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Dynamic coating for fast and reproducible determination of basic drugs by capillary electrophoresis with diode-array detection and mass spectrometry

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Abstract

The double coating principle of CEofix® buffers was evaluated for the analysis of some basic drugs by capillary electrophoresis–diode-array detection (CE–DAD) and capillary electrophoresis–mass spectrometry (CE–MS). The involatile phosphate present in original low pH CEofix®, was replaced with formic acid for hyphenation of CE with MS. The double coating produces a substantial and highly reproducible electroosmotic flow (EOF), even at low pH. The rinsing procedure and electrolyte composition were optimized for both CE–DAD and CE–MS. The system was evaluated with the analysis of a mixture of basic drugs and a spiked urine sample enriched by solid-phase extraction (SPE). The R.S.D. values on the migration time and peak area measured for 28 analyses with CE–DAD were below 0.25 and 2.40%, respectively. For CE–MS, the R.S.D. on the migration time was 0.85% or less and the area precision ranged from 5.65 to 14.33% (for seven injections). The LOD with the developed CE–MS method was below 50 ppb for all five drug standards tested.

Keywords: Dynamic coating; Basic drugs

1. Introduction

Screening methods for elucidation of drugs and drug metabolites in biological fluids are very important in analytical toxicology and forensic laboratories. In order to cover a sufficiently broad range of drugs, complementary analytical techniques are applied. Numerous papers have already been published on the analysis of basic drugs. In these papers, the drugs were either used as test compounds to evaluate analytical innovations, or they were analyzed in real samples. Most of the latter work includes solid-phase extraction (SPE) to enrich the target compounds and dispose of the matrix of the sample (usually blood, plasma, bile or urine). The extraction step is then followed by an analytical separation step. A review on chromatographic screening techniques in toxicology was recently published [1].

Liquid chromatography (LC) with diode-array detection (DAD) is a very common technique for the determination of drugs. When combined with mass spectroscopy (MS), LC becomes extremely powerful for confirmation of the identity of basic drugs in biological samples. The sensitivity and selectivity of LC–MS has made the technique very popular. Reviews on its application in forensic toxicology have been published [2,3].

Because of its speed of analysis, high efficiency and low solvent and sample consumption, capillary electrophoresis (CE) has gained popularity and is more and more used as an alternative or complementary technique to LC separations. Analyses of basic drugs were performed with micellar electrokinetic chromatography (MEKC) [4,5], capillary electrochromatography (CEC) [6] and capillary zone electrophoresis (CZE) [7–10]. Hudson et al. have attempted to develop a comprehensive screening method for over 400 basic drugs in whole blood [11]. In a later publication, this list was updated to over 550 basic and 100 acidic drugs [12]. The use of CE in forensic toxicology has been re-

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viewed by Tagliaro et al. [13]. Hyphenation of CE to MS combines the high speed and efficiency of CE with the universality, selectivity and sensitivity inherent to MS. Moreover, MS gives information on the identity of compounds. Molecular ions combined with fragment ions provide structural information. The hyphenation of CE with MS has been reviewed by the group of Bayer [14] and the potential of CE–MS for the analysis of basic drugs illustrated [15–17].

The combination of both techniques, however, is not always so successful as may seem. Main problems generally encountered in CE–MS are the increased analysis time, the lack of suitable volatile buffer systems and the poor repeatability, reproducibility and sensitivity. Therefore, CE–MS is not yet considered as a rugged technique in the pharmaceutical laboratories.

By using a buffer system that dynamically coats the inner wall of fused silica capillaries (CEofix®, US patent no. 5,611,903), several of these problems can be circumvented. A double coating principle is applied and two solutions are flushed through the capillary. The CEofix® principle is as follows. The buffer containing the polycation (this buffer is called “initiator”) is flushed through the capillary. The polycations adsorb strongly to the capillary wall due to charge interactions. The capillary is then flushed with the running buffer containing the polyanion (this buffer is called “accelerator”). These polyanions adsorb to the first layer of polycations forming a double layer. The polyanion layer contains sulphate groups and is rather insensitive to pH variations. Therefore, a large number of negative charges are incorporated on the capillary wall resulting in a stable (pH independent) and large (>0.5 cm^2/V s) EOF when an electric field is applied across the capillary. After the analysis, the coating is stripped from the wall by a short rinse with NaOH followed by water. The dynamic coating is re-applied using the above procedure before the next analysis is started. The coating and rinsing procedures take about 2 min. Since the coating is dynamic and is replaced between every run, no memory effects occur. Several CEofix® buffers were developed covering a pH-range from 2.5 to 9.2. These buffers are composed of phosphate and malic acid based electrolytes containing polycation (initiator) or polyanion (accelerator). Optimization of the buffer can easily be done by fine-tuning the pH, adding organic modifiers and/or surfactants or cyclodextrins.

The use of dynamic coating to create a high and pH-independent EOF has already been applied in the past. Bendahl et al. used a polybrene/poly(vinylsulfonate) double coating system for the analysis of basic compounds by CE–DAD, MEKC and CE–MS [18]. Graul and Schlenoff analyzed basic proteins by CE using a poly(diallyldimethyl–ammonium)/poly(styrenesulfonate) coating [19].

The performance of a volatile variant of CEofix® buffers was investigated for the analysis of basic drugs with CE–MS using CE–DAD to develop the method. The term volatile refers to the nature of the buffer ion (i.e. formic acid). The polyanion and the polycation additives are not volatile.

The high EOF helps to overcome the problem of the prolonged analysis time resulting from a combination of the long capillary lengths that are needed to couple a CE instrument to the MS and the low pH applied for the separation of basic substances in CE. A basic drugs standard mixture could be analyzed within 9 min with CE–MS with a limit of detection (LOD) below 50 ppb for all compounds. The CE–MS method was also applied to a spiked urine sample.

2. Experimental

2.1. Chemicals and standards

Separate stock solutions of 1000 ppm of each basic drug standard (Fig. 1) were prepared in methanol. The solutions were diluted with water and mixed prior to analysis. The water used for sample dilution and make-up liquid was LC-grade from Merck (Darmstadt, Germany). Other solvents were all LC-grade and purchased from Riedel-de Haën (Seelze, Germany). The water used for SPE of spiked urine was from a Milli-Q water purification system (Millipore, Bedford, MA, USA). The standard formic acid electrolytes with or without trimethylamine and the volatile and (non-volatile) commercially available CEofix® buffer solutions were from Analis S.A. (Namur, Belgium). When the term accelerator or initiator is used, this always refers to buffer solution containing polyanion or polycation, respectively. Phosphoric acid, acetic acid, trifluoroacetic acid (TFA) and ammonia were from Sigma (Bornem, Belgium).

2.2. CE–DAD

CE was carried out on a P/ACE MDQ capillary electrophoresis instrument equipped with DAD detector (Beckman Coulter, Fullerton, CA, USA). The separations were carried out in 75 μm i.d. bare fused silica capillaries (Composite Metal Services, Worcester, UK). The applied voltages were 12 or 18 kV for capillaries with a total length of 40.2 or 60.2 cm, respectively. Injections were performed hydrodynamically at 0.5 psi for 5 s. The capillary temperature was set at 25 °C and detection was performed at 200 nm. All capillary rinsing steps were performed at 20 psi. When a new capillary was installed, it was rinsed with NaOH (1N, 10 min) and water (5 min) prior to the first analysis. Between analyses, the capillary was rinsed with the running buffer (2 min) when a buffer without accelerator was applied. For an electrolyte with accelerator, the capillary was first rinsed with NaOH (0.1N, 0.5 min), water (0.5 min), initiator solution (0.2 min) and accelerator (0.5 min) before the first analysis with this buffer. Between runs, the capillary was only rinsed with accelerator (0.5 min).

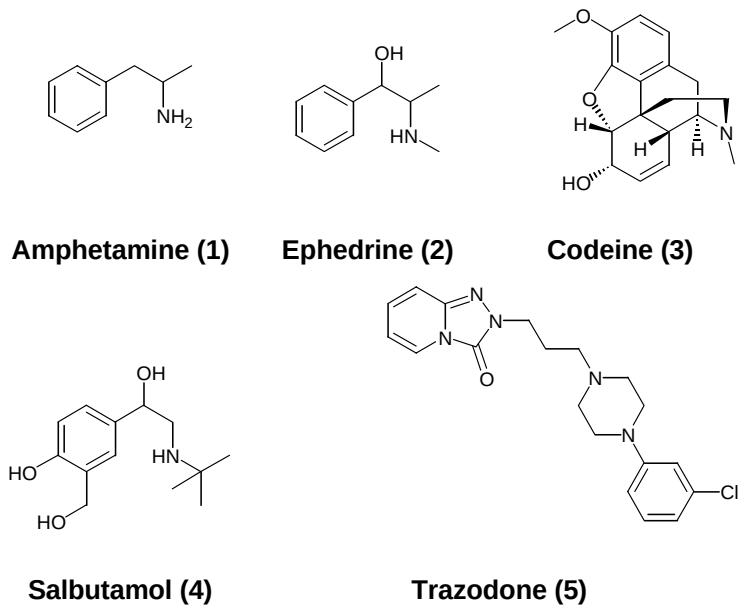

Fig. 1. Structures (peak numbers) of the selected basic drug standards.

2.3. CE–MS

For CE–MS experiments, the standard capillary cartridge was replaced with an external detector adaptor (EDA) cartridge from Beckman Coulter. The outlet of the CE capillary was inserted into the mass spectrometer spray needle. MS was performed on a LCQ ion trap mass spectrometer equipped with an electrospray ionization (ESI) source (ThermoFinnigan, San Jose, CA, USA). The ionization source was adapted for CE–MS with a special ESI needle and a micrometer to fit standard CE capillaries and to enable precise positioning of the capillary outlet, respectively (ThermoFinnigan, San Jose, CA, USA). A syringe pump installed on the MS instrument delivered the make-up liquid. The CE instrument was placed on a platform that is adjustable in height and position to avoid siphoning effects.

Analyses were carried out in 75 μm i.d. bare fused silica capillaries (Composite Metal Services, Worcester, UK). Injections were performed hydrodynamically at 2 psi for 5 s. DAD detection was bypassed. The applied CE voltage was 30 kV. Between analyses, the capillary was rinsed with the running buffer (2 min) when a buffer without accelerator was applied. If an electrolyte with accelerator was used, the capillary was first rinsed, with the ionization source open, with NaOH (0.1N, 1 min), water (1 min), initiator solution (0.5 min) and accelerator (0.5 min) before the first analysis. Then the source is closed and the first analysis is started. Before each run, the capillary was rinsed only with volatile accelerator solution (0.7 min) with the source closed. All capillary rinsing steps were performed at 20 psi.

MS detection was performed in the ESI positive ionization mode. The scan range was 100–400 atomic mass units (amu). The outlet of the capillary was precisely positioned equal with the ESI spray needle set at 5 kV (net voltage over the CE capillary is therefore 25 kV). During injection and CE voltage build-up this voltage was set to 0 kV. The heated capillary temperature was 160 °C. Nitrogen was used as sheath gas at 20 units (0.3 l/min) and no drying gas was applied. The make-up flow was composed of methanol–water (80:20, v/v) containing formic acid (0.5%, v/v) and was delivered at a flow rate of 2 μl/min. This make-up liquid was degassed daily in an ultrasonic bath. For the CE–MS^2 experiments, the trap collision induced dissociation (CID) voltage was 25% (1.25 V). The mass spectrometer was set to perform MS^2 on the molecular ion of the selected compounds.

2.4. SPE of spiked urine

For the analysis of spiked urine samples, SPE was performed based on the method developed by Logan et al. [20] using SCX Extract-Clean (500 mg, 18 ml) cartridges (Alltech, Lokeren, Belgium). Spiked urine (10 ml) was mixed with 0.5 ml of 100 mM phosphoric acid. The cartridge was conditioned with methanol (2 × 3 ml), water (Milli-Q, 2 × 3 ml) and phosphoric acid (10 mM, 3 ml). The sample was loaded on the cartridge at ca. 1 ml/min and the cartridge was left to dry for 3 min. The cartridge was rinsed with phosphoric acid (10 mM, 3 ml), acetic acid (100 mM, 2 ml) and methanol (3 ml) and was left to dry for 3 min. The compounds were eluted with ammoniacal methanol (3%, 2 × 3 ml). The collected solvent was evaporated under nitrogen after addition of 50 μl HCl (1 mM) and the residue was redissolved in 1 ml LC-grade water.

3. Results and discussion

CE analyses of basic compounds often suffer from poor migration time reproducibility due to variations in EOF and

interaction of the solutes with the capillary wall. Analysis of these compounds is mostly carried out with low pH buffers to minimize these solute–wall interactions and to charge the basic analytes. At the applied pH, the EOF is minimized leading to long analysis times and low signal-to-noise ratios.

The double coating of the CEofix® generates the same number of negative charges at the wall regardless of pH, ensuring reproducible migration times. Several CEofix® buffers were developed covering a pH-range from 2.5 to 9.2. Information on these solutions can be found on the Analis website (www.analis.be).

The commercially available CEofix® solutions are all composed of non-volatile electrolytes at high concentrations (typically 50–150 mM). They are therefore not suited for the hyphenation of CE to MS. It was the aim of this work to develop an MS compatible, i.e. volatile, CEofix® solution. An electrolyte based on formic acid was chosen for this purpose.

The performance of various electrolyte compositions and rinsing procedures on the analysis of the selected drugs was initially evaluated using CE–DAD. In a first series of experiments, a capillary was rinsed with NaOH (0.1N), water and 100 mM formic acid solution (pH 2.4) and the five standards were analyzed with the same electrolyte. The same analysis was performed using a formic acid solution that was modified for dynamic coating. Prior to analysis, the capillary was rinsed consecutively with NaOH (0.1N), water and a 100 mM formic acid initiator solution containing trimethylamine (concentration not known). Then the capillary was flushed with a 100 mM formic acid accelerator solution containing trimethylamine (concentration not known, pH 3.7) and the basic drug standards were analyzed with this electrolyte solution. The analysis time decreased ca. 2.5 times using the electrolyte solution containing the accelerator and trimethylamine. For a 60 cm capillary and an applied voltage of 18 kV, the analysis time using the accelerator solution was 5 min with baseline separation of all basic drugs compared to 12.5 min when the formic acid alone was used. The slightly higher pH of the formic acid solution with accelerator and trimethylamine (pH 3.7) compared to the formic acid solution without additives (pH 2.4) cannot account for such a drastic decrease in analysis time. When a 40 cm capillary was used with the electrolyte containing the accelerator and trimethylamine and 12 kV was applied, the analysis time was decreased even to 2.8 min, however, without baseline separation for the last two peaks (salbutamol and trazodone). The mobility of the EOF with this buffer was calculated to be ca. 0.48 $cm^2/V\,s$. The high EOF indicates that the accelerator functions adequately in a formic acid solution containing trimethylamine.

The standard rinsing procedure for analysis with CEofix® solutions involves a rinsing step with NaOH (0.1 N), water, initiator solution (polycation), and accelerator solution (polyanion). Since it was the aim to couple the CE method with MS, an alternative rinsing procedure had to be developed. The frequent introduction of NaOH and polycation into the MS is detrimental for MS detection. Therefore, if the complete rinsing procedure would be performed between runs, this would imply that the MS source would have to be opened during this rinsing procedure, or that a flow diverting system would have to be introduced between the CE capillary and the MS. For this reason, alternative rinsing procedures that do not hinder automatization and do not require hardware modifications were investigated.

The experiments were performed on the standard mixture of five basic drugs using CE–DAD. The stability of migration time was the investigated factor for each rinsing procedure. Before the start of a sequence, the complete rinsing procedure for a CEofix® buffer solution was carried out (see Section 2). Between analyses, the alternative rinsing procedure was performed. In a first test, the NaOH rinsing step was replaced by a rinsing step with NH_4OH (0.1N, 0.5 min). The migration time for the basic drugs increased by ca. 10% after 20 analyses. A second experiment was performed in which the NaOH rinsing step was left out and the rinsing step with water was prolonged (1 min instead of 0.5 min). A significant increase (ca. 20%) of migration time was observed after only five analyses. In a third test, the capillary was only rinsed with the formic acid accelerator solution containing trimethylamine (0.5 min) between runs. The resulting electropherograms are depicted in Fig. 2. With the last procedure, migration time and peak area were stable for at least 27 runs (Fig. 3). The first analysis is not taken into account since the capillary only stabilized after the first injection. The R.S.D. values on migration time and peak area were 0.25% ($n = 27$) or less and below 2.40% ($n = 27$), respectively, for all selected basic drugs.

With the volatile accelerator solution and the adapted rinsing procedure, sequences of samples could be analyzed with CE–MS without opening the MS source in between runs. In summary, the capillary is rinsed following the complete procedure before the first run with the source open and between runs, the capillary is only rinsed with the accelerator solution for 1 min without opening the ionization source. Trimethylamine that was initially added to improve the peak shape was left out of the electrolyte used for MS detection because this additive significantly reduces the sensitivity in the positive ionization mode. This modification resulted in a decreased resolution of compounds 3 and 4 in CE–DAD compared to results with a buffer containing trimethylamine. However, since MS detection will be performed, the additional selectivity of the detector will enable to separate these compounds. On the other hand, a small amount of TFA was added to the accelerator solution to improve MS sensitivity. A TFA concentration of 1 mM was chosen because this amount results in good sensitivity and acceptably low CE current. The CE current has to be kept as low as possible (preferably below 20 μA) because high currents can lead to problems at the ESI interface and the ESI needle voltage. The addition of TFA led to an increase in peak area of ca. 150–200% for the basic drugs. No ion pairing of the basic drugs with the TFA, which would lead to decreased resolution and sensitivity, was observed. The basic drug mixture

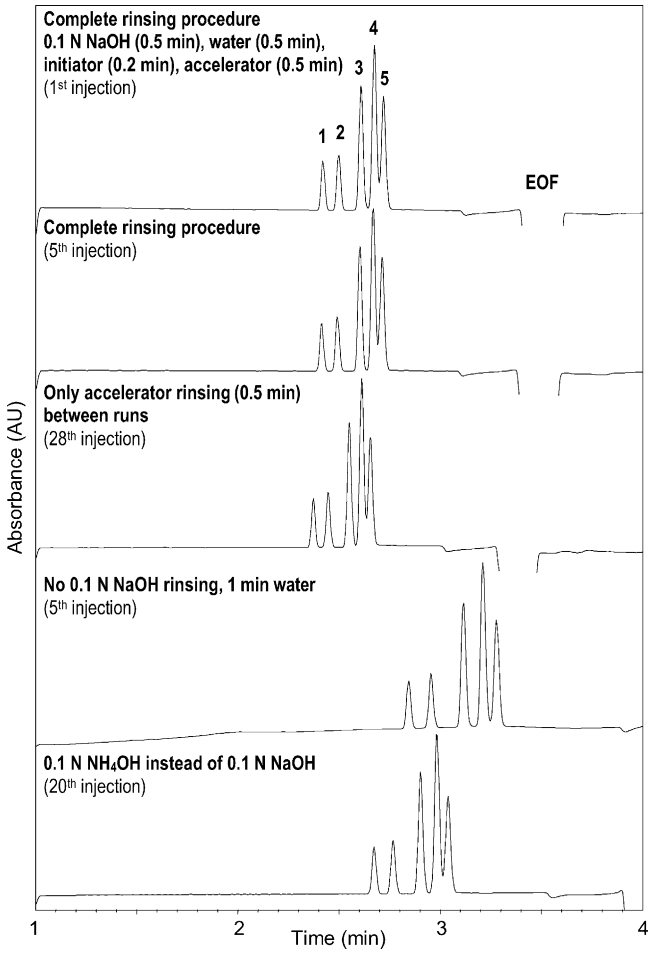

Fig. 2. Influence of various rinsing procedures on the migration time of the basic drugs (15 ppm each in water) analyzed by CE–DAD. Capillary: 75 μm i.d. × 40.2 cm total length, electrolyte: 100 mM formic acid accelerator solution containing trimethylamine (pH 3.7), voltage: 12 kV, injection: 0.5 psi for 5 s, detection: UV at 200 nm, temperature: 25 °C. Peak numbering: see Fig. 1.

was analyzed by CE–MS using a formic acid–TFA solution (100–1 mM) after flushing the capillary with only this electrolyte solution (no initiator and accelerator solutions were used). The same mixture was analyzed using a formic

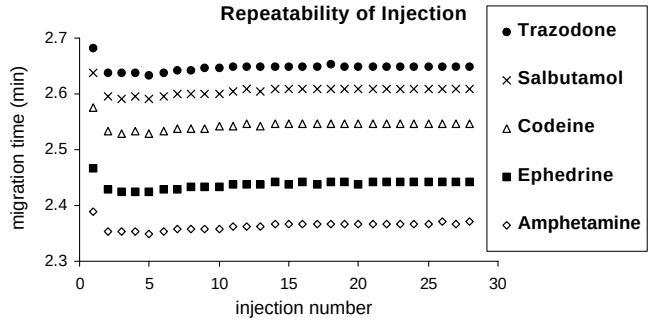

Fig. 3. Repeatability of migration time after rinsing with electrolyte accelerator solution only between CE–DAD analyses. Electrolyte: 100 mM formic acid accelerator solution containing trimethylamine (pH 3.7), other operating conditions: see Fig. 2.

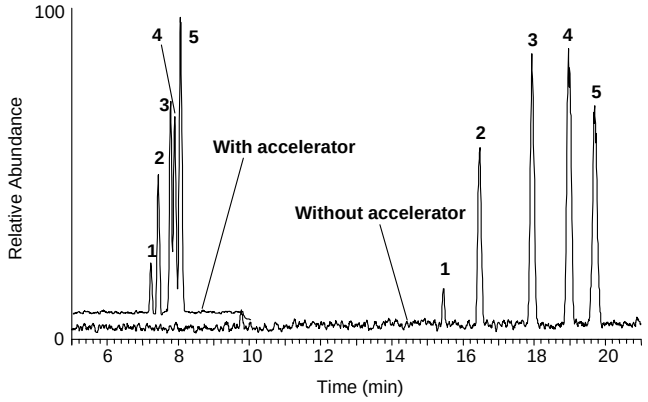

Fig. 4. Influence of accelerator on the CE–MS analysis of the selected basic drugs (2 ppm each in water). Capillary length: 93.5 cm, electrolyte: formic acid–TFA (100–1 mM) alone or formic acid–TFA (100–1 mM) accelerator solution. Other operating conditions: see Section 2. Peak numbering: see Fig. 1.

acid–TFA (100–1 mM) accelerator solution. For the latter, the capillary was first flushed with the source open with NaOH, water and the electrolyte containing initiator. The ion source was then closed and the capillary was rinsed with the run buffer containing accelerator prior to injection. The results obtained with and without accelerator are shown in Fig. 4. The gain in analysis time caused by the accelerator is obvious. The use of the accelerator also leads to an increased signal-to-noise ratio and consequently improved sensitivity compared to the analysis with the electrolyte without accelerator. It is clear that the gain in speed is counterbalanced by a decreased resolution between the analytes. However, this is not problematic since the mass spectrometer will provide the additional selectivity.

With the MS detector used in this work, the voltage in the ESI interface is applied on the spray needle while the heated capillary is grounded. Since the CE capillary is inserted into this needle, electrical contact is established between the CE inlet electrode and the MS spray needle. When no voltage is applied across the CE capillary, an electric

Table 1
Repeatability of injection and linearity data for CE–MS of a standard mixture of basic drugs (2 ppm each in water)

Compound	Migration time in min (% R.S.D.)[a]	Peak area (% R.S.D.)[a]	Correlation coefficient[b]
Amphetamine	6.996 (0.7)	28.3×10^6 (5.6)	0.9994
Ephedrine	7.171 (0.7)	84.1×10^6 (7.9)	0.9991
Codeine	7.481 (0.8)	96.6×10^6 (10.4)	0.9998
Salbutamol	7.576 (0.8)	129.7×10^6 (11.9)	0.9999
Trazodone	7.729 (0.8)	254.5×10^6 (14.3)	0.9999

Capillary: 93.0 cm L × 75 μm i.d., electrolyte: formic acid–TFA (100–1 mM) accelerator solution, injection: 5 s at 2 psi, applied voltage: 30 kV, rinsing between runs: electrolyte for 0.7 min at 20 psi, MS: ESI positive ionization 100–400 amu, make-up flow: 2 μl/min methanol–water (80:20, v/v) containing formic acid (0.5%, v/v).

[a] n = 7 (run number 4–10).

[b] 0, 0.05, 0.2, and 2 ppm (three injections for each level).

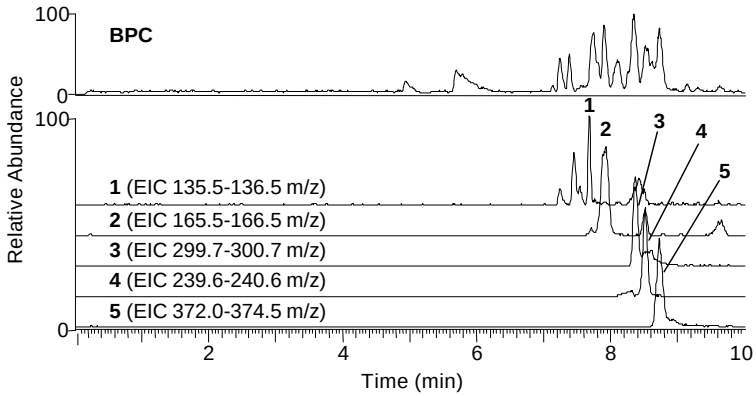

Fig. 5. CE–MS analysis of a spiked urine sample (0.2 ppm each) after SPE. Capillary length: 93.5 cm, electrolyte: formic acid–TFA (100–1 mM) accelerator solution. Other operating conditions: see Section 2. BPC: base peak chromatogram, EIC: extracted ion chromatogram. Peak numbering: see Fig. 1.

field is generated across the capillary due to the voltage present on the spray needle. This leads to the generation of an EOF and the migration of analytes towards the injector. Care has to be taken to minimize this effect in order to maintain sensitivity and repeatability. Therefore, during injection and CE voltage build-up, the spray needle voltage was set at 0 kV. If this is not done, the basic drug standards are not or hardly detected, even at the 2 ppm level.

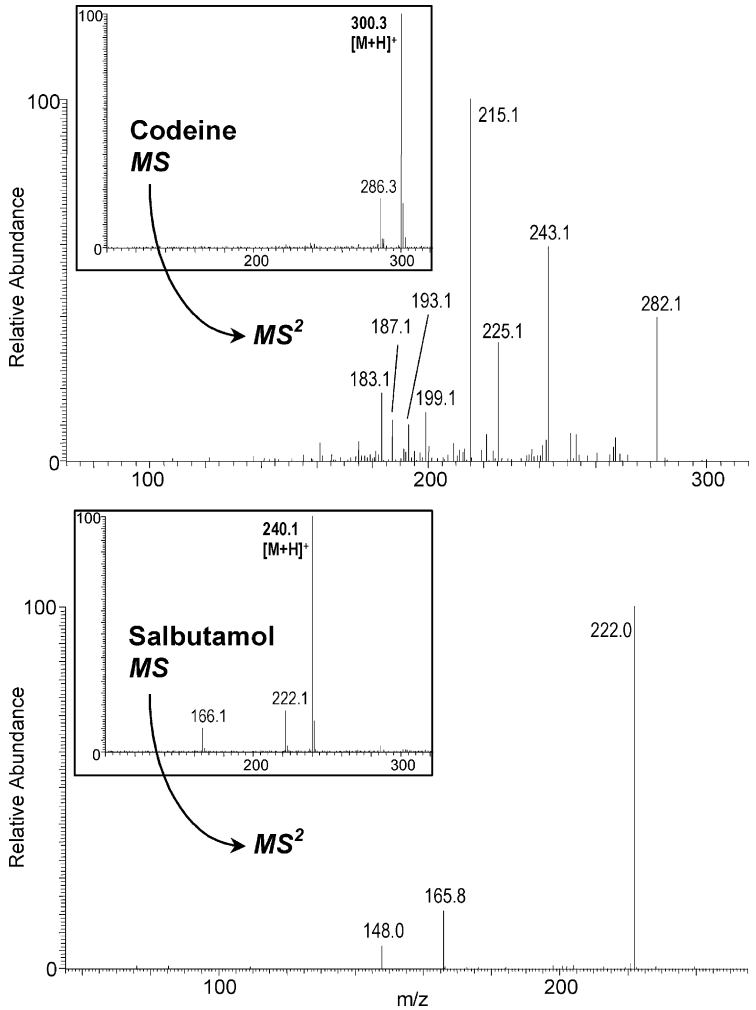

Fig. 6. Examples of MS and MS^2 spectra of a CE–MS^2 analysis, taken on the molecular ion of the compound (2 ppm in water). Capillary length: 93.5 cm, electrolyte: formic acid–TFA (100–1 mM) accelerator solution. Other operating conditions: see Section 2.

The effect is so drastic because of the high EOF and analyte mobility.

The performance of the volatile CEofix® solution and analytical method was tested by 10 consecutive injections of the 2 ppm basic drugs standard mixture with the formic acid–TFA (100–1 mM) solution containing accelerator and the adapted rinsing procedure. Migration times stabilized only after three injections (instead of 1 under the conditions for CE–DAD). The R.S.D. on migration time and peak area are depicted in Table 1. The LOD (three times signal-to-noise) with this method is below 50 ppb for all standards. The linearity of the method was evaluated using 4 concentration levels (0, 0.05, 0.2, and 2 ppm). The correlation coefficient was above 0.999 for all compounds (Table 1).

As an application of the method, a spiked urine sample was analyzed after SPE enrichment. The sample pretreatment step is necessary because various problems arise when urine is analyzed directly by CE–MS. Injection of untreated urine causes the CE current to increase to such a level that the voltage of the ESI needle rises above its set point. This adversely affects the sensitivity and repeatability. A second problem is that actual concentration levels of the target compounds might be too low to be detected. Finally, as many polar and ionic substances are present in urine in relatively high concentrations, ionization of these compounds might lead to decreased sensitivity and repeatability for the target compounds.

SPE was carried out on a urine sample (10 ml) spiked with 0.2 ppm of each basic drug. After SPE, the residue was redissolved in 1 ml water. A concentration factor of 10 is obtained in this way. The recovery of the extraction procedure was calculated by comparing the CE–MS peak area of the basic drugs in the SPE extract with the peak area of the basic drugs in a standard solution of 2 ppm of each drug. The recoveries for the selected drugs were: amphetamine: 91.5%, ephedrine: 93.7%, codeine: 93.2%, salbutamol: 89.7%, and trazodone: 88.3%. All basic drugs were detected and identified based on the migration time and molecular ion (Fig. 5).

CE–MS^2 was performed on a 2 ppm standard mixture of the basic drugs. This allows unequivocal confirmation of the presence of the substance in a sample based on the migration time, molecular weight and fragment ions. The CID voltage was optimized for optimal fragmentation of the molecular ion. This was done by infusing a 5 ppm standard solution at 2 μl/min into the mass spectrometer, and ramping the CID voltage during this operation. A voltage of 1.25 V results in adequate fragmentation with acceptable sensitivity. After CID optimization, CE–MS^2 was performed on a 2 ppm standard mixture in water using the formic acid–TFA (100–1 mM) electrolyte with accelerator and the analytical conditions described above. The mass spectrometer is set to perform MS^2 on any of the molecular ions originating from the compounds in the test mixture. For this reason, each scan is divided into two subscans. In a first stage, MS is performed. If any of the selected molecular ions is detected at a certain level, MS^2 is performed on this ion in the trap. A drawback of this operation is the significant loss of signal intensity. However, since the MS^2 spectra originate from a single ion, the background in these spectra is very low. Examples of recorded mass spectra are

Table 2
MS and MS^2 data collected with CE–MS for the selected basic drugs (2 ppm each)

Compound (mass)	MS-ions (relative intensity)[a]	MS^2-ions (relative intensity)	Identity MS^2-ions
Amphetamine (135.1)	119.0 (47) **136.0** (**100**)	119.0 (100)	$[M + H-NH_3]^+$
Ephedrine (165.1)	148.2 (56) **166.1** (**100**)	148.1 (100)	$[M + H-H_2O]^+$
Codeine (299.2)	240.2 (9) 286.3 (21) **300.3** (**100**)	183.1 (20) 187.1 (12) 193.1 (11) 199.1 (14) 215.1 (100) 225.1 (33) 243.1 (60) 282.1 (40)	$[M + H-C_3H_7N-CH_3OH-CO]^+$ $[M + H-C_3H_7N-CH_3OH-H_2O]^+$ $[M + H-C_3H_7N-CO]^+$ $[M + H-C_3H_7N-H_2O]^+$ $[M + H-C_3H_7N]^+$ $[M + H-H_2O]^+$
Salbutamol (239.2)	166.1 (11) 222.1 (17) **240.1** (**100**)	148.0 (6) 166.0 (16) 222.0 (100)	$[M + H-C_4H_8-2H_2O]^+$ $[M + H-C_4H_8-H_2O]^+$ $[M + H-H_2O]^+$
Trazodone (371.2)	176.2 (8) **372.3** (**100**) 374.3 (34)	148.0 (15) 176.1 (100)	$[M + H-C_{10}H_{12}N_2Cl-CO]^+$ $[M + H-C_{10}H_{12}N_2Cl]^+$

Capillary: 93.0 cm $L \times 75$ μm i.d., electrolyte: formic acid–TFA (100–1 mM) accelerator solution, injection: 5 s at 2 psi, applied voltage: 30 kV, rinsing between runs: electrolyte for 0.7 min at 20 psi, MS: ESI positive ionization 100–400 amu, MS^2 trap collision induced dissociation (CID) voltage: 25% (1.25 V), make-up flow: 2 μl/min methanol–water (80:20, v/v) containing formic acid (0.5%, v/v).

[a] Ion in bold (molecular ion) was taken for fragmentation in MS^2.

shown in Fig. 6 and details on the fragments are given in Table 2. The obtained spectra were in good agreement with the spectra obtained in the infusion experiments.

4. Conclusion

The application of a volatile formic acid electrolyte with accelerator results in fast and reproducible CE analyses of basic drugs at low pH. With an alternative rinsing procedure for dynamic double coating of the capillary wall, the method could be transferred to CE–MS. Sequences can be run fully automated without opening the ionization source during rinsing steps. The LOD for CE–MS was less than 50 ppb for the selected drugs and their migration time was highly reproducible (0.85% R.S.D. or lower). The method was successfully applied to the analysis of a spiked urine sample. On-line CE–MS^2 was performed on a standard mixture allowing the unequivocal confirmation of the presence of a certain drug in a biological sample.

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