Talanta 99 (2012) 897–903

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Selective extraction and determination of catecholamines in urine samples by using a dopamine magnetic molecularly imprinted polymer and capillary electrophoresis

Mohamed Bouri [a,b], M. Jesús Lerma-García [a,c], Rachid Salghi [b], Mohammed Zougagh [c,d], Angel Ríos [a,c,*]

[a] Department of Analytical Chemistry and Food Technology, University of Castilla–La Mancha, Av. Camilo José Cela 10, E-13004, Ciudad Real, Spain
[b] Laboratoire d'Ingénieries des Procédés de l'Energie et de l'Environnement, ENSA, B.P. 1136, Agadir, Morocco
[c] Regional Institute for Applied Chemistry Research, IRICA,, Av. Camilo José Cela 10, E-13004, Ciudad Real, Spain
[d] Albacete Science and Technology Park, E-02006, Albacete, Spain

ARTICLE INFO

Article history:
Received 19 March 2012
Received in revised form
14 July 2012
Accepted 19 July 2012
Available online 25 July 2012

Keywords:
Magnetic molecularly imprinted polymer
Extraction
Capillary electrophoresis
Catecholamines
Human urine

ABSTRACT

A rapid and selective method for the extraction and determination of catecholamines (CLs) from urine samples has been successfully developed using a magnetic molecularly imprinted polymer (MMIP) as a sorbent material. The MMIP has been prepared using dopamine hydrochloride (DA) as template molecule, methacrylic acid (MAA) as functional monomer, ethylene glycol dimethacrylate (EDMA) as cross-linking agent and Fe_3O_4 magnetite as magnetic component. The extraction was carried out by stirring urine samples with the magnetic polymer. When the extraction was completed, the MMIP, together with the captured analytes, was easily separated from the sample matrix by an adscititious magnet. The analytes desorbed from the MMIP were determined by capillary electrophoresis (CE). It was shown that the MMIP had high affinity and selectivity toward DA and other structurally related CLs such as 3-methoxytyramine hydrochloride (MT), DL-normetanephrine hydrochloride (NME), DL-norephinephrine hydrochloride (NE) and (±) epinephrine (E). Different parameters affecting the extraction efficiency were evaluated in order to achieve the optimal pre-concentration of the analytes and to reduce non-specific interactions. Under the optimal conditions, the CL limits of detection were at the 0.04–0.06 μM range. The relative standard deviations of migration time and response ranged from 0.7% to 1.4% and from 2.9% to 5.5%, respectively. The proposed method was successfully applied to determine CLs, including MT, NME, DA, NE and E in human urine samples.

1. Introduction

Catecholamines (CLs), such as dopamine, adrenaline and noradrenaline, are neurotransmitters in the central and peripheral nervous systems (Fig. 1). Clinical measurement of CLs and their analogs in biological samples is useful for clinical diagnosis of pheochromocytoma and neuroblastoma of Parkinson's disease and in the investigation of stress systems [1]. This fact has prompted the development of several methods for the determination of dopamine and their analogs in biological fluids such as serum, urine and plasma. Since CLs in biological fluids only occur in small quantities, the analytical methods developed for their determination should be both selective and sensitive.

Several methods have been developed to determine CLs in urine samples [2–7]. Liquid chromatography (LC) and capillary electrophoresis (CE), coupled with fluorescence and electrochemical detectors [2–4], tandem mass spectrometry [5,6,7] or ultraviolet detector [7,8], have been previously used for this purpose. As CLs are found at low concentration in urine, a preconcentration step is necessary to detect them and, thus, performing the corresponding determination. However, and since this enrichment procedure also leads to the concentration of matrix constituents, samples should be cleaned up in order to eliminate possible interferences before analysis. The clean-up step is usually performed by solid-phase extraction (SPE) with different sorbents, such as C18 [8,9], cation-exchange [10] and alumina [11], by molecularly imprinted polymers (MIPs) [12] and/or by immunosorbents (IS) [13]. Among these, the best sorbents used to perform highly selective extractions of dopamine and their analogs were IS [13]. In the case of dopamine hydrochloride (DA), an enzyme-linked immunosorbent assay (ELISA)-based plate kit is commercially available for its quantitative measurement from the distributor, ALPCO diagnostics, with 8.3 pg sample sensitivity, and sample volume 100 μL. These sorbents are based on the antigen–antibody principle and,

* Corresponding author at: Department of Analytical Chemistry and Food Technology, University of Castilla–La Mancha, Av. Camilo José Cela 10, E-13004, Ciudad Real, Spain. Tel.: +34 926 29 52 32; fax: +34 926 29 52 18.
E-mail address: angel.rios@uclm.es (A. Ríos).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.07.053

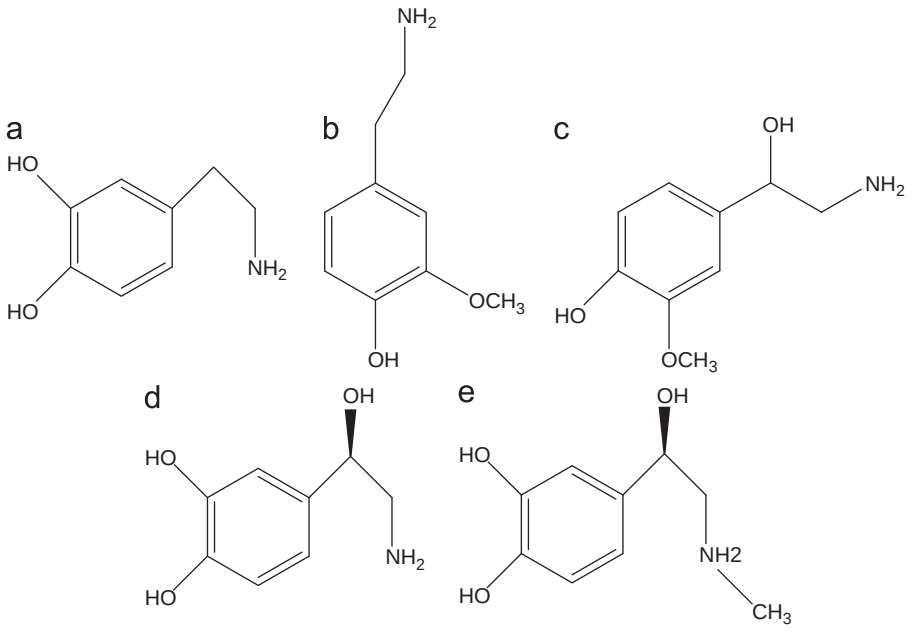

Fig. 1. Chemical structures of DA (a), MT (b), NME (c), NE (d) and E (e).

therefore, when a particular antibody in a matrix needs to be detected, its antigen is immobilised on a solid support and, once the matrix is percolated through this sorbent, the analyte of interest is retained by highly-selective interactions. However, the application of IS for selective extraction is very limited for several reasons. Among these are the high cost associated in producing IS, the very strict conditions required for their proper use, the limited number of times that IS can be reused, and the low number of molecules that can be extracted using this method. In order to overcome these drawbacks of IS to perform selective extractions, and taking the advantage provided by polymeric sorbents, a new trend for using highly selective polymeric sorbents appeared in the mid-1990s. These sorbents are known as MIPs. Even though MIPs appeared in the early 1970s, it was not until Sellergren [14,15] used them for the first time to extract pentamidine from urine samples that they became increasingly used in SPE. The use of MIPs as sorbents in SPE has led to a SPE protocol, which is known as molecularly imprinted solid-phase extraction (MISPE). Compared to natural receptors, MIPs not only demonstrate comparable molecular selectivities but they are also more robust and reusable, and less expensive to prepare [16]. However, some drawbacks to MISPE have restricted its widespread application. MISPE is normally used in cartridge mode, which often results in a tedious column packing procedure, high backpressure and a low flow rate. In order to avoid these disadvantages several attempts have been made to develop an on-line MISPE system, or to prepare a monolithic column coupled to a chromatographic system [17–20]. Providing magnetism to the MIP and then using magnetic separation is another promising alternative.

Magnetic particles have been widely applied in biological fields, such as bioseparation [21], drug delivery [22] and biomolecular sensing [23], being also recently used as SPE sorbents in environmental sample pretreatment [24–26]. The preparation of a magnetic MIP (MMIPs) has been previously reported [27–33]. Zhang et al. [30] and Hu et al. [31] have applied MMIPs to plant samples in order to perform trace analyses of auxin phytohormone and triazines, respectively. The advantage of MMIPs is obvious, as Y. Zhang et al. reported [34]: "the participation of a magnetic component in the imprinted polymer can build a controllable rebinding process and allow magnetic separation to replace the centrifugation and filtration step in a convenient and economical way". When MIP particles contain magnetic components, adsorption can be achieved by dispersing them in solution, being then easily separated from the matrix by applying an external magnet. Therefore, MISPE with magnetic separation provide a convenient and highly efficient enrichment, avoiding SPE column packing and the time-consuming process of loading a large-volume sample. However, MMIP has not been applied yet.

The aim of this study was the development of a new method to improve and simplify the determination of CLs including 3-methoxytyramine hydrochloride (MT), DL-normetanephrine hydrochloride (NME), DA, DL-norephinephrine hydrochloride (NE) and (±) epinephrine (E) in urine samples (Fig. 1). For this purpose, a new MMIP was synthesized using DA as template molecule. The characteristics of the MMIP and binding experiments were investigated. The resulting polymers were used as sorbents for the extraction of CLs from urine samples, followed by capillary electrophoresis (CE) determination. By using the proposed methodology CLs were selectively isolated and matrix interferences were eliminated in a short time, which simplifies sample treatment procedure.

2. Experimental

2.1. Chemicals, material and samples

Iron (II) chloride tetrahydrate ($FeCl_2$ 4 H_2O), iron (III) chloride hexahydrate ($FeCl_3$ 6 H_2O), γ-methacryloxypropyltrimethoxysilane (γ-MPS), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) and α,α′-azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (EtOH), methanol (MeOH), acetonitrile (ACN), hydrochloric acid, acetic acid, sodium bisulphate and ammonia were supplied by Panreac (Barcelona, spain).

The CLs standards MT, NME, DA, NE and E were purchased from Sigma-Aldrich (St. Louis, MO, USA). Individual stock standard solutions of 10 mM in HCl (0.1 M) and sodium bisulphate (0.5 mM) were prepared, stored at 4 °C in the dark and used for further dilution. The analytical grade reagents employed to

prepare carrier electrolyte were sodium tetraborate anhydrous (borate), supplied from Fluka (Buchs, Switzerland), and di-sodium hydrogen phosphate (Na_2HPO_4), supplied by Panreac. Water was purified with a Milli-Q system (Millipore). Sodium hydroxide (NaOH, Panreac) was used for capillary conditioning. All solutions prepared for CE analysis were passed through a 0.45 μm nylon filter before use, degassed by sonication and freshly prepared each day. Human urine samples were supplied by volunteers.

2.2. Apparatus

CL determination was carried out in an Agilent Model G1600AX (Palo Alto, CA, USA) CE instrument provided with a diode array detector (DAD). CE voltage and temperature were set at 20 kV and 25°C, respectively. Detection was performed at 205 ± 10 nm (450 ± 10 nm as reference). Hydrodynamic injection mode (50 mbar) was applied for 5 s. A fused-silica capillary (Análisis Vínicos, Tomelloso, Spain) of 64 cm (41 cm effective length) and 50 μm id was used. Capillary was daily conditioned by washing with freshly prepared 0.1 M NaOH (30 min) followed by deionized water (20 min) and fresh running electrolyte (20 min). Capillary was also rinsed with electrolyte for 10 min after each complete run.

2.3. Preparation of double-bond-functionalized Fe_3O_4 magnetite

Fe_3O_4 magnetic nanoparticles were prepared by the co-precipitation method according to a previously described procedure [35]. A 180 mL of an aqueous solution containing 11.2 mmol Fe^{3+} and 5.6 mmol Fe^{2+} was heated at 50 °C. Then, a 12.5 mL of ammonia was added under vigorous stirring. After 30 min, the reaction was heated and kept at 90 °C for 30 min again. N_2 was used as the protective gas in the whole experiment. After completion of the reaction, the black precipitate was collected by an external magnetic field, washed with water and ethanol, and dried in vacuum. To modify the magnetic nanoparticles with a double bond, a 4 mL γ-MPS was dropwise added into the mixture solvents of ethanol and water (1:1, v/v) containing 50 mg of dispersed Fe_3O_4 nanoparticles, and the reaction was kept for 12 h at 40 °C under N_2 gas. Then, the product was separated and washed with ethanol for several times, and dried in vacuum.

2.4. Preparation of MMIPs and binding experiments

In this study, six MMIPs (MMIP1, MMIP2, MMIP3, MMIP4, MMIP5 and MMIP6) and their corresponding magnetic non-molecularly imprinted polymers (MNIPs) (MNIP1, MNIP2, MNIP3, MNIP4, MNIP5 and MNIP6) were synthesized by preparing the polymerization mixtures included in Table 1. These polymerization mixtures were prepared by mixing a functional monomer (MAA), a cross-linker (EDMA), pore-forming solvents (mixtures of ACN/water or MeOH/water), and AIBN as thermal initiator. In all cases, 0.05 g of dispersed double-bond-functionalized Fe_3O_4 magnetic nanoparticles were added into the polymerization mixture. Polymerization was performed at 60 °C for 24 h. The resulting product was collected by the external magnetic field and washed several times with acetic acid (1%) with the aid of mechanical agitation until the template molecule could not be detected by CE. The obtained polymers were finally rinsed with water to remove the remaining acetic acid and dried in a vacuum desiccator for 24 h before use. The control MNIPs were prepared in absence of DA during the polymerization process and treated following an identical procedure. MMIPs and MNIPs did not need to be ground before they were used.

Capacity evaluation of MMIPs and MNIPs to recognize and bind template was performed in a phosphate buffer saline (PBS) at pH 8. Briefly, 20.0 mg MMIPs or MNIPs were mixed with 2.0 mL of DA at the concentration of 2 μM in PBS. The solution was incubated for 24 h at room temperature, and then the supernatant was separated and analyzed by CE. The amount of DA bound on the magnetic polymers was obtained by calculating the recovery. As it is observed in Table 1, a good recovery (98%) was obtained in the extraction of DA by MMIP2; therefore this MMIP was selected for the next experiments.

2.5. Extraction procedure

An amount of 50 mg of MMIP2 was put into a conical flask and conditioned in sequence with 3.0 mL acetic acid (1%) and 3.0 mL PBS (pH 8). The supernatant was separated from the polymers with a magnet and discarded. Then, 2.0 mL of test urine sample was added into the conical flask, and 18.0 mL of PBS (pH 8) was also added as a buffer. The solution was mixed by mechanical agitation for 15 min. After the extraction was completed, the captured DA and its analogs were rapidly separated from the solution under a strong external magnet. After discarding the supernatant solution, the analytes were eluted from the MMIP2 with 2 × 1.0 mL acetic acid (1%). The elution fraction was evaporated to dryness under a nitrogen stream at 40 °C, dissolved in 0.2 mL of phosphate–borate buffer (pH 5.5, 20 mM ionic strength) and analyzed by CE.

3. Results and discussion

First, the MMIP procedure was designed and optimized to carry out the extraction of CLs from the urine samples and, then, the electrophoretic method was also optimized to separate and quantify these analytes.

Table 1
Polymerization mixture composition and percentage of bound DA by the MMIPs and MNIPs.

	DA (mmol)	MAA (mmol)	EDMA (mmol)	AIBN (g)	ACN (mL)	MeOH (mL)	H_2O (mL)	Fe_3O_4 NPs (g)	Bound DA (%)
MMIP1	0.026	1	0.5	0.013	9	–	1	0.05	58.3
MNIP1	–	1	0.5	0.013	9	–	1	0.05	17.6
MMIP2	0.026	1	0.5	0.013	9.75	–	0.25	0.05	98.2
MNIP2	–	1	0.5	0.013	9.75	–	0.25	0.05	20.3
MMIP3	0.26	1	0.5	0.013	9	–	1	0.05	77.2
MNIP3	–	1	0.5	0.013	9	–	1	0.05	21.1
MMIP4	0.026	1	0.5	0.013	–	9	1	0.05	78.6
MNIP4	–	1	0.5	0.013	–	9	1	0.05	19.6
MMIP5	0.26	1	3.8	0.01	9	–	1	0.05	67.3
MNIP5	–	1	3.8	0.01	9	–	1	0.05	18.5
MMIP6	0.26	1	5	0.01	9	–	1	0.05	53.7
MNIP6	–	1	5	0.01	9	–	1	0.05	17.5

3.1. Evaluation of MMIP cross-selectivity

The selectivity of MMIP2 was investigated with E, NE, NME and MT as the structural analogues of DA template. The experiment was carried out by adding 20.0 mg MMIP2 or MNIP2 in a glass tube containing 2.0 mL of each stock solution at the concentration of 2 μM. The solution was incubated for 24 h at room temperature, and then, the supernatant was separated and analyzed by CE. As observed in Fig. 2, the amounts of DA and the structural analogues bounded to the MMIP2 were higher than those bounded to the MNIP2. Thus, MMIP2 provided a high selectivity for DA and its structural analogues.

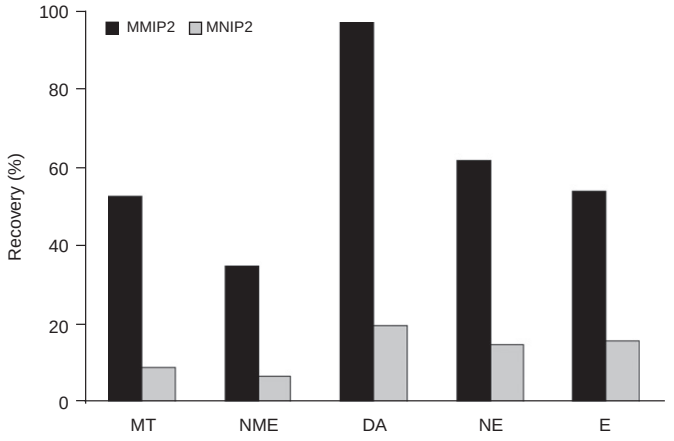

Fig. 2. Binding tests for DA and its related derivates: E, NE, NME and MT using MMIP2 and MNIP2.

3.2. Optimization of extraction conditions

The extraction conditions were optimized by analyzing a standard solution (2 μM) of the CL compounds. The parameters affecting the performance of the extraction, such as pH, adsorption time, elution solvent, sample volume and mass of sorbent, were investigated. When one parameter was changed, the other parameters were fixed at their optimal values.

The binding sites of MMIP2 are in situ synthesised by copolymerisation of functional monomers and cross-linker around the template molecule. In this work, MMA, containing carboxyl functional groups, was used as functional monomer. Template binding to the imprinted polymers is due to hydrogen bound, electrostatic force and charge transfer between the carboxyl groups and the imprinted molecules. Then, 0.1 M HCl and 0.1 M NaOH were used to control pH from 1.0 to 11.0 in order to determine 2 μM DA standard solution. The best results were achieved for pH values ranging from 8.0 to 11.0, as it is shown in Fig. 3a.

Taking into account the experimental data obtained by Sanchez-Rivera et al. [36], dopamine (X-NH_2) will be positively charged at pH < 8 and within the 8.8–11 pH range, important fractions of cationic (X-NH_3^+) (pK_{a1} (X-NH_3^+)=9.046), neutral (X-NH_2) (pK_{a2} (X-NH_2)=10.58) and anionic (X-NH^-) (pK_{a3} (X-NH^-)=12.07) species are present. On the other hand, MAA contains a carboxylic group that can be ionized and can interact with the amide group of H_3DA^+ (at pH < 8) via electrostatic forces. As a result, a pH in the range 6–8 will greatly influence the interactions between the analyte and the MMIP2. When pH > 9, the predominant species are neutral and anionic, thus any electrostatic interaction with the carboxylic group of MMA can occur. The high recoveries obtained with these high pHs can be

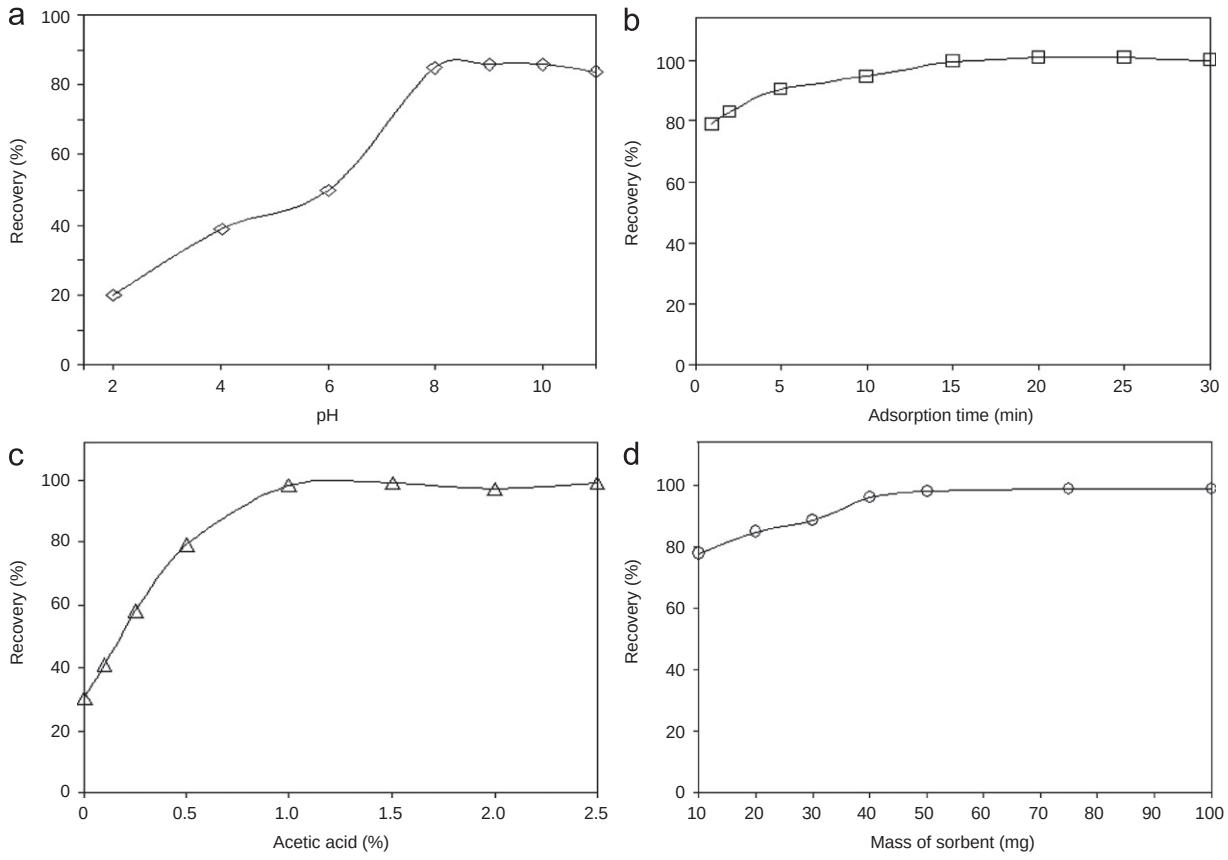

Fig. 3. Effects of sample pH (a), adsorption time (b), percentage of acetic acid (c) and mass of sorbent (d) on DA recovery by MMIP2.

explained taking into account that the hydrophobic properties of EDMA-containing polymer may promote a higher non-specific adsorption of Dopamine to the MMIP. Therefore, a phosphate buffer saline (PBS) at pH 8.0 was selected for further experiments.

Next, the effect of the adsorption time on DA recovery, which was varied between 1 and 30 min, was also examined. As observed in Fig. 3b, 15 min was enough to achieve a complete recovery, this being time selected for the next experiments.

On the other hand, desorption conditions were also optimized. Elution solvent composition, which was composed by water containing different acetic acid percentages (0–2.5%, v/v), was firstly optimized. Experiments were carried out using 50 mg of sorbent and 2 mL of DA standard solution (2 μM at pH 8). As observed in Fig. 3c, recovery increased dramatically when the percentage of acetic acid was increased from 0% to 1.0%. Recovery barely changed as the proportion of acetic acid was further increased from 1.0% to 2.5%. Therefore, 1.0% acetic acid was selected for subsequent experiments. Next, the minimum elution solvent volume needed to efficiently elute the adsorbed DA was optimized. Different volumes, ranging from 1.0 to 5.0 mL, were tested. The best results were obtained when 2.0 mL was used. Thus, 2×1.0 mL water containing 1% acetic acid under 5 min of agitation was the optimal condition selected for the desorption stage. Thus, the whole extraction procedure can be accomplished within 25 min.

Finally, the optimal amount of MMIP used to quantitatively extract DA was optimized. For this purpose, different amounts of MMIP2, ranging from 10 to 100 mg, were tried to extract DA from 2 mL standard solution (2 μM) in PBS at pH 8. As observed in Fig. 3d, recovery increased when the amount of MMIP2 was increased from 10 to 50 mg. When the amount was higher than 50 mg, any significant recovery improvement was obtained. Therefore, the optimal amount of sorbent was fixed at 50 mg. After each extraction, sorbent was easily recovered by rinsing it with acetic acid (1%). Sorbent recycling was then studied, and the results showed that the sorbent can be used at least ten times with the same extraction efficiency.

3.3. Separation and detection of CLs

The influence of several parameters was investigated to identify the key variables that affect sensitivity and separation efficiency of CLs. For this purpose, standard solutions at a concentration of 2 μM were used.

Most CL separations reported in literature [37–44] used additives to achieve good separation and to protect analytes from degradation. At lower pH buffers, in which compounds have a net positive charge, either micelle-forming surfactants [37] or electroosmotic flow (EOF)-decreasing wall modifiers [44] have been used to separate the analytes. At high pH buffers, in which compounds have a net negative charge, more favourable separation condition were obtained. However, and since compounds are less stable and easily oxidized at basic pHs, antioxidants have often been added [38–43]. In acid media, CLs are generally protonated, hence shorter and better separations are obtained. Therefore, acid pH values have been selected to separate CLs by CE. Thus, pH effect on the EOF and on the dissociation of analytes in the running electrolyte and, therefore, on electrophoretic migration and resolution of analytes was firstly adressed. pH values were varied between 4.0 and 7.0 using phosphate as the running electrolyte solution at 0.02 M ionic strength. CLs migrated in the following order of increasing migration times: MT, NME, DA, NE and E. From the pH-dependence of the apparent mobility of the five CLs, a pH value of 5.5 resulted as the optimum for their separation. Two different aqueous electrolytes were tested: phosphate and phosphate–borate buffer over the range 10–50 mM at pH 5.5. Good resolution, better sensitivity and less fluctuant baselines were obtained when a 20 mM phosphate–borate buffer was used. In order to achieve a good compromise between running time and the heat generated inside the capillary by Joule effect, a 20 mM concentration of phosphate–borate buffer was finally selected.

Next, the applied voltage for CL separation was optimized. For this purpose, different applied voltages, ranging from 10 to 25 kV, were applied. The separation voltage directly determines migration time, also affecting peak resolution. In all cases, migration time decreased when voltage was increased. Thus, a voltage of 20 kV was selected as the best compromise between migration time and separation efficiency. Probably, due to the Joule effect and capillary heating, not very reproducible results were obtained at voltages higher than 20 kV. Moreover, capillary temperature was also set at 25 °C and a good separation of CLs was obtained.

On the other hand, hydrodynamic injection was selected since more reproducible results were obtained when compared to electrokinetic injection [38–43]. Injection time and pressure were also optimized. Time was varied between 3 and 15 s. Sensitivity increased when injection time was increased. However, more than 5 s resulted in no separation of NE and E peaks. Consequently, an injection time of 5 s was selected as the optimum value. Regarding injection pressure, values ranging from 30 to 50 mbar were essayed. A pressure of 50 mbar was selected since it provides the best sensitivities for all the CLs studied.

3.4. Performance characteristics of the MMIP-CE method and application to the determination of CLs in human urine samples

Under the optimized conditions, 50 mg of MMIP2 were put into a conical flask and conditioned in sequence with 3.0 mL acetic acid (1%) and 3.0 mL PBS (pH 8). The supernatant was separated from the polymers with a magnet and discarded. Then 20.0 mL of standard solutions, prepared in PBS (pH 8) of MT, NME, DA, NE and E in the 0.25–2.50 μM concentration range, was added into the conical flask. The solution was mixed by mechanical agitation for 15 min. After the extraction was completed, the captured DA and its analogs were rapidly separated from the solution under a strong external magnet. After discarding the supernatant solution, the analytes were eluted from the MMIP2 with 2×1.0 mL acetic acid (1%). The elution fraction was evaporated to dryness under a stream of nitrogen, dissolved in 0.2 mL of phosphate–borate buffer (pH 5.5, 20 mM ionic strength) and analyzed by CE. Five CLs were separated and quantified in less than 10 min with good resolutions (see Fig. 4).

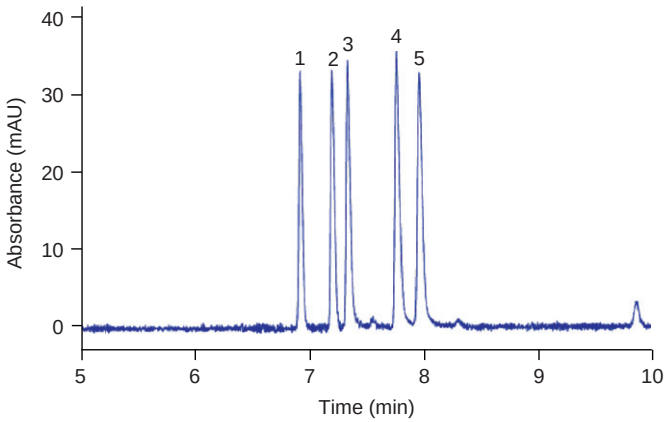

Fig. 4. Electropherogram of a 2 μM standard solution of CLs. Conditions: capillary, 64 cm id 50 μm (41 cm effective length); capillary temperature, 25 °C; hydrodynamic injection, 5 s; applied voltage, 20 kV; running electrolyte, 20 mM phosphate–borate at pH 5.5; detection, at 205 nm. Peak identification: 1, M, 2, NME, 3, DA, 4, NE and 5, E.

Table 2
Calibration data and validation parameters obtained for the CL determination by the proposed MMIP-CE method.

Analyte	Linear range (μM)	$Y=(A\pm S_A)X+(B\pm S_B)$	R^2	$S_{y/x}$	LOD (μM)	LOQ (μM)	Run-to-run precision	
							Migration time RSD (%)	Responses RSD (%)
MT	0.25–2.50	$(20.06\pm0.22)X+(0.59\pm0.33)$	0.9995	0.42	0.06	0.21	1.2	3.6
NME	0.25–2.50	$(22.39\pm0.14)+(0.52\pm0.21)$	0,9999	0.26	0.04	0.12	1.4	2.9
DA	0.25–2.50	$(39.76\pm0.39)+(0.53\pm0.58)$	0,9996	0.75	0.06	0.19	0.9	4.3
NE	0.25–2.50	$(30.40\pm0.27)-(0.88\pm0.41)$	0,9997	0.53	0.05	0.18	0.7	5.2
E	0.25–2.50	$(22.49\pm0.22)-(0.14\pm0.33)$	0,9996	0.43	0.06	0.19	1.1	5.5

A: slope, S_A: SD of the slope; *B*: intercept; S_B: SD of the intercept; *R*: regression coefficient; S_y/x: SD of residuals ($n=10$).

The figures of merit of the method, which are summarized in Table 2, were evaluated for a hydrodynamic sample injection of 5 s. External calibration curves using peak areas were constructed by injecting into the CE system the standard solutions (by triplicate) after being preconcentrated using the MMIP. Straight lines with R^2 of 0.999 were obtained in all cases (Table 2), which demonstrated that the proposed method can be used for quantitative analytical purposes. The LODs, defined as the concentration of analyte giving a signal equivalent to the blank signal plus three times its SD, are also presented in Table 2. Since the blank signal is practically the same for all analytes, intercept values and their corresponding SDs of the calibration equations were used to calculate these values. The LOD values obtained ranged from 0.04 to 0.06 μM, which presents advantages with respect to other values obtained using capillary electrophoresis after the solid-phase extraction. Thus, in a previous publication, Vuorensola et al. [8] used SPE with a polymer sorbent material and CE-DAD for the determination of 3,4-dihydroxybenzylamine, dopamine, 3-methoxytyramine, normetanephrine and metanephrine in urine samples, but the LOD ranged between 0.40 and 0.70 μM.

The precision of the method, expressed as RSD, was obtained by injecting a 2.00 μM standard solution ten times during one working day. In all cases, precision RSD values for the responses and migration times ranged between 2.9–5.5% and 0.7–1.4%, respectively.

The use of MMIP in combination with CE was tested on real sample matrices (viz. human urine samples). In the first place, urine samples (2.0 mL) were diluted with 18.0 mL of phosphate–borate buffer (pH 5.5, 20 mM ionic strength), filtered and directly injected into the CE system without being subjected to the MMIP step. Unstable baselines and no analyte separation were obtained in the electropherograms due to the interferences present in these urine samples. However, when these samples were extracted as described in Section 2.5, a stable baseline and few interferences were obtained. Fig. 5a illustrates the effect of this MMIP step on the urine samples. As any CL peak was detected in these samples, samples were spiked at different levels with the five CLs and determined by the proposed MMIP-CE method. Fig. 5b shows a representative electropherogram obtained for a human urine sample spiked with 2.5 μM of each CL. The recovery results obtained for each analyte after spiking samples at different CL concentration levels are shown in Table 3. As it is shown in this table, recovery values, which were estimated from measured versus added amounts, ranged between 91.8% and 103.6%, depending on the analytes. These recoveries can be favourably compared to other recovery values previously obtained using MIPs. Recoveries in the 81.2–95.1% range with RSD values comprised between 2.3% and 12.4% were obtained for CLs in spiked urine samples by using a SPE procedure with a chemically modified polymer resin (Amberlite XAD-4) crown ether and by reversed-phase ion-pair high-performance liquid chromatography with electrochemical detection [45], which were lightly lower than those obtained by the proposed methodology.

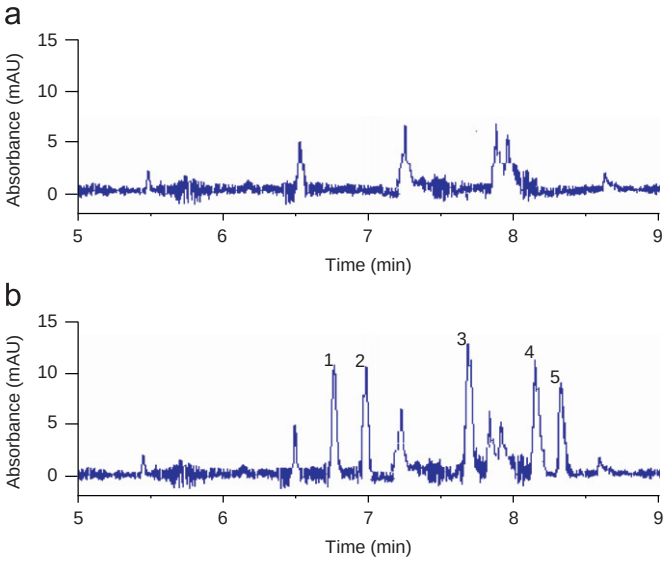

Fig. 5. Electropherogram of (A) an extract of blank human urine sample and (B) human urine sample spiked at 2.5 μM by each CL. Peak identification, separation and detection conditions as indicated in Fig. 4.

Table 3
Recovery results obtained for CLs after spiking human urine samples at different CL concentration levels.

Analyte	Added (μM)	Found (μM)	Recovery (%)
MT	2.50	2.35	94.0
	5.00	4.96	99.2
	10.00	10.16	101.6
	15.00	15.08	100.5
	20.00	19.84	99.2
NME	2.50	2.37	94.8
	5.00	5.01	100.2
	10.00	10.01	100.1
	15.00	14.79	98.6
	20.00	20.06	100.3
DA	2.50	2.30	92.0
	5.00	5.12	102.4
	10.00	9.89	98.9
	15.00	4.89	99.3
	20.00	9.65	98.3
NE	2.50	2.59	103.6
	5.00	4.92	98.3
	10.00	9.59	95.9
	15.00	14.32	95.5
	20.00	20.32	101.6
E	2.50	2.30	92.0
	5.00	5.14	102.8
	10.00	10.15	101.5
	15.00	14.91	99.4
	20.00	18.74	93.7

4. Conclusions

In this work, MMIPs with a strong magnetic responsiveness and a selective character has been successfully prepared and applied for the extraction of DA and its analogs from urine samples, followed by their separation and detection by CE. Studies of recognition properties have demonstrated high adsorption capacity and selectivity of the MMIP beads to the DA template molecule. Moreover, the imprints have also shown cross-selectivity for M, NME, NE and E. On the other hand, no interference was observed at the migration time of the analytes of interest; thus the proposed method provides improved isolation and identification of M, NME, DA, NE and E in complex urine samples. The proposed magnetic method presents several advantages when compared with the traditional SPE protocol, such as the easy collection of MMIP sorbents by an external magnetic field and the elimination of the time consuming column passing and filtration steps, among others. In addition, MMIP sorbents can be also easily recycled by rinsing with 1% acetic acid. Thus, this method can be considered as a promising and good alternative to the traditional techniques. A next step could be the on-line use of MMIPs combined with mass spectrometry detection, in order to improve sensitivity and thus expanding the applicability of the method.

Acknowledgements

Financial support from the Spanish Ministry of Science and Innovation (CTQ2010-15027) is gratefully acknowledged. The support given through an "INCRECYT" research contract to M. Zougagh and a research post-doc fellowship from Junta de Comunidades de Castilla-La Mancha and the Fondo Social Europeo to M.J. Lerma-García is also acknowledged.

References

[1] B. Kagedal, D.S. Goldstein, J. Chromatogr. 429 (1988) 177–233.
[2] J.M. Feldman, Clin. Chem. 32 (1986) 840–844.
[3] M. Candito, E. Billaud, M. Chauffert, J.M. Cottet-Emard, D. Desmoulin, J.P. Garnier, J. Greffe, C. Hirth, N. Jacob, F. Millot, A. Nignan, M.C. Patricot, L. Peyrin, P.F. Plouin, Ann. Biol. Clin. 60 (2002) 15–36.
[4] H. Nohta, H.K. Jeon, M. Kai, Y. Ohkura, Anal. Sci. 9 (1993) 537–540.
[5] K. Vuorensola, H. Sirén, U. Karjalainen, J. Chromatogr. B 788 (2003) 277–289.
[6] L.A. Kartsova, A.A. Sidorova, V.A. Kazakov, E.A. Bessonova, A.Ya. Yashin, J. Anal. Chem 59 (2004) 737–741.
[7] Z.D. Peterson, D.C. Collins, C.R. Bowerbank, M.L. Lee, S.W. Graves, J. Chromatogr. B 776 (2002) 221–229.
[8] K. Vuorensola, H. Sirén, J. chromatgr. A 895 (2000) 317–327.
[9] C. Canfell, S.R. Binder, H. Khayam-Bashi, Clin. Chem. 28 (1982) 25–28.
[10] M. Radjaipour, H. Raster, H.M. Liebich, Eur. J. Clin. Chem. Clin. Biochem. 32 (1994) 609–613.
[11] R. Zhu, W.Th. Kok, Anal. Chem. 69 (1997) 4010–4016.
[12] B. Claude, R. Nehmé, P. Morin, Anal. Chim. Acta 699 (2011) 242–248.
[13] J. Kim, M. Jeon, K.J. Paeng, I.R. Paeng, Anal. Chim. Acta 619 (2008) 87–93.
[14] B. Sellergren, Anal. Chem. 66 (1994) 1578–1582.
[15] B. Sellergren, J. Chromatogr. A 673 (1994) 133–141.
[16] C.J. Tan, H.G. Chua, K.H. Ker, Y.W. Tong., Anal. Chem. 80 (2008) 683–692.
[17] Y. Watabe, K. Hosoya, N. Tanaka, T. Kubo, T. Kondo, M. Morita., J. Chromatogr. A 1073 (2005) 363–370.
[18] M. Jiang, J.H. Zhang, S.R. Mei, Y. Shi, L.J. Zou, Y.X. Zhu, K. Dai, B. Lu, J. Chromatogr. A 1110 (2006) 27–34.
[19] J.X. He, S. Wang, G.Z. Fang, H.P. Zhu, Y. Zhang., J. Agric. Food Chem. 56 (2008) 2919–2925.
[20] J.J. Ou, L.H. Hu, L.G. Hu, X. Li, H.F. Zou, Talanta 69 (2006) 1001–1006.
[21] Z.Q. Zou, M. Ibisate, Y. Zhou, R. Aebersold, Y.N. Xia, H. Zhang, Anal. Chem. 80 (2008) 1228–1234.
[22] G. Lu, F. He, X.M. Wang, F. Gao, G. Zhang, T. Wang, H. Jiang, C.H. Wu, D.D. Guo, X.M. Li, B.A. Chen, Z.Z. Gu, Langmuir 24 (2008) 2151–2156.
[23] L.H. Zhang, B.F. Liu, S.J. Dong, J. Phys. Chem. B 111 (2007) 10448–10452.
[24] X.L. Zhao, Y.L. Shi, T. Wang, Y.Q. Cai, G.B. Jiang, J. Chromatogr. A 1188 (2008) 140–147.
[25] J.D. Li, X.L. Zhao, Y.L. Shi, Y.Q. Cai, G.B. Jiang, J. Chromatogr. A 1180 (2008) 24–31.
[26] X.L. Zhao, Y.L. Shi, Y.Q. Cai, S.F. Mou, Environ. Sci. Technol. 42 (2008) 1201–1206.
[27] H. Yuling, L. Yuanwen, L. Ruijin, T. Wei, L. Gongke, Talanta 84 (2011) 462–470.
[28] L. Xubiao, Z. Youcai, H. Yining, Y. Lixia, T. Xinman, L. Shenglian, J. Hazard. Mater. 187 (2011) 274–282.
[29] X. Zhang, L. Chen, Y. Xu, H. Wang, Q. Zeng, Q. Zhao, N. Ren, L. Ding, J. Chromatogr. B 878 (2010) 3421–3426.
[30] Y. Zhang, Y. Li, Y. Hu, G. Li, Y. Chen, J. Chromatogr. A 1217 (2010) 7337–7344.
[31] Y. Hu, Y. Li, Y. Zhang, G. Li, Y. Chen, Anal. Bioanal. Chem. 399 (2011) 3367–3374.
[32] X. Kan, Q. Zhao, D. Shao, Z. Geng, Z. Wang, J.J. Zhu, J. Phys. Chem. B 114 (2010) 3999–4004.
[33] Y. Ji, J. Yin, Z. Xu, C. Zhao, H. Huang, H. Zhang, C. Wang., Anal. Bioanal. Chem. 395 (2009) 1125–1133.
[34] Y. Zhang, R.J. Liu, Y.L. Hu, G.K. Li, Anal. Chem. 81 (2009) 967–976.
[35] C. Yang, G. Wang, Z. Lu, J. Sun, J. Zhuang, W. Yang., J. Mater. Chem. 15 (2005) 4252–4257.
[36] A.E. Sánchez, S. Corona, G. Alarcón, A. Rojas, M.T. Ramírez, M.A. Romero, Spectrochim. Acta A 59 (2003) 3193–3203.
[37] C.P. Ong, S.F. Pang, S.P. Low, H.K. Lee, S.F.Y. Li, J. Chromatogr. 559 (1991) 529–536.
[38] T. Kaneta, S. Tanaka, H. Yoshida, J. Chromatogr. 538 (1991) 385–395.
[39] P. Britz-McKibbin, A.R. Kranack, A. Paprica, D.D.Y. Chen, Analyst 123 (1998) 1461–1463.
[40] P. Britz-McKibbin, J. Wong, D.D.Y. Chen, J. Chromatogr. A 853 (1999) 535–540.
[41] P. Britz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2000) 1242–1252.
[42] R. Zhu, W.Th. Kok, Anal. Chem. 69 (1997) 4010–4016.
[43] H. Siren, U. Karjalainen, J. Chromatogr. A 853 (1999) 527–533.
[44] K. Vuorensola, H. Siren, J. Chromatogr. A 895 (2000) 317–327.
[45] M. Lee, S. Youn, T. Sankar, I.R. Paeng, B.Y. Cho, K.J. Paeng, B.Y. Cho, K.J. Paeng, J. Chromatogr. A 1160 (2007) 340–344.