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Combination of Aqueous and Non-Aqueous Capillary Electrophoresis with Electrospray Mass Spectrometry for the Determination of Drug Residues in Water

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Summary. The work presented in this paper deals with the combination of capillary electrophoresis (CE) with electrospray mass spectrometry (MS) for the determination of drug residues in water. CE/MS methods have been developed based on either aqueous or non-aqueous ammonium acetate solutions as the carrier electrolyte for the separation of selected drugs. The different separation conditions were compared in terms of selectivity and detection limits; both aqueous and non-aqueous CE proved to be suitable for the present analytical task, exhibiting detection limits between 3 and 93 $\mu g/dm^3$ (injected standard concentration) corresponding to concentrations between 5 and 19 ng/dm^3 in the sample.

A combination of liquid-liquid extraction and solid-phase extraction was investigated for sample pretreatment, yielding enrichment factors of 10000. The applicability of CE/MS was demonstrated for the analysis of several river water samples.

Keywords. Drug residues; Water analysis; Non-aqueous capillary electrophoresis; Electrospray ionization mass spectrometry.

Introduction

Capillary electrophoresis (CE) in combination with mass spectrometric detection is a powerful technique in environmental analytical chemistry for the determination of trace contaminations. Mass spectrometry (MS) offers the advantage of a sensitive detection together with an outstanding selectivity; in combination with an analytical separation technique such as gas chromatography (GC), liquid chromatography (LC), or CE, MS detection can be employed for a wide range of applications. Nevertheless, the hyphenation of CE and MS is still at its beginning, and therefore only little experience exists with this technique. The aim of the present work is to compare aqueous and non-aqueous CE in combination with electrospray ionization (ESI) MS for the analysis of drug residues in surface water and to demonstrate the applicability of CE/MS for real water samples. As recently shown, non-aqueous CE

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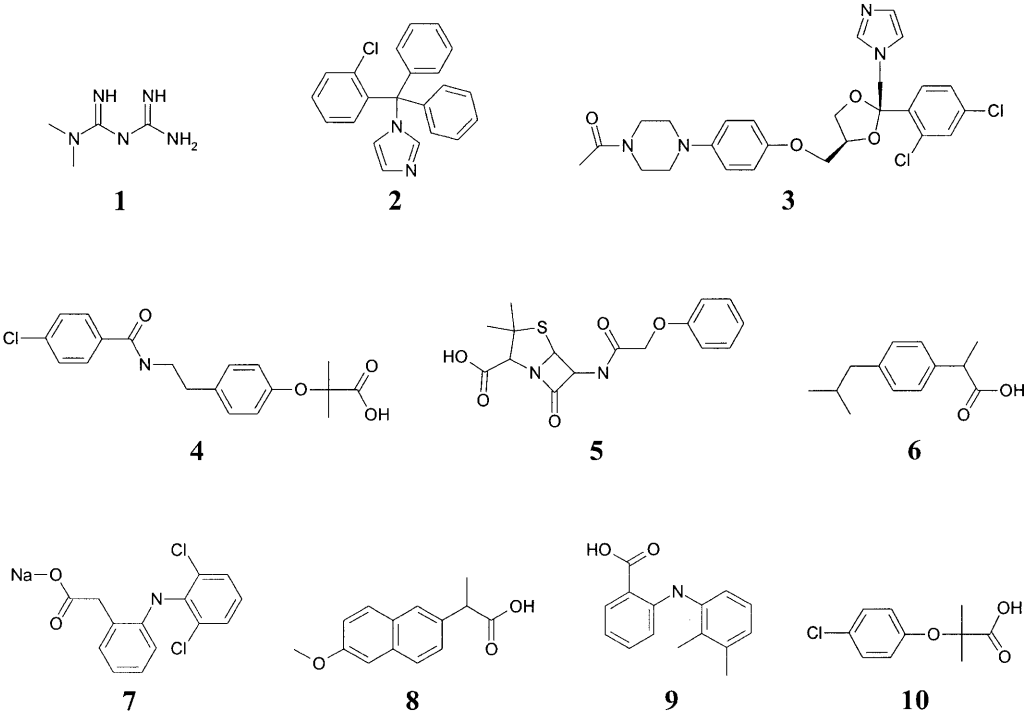
offers different selectivities compared to aqueous CE which in some cases leads to a remarkable increase in resolution of the analytes [1–4].

Drug residues have become a noteworthy contamination factor in surface water during the last years. The excretion of drugs and their metabolites together with improper waste disposal have led to considerable concentrations of various compounds. Even the treatment of communal waste water in purification plants cannot avoid the entry of some drugs because of the high stability of some of them or of their metabolites against biological degradation. Another fact for the necessity of controlling drug residues in surface water is that under certain circumstances these compounds may enter the drinking water produced from groundwater as recent studies have shown [5–7].

In terms of the analytical separation techniques, the determination of drugs in surface water is mainly carried out by GC, usually in combination with mass spectrometric detection which often requires derivatization [8–14]; up to now only little work has been done on the analysis of these compounds by the combination of a liquid phase separation technique and MS such as liquid chromatography/mass spectrometry [13, 15] or CE/MS. Taking into account the acidic or basic properties of a number of drugs, CE in combination with ESI-MS seems to be very suitable for the determination of various compounds (see Scheme 1) such as metformin (**1**), clotrimazole (**2**), ketoconazole (**3**), bezafibrate (**4**), penicillin V (**5**), ibuprofen (**6**), diclofenac (**7**), naproxen (**8**), mefenamic acid (**9**), and clofibric acid (**10**). The selection of drugs to be analyzed was made corresponding to the total amount sold and the stability against metabolization and degradation. Some of them show either



Scheme 1

an acidic site (carboxyl group or phenol), a basic site (amide), or both, which makes protonation or deprotonation and hence the detection by MS possible.

Apart from the separation technique, water samples have to be pretreated in order to get rid of matrix components and to enrich the analytes; the usual way to accomplish this aim is to perform a solid-phase extraction (SPE) employing suitable stationary phases (reversed phase materials) and conditions. In contrast to drinking water, surface water often contains a high amount of organic carbon in the form of humic substances or the like, making the sample pretreatment more difficult, especially when high enrichment factors are aspired.

Results and Discussion

Development and comparison of aqueous and non-aqueous CE methods

Ammonium acetate at a concentration of 20 m*M* was employed as the carrier electrolyte both in aqueous and non-aqueous CE. For aqueous CE, the *pH* was adjusted with 1 *M* acetic acid in a range between 4 and 6.6 in order to optimize the separation of the drugs. Figure 1 shows the dependence of the mobility of the anionic species on *pH*. The analytes may be divided into two groups: one group (consisting of compound **6**, **7**, **8**, and **9**) shows a strong increase of the mobility with *pH*, a second group (**4**, **5**, and **10**) is only little influenced by *pH*. This behaviour can be attributed to the different pK_a-values of the investigated drugs: those belonging to the first group exhibit pK_a-values between 4.2 and 4.6 [16], whereas the second group's members are characterized by pK_a-values between 2.8 (compound **5** [17]) and approximately 3.3 (compounds **4** and **10**, in analogy to chlorophenoxyacetic acid [18]). As a result, the drugs of the first group undergo increasing deprotonation with increasing *pH*; those of the second group are deprotonated all over the investigated *pH* range, hence showing a more or less constant mobility. As can be

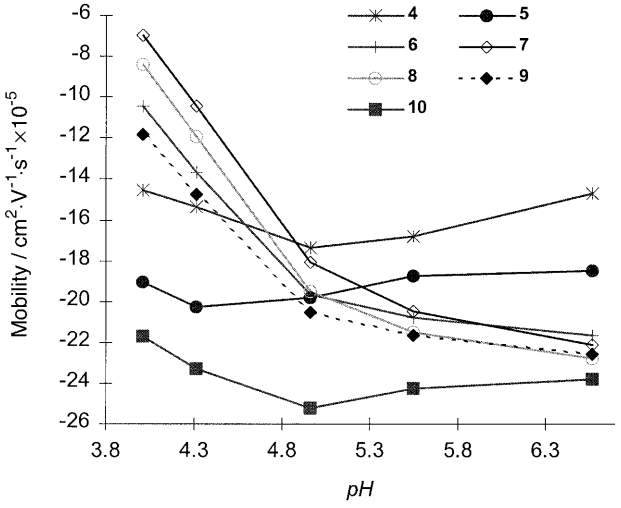


Fig. 1. Dependence of the mobilities of the selected drugs on the *pH*; carrier electrolyte: 20 m*M* ammonium acetate, *pH* adjusted with 1 *M* acetic acid; voltage: 20 kV, injection: 50 mbar, 0.3 min

Table 1. Comparison of the detection limits for selected drugs using either aqueous or non-aqueous CE in both the negative and positive detection mode for signal-to-noise ratios of 3 ($\mu g/dm^3$); conditions: carrier electrolyte: 20 m*M* ammonium acetate, $pH = 5.1$ (aqueous CE) or 20 m*M* ammonium acetate with 60 m*M* acetic acid in methanol:acetonitrile = 40:60 (v/v) (non-aqueous CE); injection: 50 mbar, 0.3 min; voltage: 20 kV (aqueous CE), −20 kV (non-aqueous CE)

	detection limits/$\mu g \cdot dm^{-3}$			
	Aqueous CE		Non-aqueous CE	
	Positive	Negative	Positive	Negative
1	3	–	–	–
2	5	–	–	–
3	9	–	–	–
4	33	27	32	11
5	59	134	91	156
6	–	47	–	40
7	33	66	43	15
8	18	93	86	82
9	25	27	57	13
10	–	60	–	11

seen from Fig. 1, the separation of the analytes would be best (within the investigated *pH* range) at a *pH* of 4, but at such a low *pH* analyte **10**, having the highest mobility against the EOF, reaches the MS only after 42 min. In order to keep the run time below 20 min, a *pH* of 5.1 was chosen, accepting a lower resolution between the analytes but gaining analysis time. With MS as a very selective detector, peak overlapping is no problem as long as the overlapping analytes have different m/z values and there is no interference with matrix components; these requirements were fulfilled for the present analytical task. Finally, a carrier electrolyte containing 20 m*M* ammonium acetate adjusted to $pH = 5.1$ with acetic acid was used for the determination of the detection limits in the aqueous CE mode. The detection limits are given in Table 1 for a signal-to-noise ratio of 3 in the positive and in the negative detection mode. Compounds **1**, **2**, and **3** could only be detected in the positive mode because these analytes exhibit only basic sites; some other analytes showing both basic and acidic sites could be detected in both the positive and the negative detection mode. Compounds **6** and **10**, on the other hand, could only be detected in the negative mode due to the absence of a basic site, making protonation and detection in the positive mode impossible. Interestingly, compound **8** showed an anomalous behaviour in so far that it could undergo protonation although obviously no basic site is present in the molecule.

For non-aqueous CE, new capillaries were treated with hexadimethrin bromide for permanent reversal of the electroosmotic flow (EOF). The acidity of the carrier electrolyte, containing 20 m*M* ammonium acetate solution in methanol, was optimized for different concentrations of acetic acid. Increasing the acetic acid concentration to 200 m*M* led to a strong decrease of the mobility of the analytes **6**, **7**, **8**, and **9**, whereas in analogy to the aqueous system compounds **4**, **5**, and **10** were

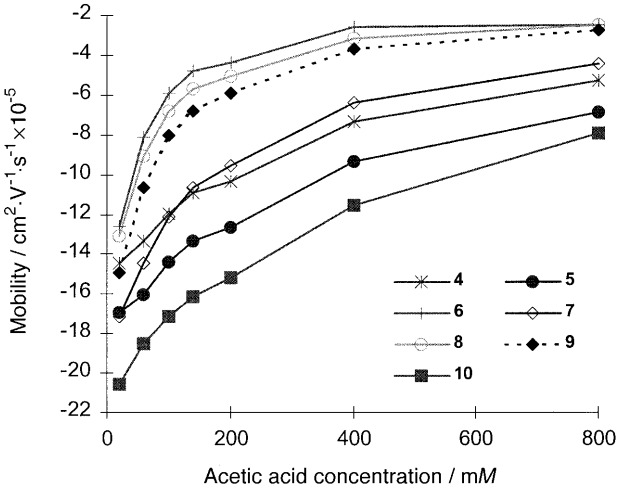


Fig. 2. Dependence of the mobility of selected drugs on the acetic acid concentration in non-aqueous CE; carrier electrolyte: 20 m*M* ammonium acetate, acetic acid; voltage: −20 kV; injection: 50 mbar, 0.3 min

less influenced (Fig. 2); this different behaviour of the two groups resulted in changes in migration order, allowing to adjust the selectivity of the analytes by choosing an appropriate concentration of acetic acid. In this case, 60 m*M* acetic acid was optimal in terms of resolution between the analytes and a short analysis time.

Another parameter being investigated was the addition of acetonitrile to the methanolic carrier electrolyte. Besides the influence on the migration times due to changes of the EOF, the addition of acetonitrile could also be used for a fine-tuning of the resolution between the analytes. At a methanol/acetonitrile ratio of 40:60 (v/v) the best resolution together with reasonable migration times was obtained. Figure 3

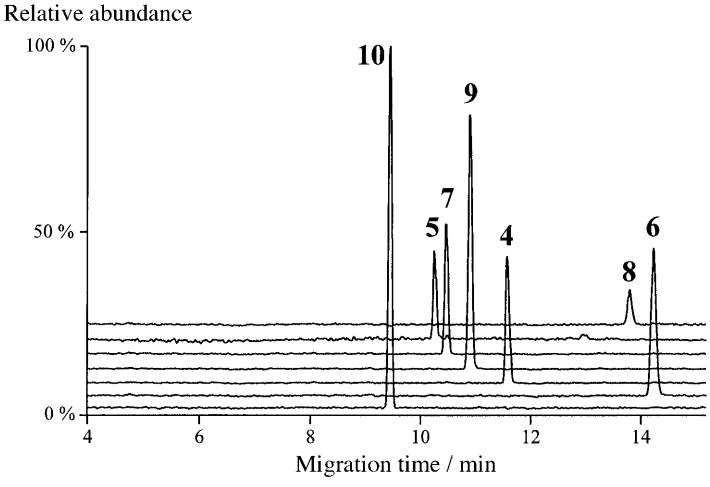


Fig. 3. Extracted ion electropherogram of a standard mixture of 7 drugs in the non-aqueous CE/MS mode; carrier electrolyte: 20 m*M* ammonium acetate, 60 m*M* acetic acid in methanol:acetonitrile = 40:60 (v/v); voltage: −20 kV, injection: 50 mbar, 0.3 min, concentrations: 10 mg/dm^3 each

shows the extracted ion electropherogram of a mixture of 7 drugs acquired in the selected ion monitoring mode by the combination of non-aqueous CE and MS. Under these non-aqueous conditions, the mobility of compound **1** against the EOF was too high to reach the detector; compounds **2** and **3**, on the other hand, could not be separated from the injection zone; therefore these drugs were excluded from further investigations.

The detection limits given in Table 1 were calculated using the same criterion as for the aqueous system; the conditions were also the same except for the separation voltage which was $-20\,\text{kV}$ instead of $+20\,\text{kV}$. The detection limits of the non-aqueous CE system were of the same order of magnitude (between 11 and 156 $\mu g/dm^3$) as for the aqueous system; again, compounds **6** and **10** could only be detected in the negative mode.

Optimization of sample pretreatment

With respect to the detection limits determined for standard solutions, a sample pretreatment was necessary in order to obtain an enrichment factor of about 10^4. The problem of such a rather high concentration was the fact that matrix components of the sample were also strongly enriched which resulted in peaks unusable for quantitation by CE/MS. As a way out a sample cleanup by liquid-liquid extraction (LLE) prior to the SPE was tested. The extraction was performed using a mixture of methyl *tert*.-butyl ether and hexane as the extraction solvent after the water sample had been acidified with hydrochloric acid (see experimental section). The organic phase was re-extracted with a 2 m*M* sodium hydroxide solution which was then brought to *pH* 2 and diluted to 250 cm^3 with Milli-Q water of *pH* 2. This dilution step was necessary in order to lower the percentage of organic solvent being dissolved in the aqueous phase which otherwise would lower the extraction efficiencies due to an undesirably high elution strength in the following SPE procedure.

In preliminary experiments various stationary phases were investigated for SPE; recoveries were determined with 100 cm^3 standard solution brought to *pH* 2 containing 4 $\mu g/dm^3$ of each drug employing 100 mg cartridges. Among LiChrolut RP 18, LiChrolut EN, Oasis, Bond-Elut C18-OH, and Bondesil ODS the latter offered the best recoveries for the selected analytes and was therefore chosen for further optimization of the SPE. Some major problems associated with the SPE of the drugs had to be faced: the SPE conditions had to meet the requirement of a quantitative adsorption to the stationary phase, and elution and redissolution may depend on the solubility of the analytes which is rather poor for some of them (especially for compounds **6**, **7**, and **9**). Adsorption to glassware during the SPE may also negatively affect the recoveries.

Using a sample volume of 500 cm^3 and SPE cartridges packed with 500 mg Bondesil ODS, the influence of the sample *pH* was checked for $pH = 2$, 5.5, and 8.5 resulting in best recoveries for $pH = 2$ for all analytes. Another precaution leading to a remarkable improvement of the overall extraction efficiencies was the silanization of the glassware getting in contact with either the water sample or the extract with dimethyldichlorosilane.

Finally, after consideration of all the factors influencing the extraction, recoveries and detection limits were determined for the drugs under investigation.

Table 2. Recoveries and detection limits for selected drugs (aqueous CE, negative mode)

Analyte	Recoveries (SPE)	Standard deviation/%	Recoveries (LLE+SPE)	Standard deviation/%	Detection limit in sample/ng · dm^{-3}
4	97.1	1.0	81.9	16.1	5.8
5	53.3	13.3	[a]	–	–
6	90.2	1.7	62.7	16.2	12.8
7	83.5	2.1	54.2	18.6	18.8
8	95.7	0.8	71.6	25.4	16.0
9	43.1	2.4	[a]	–	–
10	96.3	1.2	79.7	29.9	4.8

[a] Compounds **5** and **9** could not be extracted by the present LLE-SPE method

The results are given in Table 2; it shows the recoveries of standard solutions employing only SPE as well as the recoveries using the combination of SPE and LLE together with the detection limits obtained. Compounds **1** to **3** could not be extracted under these acidic conditions; the extraction of these analytes may be performed under basic conditions but was not further investigated in the present work. Compounds **5** and **9** could be extracted by SPE but were lost in the LLE prior to SPE. The recoveries of the other analytes using the SPE without the combination with LLE were between 43 and 97%, exhibiting standard deviations between 0.8 and 2.4% except for compound **5** (13.3%). The combination of LLE and SPE obviously led to a decrease of recoveries for all analytes, whereas the standard deviation increased which can be attributed to the three extraction steps involved during the sample pretreatment. Nevertheless, the detection limits achieved by CE/MS were sufficiently low for the analysis of real surface water samples.

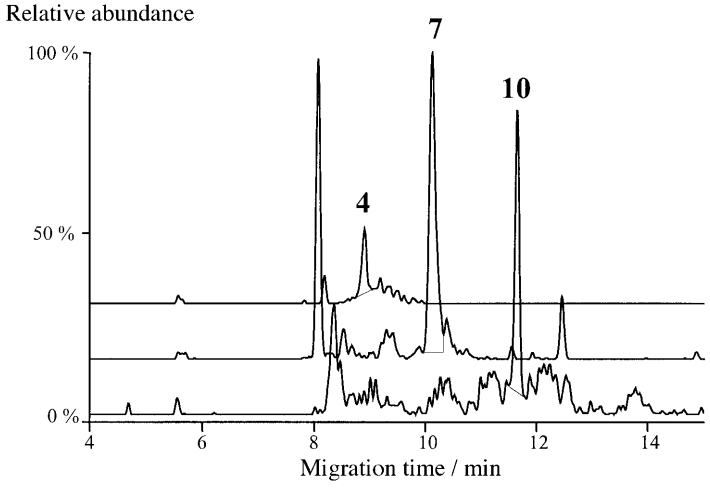


Fig. 4. Extracted ion electropherogram of a real sample (Feldaist 4) acquired by aqueous CE/MS in the negative detection mode; carrier electrolyte: 20 mM ammonium acetate, $pH = 5.1$; voltage: 30 kV; injection: 50 mbar, 0.3 min

Table 3. Results of five river water samples (in $ng \cdot dm^{-3}$) determined by aqueous CE/MS

Analyte	Feldaist 3	Feldaist 4	Gusen 2	Gusen 3	Gusen 4
4	4.8	20.1	20.4	< 4.8	10.5
7	28.3	392.1	163.5	85.0	161.4
8	< 13	< 13	< 13	< 13	38.2
10	24.8	43.5	24.4	19.3	41.8

Analysis of real surface water samples by CE/MS

Employing the combination of LLE and SPE, a sample pretreatment procedure was developed allowing the enrichment of the water sample by a factor of 10^4 and the compatibility with CE as well. The analysis of several real samples should demonstrate the applicability of CE/MS for the present analytical task; a sample electropherogram is shown in Fig. 4. In all five samples analyzed, compounds **7** and **10** were detected at concentrations between approximately 20 and 400 ng/dm^3 (see Table 3). In four samples out of the five, compound **4** was found at concentrations between 5 and 20 ng/dm^3 and, finally, in one sample compound **8** was detected at a concentration of 38 ng/dm^3. The determination was carried out employing the aqueous CE system using external calibration; additionally, the data of the analysis were confirmed by standard addition for one sample.

Experimental

Instrumentation and CE/MS conditions

A Crystal 310 instrument (Thermo CE, Franklin, MA, USA) was employed for CE/MS experiments. 50 µm i.d. fused silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). New capillaries with a length of 70 cm were conditioned by flushing with 0.1 *M* NaOH followed by water (10 min each). For permanent EOF reversal they were coated by treatment with a solution of 0.001% (m/v) hexadimethrin bromide in methanol followed by a flush with methanol and water (10 min each). Finally, the conditioning procedures were completed by flushing with the CE carrier electrolyte. Prior to each run the capillary was flushed with carrier electrolyte for 3 min. The carrier electrolyte for aqueous CE was 20 m*M* ammonium acetate; the *pH* was adjusted with 1 *M* acetic acid. For non-aqueous CE, an ammonium acetate solution in methanol and acetonitrile with various concentration of acetic acid was employed.

MS detection was performed on a quadrupole system HP 5989B (Agilent, Palo Alto, CA, USA) equipped with a radio-frequency-only hexapole (Analytica of Branford, Branford, CT, USA) using a pneumatically assisted electrospray ionization interface HP 59987A (Agilent) and a CE-probe. The sheath liquid consisting of 2-propanol:water = 80:20 (v/v) contained either 0.1% (v/v) acetic acid for the positive detection mode or 0.1% (v/v) triethylamine for the negative detection mode and was delivered by a syringe pump (Model 22, Harvard Apparatus, South Natick, MA, USA) at a flow rate of 4 mm^3/min. The drying gas flow rate was 1.4 dm^3/min (nitrogen 5.0 at a temperature of 150°C); a nebulizing gas was not applied.

Chemicals

Ammonium acetate, acetic acid, methanol, 2-propanol, and triethylamine were purchased from Merck (Darmstadt, Germany), methyl *tert*.-butyl ether (*MTBE*) from Fluka (Buchs, Switzerland), and hexane

and acetonitrile from Baker (Deventer, The Netherlands). Drug standard materials were purchased from Sigma-Aldrich (St. Louis, MO, USA). High purity water was prepared by a Milli-Q water purification system (Millipore, Milford, USA).

Pretreatment and enrichment of water samples

All glassware getting in contact with either the water sample or the extract was silanized by flushing with a 10% (v/v) solution of dimethyldichlorosilane in toluene, followed by flushing with pure toluene (twice) and methanol (twice). The glass equipment was then placed in a dryer for 3 h at 160°C.

Water samples were pretreated by a liquid-liquid extraction prior to SPE. 500 cm^3 of water sample were brought to *pH* 2 with conc. HCl; after the addition of 50 g Na_2SO_4 the sample was extracted twice with 25 cm^3 of a mixture of hexane:*MTBE* = 1:1 (v/v). The organic phase was re-extracted twice with 50 cm^3 2 m*M* NaOH solution, brought to *pH* 2 with HCl, and diluted to 250 cm^3 with water of *pH* 2. The samples were passed through an SPE cartridge (6 cm^3) packed with 500 mg Bondesil ODS 40 µm (Varian, Palo Alto, CA, USA) conditioned with acetone, MeOH, and H_2O of *pH* 2 (one cartridge volume each). The flow rate was adjusted to approximately 10 cm^3/min. After the cartridges had been allowed to dry for 30 min, the drugs were eluted using an overall volume of 2 cm^3 methanol. The extract was dried in a N_2 stream (purity 4.6), and finally the residue was redissolved in 50 mm^3 of a mixture of MeOH:carrier electrolyte = 80:20 (v/v) prior to injection (50 mbar, 0.3 min).

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