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Journal of Chromatography A, 1000 (2003) 953–961
JOURNAL OF CHROMATOGRAPHY A
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On-line micellar electrokinetic chromatography–mass spectrometry: feasibility of direct introduction of non-volatile buffer and surfactant into the electrospray interface

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Abstract

An on-line method for the coupling of micellar electrokinetic chromatography (MEKC) and mass spectrometry (MS) is presented which allows conventional MEKC conditions to be employed without further modification. The MEKC system is coupled directly to electrospray ionization (ESI) MS using a triaxial interface. A systematic study of the influence of the surfactant concentration, the nature and concentration of buffer salts and presence of organic modifier on the interface performance indicated the feasibility of the MEKC–MS approach. Effective interfacing of MEKC was achieved with both single quadrupole and ion-trap MS instruments. Using a background electrolyte containing 20 m*M* sodium dodecyl sulfate (SDS) and 10 m*M* sodium phosphate buffer, it is demonstrated that full MEKC runs of test mixtures of mebeverine and related compounds can be monitored by ESI-MS with satisfactory sensitivity. Sub-μg/ml levels of the analytes can still be detected in full scan mode, while detection limits are in the 10–50 ng/ml range when selected ion monitoring is applied. It is shown that such sensitivity would allow full-scan MS detection of 0.1% (w/w) levels of potential impurities in mebeverine. With the ion-trap instrument successful MEKC–MS/MS experiments were carried out providing information-rich MS spectra of the related compounds. Repeated MEKC–MS analyses proved that in the course of 1 day the migration time of mebeverine remained fairly constant while the MS-signal intensity only gradually decreased to approximately 65% of its original value. Once-a-day cleaning of the first part of the ion source, which takes only 5 min, suffices to preserve an optimal interface performance for a prolonged period of time.

Keywords: Surfactant concentration; Micellar electrokinetic chromatography; Interfaces, MEKC–MS; Buffer composition; Electrospray interface; Mebeverine

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a powerful separation technique providing a high efficiency, selectivity and optimization flexibility. MEKC is suitable for the separation of both neutral and charged compounds, and allows analysis of a wide range of sample constituents. This can be very useful for, e.g., drug-purity assessment where (part of) the impurities may be unknown prior to analysis [1,2]. Clearly, the coupling of micellar electrokinetic chromatography (MEKC) and mass spectrometry (MS) seems extremely attractive and advantageous, as it would combine a highly versatile separation technique with mass-selective and structure-elucidative detection. However, on-line MEKC–MS has

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0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00179-1

often been considered to be problematic due to potential interferences caused by the non-volatile surfactants and buffer salts in the background electrolyte. As a consequence, most current MEKC–MS approaches involve an adjustment or modification of the separation conditions in order to avoid ion-source contamination and loss of sensitivity caused by the micellar phase. The most common approach is the so-called partial-filling MEKC–MS technique in which the surfactant molecules are prevented from entering the mass spectrometer [3–9]. Unfortunately, under the required experimental circumstances the separation performance of these systems often is strongly compromised and specific optimization is needed for each analyte. Other approaches involve reverse migrating micelles [7,10] and the use of volatile [11] or high-molecular-mass [12–14] surfactants. Direct coupling of MEKC and MS applying sodium dodecyl sulfate (SDS) in phosphate and borate buffers has been reported using an atmospheric pressure chemical ionization (APCI) interface [15,16], but the sensitivity of the method appeared to be rather unfavourable. Using this type of interfacing, Isoo et al. [16] required a 100–600-fold preconcentration of the sample to achieve sub-μg/ml detection limits. For routine analysis, a direct coupling of conventional MEKC with electrospray-ionisation (ESI-)MS would be most convenient, but until now this option has hardly been considered or investigated. The possibility of such an approach was mentioned only briefly as part of capillary electrophoresis (CE)–MS studies [17,18]. Applying selected-ion monitoring (SIM), Tanaka et al. [17] showed the ESI-MS detection of some test drugs (50–100 μg/ml) which were separated by MEKC using 80 m*M* SDS in a 50-m*M* ammonium carbonate buffer (pH 8.5). During analysis, however, the running buffer in the inlet vial did not contain SDS. Cheng et al. [18] indicated the possible analysis of drugs (1 mg/ml) by direct conjunction of MEKC and ESI-MS with a background electrolyte containing 20 m*M* SDS and 40 m*M* ammonium acetate (pH 9).

In this paper the possibility of directly introducing non-volatile buffers containing SDS into the mass spectrometer is studied using a triaxial ESI interface. With the drug mebeverine as test compound, the influence of buffer salts, surfactant concentration and presence of organic modifier on the interface performance and MS sensitivity is examined. The separation and detection of mebeverine and six related compounds at various concentration levels using on-line MEKC–MS is investigated. Furthermore, the utility of an ion-trap MS instrument to provide MS/MS spectra of the related compounds after their separation by MEKC is evaluated.

2. Experimental

2.1. Chemicals and materials

Sodium dodecyl sulfate, boric acid, phosphoric acid, sodium hydroxide, formic acid, acetic acid, ammonium acetate and disodium hydrogenphosphate were purchased from Merck (Darmstadt, Germany). Methanol and acetonitrile were from Biosolve (Valkenswaard, The Netherlands). Mebeverine ((±)-4-[ethyl[2-(4-methoxyphenyl)-1-methylethyl]amino]-butyl-(3,4-dimethoxy) benzoate; Fig. 1) and six related compounds were a gift from Solvay Pharmaceuticals (Weesp, The Netherlands). Deionized water was filtered and degassed before use. Fused-silica capillaries were from Composite Metal Services (The Chase, Hallow, UK) and flushed with 1 *M* sodium hydroxide (10 min) and water (10 min) prior to use.

For the infusion experiments, 1-μg/ml mebeverine solutions were prepared in, respectively, formic acid (50 and 100 m*M*), ammonium acetate

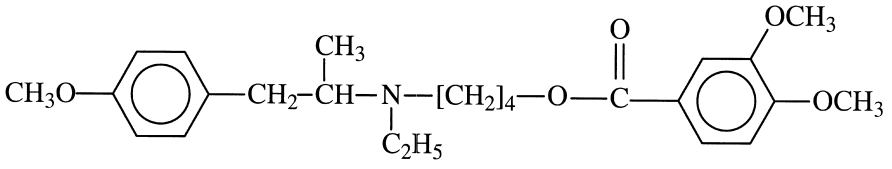

Fig. 1. Molecular structure of mebeverine.

(25 and 50 m*M*), sodium phosphate buffer, pH 2.1–2.5 (5 and 25 m*M*), sodium phosphate buffer, pH 7.5 (5 and 25 m*M*), and sodium borate buffer, pH 9.3 (10 m*M*). In addition, solutions of 1 μg/ml mebeverine in 100 m*M* formic acid and 10 m*M* sodium phosphate buffer (pH 7.5) each with 1, 5, 10, 20 or 50 m*M* SDS were made. Test mixtures of mebeverine and related compounds were prepared in water at the following concentrations: (i) 10 μg/ml each, (ii) 1 mg/ml mebeverine and 10 μg/ml of each related compound, and (iii) 1 mg/ml mebeverine and 1 μg/ml of each related compound. For the MEKC experiments the background electrolyte contained 10 m*M* sodium phosphate (pH 7.5), 20 or 50 m*M* SDS and 25% acetonitrile. The composition of the sheath liquid was acetic acid–methanol–water (1:50:50, v/v/v).

2.2. CE systems

MEKC–MS was performed using a PrinCE CE system (Prince Technologies, Emmen, The Netherlands) with a 75-μm I.D. capillary of 75 or 100 cm. MEKC separations were always carried out at a voltage of 30 kV. In combination with the Platform MS, samples were typically injected using a pressure of 110 mbar for 18 s. During MEKC analysis with the Platform, a pressure of 75–90 mbar was applied to overcome the overpressure in the ion source (which occurred when the triaxial interface was used) and to assure a flow towards the capillary outlet. Prior to each analysis the capillary was flushed with fresh background electrolyte for 1 min at 1000 mbar. During infusion experiments, the mebeverine solution under study was continuously pumped from the inlet vial through the capillary to the ESI interface applying a pressure of 150 mbar. In combination with the Agilent MS, samples were injected for 6 s at 25 mbar and no extra pressure was applied during analysis. In this case, the capillary was flushed with fresh buffer for 0.6 min at 2000 mbar before every analysis.

MEKC with UV absorbance detection was carried out on a P/ACE MDQ system (Beckman Coulter, Fullerton, CA) equipped with a diode array detector. The capillary (75 μm I.D.×57 cm) was thermostated at 25 °C. Sample was injected by applying a pressure of 35 mbar for 4 s, and the separation voltage was 30 kV.

2.3. MS systems

Coupling of MEKC and MS was performed utilizing triaxial interfaces in which the capillary effluent is mixed with a sheath liquid and nebulized by nitrogen gas. The flow-rate of the sheath liquid was 5 μl/min in all cases. Initial experiments were carried out on a Platform quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an ESI source. The electrospray voltage was 3.3 kV and the nebulizing gas rate was 50 l/h. Data were collected in full scan (180–600 m/z) at a scan rate of 3 s. In a later stage also an Agilent 1100 Series LC/MSD SL ion-trap mass spectrometer (Agilent Technologies, Waldbronn, Germany) equipped with an ESI source was used. With this instrument the electrospray voltage was 5.0 kV and the nebulizing gas pressure was adjusted to 15 p.s.i. In full scan mode, the scan range was 150–600 m/z and three scans were averaged for one spectrum. To avoid overloading of the ion-trap, the ion accumulation time was automatically adjusted using the Ion-Charge-Control option of the instrument. In MS–MS analysis, the voltage for the collision energy ranged from 0.45 to 1.70 V depending on the parent ion.

3. Results and discussion

3.1. Infusion experiments

The influence of various buffers, SDS and acetonitrile on the MS signal of the test compound mebeverine (m/z 430) was determined by infusion of solutions into the Platform mass spectrometer. In order to mimic MEKC–MS conditions as much as possible, the mebeverine solutions in each respective buffer were pushed through the CE capillary to the ESI interface which merges the capillary effluent with sheath liquid prior to nebulization. Applying 150 mbar to the inlet, the resulting flow through the capillary was similar to a common electro-osmotic flow (EOF). No separation voltage was applied in this instance because it would have led to different flow-rates and mobilities of mebeverine amongst the

various tested buffers, thereby hindering a proper comparison. For consistency, the mebeverine intensity measured for a specific buffer solution was always expressed relative to the signal obtained for a solution in reference buffer analysed just before or after the solution under study.

The effect of formic acid, ammonium acetate, sodium phosphate buffers and sodium borate buffer on the MS response of a 1-μg/ml mebeverine solution was studied (Fig. 2). As expected, the volatile formic acid and ammonium acetate show good MS compatibility. Although in principle MEKC can also be performed at low pH (applying a negative voltage), the use of phosphate and borate buffers at medium or high pH is much more common and often required to achieve and maintain a good separation performance. However, phosphate and borate clearly reduce the MS signal of mebeverine (Fig. 2). This reduction can be fully attributed to ion suppression effects during ESI, and not to source fouling as the intensity was fully restored when the mebeverine solution in formic acid was measured again. The adverse effect of the sodium phosphate buffer increases with both its concentration and its pH. The latter phenomenon is probably caused by the presence of doubly charged phosphate and higher concentrations of sodium ions at pH 7.5. It should be noted, however, that even at 25 m*M* sodium phosphate (pH 7.5) the MS signal of mebeverine was still significant (about 10% of the signal obtained with formic acid) and could be measured reliably.

The effect of SDS (0–50 m*M*) on the MS signal of mebeverine was also studied by infusion. SDS is a well-known and notorious suppressor of analyte-ion signals measure in positive-ion mode ESI-MS [11,19–21]. The addition of 1 m*M* SDS to a mebeverine solution (1 μg/ml in 100 m*M* formic acid (pH 2.4)) indeed seriously reduces the MS response. At higher SDS concentrations the mebeverine response further decreases (Fig. 3A), but the signal decline quickly becomes rather gradual and even with 50 m*M* SDS the molecular ion of mebeverine was still measurable (over 20% of the signal obtained without SDS). Although the ESI process is well understood [22], the exact mechanism of analyte signal quenching by SDS has not yet been addressed. ESI essentially results from a continuous

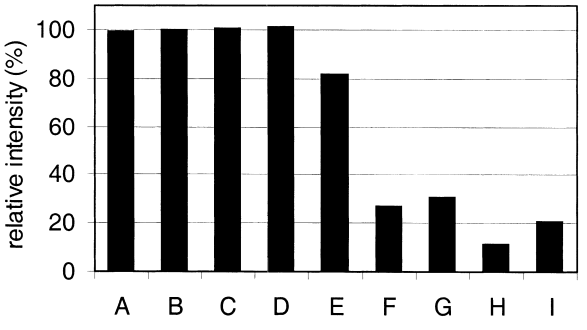

Fig. 2. Relative MS signal of 1 μg/ml mebeverine (monitored at *m*/*z* 430) in various buffers measured by infusion. The signal obtained for 1 μg/ml mebeverine in 100 m*M* formic acid (pH 2.6) was set at 100%: (A) 50 m*M* formic acid (pH 2.6), (B) 100 m*M* formic acid (pH 2.4), (C) 25 m*M* ammonium acetate (pH 6.6), (D) 50 m*M* ammonium acetate (pH 6.7), (E) 5 m*M* sodium phosphate (pH 2.5), (F) 25 m*M* sodium phosphate (pH 2.1), (G) 5 m*M* sodium phosphate (pH 7.5), (H) 25 m*M* sodium phosphate (pH 7.5), and (I) 10 m*M* sodium borate (pH 9.3).

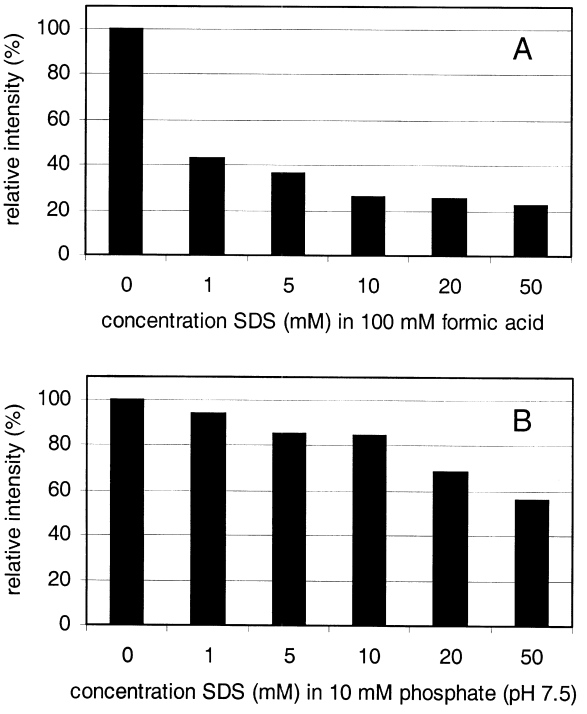

Fig. 3. Influence of the SDS concentration on the relative MS signal of 1 μg/ml mebeverine (monitored at *m*/*z* 430) in (A) 100 m*M* formic acid (pH 2.4), and (B) 10 m*M* sodium phosphate buffer (pH 7.5). Note that the 100% levels in (A) and (B) refer to different systems which have different absolute signal intensities (see Fig. 2).

fission of charged parent droplets into smaller and smaller droplets from which finally analyte ions are transferred to the gas phase. In the process of droplet formation, the parent droplets first take an elongated shape forming a tail. Subsequently, smaller droplets with higher charge-to-mass ratio emerge from the tail. To explain the suppression effects of anionic surfactants in the positive ion mode, Rundlett and Armstrong [19] have suggested that the offspring droplets contain the species that reside on the surface of the parent droplets. As the surfactant preferentially stays at the liquid–air interface, the droplets will be enriched in surfactant ions. Coulombic interactions of the oppositely charged surfactant and analyte ions will inhibit the transfer of analyte ions into the gas phase and, consequently, reduce the MS signal. With this suppression model in mind, one might speculate on why the adverse effect of an increasing SDS content slowly diminishes. Maybe a relatively small amount of SDS already suffices to largely saturate the surface of the charged electrospray droplets. Increase of the SDS concentration then does not lead to a proportionally higher surface coverage, and thus not to a proportional decrease of signal.

MEKC with SDS as surfactant is commonly carried out at pH 6–10 using inorganic buffers, and also a certain percentage of organic modifier is often added to the background electrolyte. Therefore, the combined effect of SDS and sodium phosphate, as well as the influence of acetonitrile on the MS response of mebeverine (1 μg/ml), was investigated by infusion. A 10 m*M* sodium phosphate buffer (pH 7.5) by itself already causes a loss in the MS response of mebeverine of about 75% when compared to a solution in 100 m*M* formic acid. Subsequent addition of SDS (1–50 m*M*) to the mebeverine solution in phosphate buffer shows a further MS-signal decrease with increasing SDS concentration (Fig. 3B), however, the extent of signal suppression was not very dramatic. Apparently, the ion-suppression effects of sodium phosphate and SDS are not cumulative. As expected, no negative or positive effects of acetonitrile on the MS detection of mebeverine were observed for electrolytes containing 10 m*M* sodium phosphate buffer (pH 7.5), 20 m*M* SDS and 5–25% acetonitrile. In comparison with the volume of organic solvent provided by the sheath liquid, the small volumes of acetonitrile from the background electrolyte flowing to the interface are negligible.

3.2. On-line coupling of MEKC with single quadrupole MS

The infusion experiments described above indicate that it should be possible to record significant MS signals from analytes under conventional MEKC conditions. In order to evaluate this, a test mixture of mebeverine and related compounds was analysed. As was determined by MEKC with UV absorbance detection (Fig. 4), this mixture could be separated using a background electrolyte of 10 m*M* sodium phosphate (pH 7.5), 20 m*M* SDS and 25% acetonitrile. This separation system allowed us to study the combined effects of SDS, phosphate and acetonitrile in on-line MEKC–MS. Although the concentration of organic modifier is relatively high and the SDS concentration rather low, micellar distribution still plays a role under these circumstances [23]. Besides, at the end it is the amount of surfactant (irrespective of what form) reaching the interface that is responsible for signal suppression [19]. For on-line MS detection, the separation system was coupled directly to the Platform mass spectrometer via the triaxial

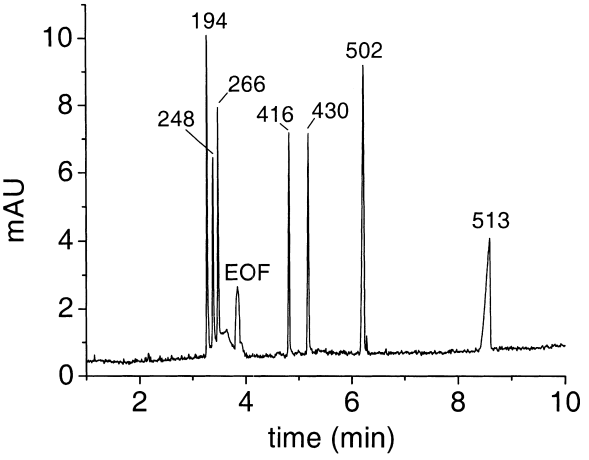

Fig. 4. MEKC–UV of a mixture of mebeverine and related compounds (10 μg/ml each) using a background electrolyte containing 10 m*M* sodium phosphate (pH 7.5), 20 m*M* SDS and 25% acetonitrile. The compounds are designated by the m/z values of their protonated molecular ions ($[M+H]^+$).

ESI interface without taking any precautions to prevent surfactant or buffer from entering the mass spectrometer. In the positive ion mode, clusters of SDS, sodium, phosphate and/or acetate ions revealed some discrete but intense signals (mainly at m/z 105, 311, 393, 475 and 599) which, together with the stream of buffer ions, caused a continuously high total ion current (TIC). As a consequence, no apparent analyte signals were observed in the TIC trace when the mebeverine test mixture with component concentrations of 10 μg/ml each was analysed by MEKC–MS. In the mass spectra recorded during the run, however, the pseudo-molecular ions ($[M+H]^+$) of the analytes could be discerned, although their intensity was relatively small when compared with the signals caused by the ion clusters from the background electrolyte (Fig. 5). Nevertheless, the significance of the analyte signals could be clearly demonstrated by the extracted-ion chromatograms constructed for the respective m/z values of the mixture compounds which show clear peaks with a satisfactory signal-to-noise ratio (Fig. 6A). Somewhat surprisingly under the applied ESI conditions the last migrating substance (a diamino compound) is mainly detected in its singly charged form (at m/z 513), although in solution at pH 7.5 it is diprotonated.

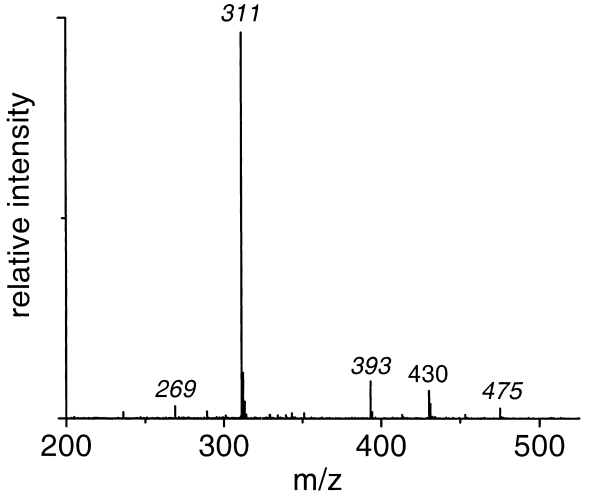

Fig. 5. Mass spectrum recorded at the apex of the mebeverine peak during MEKC–MS of a mixture of mebeverine and related compounds (10 μg/ml each). Background electrolyte, see Fig. 4. The m/z values of ion clusters from the background electrolyte are printed italic.

Since the TIC trace did not reveal the analytes and the construction of the extracted-ion chromatograms of Fig. 6A required foreknowledge on the sample constituents, one may ask whether it is possible to detect unknown compounds. In this respect it is important to note that distinct peaks were only observed in the extracted-ion chromatograms of m/z values corresponding to the test compounds. Other m/z values yielded chromatograms consisting of mere base lines with random noise. In order to reveal unknown compounds detected by MEKC–MS, we now envision a software program that constructs all extracted-ion chromatograms in a predefined region (e.g., m/z 100–600) and subsequently checks each chromatogram for the presence of peaks (applying a common integration routine). The selection of chromatograms with discrete peaks in principle should represent all detected components of an analysed sample, including unknowns. We will investigate the feasibility of this data-handling approach in the near future.

From the viewpoint of both achievable sensitivity and impurity profiling, it is interesting to see if low levels of analytes can be detected in an excess of the parent drug. Therefore, a 1-mg/ml solution of mebeverine was prepared with the related compounds at 1 μg/ml, mimicking a 0.1% (w/w) level of impurities. MEKC–MS analysis of this sample revealed that all related compounds can still be detected (Fig. 6B). The peak of the related compound with m/z 416 is now broadened, probably because it partly overlaps with the overloaded mebeverine band. The wide band in the m/z-248 extracted-ion trace originates from a minor fragment of mebeverine that arises during ESI. The analyte detectability could be further improved by SIM. Using this mode with the ion-trap mass spectrometer (see below), the MS detection limits for the related compounds were in the 10–50-ng/ml region, which is quite favourable for a MEKC-based method permitting detection of specified impurities below the 0.01% level. The MEKC–MS system was further challenged by raising the concentration SDS up to 50 m*M* and leaving the further conditions unchanged. With this system the detectability in the extracted-ion chromatograms (obtained in the full

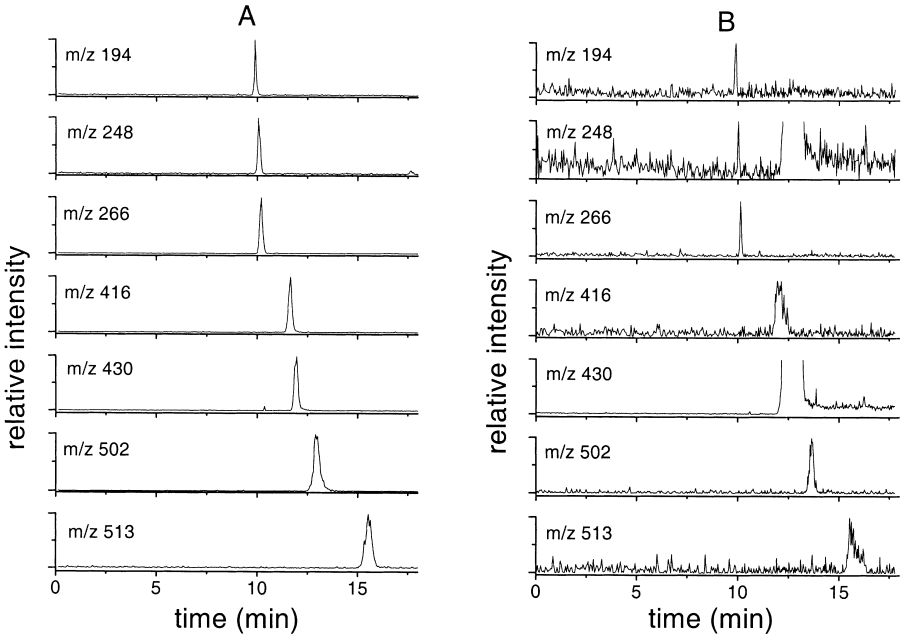

Fig. 6. MEKC–MS of a mixture of mebeverine and related compounds. Background electrolyte, see Fig. 4. Compound concentrations: (A) 10 μg/ml each, and (B) 1 mg/ml of mebeverine and 1 μg/ml of every related compound.

scan mode) somewhat deteriorated by enhanced suppression effects so that some related compounds in the 0.1% mixture could no longer be detected.

Comparison of Figs. 4 and 6A indicates that in the MEKC–MS system some extra band broadening occurs which, however, does not seem essentially different from broadening more often observed in CE–MS using a triaxial interface (see, e.g., Ref. [18]). The major sources of the extra band broadening most probably are the mixing of the capillary effluent with sheath liquid and the laminar flow resulting from pressure differences across the capillary during analysis. The latter is quite likely to occur in CE–MS or MEKC–MS. In this particular case, the design of the Platform ion source is such that an overall overpressure arises at the capillary outlet when used with a triaxial interface. It appeared not easy to precisely compensate this overpressure. Therefore, during MEKC–MS analysis with the Platform instrument, a relatively large hydrodynamic pressure was always applied at the inlet vial in order to ensure a continuous flow towards the MS detector under all circumstances. In other cases, like with the Agilent ion-trap set-up, the nebulizing gas causes an underpressure at the capillary outlet and, consequently, a continuous suction of background electrolyte. Preliminary experiments in our laboratory indicate that accurate cancelling of these pressure differences over the capillary may lead to significantly improved plate numbers in CE–MS and MEKC–MS. We are currently studying this remedy in more detail and will report on it in another paper.

In order to check whether the MS system is capable of handling the continuous supply of non-volatile surfactants and salts in the course of 1 day, a mebeverine solution (25 μg/ml) was repeatedly analysed by MEKC–MS using a background electrolyte of 10 m*M* sodium phosphate (pH 7.5), 20 m*M* SDS and 25% acetonitrile. Like in normal MEKC, before every analysis (runtime, 30 min) the capillary was flushed with fresh background electrolyte without decoupling the capillary outlet from the interface. Each injection of mebeverine yielded a good-quality extracted-ion chromatogram (m/z 430) with a prominent peak. During the day, the migration time of mebeverine remained fairly constant, while

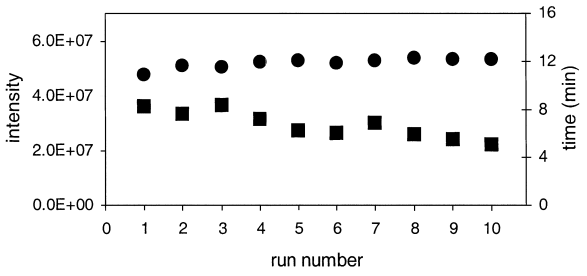

Fig. 7. Repeated MEKC–MS analysis of 25 μg/ml mebeverine. Background electrolyte, see Fig. 4. (■) Signal intensity of mebeverine monitored at 430 m/z; (●) migration time of mebeverine. The mebeverine solution was injected at 45-min intervals.

the absolute peak intensity showed a slow decrease in time (Fig. 7). This loss of sensitivity might be caused by a gradual fouling of the ion source. Of course, it will be inevitable that some part of the non-volatile salts that are led into the MS will deposit in the ion source. The sensitivity was simply restored by removing the white deposit from the first part of the ion source with water and ethanol (a 5-min procedure). This cleaning was carried out after each day or every 10–15 MEKC–MS runs. If required, e.g., after a full week of extensive use of SDS-containing buffers, the complete ion source was cleaned to regain optimal MS performance.

3.3. On-line coupling of MEKC with ion-trap MS

As the results obtained with on-line MEKC–MS using a single-stage mass spectrometer were quite promising, we decided to study the direct coupling of MEKC to an ion-trap mass spectrometer as well. With this instrument MS^n experiments can be carried out, allowing the gain of structural information (and possibly structural elucidation) of unknown compounds. Obviously, the successful on-line coupling of MEKC with such an instrument would be very beneficial. Since the used Platform and Agilent ion trap mass spectrometers considerably differ in design of the ESI interface (in-axis versus orthogonal spray), ion source and mass analyzer, the feasibility of feeding SDS and phosphate-containing buffers directly into the ion-trap instrument was first checked by infusion experiments. Similar results were obtained as described in Section 3.1 leading to the same overall conclusion: SDS and sodium phosphate seriously suppress analyte signals measured by the ion trap system, but the signals remain sufficiently high to allow detection of relevant analyte concentrations.

Using the ion-trap instrument, on-line MEKC–MS analysis of the test mixture (each component 10 μg/ml) with the background electrolyte containing 10 m*M* sodium phosphate, 20 m*M* SDS and 25% acetonitrile provided results analogous to Fig. 6A. In this instance, however, the last migrating compound was mainly detected as doubly charged species at m/z 257. MS–MS detection of the mixture constituents separated by MEKC yielded some good-quality spectra for which the major fragment ions are listed in Table 1. In this preliminary stage, the conditions for collision-induced dissociation were chosen such that the parent ions remained detectable in the product spectrum. The obtained intensities of the fragment ions indicated that useful spectra can be obtained down to 1–5 μg/ml injected concentration. Clearly, the obtained spectra reflect the common origin of the related compounds which almost all show fragments at m/z 121 and 149. Based on the molecular structure of mebeverine, a structure proposal for the fragments observed at m/z 121, 149, 237, 248 and 365 could be given. Such MS/MS information evidently would be very useful when the presence of a specific impurity has to be determined with high reliability, or when identification of unknown compounds is pursued.

Table 1
Pseudo-molecular ions and their major collision-induced fragment ions for mebeverine and related products as recorded during MEKC–MS/MS of test mixture containing 10 μg/ml of each component[a]

Pseudo-molecular ion (m/z)	Major fragment ions (m/z)
194	149, 121
248	149, 121
266	149, 121
416	149, 121
430	248, 149, 121
502	248, 237, 149
257	365, 248, 149, 121

[a] Background electrolyte, 10 m*M* sodium phosphate (pH 7.5), 20 m*M* SDS and 25% acetonitrile.

4. Conclusions

On-line MEKC–MS by direct introduction of the background electrolyte into an electrospray ion source was accomplished. The coupling was employed successfully under conventional MEKC conditions with a background electrolyte containing SDS, sodium phosphate buffer and organic modifier. Although significant suppression of the analyte signal occurs, using extracted-ion chromatograms (obtained in the full scan mode) detection limits of about 1 μg/ml are achieved, whereas MS detection below 100 ng/ml is possible when SIM is used. Such a sensitivity will be quite sufficient for several relevant analytical queries like, as indicated in this study, the impurity profiling of drugs. The direct coupling of MEKC with an ion-trap instrument with MS^n capabilities is also demonstrated yielding a hyphenated technique with very interesting analytical potential.

The considerable analyte signals that we have observed in the presence of SDS, seem contradictory to the rather common perception that SDS completely destroys analyte ion signals [18]. However, the latter may be only valid for ESI set-ups that do not encompass the low volume flow in MEKC and the influence of a sheath liquid. Without sheath liquid, the surfactant–analyte ratio entering the ion source is actually much larger, which may lead to a more extensive loss of signal. The fact that we have obtained similar MEKC–MS results with instruments with quite different ion-source designs, further supports our conclusion that coupling of MEKC and ESI-MS by direct introduction of the background electrolyte is feasible. Various aspects such as quantitative analysis, detection of unknown compounds and data handling to distinguish analyte signals from background noise, still need attention. Also the sheath liquid composition was not yet really optimized for MEKC–MS purposes. Especially the possible addition of substances to the sheath liquid that would attenuate the adverse effects of SDS in ESI might be intriguing.

Acknowledgements

The authors are grateful to Drs. J.T. Op't Land of Solvay Pharmaceuticals (Weesp, The Netherlands) for providing mebeverine and related compounds. We wish to thank Dr. J.C.M. Waterval (Organon), Dr. P.G. Muijselaar (Solvay Pharmaceuticals), Dr. C.E. Sänger (AstraZeneca), Dr. C.M.B. van den Beld (Yamanouchi Europe), Dr. M. Jimidar (Janssen Pharmaceutica) and Dr. T. Eichinger (Aventis) for stimulating discussions, and their companies for financially supporting this study.

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