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Determination of catecholamines and metanephrines in urine by capillary electrophoresis–electrospray ionization–time-of-flight mass spectrometry

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Abstract

A method successfully coupling capillary electrophoretic separation to time-of-flight mass spectrometric (TOFMS) detection for the simultaneous analysis of catecholamines (dopamine, norepinephrine, and epinephrine) and their *O*-methoxylated metabolites (3-methoxytyramine, normetanephrine, and metanephrine) is described. The inner capillary wall was coated with polyvinyl alcohol in order to obtain baseline resolution of catecholamines and metanephrines and to ensure reproducibility without extensive restorative washing of the capillary. Using electrokinetic injection, detection limits of 0.3 μM for dopamine and norepinephrine, 0.2 μM for 3-methoxytyramine and normetanephrine, and 0.1 μM for epinephrine and metanephrine were achieved with standard solutions. The usefulness of this approach was demonstrated by applying the developed method to the analysis of a spot collection of human urine from a healthy volunteer. The catecholamines and metanephrines were removed from the urine samples and preconcentrated by simultaneous SPE on cation-exchange sorbents. The recoveries of all analytes, with the exception of epinephrine (75%), were over 80%. Catecholamines and metanephrines in the urine samples were quantitated using 3,4-dihydroxybenzylamine as an internal standard. Submicromolar concentrations, consistent with the catecholamine and metanephrine levels reported for normal human urine, were detected.
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1. Introduction

Catecholamines represent a class of small, potent bioregulatory molecules. They act as neurotransmitters in the central and peripheral nervous systems and serve as hormones in the systemic circulation, regulating heart rate and blood pressure [1].

Clinical measurements of plasma or urine levels of catecholamines and their metabolites, metanephrines, are crucial for the proper diagnosis and treatment of certain potentially fatal catecholamine-producing tumors, e.g. pheochromocytoma [1]. Most current clinical assays for catecholamines and metanephrines

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use HPLC with electrochemical detection [2–6]. However, these assays are not completely specific and can also suffer from insufficient sensitivity.

In the last few years, the coupling of various separation techniques to mass spectrometry has become a focus of substantial research. The mass spectrometer can identify the analytes based on their mass-to-charge ratios and thus provide the detection specificity that most other types of detectors lack. GC–MS has been used for the quantitation of urinary normetanephrine and metanephrine [7]. Recently, some authors reported the application of HPLC–MS to the analysis of 9-fluorenylmethyloxycarbonyl derivatives of catecholamine standards [8] and underivatized catecholamines and metanephrines in a spot collection of urine [9]. Kushnir et al. have recently published an HPLC–MS–MS method for the determination of catecholamines in patient specimens (24-h urine collection) [10].

Applications of other separation methods for the analysis of catecholamines have been proposed, namely electrophoretic techniques offering higher resolution than HPLC. While there have been many papers reporting the use of capillary electrophoresis (CE) with UV or fluorescence detection for the determination of metanephrines and/or catecholamines [11–18], very few authors have used CE–MS for the analysis of these compounds [19–22]. Three of these reports were limited to analysis of solutions of standard compounds [18,19,22]. Jäverfalk-Hoyes et al. analyzed an extract of brain tissue, however, dopamine was the only catecholamine assayed [21]. To our knowledge, assays employing electrophoretic separation coupled with MS detection have not been applied to the simultaneous determination of catecholamines and metanephrines in human urine or plasma.

The major advantage of the time-of-flight mass spectrometer (TOFMS) is its ability to detect ions of different mass-to-charge ratios (m/z) simultaneously and, therefore, resolve incompletely separated or co-eluting peaks, unless the eluting compounds form ions of identical m/z ratios.

In this study, we have sought to combine the advantages of the high separation efficiency of capillary electrophoresis (CE) and the specificity of TOFMS detection for the development of a method for simultaneous determination of catecholamines and metanephrines. We have applied the developed method to the determination of analytes of interest in a spot collection of human urine with concentrations within the reference interval for healthy individuals [1]. Patients with pheochromocytoma have concentrations at least double those of healthy individuals and commonly have concentrations more than 10 times the upper limit of the reference interval [1]. Sample clean-up was carried out by solid-phase extraction (SPE) on cation-exchange sorbents.

2. Experimental

2.1. Materials and chemicals

Catecholamines and their *O*-methoxylated metabolites (3-hydroxytyramine HCl (dopamine, D), 3-methoxytyramine HCl (3MT), (±)-norepinephrine[+]-hydrogentartrate (NE), DL-normetanephrine HCl (NM), (±)-epinephrine HCl (E), and DL-metanephrine HCl (M)) were purchased from Sigma (St Louis, MO, USA). Internal standards (3,4-hydroxybenzylamine HBr (DHBA), 4-hydroxy-3-methoxybenzylamine HCl (HMBA)) and polyvinyl alcohol (PVA, 99+%, average M_r 89 000–98 000 g/mol) were purchased from Aldrich (Milwaukee, WI, USA). Ammonium acetate, sodium acetate, HPLC-grade methanol and glacial acetic acid were from Mallinckrodt (Paris, KY, USA). HPLC grade water (pH 5.2) was obtained from Fisher (Fair Lawn, NJ, USA) and was used for the preparation of all solutions used in this work.

2.2. Buffers and standard solutions

Ammonium acetate and sodium acetate buffers were prepared at 30 mM concentration and adjusted to a final pH of 4.2–4.5 with acetic acid. Acetic acid (1%) was prepared by mixing glacial acetic acid with water in a ratio of 1:99 (v/v). All buffers were filtered using Acrodisc® syringe filters with 0.2 μm HT Tuffryn® membrane (Pall Gelman Laboratory, Ann Arbor, MI, USA) and degassed under house vacuum and/or by sonication. Stock solutions of D, NE, E, NM, M, HMBA, and DHBA were prepared in 0.1 M acetic acid at 10 mM and were kept in the dark at −20 °C. Under these conditions, the solu-

tions were stable for at least 1 month. The stock solutions were thawed and further diluted to the desired concentrations with water immediately prior to use.

2.3. Instrumentation

A Crystal CE Model 300 (UNICAM, Madison, WI, USA) capillary electrophoresis apparatus capable of performing both electrokinetic and hydrodynamic injections was used for all separations. The temperature during runs was set at 22 °C. For CE with UV–Vis detection, a Model 759 A UV absorbance detector (Applied Biosystems, Foster City, CA, USA) was used. ChromPerfect 3.54 (Justice Laboratory Software, Palo Alto, CA, USA) was used for data acquisition from the CE–UV analyses. A commercial Jaguar™ time-of-flight mass spectrometer (LECO, St Joseph, MI, USA) was used as the detector for all CE–TOFMS separations. The Jaguar™ uses orthogonal acceleration architecture with a multi-channel plate and a multi-anode detector. Spectra were collected at a rate of 5000 Hz, with a sum rate of 1600, which resulted in 3.1 spectra s^{-1}. The nozzle board was heated to 80 °C.

2.4. Electrospray interface

Ionization was accomplished by electrospray. The liquid sheath flow-rate was controlled using a syringe pump (Model 11, Harvard Apparatus, Holliston, MA, USA) with a 250-μl glass syringe fitted with a two-inch 22-gauge stainless steel needle (Hamilton, Reno, NV, USA). The syringe was connected to a 50 μm I.D.×187 μm O.D. fused-silica capillary transfer line (Polymicro Technologies, Phoenix, AZ, USA) using a Chemfluor PTFE straight union (Norton Performance Plastics, Akron, OH, USA). A Chemfluor tee connected the transfer line to the analytical CE column. As the liquid sheath entered the tee, it flowed along the outside of the CE column and inside the 26-gauge stainless steel electrospray needle, which was held by the tee. The end of the electrospray needle was tapered using sandpaper to aid in the production of a Taylor cone. The interface was mounted on an XYZ-stage (Series 462, Newport, Englewood, CO, USA) to optimize the electrospray needle position relative to the TOFMS sample orifice. A microscope fitted with a 20× eyepiece and a 15–60× adjustable objective (Edmund Scientific, Barrington, NJ, USA) was used together with a Series 41722 fiber optic illuminator (Cole-Palmer, Arcade, NY, USA) to visually observe the integrity of the Taylor cone at the electrospray tip.

2.5. Preparation of CE capillaries

CE capillaries (75 μm I.D.×365 μm O.D. and 50 μm I.D.×187 μm O.D., Polymicro Technologies, Phoenix, AZ, USA) were coated with polyvinyl alcohol (PVA) prior to use to suppress the electroosmotic flow. The procedure was similar to that used by Clarke et al. [23]. Briefly, a solution of 6% PVA in water was thoroughly degassed using sonication. Then 2.5 m of the fused-silica capillary was attached to a small in-house made pressure vessel containing a vial with the 6% PVA solution. The PVA solution was passed through the capillary column at 100 p.s.i. for 1 h. The column was then emptied at 30 p.s.i. and dried under nitrogen for 1 h. Finally, the column was placed in a GC oven (HP 5890, Hewlett-Packard, Palo Alto, CA, USA) under a stream of helium (20 p.s.i.) and subjected to a temperature program of 40 °C ramped to 145 °C at 5 °C min^{-1}, followed by holding at 145 °C for 5 h. The entire process was repeated while filling the capillary from the opposite end to ensure sufficient and uniform coating.

2.6. Solid-phase extraction

A modified Waters Generic Oasis MCX method for extraction of basic compounds was used. Urine samples collected from healthy volunteers were acidified with HCl to pH 2 and centrifuged at 1600 *g* for 3 min. To 2- and 5-ml aliquots of urine, 100 μl of 0.2 *M* EDTA/ml of urine and 10 μl of 0.5 *M* ascorbic acid/ml of urine were added. For CE–UV comparison of spiked and non-spiked urine samples, some of the 2-ml aliquots were enriched with catecholamine and metanephrine standards to a final concentration of 5 μ*M*. For MS detection, 5-ml aliquots of urine were spiked with 50 μl of 100 μ*M* internal standard (DHBA) to give a final concentration of 1 μ*M*. The specimens were adjusted to pH 7 with sodium hydroxide and immediately applied to the SPE cartridge. One-milliliter Oasis MCX car-

tridges filled with 30 mg of sorbent (Waters, Milford, MA, USA) were conditioned with 1 ml of methanol and 1 ml of water. After sample application, the cartridges were washed with 1–2 ml of 0.1 *M* HCl and 1–2 ml of methanol. The analytes were eluted with 1.4 ml of 5% ammonium hydroxide in methanol into vials containing 30 μl of glacial acetic acid. The eluates were dried using vacuum centrifugation and reconstituted in 200 μl (for the 2-ml urine aliquots) or 250 μl (for the 5-ml urine aliquots) of water.

2.7. Recovery studies

Human urine samples supplemented with catecholamine and metanephrine standards at concentrations of 10 and 2 μ*M* ($n=6$) and samples without the addition of standards were used to evaluate extraction recoveries. All samples were extracted by SPE and analyzed by CE–UV at the same time. The analyte signal from the non-spiked samples was subtracted from the average analyte signal from the supplemented samples and the recovery was calculated by comparing the difference between the spiked and non-spiked samples to the signal of a mixture of standards of corresponding concentrations.

2.8. Linearity, detection limits, and quantitation

For UV detection, linearity was evaluated by analyzing solutions of individual catecholamines and metanephrines at concentrations of 0.5, 1.0, 10.0, and 100.0 μ*M*. The limits of detection (LOD) were determined as the concentrations corresponding to a signal-to-noise ratio (S/N) of 3. The noise was determined as the root mean square (RMS) noise using ChromPerfect 3.54 data acquisition software. The concentrations were calculated using the calibration curves constructed from the linearity data for each individual analyte. For MS detection, because of the non-linear character of electrokinetic injection, an internal standard was used instead of a calibration curve for determining the concentrations of the analytes. The LODs were determined by analyzing mixtures of catecholamines and metanephrines of progressively lower concentrations until the signal of each analyte was 3× the S/N ratio. The concentrations of the analytes, C_{anal}, were calculated using the following equation:

$$C_{anal} = [\mathrm{Signal}_{anal}/(\mathrm{I.E.}_{anal} \times \mathrm{Recovery}_{anal})]/[(\mathrm{Signal}_{IS}/(\mathrm{I.E.}_{IS} \times \mathrm{Recovery}_{IS})] \times C_{IS} \quad (1)$$

where Signal_{anal} and Signal_{IS} are the peak areas of the analytes and the internal standard, respectively, $\mathrm{I.E.}_{anal}$ and $\mathrm{I.E.}_{IS}$ are the ionization efficiencies, $\mathrm{Recovery}_{anal}$ and $\mathrm{Recovery}_{IS}$ are the SPE recoveries, and C_{IS} is the concentration of the internal standard.

3. Results and discussion

3.1. CE separation of catecholamines and metanephrines

The conditions for the electrophoretic separation of the analytes were chosen with the limitations posed by ESI-MS detection in mind. Electrospray ionization requires the use of volatile buffers free of any non-volatile additives. However, most catecholamine and metanephrine separations reported in the literature [11–18] used such additives to achieve good separation or to protect the analytes from degradation. In lower pH buffers, in which the compounds have a net positive charge, either micelle-forming surfactants [11] or electroosmotic flow (EOF)-decreasing wall modifiers [18] have been used to separate the analytes. High pH buffers, in which the compounds have a net negative charge, yield more favorable separation conditions. However, because the compounds are less stable and easily oxidized under basic conditions, non-volatile antioxidants have often been used [12–17].

Using CE–UV, we evaluated 30 m*M* sodium acetate and ammonium acetate buffers, both at pH 4.2–4.5, as well as 1% acetic acid for the separation of a standard mixture of eight compounds consisting of three catecholamines, three methanephrines, and two internal standards. The resolution achieved with acetic acid was lower than that obtained with the acetate buffers, nevertheless all compounds were baseline resolved. To avoid using EOF-reducing buffer additives, we permanently coated the capillary inner wall with polyvinyl alcohol (PVA). This coating markedly reduced the EOF and significantly improved the separation (Fig. 1). Additionally, it was easy to apply and was stable after application, providing good reproducibility.

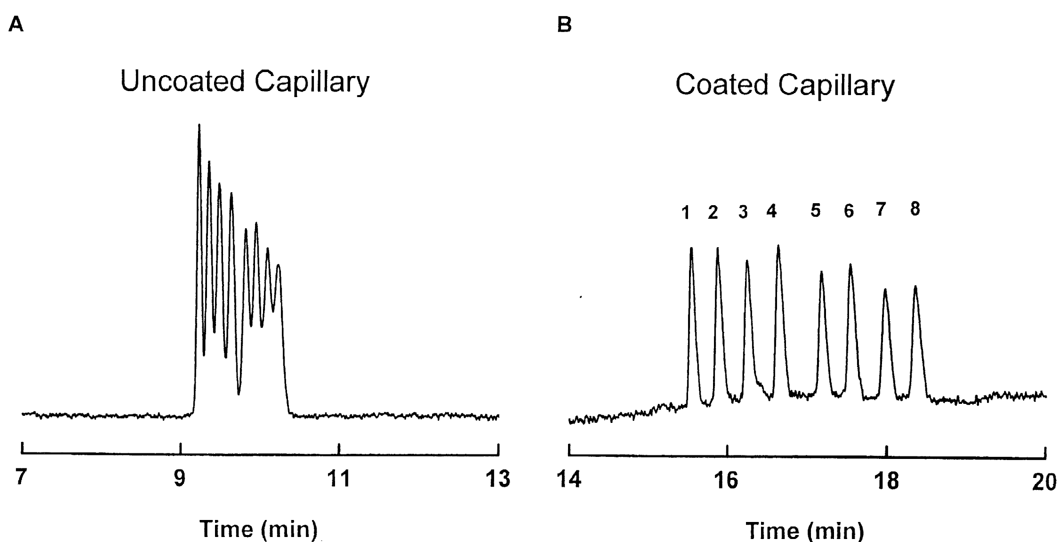

Fig. 1. CE separation of 1 μ*M* catecholamine standards on (A) uncoated and (B) PVA-coated capillaries using UV detection. Conditions: buffer, 1% acetic acid (pH 2.8); capillary, 80/92 cm (L_{det}/L_{tot}), 75 μm I.D., 360 μm O.D.; injection, 0.1 min at 20 kV; run voltage, 25 kV; current, 8.8 μA; $\lambda=215$ nm. Peak assignments: 1=DHBA, 2=HMBA, 3=D, 4=3-MT, 5=NE, 6=NM, 7=E, 8=M.

The linearity of the CE–UV of catecholamines and metanephrines was determined in the range of 0.5–100 μ*M* from three replicate measurements. The limits of detection (LOD) were determined as concentrations corresponding to $S/N=3$. The correlation coefficients, *R* (range), regression equations, and LODs are listed in Table 1.

3.2. CE-TOFMS analysis of standard compounds

The same standard mixture was analyzed using CE coupled to the TOFMS with electrospray ionization.

Table 1
Correlation coefficients, regression equations, and detection limits for the catecholamine and metanephrine standards obtained with UV detection

Compound	*R* (0.5–100 μ*M*)	Regression equation	LOD (μ*M*)
DHBA	0.9995	$y=868x+88$	0.11
HMBA	0.9999	$y=948x+81$	0.10
D	0.9996	$y=974x+50$	0.11
3MT	0.9995	$y=1069x+137$	0.10
NE	0.9999	$y=874x+169$	0.12
NM	0.9999	$y=1064x+140$	0.10
E	0.9999	$y=1008x+176$	0.11
M	0.9999	$y=1136x+193$	0.11

R, correlation coefficient (range).

Ammonium acetate and sodium acetate buffers that had worked well for CE–UV separation were found to suppress ionization of the analytes in the electrospray. We, therefore, used 1% acetic acid as the separation medium. It yielded a stable electrospray and baseline separation of the analytes (Fig. 2). While for CE–UV 75 μm I.D./360 μm O.D. capil-

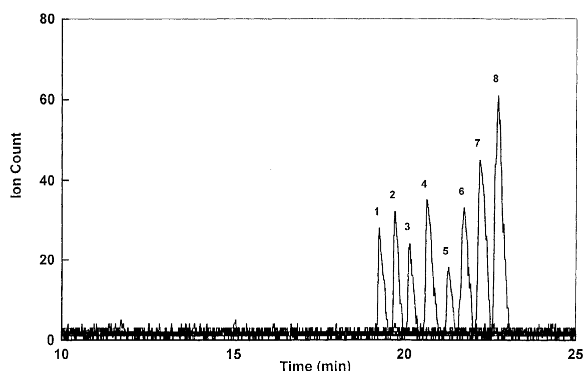

Fig. 2. CE-TOFMS analysis of 1 μ*M* catecholamine standards. CE conditions: buffer, 1% acetic acid (pH 2.8); capillary, 91 cm length, 50 μm I.D., 187 μm O.D., PVA coated; injection, 0.2 min at 20 kV; run voltage, 23 kV; current, 3.0 μA. MS conditions: ES voltage, 3.1 kV; liquid sheath flow and composition, 1.5 μl/min, 75:25:0.1 methanol/water/acetic acid (v/v). Peak identification as in Fig. 1.

laries were used, to maximize the signal magnitude without compromising the separation efficiency, for CE–MS, we chose a capillary of a smaller O.D. to achieve a smaller electrospray tip area. This allowed for a better focusing of the electrospray voltage and higher electric field on the tip, ultimately aiding the formation of ions. We had the choice between 50/187 and 75/150 (I.D./O.D. in μm) capillaries. The 50 μm I.D. was chosen over the 75 μm I.D., because the 50/187 capillary has a thicker wall than the 75/150 capillary and is therefore more resistant to breaking in the presence of accidental gas bubbles formed within the capillary.

Under the experimental conditions used, each of the analytes yielded two types of ions: a protonated molecular ion (Table 2) and a daughter ion formed by the loss of either a water molecule or an ammonia molecule from the protonated molecular ion. However, for all of the compounds studied here, the relative abundances of the daughter ions were less than 10% of the relative abundances of the molecular ions and thus, detection was based on the signal produced by the molecular ions. The mass resolution was calculated to be 928 at m/z 170.

As observed by other authors [22], the ionization efficiencies of the individual analytes were not equal. For the purposes of quantitation using the CE–MS method, the ionization efficiencies were calculated as ratios of the peak areas of 1-μ*M* standards of each catecholamine and metanephrine to the peak area of the internal standard.

Due to the inherent dilution of the analyte zones by the liquid sheath as they exited the CE column, the detection limits of this technique were substantially higher than those obtained with a UV detector under the same pressure injection conditions. For a 10-μ*M* mixture of standards, the signals of four of the compounds (DHBA, HMBA, D, and NE) were only slightly higher than the LODs obtained using the maximum pressure injection (1.5% of the length of the capillary) that still yielded reasonable separation efficiency (results not shown). We, therefore, used electrokinetic injection, which allowed for the introduction of narrow, highly concentrated sample zones into the capillary and, thus, yielded significantly lower LODs [24,25]. The LODs were determined by analyzing progressively lower concentrations of catecholamines and metanephrines until the signals of the individual analytes were 3× the S/N ratio. Mixtures of the analytes at concentrations 1.0, 0.7, 0.4, 0.3, 0.2, and 0.1 μ*M* were thus analyzed. The detection limits for the analyte standards are summarized in Table 2.

3.3. Sample clean-up

The urine matrix is complex and, therefore, sample clean-up is a necessary part of current clinical assays. Both CE separation and electrospray ionization–TOFMS detection are very sensitive to the presence of high concentrations of salts; thus, while reduction of potential interferences was important, the primary goal of our sample pretreatment was to minimize the salt content.

Although catecholamines and metanephrines have very similar structures, their chemical behaviors, namely interactions with various SPE sorbents, differ significantly. The catecholamines contain a catechol moiety, whereas in the metanephrines, position three of the benzene ring is methoxylated, which produces significant differences in hydrophobicity and pK_a values between these two groups of compounds.

Table 2
Mass-to-charge ratios, ionization efficiencies, and CE–MS LODs of protonated analyte molecular ions

Compound	m/z (M+1 ions)	Ionization efficiencies	LODs (μ*M*)
DHBA	140.1	1.00	0.2
HMBA	154.1	1.14	0.2
D	154.1	0.86	0.3
3MT	168.1	1.25	0.2
NE	170.1	0.71	0.3
NM	184.1	1.18	0.2
E	184.1	1.62	0.1
M	198.1	2.56	0.1

Several papers have described the analysis of both catecholamines and metanephrines in urine [2–5] and plasma [6] using one set of chromatographic conditions; however, in these reports, the two groups of compounds were extracted by separate SPE procedures and the extracts were chromatographed separately. For the SPE of catecholamines, alumina or alumina followed by a cation exchanger have been used [3,5,6]. Metanephrines have been most frequently extracted using a cation exchanger or a combination of cation and anion exchangers [2–6]. A few papers have reported simultaneous extraction and determination of catecholamines and metanephrines in urine and/or plasma [26–30]. Most of these methods [26–29] employed on- or off-line strong cation exchangers to extract all analytes. The specificity and sensitivity were then achieved by post-column coulometric [26,28,29] or chemical [27] oxidation followed by derivatization of the analytes with a fluorogenic reagent. The set-ups used for these techniques represent complex, multicomponent systems that require optimization of many parameters. Burke et al. [30] published an HPLC–EC method with an extraction procedure for catecholamines, metanephrines and other metabolites using alumina B and N with a diethylether wash and an ethyl acetate elution. In our hands, this approach was not sufficiently reproducible or efficient. Recently, Chan et al. [9] published a method for coupling HPLC to atmospheric pressure chemical ionization mass spectrometry for simultaneous analysis of catecholamines (D, NE, E) and metanephrines (NM, M) in human urine after SPE on Bio-Rex 70 cation-exchange resin. They applied the developed method to a spot collection of healthy human urine; however, they did not attempt any quantitation of the analytes in the specimen.

We have evaluated several solid-phase sorbents using the simple CE–UV set-up and determined that the Oasis MXC cation-exchange cartridges allowed for simultaneous SPE of all six catecholamines and metanephrines and the two internal standards with good recoveries. The manufacturer's extraction method suggested loading an acidified sample, washing the cartridge with 0.1 *M* HCl and methanol and eluting the analytes with 5% ammonium hydroxide in methanol. Under these conditions, peaks for the compounds of interest were completely masked by interfering substances present in urine. We tested another suggested wash step that used 5% TEA in methanol. This step should eliminate tertiary and aromatic amines. Using this modification, we obtained a significantly cleaner extract. However, the volume of 5% TEA necessary for adequate reduction of interferences caused analyte losses as high as 10%. By applying samples at several pH values, we found that the interferences decreased with increasing pH. Consequently, we used samples adjusted to pH 7 and obtained a reduction of interferences equal to or better than the results with the TEA wash step, while recoveries of the analytes were not adversely affected. Yet higher sample pH was avoided to prevent analyte degradation. Recoveries of the analytes from human urine spiked with catecholamine and metanephrine standards at 2 and 10 μ*M* concentrations are summarized in Table 3.

3.4. SPE–CE-TOFMS of catecholamines and metanephrines in urine

Having identified SPE conditions for the catecholamines and metanephrines, urine extracts were analyzed by CE-TOFMS. A selected-ion plot for a representative analysis is shown in Fig. 4. It is clear from Figs. 3 and 4 that TOFMS detection using selected-ion monitoring yields markedly simplified electropherograms in comparison with non-selective UV detection. The analytes are identified on the basis of their characteristic m/z ratios.

With electrokinetic injection, quantitation is not as straightforward as with pressure injection. The

Table 3
SPE recoveries of catecholamines and metanephrines from spiked urine samples

Compound	Recovery (%)[a]	
	2 μ*M* urine sample	10 μ*M* urine sample
DHBA	86.7±2.5	93.2±4.7
HMBA	89.5±2.5	93.5±4.0
D	83.4±3.1	88.5±4.7
3MT	87.5±3.7	89.3±5.8
NE	81.9±4.1	85.5±4.1
NM	87.3±5.7	92.1±8.8
E	75.5±4.5	75.9±9.4
M	91.2±3.6	89.3±3.5

[a] Results are given as the mean±SD for six sets of experiments.

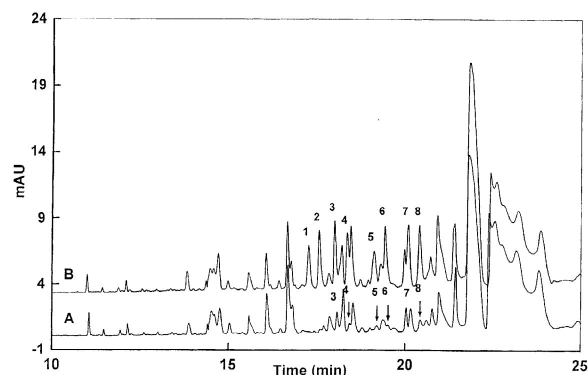

Fig. 3. CE–UV analysis of Oasis MCX extracts of 2 ml of (A) blank urine, and (B) urine spiked with catecholamine and metanephrine standards. The extracts were reconstituted in 0.2 ml of water. Conditions: buffer, 30 m*M* sodium acetate (pH 4.5); capillary, 90/101 cm (L_{det}/L_{tot}), 75 μm I.D., 360 μm O.D., PVA coated; injection, 0.2 min at 100 mbar; run voltage, 30 kV; current, 40 μA; λ=215 nm. Peak identification as in Fig. 1.

amount injected is influenced by the ionic strength of the sample and is not a linear function of the analyte concentration. To solve this problem, we employed an internal standard and calculated the concentrations of the analytes using Eq. (1). We tested two compounds for use as an internal standard, namely DHBA and HMBA. These compounds gave similar ionization efficiencies and SPE recoveries. However, HMBA was ionized with the same m/z ratio as D and migrated next to D, whereas DHBA has a unique

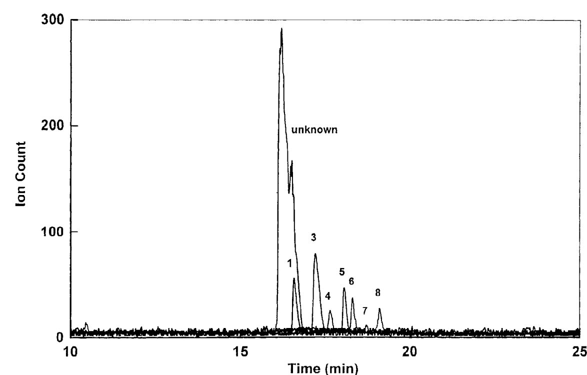

Fig. 4. CE-TOFMS analysis of an Oasis MCX extract of 5 ml of non-spiked urine reconstituted in 0.25 ml of water. CE conditions: buffer, 1% acetic acid (pH 2.8); capillary, 86 cm length, 50 μm I.D., 187 μm O.D., PVA coated; injection, 0.2 min at 20 kV; run voltage, 23 kV; current, 3.5 μA. MS conditions as in Fig. 2. Peak identification as in Fig. 1.

Table 4
Catecholamine and metanephrine levels in a spot collection of human urine

Compound	μmol/l of urine
D	1.89
3MT	0.31
NE	0.78
NM	0.51
E	0.07
M	0.20

m/z ratio and could be easily distinguished from other catecholamines and metanephrines. In order to avoid the possibility of a partial overlap of the peaks of D and HMBA, the two compounds giving ions of the same m/z ratio, DHBA was chosen as the internal standard.

Using this method, we analyzed a 5-ml aliquot of a spot collection of human urine spiked with the internal standard (DHBA) to a concentration 1 μ*M*. The urine sample was subjected to SPE and the dried eluate was reconstituted in 0.25 ml of water, which represented a 20-fold preconcentration of the analytes. The levels of catecholamines and metanephrines were then calculated using Eq. (1), with the internal standard at 1 μ*M*, and the values of the ionization efficiencies and recoveries for the individual analytes taken from Tables 2 and 3, respectively. The calculated concentrations are summarized in Table 4 and are consistent with values reported for healthy individuals [1,30].

4. Conclusions

In this paper, we have described the successful coupling of CE to TOFMS for the simultaneous analysis of catecholamines and metanephrines. This method combines the advantages of high separation efficiency of CE and detection specificity of TOFMS. Coating of the CE capillary with PVA allowed for baseline separation of all analytes and increased the reproducibility of analysis. This also circumvented the need for extensive between-run washing of the capillary with hydroxides, which is necessary to ensure reproducibility with the use of uncoated capillaries.

We have demonstrated the usefulness of this

approach by applying this assay to a spot collection of normal human urine from healthy volunteers. The catecholamines and metanephrines were removed from the urine samples simultaneously by SPE on cation-exchange sorbents. The recoveries of all analytes, with the exception of epinephrine, were over 80%. The individual analytes were identified both by location in the electropherogram and by their respective m/z ratios measured by the TOFMS.

Since pressure injection in our case did not yield sufficiently low LODs, we employed electrokinetic injection. Using a quantitation method based on the calculation of analyte concentration by comparison to an internal standard, we were able to measure catecholamine and metanephrines levels consistent with values reported for healthy individuals.

While the presented assays could form the basis for a method to be used in clinical practice, improvement of the electrospray ionization efficiency to achieve lower detection limits with pressure injection, which is the preferred mode of injection for quantitation purposes, and thorough validation of this methodology, are still needed. Work is currently in progress to improve the electrospray interface design in order to achieve lower analyte detection limits.

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References

[1] E.G. Biglieri, J.D. Baxter, in: P. Felig, J.D. Baxter, A.E. Broadus, L.A. Frohman (Eds.), Endocrinology and Metabolism, McGraw-Hill, New York, 1981, Chapter 14.
[2] N.C. Parker, C.B. Levtzow, P.W. Wright, L.L. Woodard, J.F. Chapman, Clin. Chem. 32 (1986) 1473.
[3] R.T. Peaston, T.W.J. Lennard, L.C. Lai, J. Clin. Endocrinol. Metab. 81 (1996) 1378.
[4] M. Hay, P. Mormède, J. Chromatogr. B 703 (1997) 15.
[5] A. Ciofu, E. Baudin, P. Chanson, A.F. Cailleux, E. Comoy, J.C. Sabourin, M. Ducreux, G. Schaison, M. Schlumberger, Eur. J. Endocrinol. 140 (1999) 434.
[6] E. Grouzmann, M. Fathi, M. Gillet, A. de Torrenté, C. Cavadas, H. Brunner, T. Buclin, Clin. Chem. 47 (2001) 1075.
[7] C. Canfell, S.R. Binder, H. Khayam-Bashi, Clin. Chem. 28 (1982) 25.
[8] S. Chen, Q. Li, P.M. Carvey, K. Li, Rapid Commun. Mass Spectrom. 13 (1999) 1869.
[9] E.C.Y. Chan, P.C. Ho, Rapid Commun. Mass Spectrom. 14 (2000) 1959.
[10] M.M. Kushnir, F.M. Urry, E.L. Frank, W.L. Roberts, B. Shushan, Clin. Chem. 48 (2002) 323.
[11] C.P. Ong, S.F. Pang, S.P. Low, H.K. Lee, S.F.Y. Li, J. Chromatogr. 559 (1991) 529.
[12] T. Kaneta, S. Tanaka, H. Yoshida, J. Chromatogr. 538 (1991) 385.
[13] P. Britz-McKibbin, A.R. Kranack, A. Paprica, D.D.Y. Chen, Analyst 123 (1998) 1461.
[14] P. Britz-McKibbin, J. Wong, D.D.Y. Chen, J. Chromatogr. A 853 (1999) 535.
[15] P. Britz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2000) 1242.
[16] R. Zhu, W.Th. Kok, Anal. Chem. 69 (1997) 4010.
[17] H. Sirén, U. Karjalainen, J. Chromatogr. A 853 (1999) 527.
[18] K. Vuorensola, H. Sirén, J. Chromatogr. A 895 (2000) 317.
[19] R.D. Smith, C.J. Baringa, H.R. Udseth, Anal. Chem. 60 (1988) 1948.
[20] Y. Takada, M. Yoshida, M. Sakairi, H. Koizumi, Bunseki Kagaku 44 (1995) 241.
[21] E.M. Jäverfalk-Hoyes, U. Bondesson, D. Westerlund, P.E. Andrén, Electrophoresis 20 (1999) 1527.
[22] K. Vuorensola, J. Kokkonen, H. Sirén, R.A. Ketola, Electrophoresis 22 (2001) 4347.
[23] N.J. Clarke, A.J. Tomlison, G. Schomburg, S. Naylor, Anal. Chem. 69 (1997) 2786.
[24] M. Albin, P.D. Grossman, S.E. Moring, Anal. Chem. 65 (1993) 489A.
[25] G. Hempel, Electrophoresis 21 (2000) 691.
[26] H. Nohta, E. Yamaguchi, Y. Ohkura, H. Watanabe, J. Chromatogr. 493 (1989) 15.
[27] H.-K. Jeon, H. Nohta, Y. Ohkura, Anal. Biochem. 200 (1992) 332.
[28] M. Tsunoda, K. Takezawa, T. Santa, K. Imai, Anal. Biochem. 269 (1999) 386.
[29] K. Takezawa, M. Tsunoda, N. Watanabe, K. Imai, Anal. Chem. 72 (2000) 4009.
[30] W.J. Burke, H.D. Chung, S.W. Li, Anal. Biochem. 273 (1999) 111.