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Analysis of basic pharmaceuticals by capillary electrophoresis in coated capillaries and on-line mass spectrometric detection

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Abstract

Basic pharmaceuticals were separated using capillary electrophoresis (CE) and positively identified with the aid of an on-line coupled sectorfield mass spectrometer (MS). The coupling device consisted of a modified electrospray (ES) ion source with coaxial sheath liquid. The adaptation of the CE-conditions for the particular needs of ES-MS is discussed in brief. Applying volatile buffers of low ionic strength and using uncoated capillaries in CE causes limited sample loadability, poor peak shapes and decreases resolution. The separation performance can be significantly improved if the capillaries are coated with poly(ethylene glycol) (PEG) or poly(vinyl alcohol) (PVA). As extracted from the reconstructed ion current of the mass spectra, the baseline separated signals of structurally very similar compounds can be achieved. Signals due to the protonated and Na^+-attached molecules have been observed. Nozzle-skimmer dissociation may be a versatile tool for additional molecular structure information.

Keywords: Mass spectrometry; Ephedrine; Tocainide; Poly(ethylene glycol); Poly(vinyl alcohol); Nozzle-skimmer dissociation

1. Introduction

Capillary electrophoresis–mass spectrometry (CE–MS) is an attractive combination of two high-resolution techniques with the potential to solve complex analytical problems [1–5]. CE permits fast and efficient separations of a wide variety of charged [6–8] and even uncharged species [9,10] whereas MS provides information about the mass and, potentially, the structure of the separated compounds [11–13]. A key point of the coupling of these two techniques is the specific interface design which serves as the transport, vaporisation and ionisation of the species. Within this regard, the electrospray (ES) interface with a coaxial liquid sheath arrangement is reported to be very effective [14–17]: The end of the fused-silica CE capillary is provided with a make-up flow of an electrolyte (sheath liquid) enabling the electrical contact between the ES probe-tip and the high-voltage power supply of the CE. However, there are a some difficulties and particularities to be considered when CE and MS are combined on-line: (i) If the composition of the sheath liquid differs from that of the CE electrolyte, moving ionic boundaries become visible by the UV detection of the CE [17]. (ii) The buffer system for the CE-

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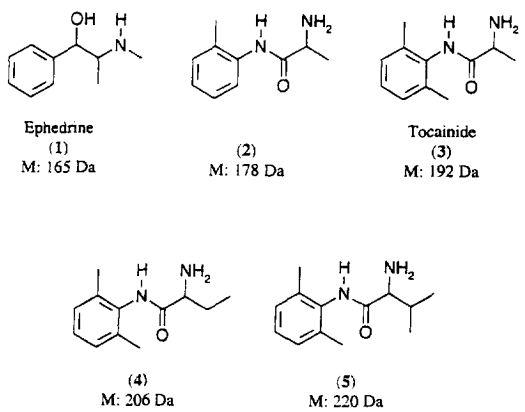

Scheme 1.

separation has to be compatible to the ES process. In CE the separation performance is increased with increasing ionic strength of the electrolyte whereas the ion-production process in ES is impaired with increasing ionic strength [18]. The most commonly used buffer systems in CE, aqueous electrolytes with non-volatile buffer components (e.g. Na-phosphate, Na-borate) are not MS-compatible because non-volatile salts can contaminate the MS ion optics and furthermore, cause increased background signals followed by a decreased sensitivity. For the transfer of standard CE methods to CE–MS one has to compromise the electrolyte systems to get both, sufficient CE separations and high sensitivity in the ES–MS detector [19].

In the present contribution, the modification of a standard CE method to CE–MS compatible conditions is summarized. The limitations of volatile acetic acid CE electrolytes and ammonium acetate buffers with regard to resolution and sample loadability in CE separations are outlined. The benefit of poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) coated capillaries in the CE–MS analysis of basic tocainide-type drugs (Scheme 1) with volatile electrolytes of low ionic strength is discussed.

2. Experimental

2.1. Chemicals

Ephedrine (**1**), tocainide (**3**) and structural analogues (**2**), (**4**) and (**5**) (see Scheme 1) were kindly provided by ASTRA-HÄSSLE (Göteborg, Sweden). HPLC-grade methanol (Lichrosolv) and acetic acid p.A. were taken as supplied from Merck (Darmstadt, Germany), ammonium acetate (microselect grade >99%) and ammonium hydroxide p.A. 25% in water purchased from Fluka (Buchs, Switzerland) were used without further purification.

2.2. Capillaries

Fused-silica capillaries of 75 μm inner diameter were obtained from Polymicro (Phoenix, AZ, USA). The thermally immobilised PVA coating was generated according to Gilges et al. [20]. The PEG coating was generated statically and crosslinked by using the PE-1-M-100 coating material from Innophase (Westbrook, CT, USA).

2.3. Instrumentation

CE experiments without MS were executed in laboratory-made equipment with UV detection which has been described in detail previously [21].

On-line CE–MS separations were performed on a BioFocus 2000 (BioRad GmbH, München, Germany) with special CE–MS equipment for connection of the liquid cooled capillary to the ES source. The capillary temperature was maintained at 20°C.

The mass spectrometric detection in the positive ion mode was performed on a Finnigan MAT (Bremen, Germany) Model 95 forward-geometry sectorfield mass spectrometer upgraded with the API-II ion source [22–24] operating in the electrospray (ES) ionisation mode. The commercially available ES stainless steel probe tip was replaced by a home-built one (800 μm I.D.). The stainless steel needle, fitting in the probe consisted of a commercially available syringe needle with 400 μm I.D. and 700 μm O.D. The standard ES conditions were as follows: spray potential U_{ES}=3.5 kV, spray current I_{ES}=3–4 μA (CE on), temperature of the aluminium capillary T_{cap}=200°C, sheath liquid [methanol–10 mmol l^{-1} acetic acid in water (9:1, v/v)]; flow-rate 2–4 μl/min delivered by a Harvard Apparatus 22 syringe pump (South Natick, Ma, USA). An increase of the spray current was observed in CE–MS

experiments as expected because in this case both power supplies, the CE and that of the ESI, worked in parallel. Preceding sample injection, the ES unit was switched off and the potential of the ES needle was held at ground potential by connecting the syringe pump needle electrically to ground. The potentials of the aluminium capillary, the tube lens, the skimmer and the rf-only octapole ion-guide were adjusted in order to induce 10–20% nozzle-skimmer dissociation [25,26] products of the protonated ephedrine ($[\mathbf{1}+H]^+$). The mass spectrometer was run at a mass resolution $m/\Delta m$ 2000, with an accelerating voltage of 5 kV. Scanning was performed from m/z 90 to 275 with 3 dec/sec. The electron multiplier was set at 2.8 kV. For the data acquisition and presentation the standard proprietary Digital DEC-Station-based ICIS-2 system provided by Finnigan MAT and the MassLib V 8.1b mass spectra evaluation program package (Max-Planck-Institut für Kohlenforschung, Mülheim, Germany) were employed.

3. Results and discussion

3.1. Optimization of capillary-electrophoretic separations

In order to transfer an established standard CE separation to CE–MS compatible conditions, it is advantageous to change the buffer composition to volatile components in order to avoid any contamination of the ES device. Additionally, it is desirable to inject as much sample as possible, i.e. balancing the highest possible sensitivity against loss of resolution [2]. Since the efficient separation of a mixture of basic drugs with phosphate buffer systems and UV detection has been described previously [27], we focus on the alterations necessary for CE–MS compatible conditions. The following results have been obtained and are described starting with the employment of uncoated capillaries.

(i) The use of aqueous acetic acid instead of a Na-phosphate buffer leads to separations of limited resolution and poor peak shape resulting from the mismatch of electrolyte and analyte mobility [28] as well as overloading. The increase of acetic acid concentration improved resolution as expected. However, high ionic strengths of the electrolytes are disadvantageous in ES-MS because of decreased ion sensitivity as described by Kebarle and Tang [18]. Furthermore, separations performed in a solution of acetic acid permit lower reproducibility in numerous runs compared to separations in buffered electrolytes due to pH shifts during and in-between the runs.

(ii) The separation performance of an uncoated capillary is much better in a volatile ammonium acetate buffer compared to an aqueous acetic acid electrolyte. Nevertheless, the resolution is lower, the sample loadability is decreased and the peak shape is poorer compared to the results employing a non-volatile Na-phosphate buffer [27].

(iii) Because of the limited separation performance obtained in the CE–MS compatible buffers it is essential to gain as much resolution as possible by the use of an appropriate column technology. The resolution of basic compounds can be significantly enhanced utilising hydrophilic coated capillaries [27,29]. The benefit of appropriate capillary coatings is even more pronounced in typical volatile CE–MS buffers of low ionic strength. The CE-experiments employing NH_4OAc-buffers and HOAc-electrolytes have been repeated in bare FS, in PVA- as well as in PEG-coated capillaries and similar results have been obtained using the latter two types of hydrophilic coated capillaries. The experiments performed with the coated capillaries resulted in a significantly higher sample loadability and resolution compared to the uncoated ones as shown in Fig. 1. In the case of the PEG-coated capillary a baseline resolution of all compounds is obtained whereas low resolution and even peak splitting of the last migrating compound is observed by the use of an uncoated capillary. Due to the suppressed electroosmotic flow in hydrophilic coated capillaries at low pH values [30], the migration time in separations of cationic compounds is increased, however.

Since a high analyte signal is achieved in ES-MS at high sample concentrations and low ionic strength of the electrolyte [2], the conclusive compromise of the buffer system and the load of sample to get the required resolution in CE at maximum sensitivity in the MS is as follows. Employing aqueous 10 mmol l^{-1} ammonium acetate and PEG- or PVA-coated capillaries leads to optimized peak resolution, sample loadability and anticipated MS signal intensity for the separation of the five basic drugs (**1–5**) given in Scheme 1.

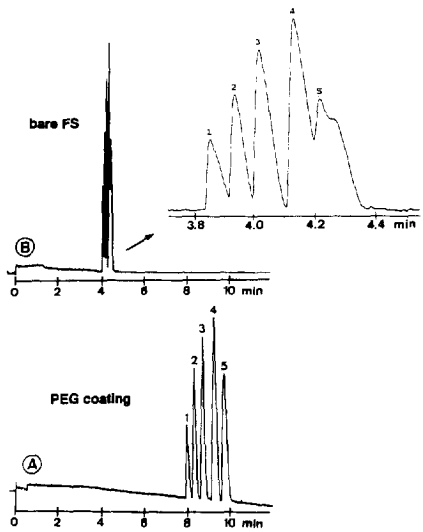

Fig. 1. Impact of capillary coating on CE separation in a 10 mmol l^{-1} NH_4OAc-buffer of pH 4. Capillaries: (A) bare FS and (B) PEG coated; Samples as in Scheme 1, 0.02 mg ml^{-1} of each compound dissolved in H_2O. Capillary: 66 cm total length, 53 cm effective length, 0.05 mm I.D., PEG coated; voltage, 30 kV; aqueous buffer systems; vacuum injection (ΔP=8.5 kPa), 10 s; UV detection, 200 nm, temperature, 20°C.

3.2. On-line CE–MS

The operating conditions of the ES-MS have been optimised as follows. A solution of 0.1 mg/ml ephedrine (**1**) in the CE-running buffer was introduced continuously from the CE system by electrophoretic migration at 20 kV. The instrumental parameters such as capillary positioning, sheath flow-rate, ES-source voltages and MS ion optics, have been optimised to produce a signal of maximum sensitivity and stability.

An example of the CE–MS analysis of the basic test mixture is given in Fig. 2. The UV signal detected with the CE instrument is shown in the upper part (Fig. 2A), the reconstructed ion current (RIC) in the middle one (Fig. 2B). The total length of the capillary was 78 cm, in-capillary UV detection was performed at 21 cm resulting in reduced migration times and diminished resolution in the UV detection mode compared to the MS signals. The signal-to-noise ratio of the reconstructed ion current is lower compared to the UV detection at 200 nm because of the background signals present in the

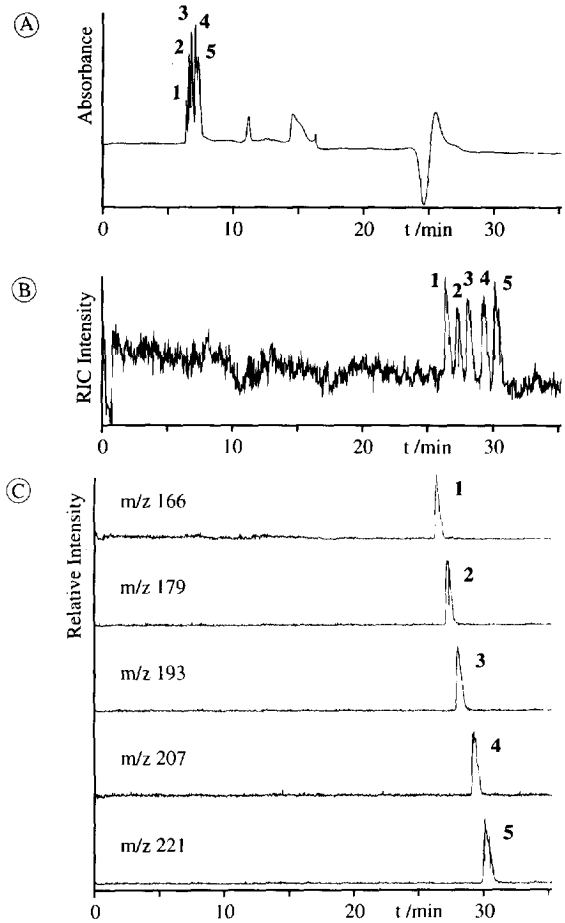

Fig. 2. Example of a CE–ES-MS analysis of the basic pharmaceuticals (**1–5**). In part A the UV detection at 200 nm is displayed. In the lower part (B) the uncorrected, unsmoothed reconstructed ion current (RIC) is shown. The individual mass traces of the protonated molecules are shown in part C. CE conditions: aqueous 10 mmol l^{-1} ammonium acetate buffer pH 4; capillary: PEG coated; effective length for UV-detection l_{UV}: 21 cm; total length l_{tot}: 78 cm; effective voltage 22.5 kV; current 8 μA; sheath liquid: methanol–10 mmol l^{-1} aqueous acetic acid (90:10, v/v).

scanned mass range. These background signals originate from electrolyte and sheath liquid cluster species, e.g. $[3\ AcOH+H]^+$ at m/z 181. Therefore, the signal-to-noise ratio depends upon the sheath-flow composition and the electrolyte concentration. It turned out, that the optimum sheath-flow composition consisted of methanol–aqueous 10 mmol l^{-1} acetic acid (9:1, v/v). Replacing the aqueous acetic

acid portion of the sheath liquid by aqueous ammonium acetate, which is supposed to be more compatible with the CE electrolyte, resulted in a lower signal-to-noise ratio in the RIC. The signal-to-noise ratio decreased also with increasing ionic strength of the CE electrolyte and sheath-liquid.

Obviously, the mass traces corresponding to the protonated molecules ($[M+H]^+$) are characterised by much higher signal-to-noise ratios (Fig. 2C) compared to the RIC. Considering the analysis of a mixture of unknown compounds it is important to get a significant signal in the RIC. The RIC given in Fig. 2B has not been corrected by any data-processing algorithm, it is displayed without any background correction or data smoothing. The broad signal at 25 min in Fig. 2A originates from the bulk flow in the capillary. This liquid flow is most probably induced by the sheath-flow, the electrospray process and by siphoning as the result of not correctly levelled inlet and "outlet" reservoirs ("outlet"=ES needle). Electroosmosis is negligible in PEG-coated capillaries at acidic pH [30].

The uncorrected single-scan ES mass spectra of the basic pharmaceuticals are shown in Fig. 3. For each compound **1–5** the mass of the protonated molecule ($[M+H]^+$) was observed to dominate the mass spectrum, e.g. m/z 193 in the case of tocainide (**3**). One peak in the RIC trace consisted of ca. 15 to 20 mass spectra. By adding these mass spectra and subtracting the background for each peak in the RIC, one obtains "background corrected" mass spectra (e.g. for **3** in Fig. 4). The latter are characterised by (i) a signal-to-noise ratio of better than 100:1, (ii) a signal due to the sodium adduct ($[M+Na]^+$, M=**2–5**, relative intensity 10–20%), (iii) solvent clusters ($[M+Na+S]^+$, M=**2–5**, S=MeOH and H_2O, relative intensity less than 5%), (iv) the absence of sodium adducts and solvents clusters if M=**1**. Therefore, the Na^+ affinity of ephedrine (1) seems to be lower compared to the one of the tocainide-type pharmaceuticals **2–5**. The adduct and cluster ions reveal further evidence for the molecular mass of the analyte, because the mass spectra including $[M+H]^+$, $[M+Na]^+$, $[M+Na+H_2O]^+$ and $[M+Na+MeOH]^+$ are characterised by typical peak distances, i.e. $\Delta m/z=22$ belongs to $\Delta([M+H]^+-[M+Na]^+)$ and $\Delta m/z=40$ belongs to $\Delta([M+H]^+-[M+Na+H_2O]^+)$ and so forth.

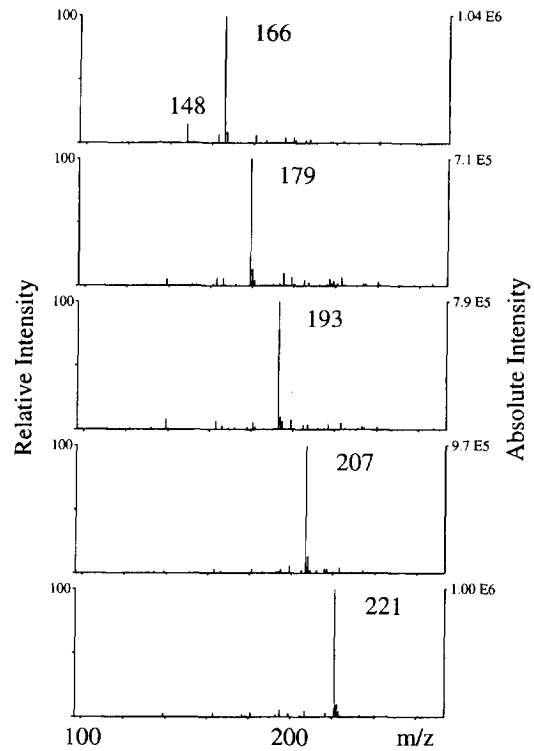

Fig. 3. Unprocessed single scan ES mass spectra of the basic pharmaceuticals (**1–5**) obtained from the CE–ES-MS analysis. CE–ES conditions as in Fig. 2.

In order to obtain information concerning the structure of the $[1+H]^+$ ion, 10–20% nozzle-skimmer dissociation (NSD [25,26]) was induced. A

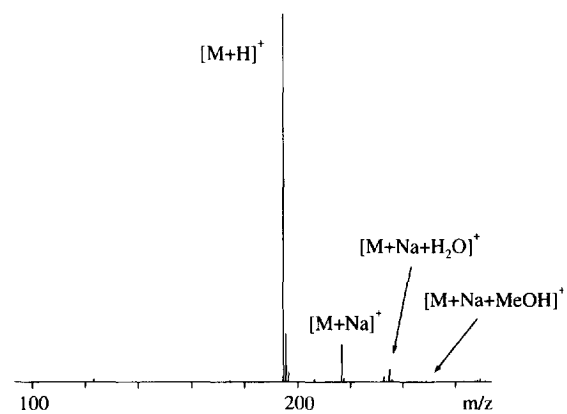

Fig. 4. ES mass spectrum of tocainide (**3**, $[M+H]^+$ at m/z 193) obtained from the CE–ES-MS analysis. The centroid data of 15 spectra have been added and subtracted by 15 adjacent mass spectra. CE–ES conditions as in Fig. 2 and in Section 2.

signal at m/z 148 appeared due to the expulsion of water. By applying the identical ES conditions, i.e. in the same CE–ES-MS analysis, the loss of $\Delta m/z = 18$ or any other fragment formation did not occur for **2–5** as indicated in Fig. 3 or one example of the "background corrected data" (Fig. 4). With regard to protonated ephedrine, the latter process is not surprising since the phenyl group or the lone pair of the amine are capable of stabilising the adjacent carbonium ion due to a mesomeric effect. Therefore, the loss of H_2O, NH_3 or CH_4 from the protonated species of **2–5** is not observed under identical CE–ES-MS conditions since potential adjacent centres for stabilising the carbonium ion are missing. The loss of water from $[1+H]^+$ is a valuable hint for a further structural characterisation[1] by using CE on-line coupled to MS. The differentiation of isomers in CE–ES-MS becomes possible according to their NSD pattern, similarly to collisional induced dissociation (CID) mass spectrometry [31,32]. The amount of dissociation product can be varied by changing the difference of the potentials of the nozzle relative to the skimmer. Considering purity determinations, the latter findings demonstrate the necessary careful inspection of data obtained with electrospray ionisation in the direct infusion mode. The on-line coupling of CE with ES-MS allows the differentiation between impurities (different retention times in CE), ion-solvent clusters (same migration time in CE) and isomer assignment (same m/z value of $[M+H]^+$ but different NSD pattern).

4. Conclusions

The coupling of capillary electrophoresis and mass spectrometry is shown to be very useful for the separation and identification of basic pharmaceuticals with similar structures as demonstrated by using ephedrine (**1**), tocainide (**3**) and three tocainide-type derivatives (**2**), (**4**) and (**5**). The separation performance in CE–MS experiments with volatile buffers of low ionic strength can be significantly enhanced by the use of PVA- and PEG-coated capillaries. The selectivity provided by a sectorfield mass spectrometer gives unequivocal support needed to verify the molecular mass (number of charges per molecule) and structure-specific fragmentation pattern of a given analyte. Comparing the application of a sectorfield mass spectrometer to that of a quadrupole mass analyzer in CE, the mass accuracies as well as the increased resolution provides more reliable information using a sectorfield mass spectrometer.

[1] Similarly, by applying NSD to norephedrine an intense loss of water from the protonated molecule is observed as well.

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