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General approach for the analysis of various alkaloid classes using capillary electrophoresis and capillary electrophoresis–mass spectrometry

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Abstract

The analysis of various alkaloid classes employing capillary electrophoresis (CE) and on-line combined CE–mass spectrometry (CE–MS) is described. A CE method is presented for the analysis of alkaloids without derivatisation or purification. The separation of four different groups of alkaloids consisting of monoterpenoid indole alkaloids, protoberberines/benzophenanthridines, β-carboline alkaloids, and isoquinolines from poppy by free zone capillary electrophoresis has been obtained using a 1:1 mixture of 100 mmol l^{-1} ammonium acetate (pH 3.1) and acetonitrile. The influence of alkaloid structure on the electrophoretic mobility is discussed. The CE–MS reconstructed total ion current (RIC) of the indole- and the opium-type standard alkaloids shows a decreased signal-to-noise ratio compared to CE using only UV detection. As expected the single-ion traces (or individual mass traces) of the $[M+H]^+$ ions show higher signal-to-noise ratios than the RIC. The electrospray MS data of the alkaloids are dominated by the protonated molecules and the Na^+-, and K^+-adducts. They display the typical pattern resulting from cluster formation or doubly charged species.

Keywords: Electrophoretic mobility; Buffer composition; Alkaloids

1. Introduction

Alkaloids represent one of the largest and most interesting groups of plant secondary metabolites. One reason for the growing attention concerning these natural compounds is their pharmaceutical and therapeutic value, such as the analgesic and antitussivic opium, antihypertonic or antiarrhythmic *Rauwolfia* or the dimeric *Catharanthus* alkaloids which are important compounds in cancer treatment. At present, the most commonly used method for qualitative and quantitative analysis of alkaloids is reversed-phase high-performance liquid chromatography (HPLC). This technique can be coupled to mass spectrometry (MS) to provide both analytical separation and structural determination of unknown

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bioactive compounds [1–4]. In addition, gas chromatography (GC) or GC–MS is applicable to alkaloid determination. This is a limited alternative since derivatisation of complex alkaloid mixtures is usually necessary but not easily performed. Further, general derivatisation procedures are not applicable to certain alkaloid groups.

In recent years capillary electrophoresis (CE) has become a promising technique which can also be applied to the analysis of plant secondary metabolites. In a recent review [5], CE applications on nearly all groups of plant natural products are summarised including the analysis of some alkaloids from opium [6,7], and from *Pilocarpus* (like pilocarpine and its isomer) [8], oxindole alkaloids from *Uncaria* [9], some isoquinoline alkaloids of different plant sources [10,11], and a few protoalkaloids from *Ephedra* [12] or even purine alkaloids – as active constituents of theophylline tablets [13].

CE–MS is an attractive combination of two high-resolution analytical techniques with the potential to solve complex biological problems [14–18]. CE permits fast and efficient separation of a wide variety of charged [19–21] and even uncharged species [22,23], whereas MS provides information about the mass and, potentially, the structure of the separated compounds [15,24,25]. A key point of the coupling of these two techniques is the specific interface design of the electrospray (ES) ion source with a coaxial liquid sheath arrangement [26–29]: The end of the fused-silica CE capillary is provided with a make-up flow of an electrolyte (sheath liquid) forming the electrical contact between the ES probe-tip and the high-voltage power supply of the CE. However, there are some difficulties and constraints 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 with UV detection in CE [29] and may impair the separation; (ii) the buffer system for the CE analysis has to be compatible with the ES process. The CE separation performance is improved with increasing ionic strength of the electrolyte, whereas the ion-production process in ES is impaired with increasing ionic strength [30]. The most commonly used buffer systems in CE, i.e., aqueous electrolytes with non-volatile buffer components like sodium phosphate or sodium borate, are not MS-compatible because non-volatile salts can contaminate the ES interface and the MS ion optics. Furthermore, they can cause intense background signals resulting in a decreased sensitivity in the total reconstructed ion current (RIC). For the transfer of standard CE methods to CE–MS, adequate electrolyte systems have to be chosen in order to get both analyte resolution and high sensitivity in the ES-MS detector [31].

In the present contribution, the successful optimisation of a CE separation method to CE–MS compatible conditions is summarised. As reviewed by Tomás-Barberán, CE offers considerable advantages concerning the separation of plant secondary metabolites compared to HPLC [5]. The CE technique provides a higher efficiency, a better peak shape, and an increased number of theoretical plates. Finally, the run time of one LC analysis is longer if chemically similar alkaloid components have to be resolved. Accordingly, we decided to explore the use of CE–MS instead of LC–MS for the analysis of structurally related alkaloids of certain plant origin. Therefore, four different classes of alkaloids, i.e., monoterpenoid indole alkaloids, protoberberines/benzophenanthridines, β-carboline alkaloids and isoquinolines from poppy, have been analysed by means of CE and CE–MS. The results suggest that the described method may be generally applicable to the analysis of alkaloids in standards and natural matrices.

2. Experimental

2.1. Chemicals

2.1.1. CE–UV

Acetonitrile, ammonium acetate, methanol, and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Acetic acid was from Applichem (Darmstadt, Germany). All reagents used were of analytical-reagent grade. The following alkaloids were kindly provided by Professor Dr. M. H. Zenk (Munich, Germany): canadine, columbamine, coptisine, jatrorrhizine and stylopine. Chelidonine, palmatine chloride, sanguinarine chloride, harmine, harmaline, harmol and harmalol were purchased from Sigma (Deisenhofen, Germany). Berberine

chloride, scoulerine, harmane and norharmane were obtained from Roth (Karlsruhe, Germany).

2.1.2. CE–MS

HPLC-grade methanol (LiChrosolv), acetonitrile and acetic acid (analytical-reagent grade) were used as supplied by Merck, ammonium acetate (microselect grade >99%) and ammonium hydroxide (analytical-reagent grade) 25% in water purchased from Fluka (Buchs, Switzerland) were used without further purification.

2.2. Sample preparation

All standards used were dissolved in methanol and diluted to a final concentration of 25–125 μg/ml.

2.3. Instrumentation

For CE–UV a BioRad BioFocus 3000 apparatus (Munich, Germany) equipped with a fast scanning UV detector and a liquid cooling system for the capillary was used. Samples were injected by pressure. The column dimensions of the bare fused-silica (FS) capillary were 55 cm (50 cm to detector)×50 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA).

The capillary was stored dry over night. Prior to starting a series of analyses, the capillary was preconditioned with 1 mol l^{-1} sodium hydroxide for 10 min, followed by 10 min with water and 15 min with running buffer. Between runs the capillary was purged for 2 min with 1 *M* sodium hydroxide, 2 min with water and finally 3 min with running buffer. The applied voltage was 15 kV and the temperature of the capillary was set to 25°C unless otherwise stated. For sample injection 345 mbar s was applied at the capillary inlet. The running buffer consisted of a 100 mmol l^{-1} solution of ammonium acetate adjusted to a pH value of 3.1 with acetic acid and diluted to a final concentration of 50% (v/v) with acetonitrile [32]

On-line CE–MS separations were performed on a BioFocus 2000 (BioRad) using a CE–MS interface designed for the connection of the liquid-cooled CE capillary to the ES source. FS capillaries of 50 μm inner diameter and 375 μm outer diameter were obtained from Polymicro Technologies. The capillary temperature was maintained at 20°C. The temperature of the samples, electrolytes and washing solutions were kept at 10°C. Every run was repeated at least three times under identical conditions. The MS detection in the positive ion mode was performed on a Finnigan MAT Model 95 forward-geometry sector-field mass spectrometer (Finnigan MAT, Bremen, Germany) upgraded with an API-II ion source (Finnigan MAT) [33–35] operating in the ES ionisation mode. Details of the operation of the sector mass spectrometer with the CE–ES device have been published elsewhere [36,37]. The standard ES conditions were as follows: electrospray potential, U_{ES}=3.0–3.5 kV; electrospray current, I_{ES}=6–9 *m*A (CE on); temperature of the aluminium capillary, T_{cap}=200–250°C; coaxial sheath liquid, methanol–water (9:1) with 1% acetic acid, methanol–water (8:2) with 1% acetic acid, acetonitrile–water (9:1) with 1% acetic acid; flow-rate, 1–2 μl min^{-1} delivered by a Harvard Apparatus 22 syringe pump (South Natick, MA, USA). The mass spectrometer was run at a mass resolution of at least $m/\Delta m$ 2000, with an accelerating voltage of ca. 5 kV. Scanning was performed from m/z 100 to 1000 with 3 dec/s or 5 dec/s. The electron multiplier was set at 1.6 kV. For data acquisition and presentation, the standard Digital Alpha Station-based ICIS-2 system provided by Finnigan MAT and the MassLib V 8.3 mass spectra evaluation program package (Max-Planck-Institut für Kohlenforschung, Mülheim, Germany) were employed.

3. Results and discussion

3.1. Development of CE separation conditions for alkaloids

In order to optimise the CE separation of alkaloids, a mixture of 15 indole alkaloids and biogenic amines (Fig. 1) was analysed. The electrolyte concentration and the pH values have significant effects on absolute and relative electrophoretic mobilities [19–21]. During method development the electrolyte composition was altered using 25, 50 and 100 mmol l^{-1} sodium dihydrogenphosphate at pH values of 2.0–3.5 and monohydrogenphosphate be-

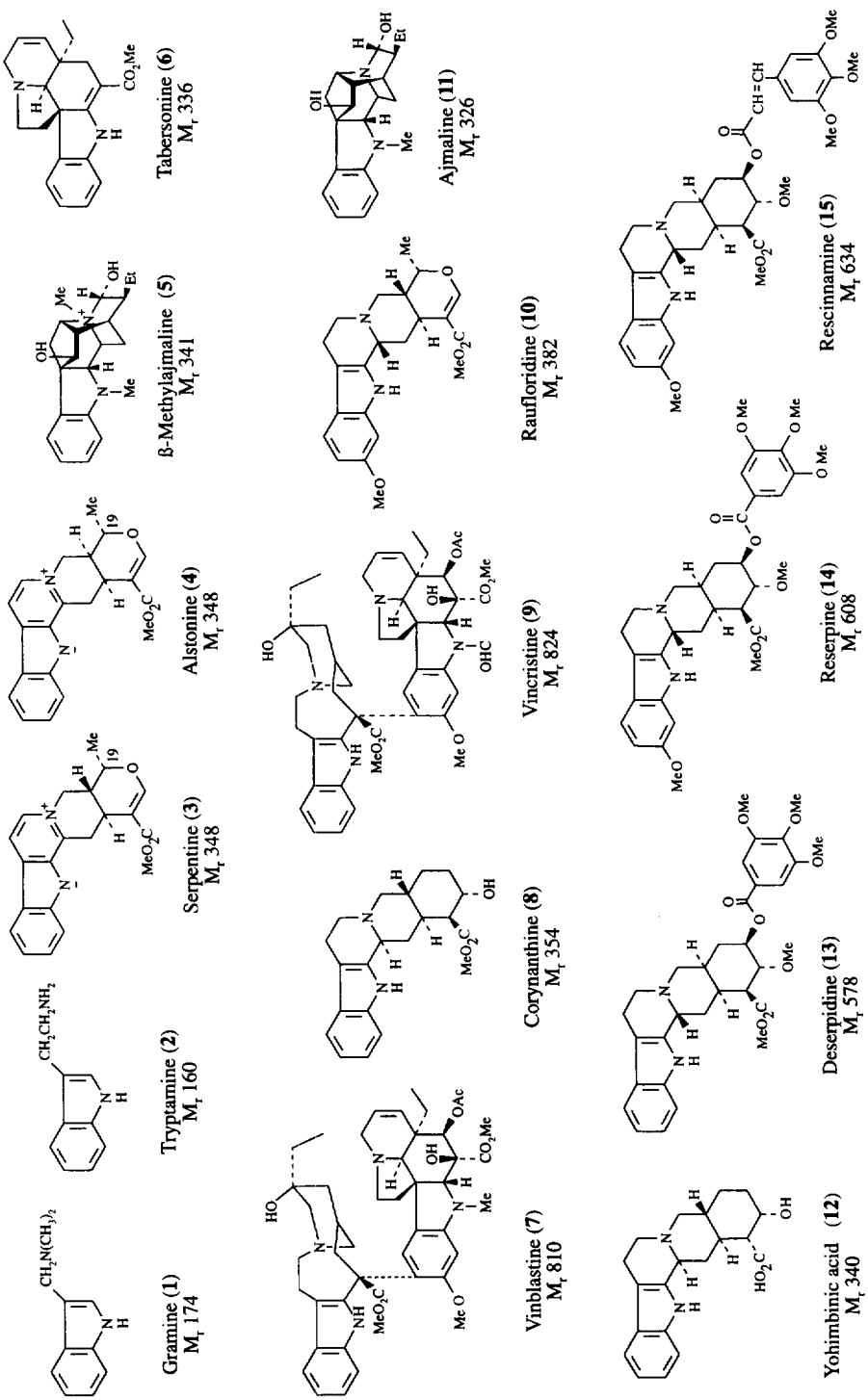

Fig. 1. Names, chemical formulas and molecular masses (M_r) of 15 indole alkaloids and biogenic amines (1–15).

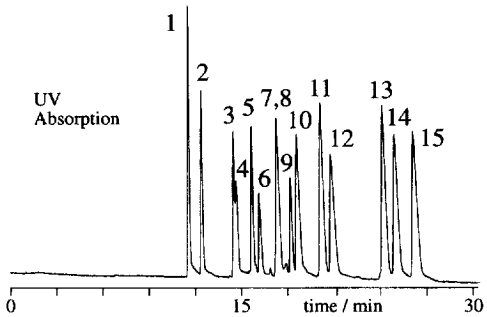

Fig. 2. CE separation of the 15 indole alkaloids and biogenic amines (1–15). Conditions: U_{CE} 15 kV, detection UV at 200 nm; capillary dimensions, 55 cm (50 cm to UV detector) × 50 μm I.D., uncoated, 25°C; electrolyte buffer, CH_3CN–100 mmol l^{-1} NH_4AcO pH 3.1 in water (1:1, v/v).

tween pH 5.0–6.5. The most efficient separation was observed at buffer concentrations of 50 mmol l^{-1} and at pH 3 [32,38].

Second, a similar approach was applied using a volatile buffer that is advantageous for the CE–MS coupling [14–16]. Ammonium acetate buffer (50 mmol l^{-1}) adjusted with acetic acid to pH 3.1 yielded a good analyte separation. The addition of different amounts of acetonitrile (10 to 80%, v/v) was then tried, and a 1:1 (v/v) dilution of the buffer with the organic solvent was found to give excellent separation. Under these conditions 14 of the 15 alkaloidal compounds were separated, 13 of them with baseline resolution (Fig. 2).

As illustrated in Fig. 2, the indole alkaloids migrate in three distinct groups consisting of the structurally simple biogenic amines tryptamine (2) and gramine (1) followed by a group consisting of ten alkaloids (3–12) of which the epimeric indole alkaloids serpentine (3) and alstonine (4) were not completely resolved. Only corynanthine (8) and the dimeric monoterpenoid alkaloid vinblastine (7) comigrated. The *Rauwolfia* alkaloids of the reserpine group migrated in the third group appearing after 24 min.

3.2. Separation of structurally different alkaloid groups

The electrophoretic method described above was applied to a more general separation of alkaloids using three standard alkaloid mixtures, i.e., protoberberines/ benzophenanthridines, β-carbolines, and isoquinoline alkaloids from opium.

Applying a separation voltage of 18 kV and a column temperature of 15°C, nine alkaloids of a group of ten protoberberines/benzophenanthridines (Fig. 3) could be sufficiently separated within 17 min without further modification of the electrophoretic parameters described above (Fig. 4). As observed for the indole-alkaloid separation, the compounds can be separated into different groups. The protoberberine-type compounds (16–25), i.e., the quaternary benzophenanthridine-type sanguinarine (16), the methyldioxylated constituents coptisine (17) and berberine (18), are baseline separated between 11.5

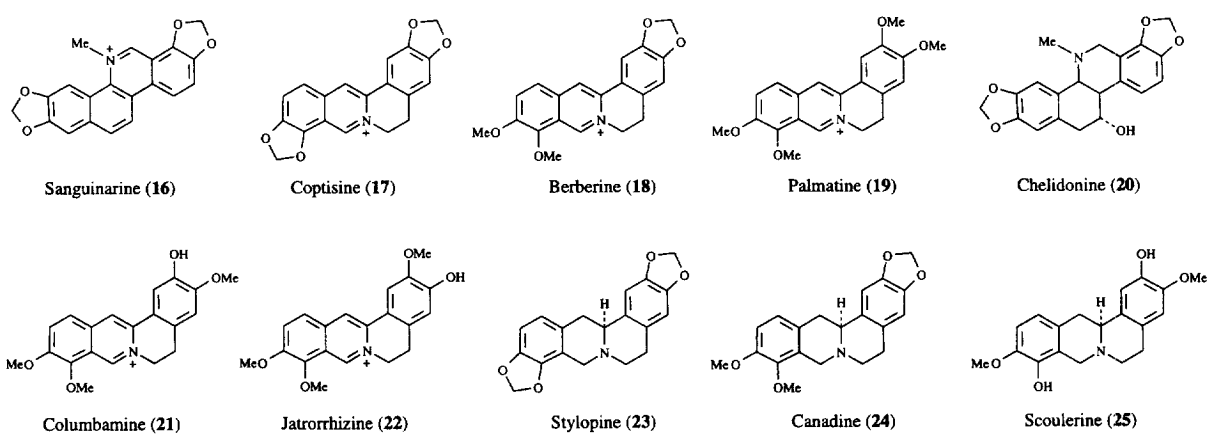

Fig. 3. Names, chemical formulas and molecular masses (M_r) of 10 protoberberine/benzophenanthridine alkaloids (16–25).

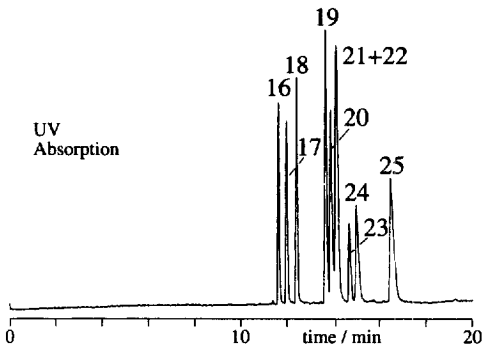

Fig. 4. CE separation of the 10 protoberberine/benzophenanthridine alkaloids (16–25). Conditions: U_{CE} 18 kV, detection UV at 240 nm; capillary dimensions, 55 cm (50 cm to UV detector)×50 μm I.D., uncoated, 15°C; electrolyte buffer, CH_3CN–100 mmol l^{-1} NH_4AcO pH 3.1 in water (1:1, v/v).

and 12.5 min. A second group appeared around a migration time of 14 min, consisting of four alkaloids: the methoxylated protoberberine-type palmatine (19), the benzophenanthridine chelidonine (20) and the isomeric protoberberines columbamine (21) and jatrorrhizine (22). The latter two comigrated, probably because the two alkaloids differ only in the position of the phenolic hydroxy group at ring D, resulting in very similar electrophoretic mobilities. Concerning the tertiary alkaloids of the tetrahydroprotoberberine-type, stylopine (23), canadine (24) and scoulerine (25), the first two alkaloids are baseline separated around a migration time of 15 min, and the dihydroxylated alkaloid (25) appeared much later at 16.5 min (Fig. 4).

Next, we tried to resolve a group of six β-carboline alkaloids (26–31) (Fig. 5) with the same electrophoretic system. Fig. 6 illustrates the complete baseline separation of all these harmane alkaloids within less than 14 min (migration times ranging from 10.78 to 13.60 min). To our knowledge this is the first demonstration of CE–UV for the analysis of a group of harmanes and excellent resolution of all constituents was obtained under the present conditions.

Additionally, a standard mixture of six typical opium alkaloids (32–37) (Fig. 7) was investigated employing the same electrophoretic conditions. Again thebaine (32), codeine (33), papaverine (34), morphine (35), narcotine (36), and narceine (37) are baseline resolved as depicted in Fig. 8. The first five

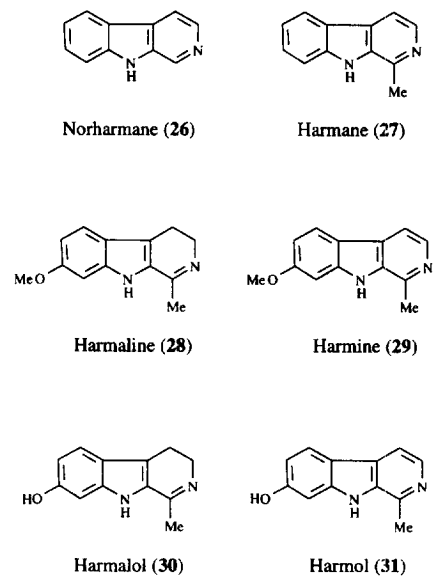

Fig. 5. Names, chemical formulas and molecular masses (M_r) of six β-carboline alkaloids (26–31).

analytes (32–36) appeared between 14.4 and 16.8 min. Due to its amphoteric character, narceine (37) has a significant lower electrophoretic mobility than the other related opium alkaloids (32–36). Correspondingly, it was detected at ca. 24 min.

3.3. Structure and electrophoretic mobility

The electrophoretic mobility μ_i of a molecule i is defined according to Eq. (1) (E, applied electrical

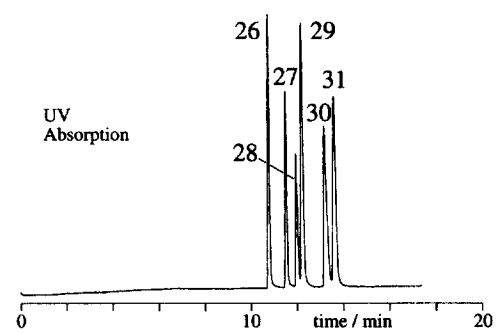

Fig. 6. CE separation of the six β-carboline alkaloids (26–31). Conditions: U_{CE} 15 kV, detection UV at 260 nm; capillary dimensions 55 cm (50 cm to UV detector)×50 μm I.D., uncoated, 15°C; electrolyte buffer, CH_3CN–100 mmol l^{-1} NH_4AcO pH 3.1 in water (1:1, v/v).

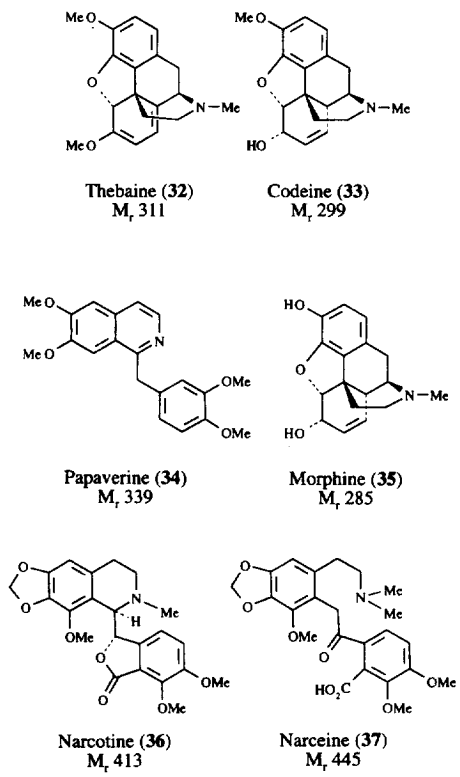

Fig. 7. Names, chemical formulas and molecular masses (M_r) of six isoquinoline alkaloids from opium (32–37).

field; v_i, migrating velocity of i; $z_i e_o$, net charge of i; f_i, translational friction coefficient of i, being a function of the molecular geometry of i [20]). Clearly, differences in the electrophoretic mobility of molecules arise as a result of differences in frictional properties, i.e., size or shape, or as a result of differences in the net charge on the molecule. If all parameters are kept constant, however, and only one functional group of the molecule under investigation is changed, insight into the structure-mobility relationship within one alkaloid class may be obtained as discussed in the following paragraph.

$$\mu_i = \frac{v_i}{E} = \frac{z_i e_o}{f_i} \quad (1)$$

For monoterpenoid indole alkaloids (1–15), protonation at low pH takes place preferentially on the N_β-nitrogen of the β-carboline substructure rather than on the N_α- indolic nitrogen. Due to this and other properties, the indole alkaloids can be separated into four different groups with decreasing electrophoretic mobility: (i) alkaloids of low molecular mass and medium basic character, i.e., gramine (1) and tryptamine (2); (ii) alkaloids of medium molecular mass but strong basic character, like alstonine (4) and serpentine (3); (iii) constituents of medium molecular mass and medium basic properties, e.g., corynanthine (8) and raufloridine (10); (iv) indole alkaloids characterised by a relatively high molecular mass and low basic properties, like deserpidine (13) and reserpine (14). An exception would be the bisindole alkaloids from *Catharanthus* vinblastine (7) and vincristine (9). Because of the possible double protonation of these bisindoles in the pH regime under investigation, these species are characterised by a higher charge than monoindoles and consequently show a lower migration time than one would expect from their molecular mass and shape.

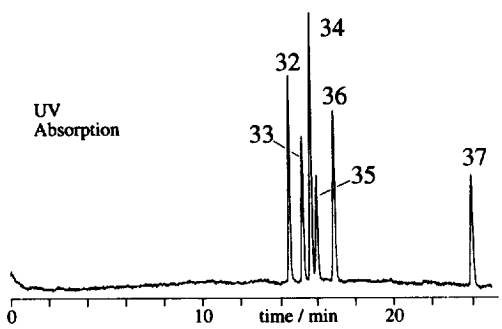

Fig. 8. CE separation of the six isoquinoline alkaloids from opium (32–37). Conditions: U_{CE} 15 kV, detection UV at 224 nm; capillary dimensions 55 cm (50 cm to UV detector) × 50 μm I.D., uncoated, 25°C; electrolyte buffer, CH_3CN–100 mmol l^{-1} NH_4AcO pH 3.1 in water (1:1, v/v).

The influence of the basic properties on the separation performance is not readily predictable for the alkaloids of the isoquinoline, β-carboline or morphinane groups. Considering these compounds, not only the pK values of the conjugated acids but also the distinct nature of the functional groups significantly influence the electrophoretic mobilities. For instance, within the group of protoberberine alkaloids (16–25) the migration times increase as the substituents change from methylendioxy- to methoxy- and hydroxy-groups, cf. coptisine (17), berberine (18), palmatine (19), columbamine (21) and jatrorrhizine (22) or the series stylopine (23),

canadine (24) and scoulerine (25). Obviously, the increased hydrophilicity of the alkaloids leads to increased migration times in this buffer system, contrary to the results on the CE analysis of peptides given in Ref. [39]. These differences may result from the different buffer systems (CH_3CN–aq. NH_4AcO vs. a non-organic solvent electrolyte) that have been applied.

With regard to the group of harmane alkaloids (26–31), again the substitution pattern has a pronounced effect on the separation. The electrophoretic mobility of the methoxylated compounds harmaline (28) and harmine (29) are higher than the corresponding hydroxylated alkaloids harmalol (30) and harmol (31). Comparing (28) vs. (29) and (30) vs. (31), the more basic character of harmaline (28) and harmalol (30) leads to shorter migration times.

A further structure–mobility relationship comparing structurally very similar alkaloids, is obtained in the case of the opium-alkaloids (32–37). The lipophilic, methoxylated thebaine (32) migrates fastest, followed by codeine (33) with one OH-group and the dihydroxylated morphine (35). As mentioned above, the increased hydrophilicity of the alkaloids leads to increased migration times under the present conditions[1]. For some of the protoberberines similar results were obtained using a sodium acetate–acetonitrile buffer [10,32].

3.4. On-line CE–MS analysis

In order to transfer an established standard CE method to CE–MS compatible conditions, it is most advantageous to change the buffer composition to volatile components in order to avoid contamination of the MS. Additionally, it is desirable (i) to inject as much sample as possible, while balancing the highest possible sensitivity against the loss of electrophoretic resolution, (ii) to decrease the ionic strength of the CE electrolyte and (iii) to minimise the electrical current generated between the inlet vial (+30 kV) and the sector-MS electrospray tip (+8 kV).

Usually, the operating conditions of the ES-MS system are optimised prior to the installation of the CE–ES-MS coupling. Accordingly, a solution of 20 μg/ml norephedrine in MeOH–water (9:1, v/v) was continuously introduced into the ES source via the coaxial sheath-liquid inlet system with a flow-rate of 1 μl min^{-1}. The instrumental parameters such as FS capillary positioning, ES source potentials and MS ion optics, have been optimised in order to achieve a signal characterised by maximum sensitivity and stability. The CE–MS analysis of the indole- and opium-type alkaloid standards are shown below[2].

An example of the CE–MS analysis of a mixture of indole alkaloids is given in Fig. 9. The UV signal detected with the CE instrument is shown in the upper part (Fig. 9A) and the RIC in the middle one (Fig. 9B). The capillary dimensions are 78 cm (21 cm to UV and 78 cm to ES-MS detector)×50 μm resulting in reduced migration times and diminished resolution in the UV detection mode compared to the RIC. The signal-to-noise ratio of the reconstructed total ion current is lower than that for the UV detection at 200 nm because of the background-electrolyte signals present in the scanned mass range. These background signals originate from electrolyte and sheath liquid cluster species, e.g., $[3AcOH+H]^+$ at m/z 181. Thus, the signal-to-noise ratio depends upon the sheath flow-composition and the CE-electrolyte concentration [41]. Lowering the sheath flow-rate from 2 to 1 μl min^{-1} leads to an increase of the signal-to-noise ratio of the RIC by a factor of 2. However, further lowering of the sheath flow-rate down to 0.5 μl min^{-1} results in an unstable RIC signal due to the diminished electrical contact between the end of the CE fused-silica capillary and the ES-probe tip held at 3 kV. The sheath flow-composition consisted of methanol–water (9:1) with ca. 1% acetic acid. The injected volume was ca. 20 nl. The sample concentration was chosen in order to yield similar peak areas in the CE–UV electropherogram, e.g., the concentration of gramine (1) and tryptamine (2) was 30 μg/ml.

Comparing the peak areas in Fig. 9A and Fig. 9B, the different intensities of the compounds' signals

[1] For seven structurally similar peptides, the CE-separation selectivity in an aqueous electrolyte buffer was shown to depend on the peptide's hydrophobicity: the higher their hydrophobicity, the lower their electrophoretic mobility [39].

[2] For the CE–MS analysis of alkaloids from *Rauwolfia serpentina* root extracts, *tinctura opii*, *Cortex quebracho*, and *Rauwolfia serpentina* cell suspensions, see Ref. [40].

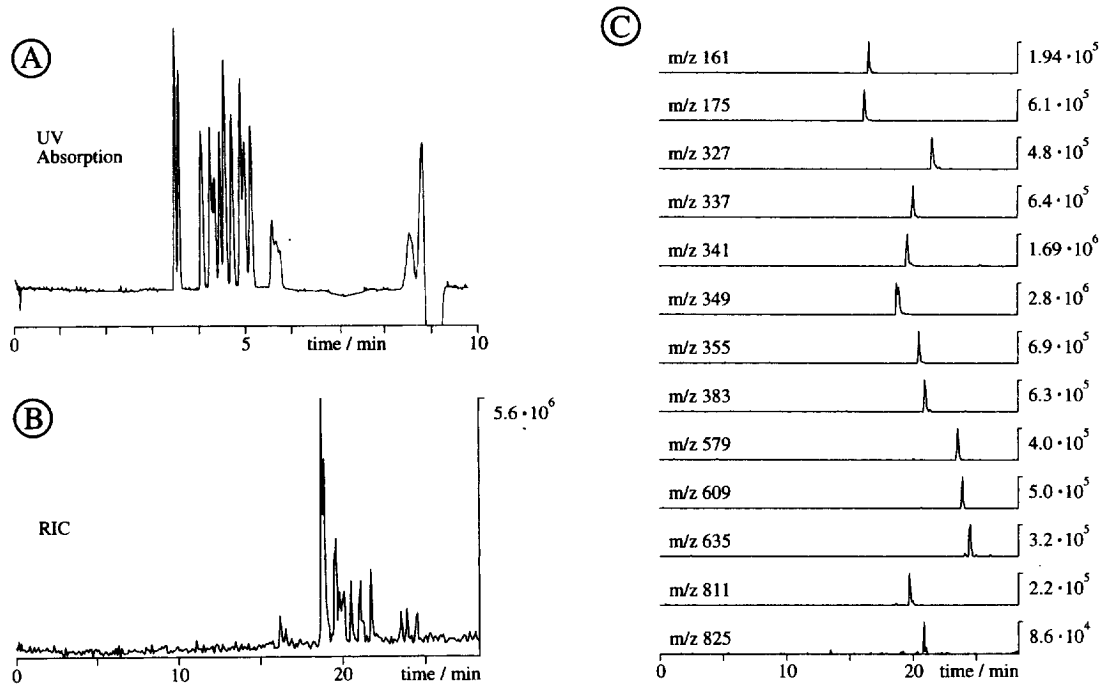

Fig. 9. CE–ES-MS analysis of a standard mixture of indole-type alkaloids (1–15). (A) UV signal at 200 nm of the CE–MS on-line coupling. (B) The uncorrected, unsmoothed reconstructed total ion current (RIC) of the CE–MS on-line coupling. (C) The individual mass traces of the protonated molecules as obtained from CE–MS on-line coupling. CE conditions: CH_3CN–aqueous 80 mmol l^{-1} NH_4AcO buffer pH 4 (1:1, v/v); capillary dimensions 75 cm (20 cm to UV detector)×50 μm I.D., uncoated fused-silica, 20°C, effective voltage 22 kV, current 8 μA; pressure injection 1.73 bar s. ES conditions: sheath liquid, methanol–water–acetic acid (89:10:1), 1 μl min^{-1}; U_{ES} 3.5 kV. MS, scanning speed 5 dec/s, mass range m/z 100 to 900, for other conditions see Section 2.

are due to the different extinction coefficients at 200 nm and the different proton affinities. Obviously, the extracted single-ion electropherograms corresponding to the protonated molecules ($[M+H]^+$) are characterised by much higher signal-to-noise ratios (Fig. 9C) compared to the RIC. Considering the analysis of a mixture of unknown compounds it is important to get significant signals in the RIC. The RIC given in Fig. 9B has not been corrected by any data-processing algorithm, it is displayed without any background correction or data smoothing. The broad signal at ca. 9 min in Fig. 9A originates from a contribution of the electroosmotic flow, the sheath-flow, the electrospray process and by siphoning as the result of not identically levelled inlet and outlet reservoirs (outlet=ES needle) [36,37].

With regard to the CE–MS results of the indole-alkaloid analysis (Fig. 9A), 13 of the 15 compounds shown in Fig. 1 have been baseline separated applying the CE–MS conditions described above. The epimeric alkaloids alstonine (4) and serpentine (3) differ only in the configuration at carbon 19 and are therefore difficult to resolve from each other. Employing the CE–UV only conditions outlined above, both alkaloids also appear at identical migration times. In addition, a suitable separation of vincristine (9) and raufloridine (10) was not possible in the CE–MS coupling under the present conditions. Due to the slightly modified CE conditions used for the CE–MS and the CE–UV systems, vinblastine (7) and corynanthine (8) can be distinguished in the RIC trace but not in the CE–UV data (Fig. 2). Further, yohimbinic acid (12) migrates slowest, appearing after ca. 25 min in the RIC, probably as a result of the higher pH value of the electrolyte buffer in CE–MS compared to CE–UV only. The remaining alkaloids are also well-separated and yielded abundant protonated molecules by the ES-MS (Fig. 10).

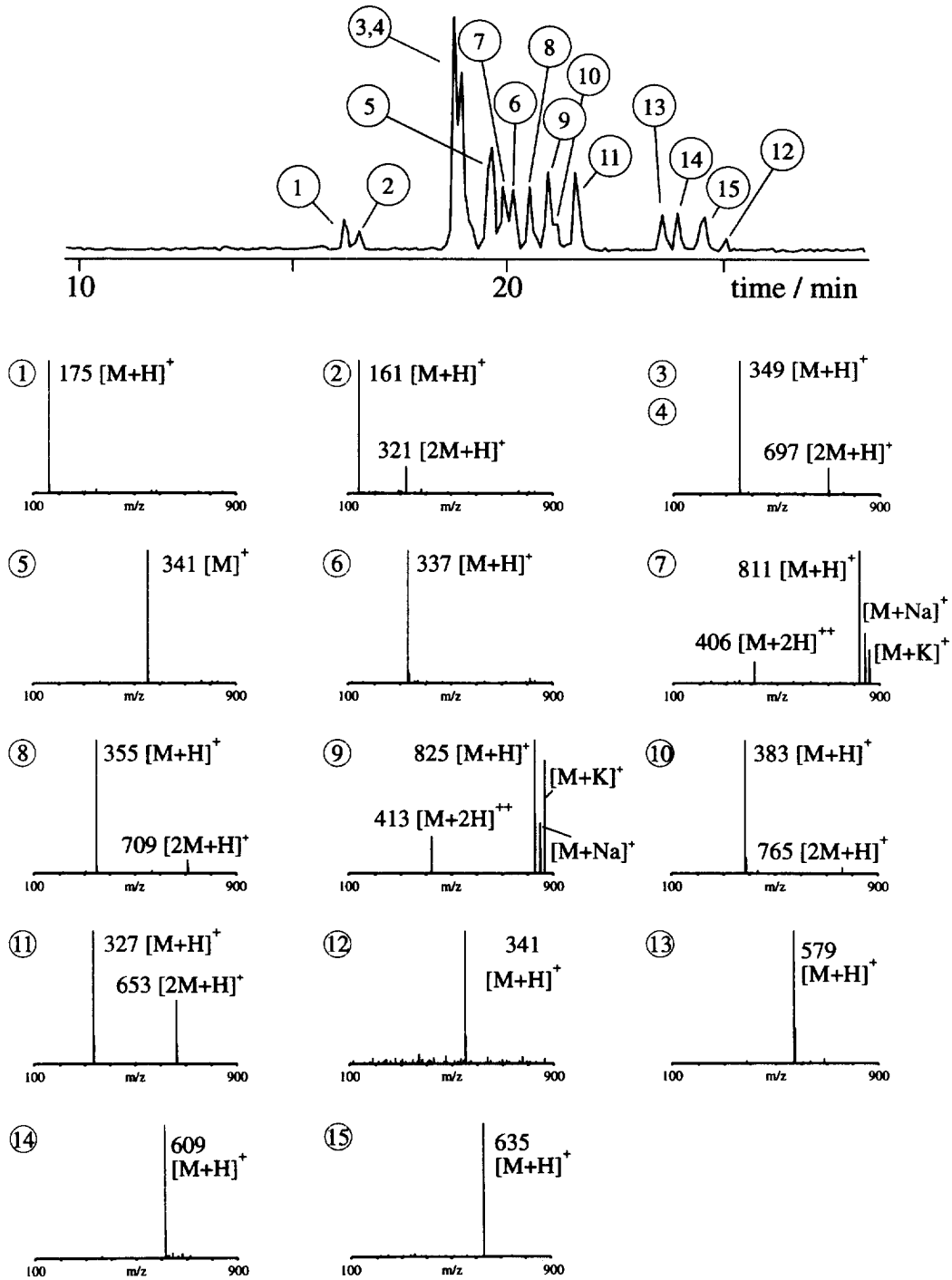

Fig. 10. Summed ES mass spectra of the indole-type alkaloids (1–15) as obtained from the CE–ES-MS analysis shown in Fig. 9. The circled numbers refer to the indole alkaloids as given in Fig. 1 and the numbers in front of the brackets refer to the m/z values (for details, see text).

Under the given conditions, the protonated molecule ($[M+H]^+$) dominated the ES mass spectrum of each compound (1–15), e.g., *m/z* 811 in the case of vinblastine (7). The acquisition of three to five MS scans per electrophoretic peak was obtained and the summed mass spectra are presented in Fig. 10. They are characterised by (i) a signal-to-noise ratio of ca. 100:1; (ii) a dominating signal corresponding to $[M+H]^+$; (iii) additional signals indicating the sodium and potassium adducts ($[M+Na]^+$, $[M+K]^+$, relative intensity 0–20%); (iv) solvent clusters ($[M+Na+S]^+$, S=MeOH, MeCN and water, relative intensity less than 3%); (v) alkaloid dimer cluster ions $[2M+H]^+$ and doubly protonated molecules $[M+2H]^{2+}$. The adduct and cluster ions reveal further evidence for the molecular mass of the analyte.

An example of the CE–MS analysis of the opium-alkaloid standard is given in Fig. 11. The UV signal detected with the CE instrument is shown in the upper part (Fig. 11A) and the RIC in the middle one (Fig. 11B). The in-capillary UV detection was performed as mentioned above resulting in reduced migration times and diminished resolution in the UV detection mode compared to the RIC trace. The signal-to-noise ratio of the reconstructed total ion current is lower than that from the UV detection at 200 nm because of the background-electrolyte signals present in the scanned mass range (see above). The mass traces corresponding to the protonated molecules ($[M+H]^+$) are characterised by much higher signal-to-noise ratios (Fig. 11C) compared to the RIC. The broad signal between 8 and 10 min in Fig. 11A originates from the bulk flow in the capillary (see above).

Each peak in the RIC trace of Fig. 11B consisted of ca. two to four mass spectra. The corresponding single-scan ES mass spectra of each compound (32–36) are presented in Fig. 12. They are characterised by (i) signal-to-noise ratios of ca. 20:1 (morphine, 35) up to greater than 100:1 (papaverine, 34), (ii) a dominating signal corresponding to $[M+H]^+$, e.g.,

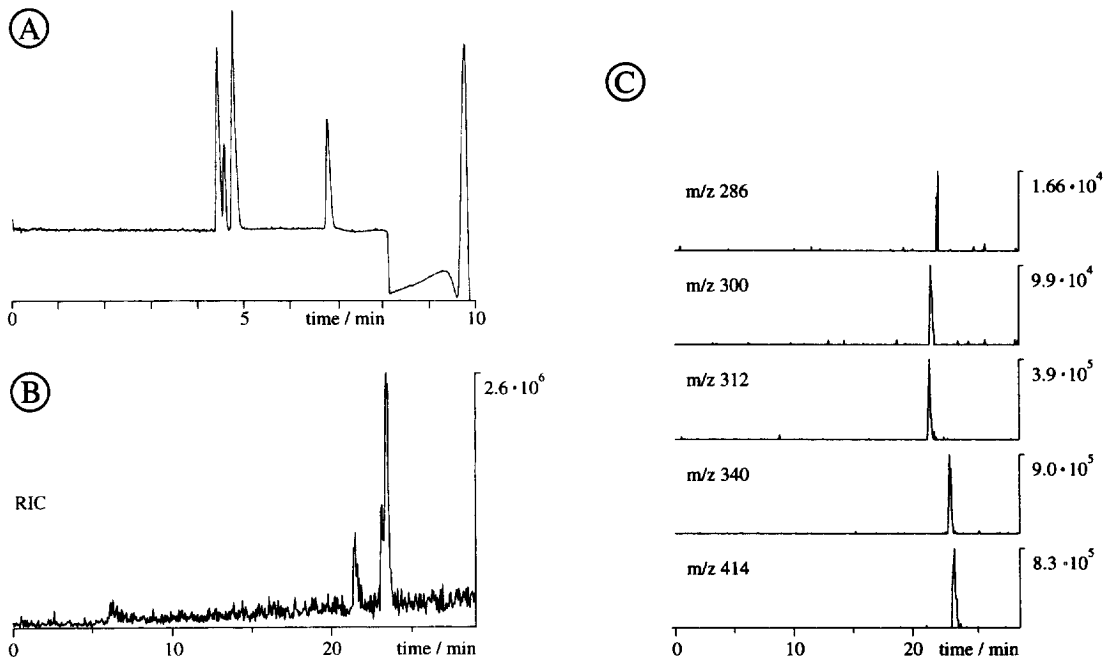

Fig. 11. Example of a CE–ES-MS analysis of a standard mixture of isoquinoline alkaloids from opium (32–36). (A) The UV signal at 200 nm of the CE–MS on-line coupling. (B) The uncorrected, unsmoothed reconstructed total ion current (RIC) CE–MS on-line coupling. (C) The individual mass traces of the protonated molecules as obtained from the CE–MS on-line coupling. MS: scanning speed 3 dec/s, mass range *m/z* 150 to 1000, other conditions as in Fig. 9.

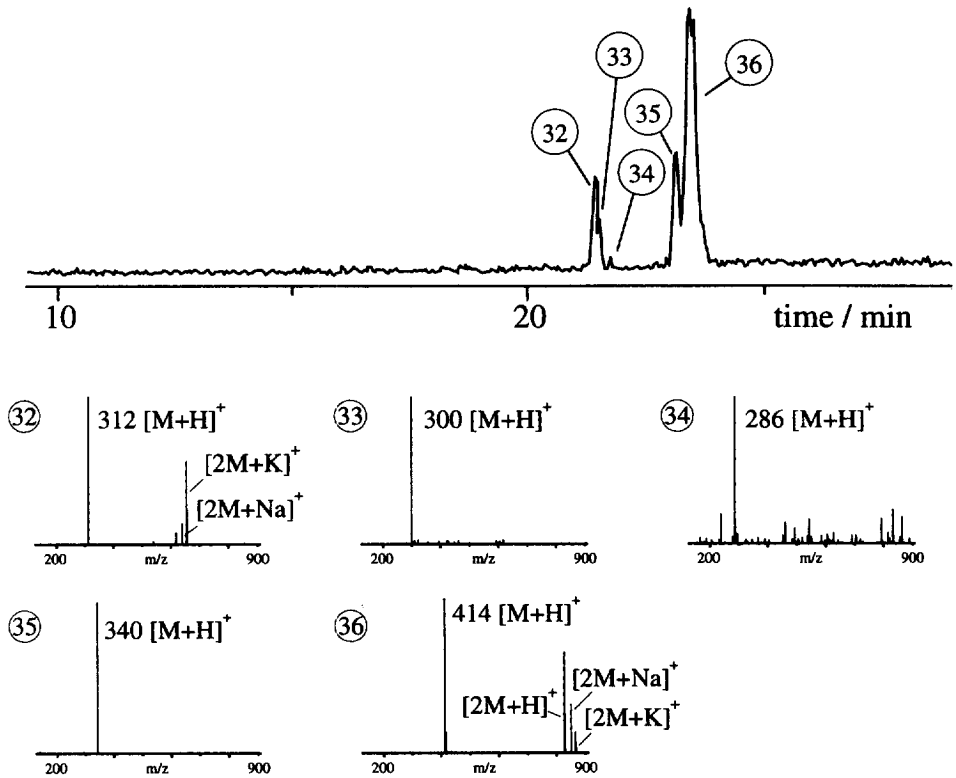

Fig. 12. Single-scan ES mass spectra of the isoquinoline-type alkaloids from opium (32–36) as obtained from the CE–ES-MS analysis shown in Fig. 11. The circled numbers refer to the indole alkaloids as given in Fig. 8 and the numbers in front of the brackets refer to the m/z values (for details, see text).

m/z 312 in the case of thebaine (32), (iii) alkaloid cluster ions $[2M+H]^+$, $[2M+Na]^+$ and $[2M+K]^+$ with varying relative intensities.

3.5. CE–UV vs. CE–MS

Comparing the CE–UV and the CE–MS electropherograms, the following points have to be emphasised: (i) the RIC is the summation of the MS signal intensities over a certain mass range as shown in Fig. 9B and Fig. 11B; (ii) the individual mass traces (Fig. 9C and Fig. 11C) have been obtained by extracting the respective m/z values from the RIC data; (iii) in UV a single wavelength is used for detection (Figs. 2,4,6,8 and 9A, Fig. 11A); (iv) the detection occurs at different positions: UV only at 50 cm, UV in CE–MS at 20 cm and ES-MS at 75 cm; (v) the detection limit of each alkaloid depends on certain molecular constants, the extinction coefficient (UV detection) and the proton affinity (ES-MS detection). The ES ionisation process depends upon the amount of other ions present in the analyte. The higher the ionic strength of the probe, the lower the signal response [30].

The RIC is always characterised by a smaller signal-to-noise ratio than the UV signal and the individual mass trace, whereas the signal-to noise ratio is quite similar for the latter two. The separation efficiencies are diminished in the RIC MS due to the presence of dead volumina in the ES-MS device, i.e. the back-diffusion of analyte ions into the sheath flow. The latter disadvantage can be compensated for by the single-ion extraction from the full-scan RIC data if incompletely resolved analytes posses different m/z values. The single-ion extraction does not make sense, however, in cases of analytes with the same nominal m/z values, cf. m/z 341 and m/z 349 in Fig. 9C. From the present

analyses, the CE-detection limit of gramine (1), for example, is estimated to be ca. 0.25 μg/ml or 2.5 pg for the UV only (223 nm) and the extracted single-ion trace at m/z 175.

4. Conclusions

CE is shown to be a very effective tool for the separation of a number of alkaloids as demonstrated by the examples of indole-, protoberberines/benzophenanthridines-, β-carboline- and opium-alkaloid standards. By using the buffer electrolyte acetonitrile–100 mmol l^{-1} NH_4AcO pH 3.1 (1:1, v/v), an applied electrical field of ca. 0.3–0.4 V/cm, an uncoated FS capillary of 50 μm I.D. and a temperature of 15–25°C, the CE separation of almost all alkaloid constituents within one class could be achieved. The separation performance of the on-line coupled CE and MS demonstrates the possibility of identifying the alkaloids by combinations of the electrophoretic mobility and the molecular mass. The specificity provided by a sector mass spectrometer gives unequivocal support needed to verify the molecular mass and the number of charges per molecule. These series of experiments illustrate the benefit of this separation and identification method which allows the analysis of different alkaloid classes without major changes of the instrumental conditions. The experimental results strongly suggest that the present CE conditions should prove generally applicable to the combined CE separation and the detection of alkaloids in plant extracts as well as in standards [40].

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