               |           |
| Linearity/ng mL⁻¹  | 20 – 500  | 10 – 500      | 100 – 500 |
| Calibration equation | $y = -62.1 + 2973x$ | $y = 10.2 + 4595x$ | $y = 40.6 + 265x$ |
| $r^2$               | 0.9975    | 0.9978        | 0.9967    |
| Reproducibility (RSD, %) | 9.1      | 8.5           | 7.2       |
| 100 ng mL⁻¹         | 9.1        | 8.5           | 7.2       |
| 300 ng mL⁻¹         | 8.9        | 9.8           | 10.4      |
| Repeatability (RSD, %) | 7.2      | 5.2           | 6.3       |
| 100 ng mL⁻¹         | 7.2        | 5.2           | 6.3       |
| 300 ng mL⁻¹         | 8.0        | 6.9           | 8.6       |
| LOD/ng mL⁻¹         | 10.0       | 5.0           | 60.0      |
| LOQ/ng mL⁻¹         | 20.0       | 10.0          | 100.0     |

a. $y$: peak area value (mAU $\times$ seconds); $x$: concentration (ng mL⁻¹).
b. interday analysis ($n = 5$).
c. intraday analysis ($n = 5$).
Validation

The proposed in-line SPE-CE method was evaluated in terms of linearity, repeatability, reproducibility, LOQs (calculated using $S/N = 10$) and LODs (calculated using $S/N = 3$). For this evaluation, standard solutions were prepared for the three barbiturates at different concentrations in MilliQ water at pH 6. The obtained values are summarized in Table 1. Sensitivity enhancement factor (SEF) values were calculated according to

$$\text{SEF}_{\text{height}} = \frac{h_{\text{prec}}}{h_{\text{HD}}} f,$$

where $h_{\text{prec}}$ is the peak height of the preconcentrated analyte, $h_{\text{HD}}$ the peak height of the non-preconcentrated analyte detected after a conventional hydrodynamic injection of 10 s at 50 mbar, and $f$ is the dilution factor.

The calibration graphs generated were linear in the tested range with determination coefficients greater than 0.9989. The repeatability and reproducibility between days of the method, expressed as the relative standard deviation (RSD, %) of five analyses of standard samples containing the analytes at two concentration levels (100 and 300 ng/mL), were lower than 4.4 and 8.9%, respectively, for all of the compounds. The SEFs (relating to the peak height) achieved were 1410, 1840 and 173 for secobarbital, phenobarbital and barbital, respectively. The LODs achieved in standard samples were in the range of 0.5 to 5 ng/mL.

Figure 3 shows the electropherogram obtained from a standard sample containing the analytes at a concentration of 20 ng/mL for secobarbital, 5 ng/mL for phenobarbital and 50 ng/mL for barbital at pH 6, analysed by in-line SPE-CE-UV.

Urine samples

Once the performance of the developed method based on in-line SPE-CE for preconcentration and separation of a group of barbiturates had been demonstrated, the method was tested on urine samples. The reason for selecting this type of matrix was based on the fact that urine analysis constitutes a good way of monitoring the intake of drugs, since this biological sample is one of the main routes of excretion in the body. Because the
complexity of this kind of matrix may affect the performance of the SPE device, a simple liquid–liquid extraction (LLE) pretreatment was carried out prior to the analysis in order to clean up the samples. The LLE procedure was based on a method previously reported in the literature. Figure 4A shows the electropherogram obtained from an extract of a blank urine sample following the optimized in-line SPE-CE method; no interfering peaks were observed. This can be attributed to the good performance of the previous clean-up of the sample, even though this step contributes to an enlargement of the analysis time. However, this previous clean-up procedure is necessary to preconcentrate the sample in a large extent by the in-line SPE. Figure 4B shows the electropherogram obtained for an extract of urine sample spiked at a concentration of 50 ng/mL for secobarbital, 50 ng/mL for phenobarbital and 400 ng/mL for barbital. Differences in the migration times of the analytes in

| Substance                  | Technique       | LOD             | Sample studied | Sample pretreatment | Ref. |
|----------------------------|-----------------|-----------------|----------------|---------------------|-----|
| Barbital acid              | CZE-UV          | 0.87 - 3.50 mg/L| Urine          | Filtration          | 19  |
| Barbital                   |                 |                 |                |                     |     |
| Pentobarbital              |                 |                 |                |                     |     |
| Amobarbital                |                 |                 |                |                     |     |
| Thiobarbituric acid        |                 |                 |                |                     |     |
| Butobarbital               |                 |                 |                |                     |     |
| N-methyl-5-phenyl-ethyl barbital acid |                 |                 |                |                     |     |
| 5-cyclohexenyl-5-ethyl barbital acid |                 |                 |                |                     |     |
| Barbital                   | MRB-CZE-UV      | 0.26 - 0.27 µg/mL | Urine          | LLE                 | 21  |
| Phenobarbital              |                 |                 |                |                     |     |
| Secobarbital               |                 |                 |                |                     |     |
| Amobarbital                |                 |                 |                |                     |     |
| Hexobarbital               |                 |                 |                |                     |     |
| Barbital                   | CZE-UV          | 5 µg/g          | Meconium       | SPE                 | 20  |
| Phenobarbital              |                 |                 |                |                     |     |
| Secobarbital               |                 |                 |                |                     |     |
| Barbital                   | MRB-CZE-UV      | 0.26 - 0.27 µg/mL | Urine          | LLE                 | 21  |
| Metharbital                |                 |                 |                |                     |     |
| Primidione                 |                 |                 |                |                     |     |
| Phenobarbital              | HPLC-UV         | 0.1 - 0.2 µg/mL | Urine          | 10-fold dilution    | 14  |
| Mephobarbital              |                 |                 |                |                     |     |
| Pentobarbital              | HPLC-UV         | 0.1 - 0.2 µg/mL | Urine          | 10-fold dilution    | 14  |
| Barbital                   | HPLC-MS         | 4 - 5 ng/mL     | Plasma         | LLE                 | 15  |
| Phenobarbital              |                 |                 |                |                     |     |
| p-hydroxyphenobarbital     | SRMP-MEEKC-UV   | 16.8 ng/mL      | Urine          | SPE                 | 26  |
| Barbital                   | EKS-CZE-UV      | 8 - 15 ng/mL    | Urine          | LLE                 | 27  |
| Metharbital                |                 |                 |                |                     |     |
| Primidione                 |                 |                 |                |                     |     |
| Phenobarbital              | HPLC-UV         | 0.1 - 0.2 µg/mL | Urine          | 10-fold dilution    | 14  |
| Mephobarbital              |                 |                 |                |                     |     |
| Pentobarbital              | HPLC-MS         | 4 - 5 ng/mL     | Plasma         | LLE                 | 15  |
| Barbital                   | HPLC-UV         | 0.13 - 2.7 µg/mL | Urine          | Filtration          | 16  |
| Diallyl barbituric acid    |                 |                 |                |                     |     |
| Phenobarbital              |                 |                 |                |                     |     |
| Butabarbital               |                 |                 |                |                     |     |
| Barbital                   | HPLC-UV         | 0.5 µg/kg       | Pork           | ASE and SPE         | 17  |
| Phenobarbital              |                 |                 |                |                     |     |
| Butabarbital               |                 |                 |                |                     |     |
| Barbital                   | GC-MS           | 0.5 µg/kg       | Pork           | ASE and SPE         | 17  |
| Phenobarbital              |                 |                 |                |                     |     |
| Barbital                   | GC/MS/MS        | 0.1 - 0.2 µg/kg | Pork           | Ultrasonic and SPE  | 18  |
| Phenobarbital              |                 |                 |                |                     |     |
standard samples and urine samples can be observed by comparing Figs. 3 and 4B. This suggests that some endogenous compounds that are also retained in the concentrator device and subsequently eluted may influence the behavior of the tested analytes.

Table 1 gives the validation parameters obtained for urine samples. The linearity was determined from triplicate injections of the samples spiked with the three compounds prior to the LLE process. The RSD in terms of repeatability and reproducibility for peak areas were below 8.6 and 10.4%, respectively, these values being higher than the obtained for standard samples; this can be explained as being due to the presence of the additional sample pretreatment step. The LODs achieved were between 5 and 60 ng/mL, calculated based on a 3:1 signal-to-noise ratio. It should be mentioned that these LODs were higher by one order of magnitude than in standards. This fact could be attributed to different factors, such as the complexity of the sample matrix, and also to a slight broadening of the electrophoretic peaks.

In the literature, different techniques, such as LC, GC and CE, have been used for the determination of barbiturate drugs in biological samples,14-22,26,27 as summarized in Table 2. In these papers, samples such as urine,14,16,19-21,26 plasma,15 serum,22 meconium20 were analysed. As can be observed from the table, the LODs obtained in our study are generally lower than those reported in other papers that focused on the determination of barbiturate drugs in urine samples by CE, except for the case of Kadi et al.28 and Botello et al.27 who reported similar LODs. In those papers the authors use a stacking technique, SRMP or EKS, in combination with CE; in both cases a sample pretreatment technique, as LLE or SPE, was needed, as in our case, to obtain a clean extract in order to be able to apply a preconcentration strategy in the same separation capillary.

Conclusions

In this study, a method based on in-line SPE-CE was successfully established to analyse three barbiturates in urine samples at low ng/mL, which are suitable for monitoring these compounds at therapeutic and toxicological levels. This method presents several advantages as the complete automation of the in-line SPE-CE process, the obtained sensitivity and the low cost of the reported strategy; however, further research should be done in the future to overcome some drawbacks as the large analysis time. In this sense, the application of pressure during the sample loading time step can be considered.

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References

1. L. A. Kartsova and E. A. Bessonova, J. Anal. Chem., 2009, 64, 326.
2. M. C. Breadmore, M. Dawod, and J. P. Quirino, Electrophoresis, 2011, 32, 127.
3. R. Ramautar, G. J. de Jong, and G. W. Somsen, Electrophoresis, 2012, 33, 243.
4. P. Puig, F. Borrull, M. Calull, and C. Aguilar, TrAC, Trends Anal. Chem., 2007, 26, 664.
5. R. Ramautar, G. W. Somsen, and G. J. Jong, Electrophoresis, 2010, 31, 44.
6. P. Puig, F. Borrull, M. Calull, and C. Aguilar, Anal. Chim. Acta, 2008, 616, 1.
7. I. Botello, F. Borrull, C. Aguilar, and M. Calull, Electrophoresis, 2012, 33, 528.
8. I. Maijó, F. Borrull, M. Calull, and C. Aguilar, Electrophoresis, 2011, 32, 2114.
9. S. Almeda, L. Arce, and M. Valcárcel, Curr. Anal. Chem., 2010, 6, 126.
10. I. Botello, F. Borrull, M. Calull, C. Aguilar, G. W. Somsen, and G. J. de Jong, Anal. Bioanal. Chem., 2012, 403, 777.
11. A. Ashnagar, N. G. Naseri, and B. Sheeri, Chin. J. Chem., 2007, 25, 382.
12. G. A. Shabir, T. K. Bradshaw, S. A. Arain, and G. Q. Shar, J. Liq. Chromatogr. Relat. Technol., 2010, 33, 61.
13. M. Iwai, H. Hattori, T. Arinobu, A. Ishii, T. Kumazawa, H. Noguchi, O. Suzuki, and H. Seno, J. Chromatogr. B, 2004, 806, 65.
14. S. Kanno, K. Watanabe, S. Hirano, I. Yamagishi, K. Gomnomi, K. Minakata, and O. Suzuki, Forensic Toxicol., 2009, 27, 103.
15. F. Q. Ye, J. C. Pan, G. X. Hu, D. Lin, J. Y. Zhu, and X. Q. Wang, Chromatographia, 2010, 72, 743.
16. Y. Martin-Biosca, S. Sagrado, R. M. Villanueva-Camans, and M. J. Medina-Hernández, J. Pharm. Biomed. Anal., 1999, 21, 331.
17. H. X. Zhao, Y. M. Qiu, L. P. Wang, J. Qiu, W. K. Zhong, Y. Z. Tang, D. N. Wang, and Z. Q. Zhou, Chin. J. Anal. Chem., 2005, 33, 777.
18. H. X. Zhao, L. P. Wang, Y. M. Qiu, Z. Q. Zhou, W. K. Zhong, and X. Li, Anal. Chim. Acta, 2007, 586, 399.
19. T. F. Jiang, Y. H. Wang, Z. H. Lv, and M. E. Yue, Chromatographia, 2007, 65, 611.
20. D. C. Delinsky, K. Srinivasan, H. M. Solomon, and M. G. Bartlellt, J. Liq. Chromatogr. Relat. Technol., 2002, 25, 113.
21. Q. L. Wang, L. Y. Fan, W. Zhang, and C. X. Cao, Anal. Chim. Acta, 2006, 580, 200.
22. K. Ohyama, M. Wada, G. A. Lord, Y. Ohba, O. Fujishita, K. Nakashima, C. K. Lim, and N. Kuroda, Electrophoresis, 2004, 25, 594.
23. S. Li and S. G. Weber, Anal. Chem., 1997, 69, 1217.
24. K. E. Ferslew, A. N. Hagardorn, and W. F. McCormick, J. Forensic Sci., 1995, 40, 245.
25. Y. Kataoka, K. Makino, and R. Oishi, Electrophoresis, 1998, 19, 2856.
26. A. Kadi, M. Hefnawy, S. Julkhuf, M. Abounassif, G. Mostafa, M. G. Kassem, S. Attia, and A. Al-Ghamdi, Analyst, 2011, 136, 2858.
27. I. Botello, F. Borrull, M. Calull, and C. Aguilar, J. Sep. Sci., 2013, 36, 524.
28. M. L. Luis, D. Blanco, J. J. Arias, F. Jiménez, A. Jiménez, and J. M. G. Fraga, J. Liq. Chromatogr. Relat. Technol., 2001, 24, 1921.
29. Y. Horii, M. Fujisawa, K. Shimada, Y. Hirose, and T. Yoshioka, Biol. Pharm. Bull., 2006, 29, 7.
30. J. Sheng, J. P. Lei, H. X. Ju, C. J. Song, and D. M. Zhang, Anal. Chim. Acta, 2010, 679, 1.
31. Oasis (R) Applications Notebook. Family of sample extraction products, Waters Corp., 2003.