ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 815–825

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Capillary electrophoresis for the analysis of tropane alkaloids: pharmaceutical and phytochemical applications

L. Mateus, S. Cherkaoui, P. Christen, J.-L. Veuthey *

Laboratory of Pharmaceutical Analytical Chemistry, University of Geneva, Bd d'Yvoy 20, 1211, Geneva 4, Switzerland

Received 15 May 1998; received in revised form 15 July 1998; accepted 14 August 1998

Abstract

Three capillary electrophoresis methods, using UV detection, were developed for the simultaneous determination of several tropane alkaloids, including atropine, scopolamine and synthetic derivatives. After optimization, the validated capillary zone electrophoresis methods were applied to the determination of these compounds in various pharmaceutical forms, such as ophthalmic and injection solutions, tablets, suppositories and aerosols. Capillary electrophoresis in the micellar mode was found to be more appropriate for the analysis of hyoscyamine and scopolamine in plant material. These two compounds are generally found together with other tropane alkaloids which present similar structures and charge to mass ratio. Furthermore, the separation of positional isomers, such as hyoscyamine and littorine generally encountered in plant extracts, was also considered. The developed method was applied to the analysis of hairy root extracts of *Datura candida* x *Datura aurea*, *Datura quercifolia* and *Hyoscyamus albus*.

Keywords: Tropane alkaloids; Atropine; Scopolamine; Alkaloid derivatives; Pharmaceuticals; Capillary electrophoresis; Plant extract

1. Introduction

For centuries, plants have been a unique source of drugs such as alkaloids. In particular, species belonging to the *Solanaceae* family produce a variety of alkaloids of considerable therapeutic importance. Tropane alkaloids mainly occur in the genera *Atropa*, *Datura*, *Duboisia* and *Hyoscyamus* [1,2]. Hyoscyamine (**1**), its racemate atropine and scopolamine (**2**), are among the principal natural alkaloids of medicinal interest in this group. They are extensively used in ophthalmic diagnosis as mydriatic as well as anticholinergic, antispasmodic and preanesthesic agents [3]. Littorine (**8**) and 6β-hydroxyhyoscyamine (**9**) are compounds frequently encountered in the Solanaceae family. The latter alkaloid is the key intermediate in the biosynthesis of scopolamine from hyoscyamine.

Alkaloids of natural origin serve as a model for the synthesis of several derivatives which have

* Corresponding author. Tel.: +41-22-7026336; fax: +41-22-7815193; e-mail: jean-luc.veuthey@pharm.unige.ch.

0731-7085/98/$ - see front matter
PII: S0731-7085(98)00223-4

improved pharmacokinetic properties, a higher efficacy and/or less toxicity. Homatropine (**3**) is prepared synthetically by the esterification of mandelic acid with 3α-tropine. Its effects correspond to those of atropine but are ten times less pronounced. Quaternary ammonium derivatives such as oxitropium (**4**), ipratropium (**5**) and flutropium (**7**) are mainly used in the treatment of chronic obstructive pulmonary diseases, whereas *N*-butylscopolamine (**6**) is used as a spasmolytic agent.

During recent years, thin-layer chromatography (TLC) [4], gas chromatography (GC) [5,6], UV spectrophotometry [7,8], fluorimetry [9,10] and high-performance liquid chromatography (HPLC) [11–14] have been widely used for the analysis of tropane alkaloids. However, due to the low volatility and thermosensitivity of these compounds, GC has so far found only limited applications [15]. Atropine and scopolamine can be analyzed by GC without derivatization, but their analysis induces some problems because they partially dehydrate at high temperature in the injection chamber, yielding apoatropine (**10**) and aposcopolamine, respectively. HPLC analysis frequently requires the addition of a basic component as a masking agent (e.g. triethylamine), the use of shielded phases, or the addition of a pairing reagent in the mobile phase in order to reduce the peak tailing and improve the column efficiency and peak shapes [16–18]. Furthermore, complex sample purification steps are frequently needed. Immunological methods can be utilized in the analysis of crude plant samples [19,20]. Nevertheless, such methods are expensive and do not allow the simultaneous analysis of several compounds.

Capillary electrophoresis (CE) represents an interesting alternative for the analysis of pharmaceutical compounds because of its efficiency, flexibility, accuracy and very high resolution [21]. CE has been successfully applied to the analysis of pharmaceutical products [22–26], but the first review on the application of CE to the analysis of plant secondary metabolites has only been published recently [27] and, surprisingly, there have been only few reports concerning the analysis of tropane alkaloids [28–30].

This paper focuses on the application of different capillary electrophoretic methods to the analysis of tropane alkaloids, both in pharmaceutical preparations and plant extracts.

2. Experimental

2.1. Chemicals

Atropine sulfate, scopolamine hydrobromide, homatropine hydrobromide, ipratropium bromide and *N*-butylscopolamine bromide were purchased from Sigma (St. Louis, MO). Oxitropium bromide and flutropium bromide were kindly provided by Boehringer (Ingelheim, Germany). Apoatropine and tropic acid were supplied by Fluka (Buchs, Switzerland). Littorine was a gift of Dr K. Shimomura (Tsukuba Medicinal Plant Research Station, Japan). 6β-Hydroxyhyoscyamine was obtained from Dr M.F. Roberts (University of London). Hydroxypropyl-β-cyclodextrin (HP-β-CD), with a degree of substitution of 0.6, was obtained from Roquette (Lestrem, France). All other chemicals were of analytical grade and were purchased from Fluka (Buchs, Switzerland). Ultrapure water, provided by a Milli-Q RG unit from Millipore (Bedford, MA), was used for standard and sample preparation. Electrolyte and sample solutions were filtered through a 0.20 μm microfilter (Supelco, Bellefonte, PA) before use.

2.2. Instrumentation and electrophoretic procedure

CE data was generated in a HP ^{3D}Capillary Electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode-array detector, an autosampler and a power supply able to deliver up to 30 kV. The total capillary (Hewlett-Packard) was 64.5 cm long, while the length to the detector was 56 cm, with a 50 or 75 μm internal diameter. An alignment interface, containing an optical slit matched to the internal diameter, was used and the detection was performed at 195 nm with a bandwidth of 2 nm, unless otherwise stated. A CE Chemstation (Hewlett-Packard) was used for instrument control, data acquisition and data handling.

All experiments were carried out in cationic mode (anode at the inlet and cathode at the outlet). The capillary was thermostated at 25°C. A constant voltage of 30 kV, with an initial ramping of 500 V s^{-1}, was applied during analysis. Sample injections were achieved using the pressure mode.

Before use, the capillary was washed with 0.1 M sodium hydroxide for 20 min, followed by water for 10 min. To achieve high migration time reproducibility and avoid solute adsorption, the capillary was washed between analyses successively with 0.1 M sodium hydroxide and water for 2 min each, then equilibrated with the running buffer for 3.5 min.

As electrolysis can alter the running buffer and subsequently change the electroosmotic flow (EOF), a replenishment system was also used to maintain a high reproducibility. Prior to each sequence, two blank injections were performed to stabilize the capillary wall surface, and allow the buffer and sample solutions to reach a constant temperature on the autosampler tray. Finally, triplicate injections were performed.

All buffers used as running electrolytes, were prepared using the Phoebus software 1.0 (Centre Analyse, Orleans, France).

Stock standard solutions were prepared by dissolving each compound in methanol (1 mg ml^{-1}) and were then suitably diluted in water in order to obtain appropriate standard solutions. The use of water as a dissolving agent allowed sample stacking which was effective in enhancing sensitivity by on-column preconcentration within the capillary.

2.3. Ophthalmic solutions

Each ophthalmic solution was composed of the alkaloid, benzalkonium chloride as preservative, hydroxypropylmethylcellulose as viscosity-increasing agent and sodium chloride as isotonic agent. The analysis was carried out without an extraction process; the aqueous solution was only diluted with water to the desired concentration (~ 0.05 mg ml^{-1}).

2.4. Pharmaceutical formulations

Liquids were diluted with water in order to obtain a 100 μg ml^{-1} final concentration of the analyte in the solution. In all cases, the internal standard (oxitropium) was present at a concentration of 100 μg ml^{-1}. The solution was homogenized by shaking, and was injected after filtering through 0.2 μm filters.

Three tablets were finely powdered and the equivalent of one tablet was accurately weighed and quantitatively extracted three times with 10 ml of water, with sonication for 15 min and vortex mixing at 5 min intervals to avoid aggregation of the powdered sample. After centrifugation ($2750 \times g$ for 5 min), supernatants were collected and diluted in a 100 ml volumetric flask and the internal standard was added.

A suppository was dissolved in 2 ml of dichloromethane and extracted as described above for the tablets.

Five doses of the aerosol were collected in a 2 ml vial and 1.5 ml of water containing the internal standard, was added. The aqueous solution was then filtered through a 0.2 μm filter and injected.

2.5. Plant extracts

The culture of *Datura candida x D. aurea* [31], *Datura quercifolia* [32] and *Hyoscyamus albus* [33] hairy roots was established after infection with *Agrobacterium rhizogenes* and samples of powdered roots were extracted according to the procedure described elsewhere [31].

3. Results and discussion

Two matrices were of special interest for the determination of tropane alkaloids. In the case of simple pharmaceutical formulations such as ophthalmic solutions or aerosols, where only one tropane alkaloid is present, capillary zone electrophoresis (CZE) methods were developed. In complex plant extracts, scopolamine and hyoscyamine are generally present together with other tropane alkaloids having similar structure and charge to mass ratio and therefore, micellar

electrokinetic chromatography (MEKC) was found to be more appropriate. By manipulating the experimental conditions, MEKC is able to separate neutral and ionic analytes in the same run.

3.1. Determination of atropine, homatropine and scopolamine in ophthalmic solutions

CZE was investigated for the simultaneous determination of three tropane alkaloids found in ophthalmic solutions: atropine, scopolamine and homatropine (Fig. 1). Several electrophoretic parameters, including buffer concentration and pH, were optimized to determine simultaneously the three compounds [34]. The best conditions were achieved using a 50 μm uncoated fused silica capillary, in the presence of 100 mM Tris-phosphate buffer at pH 7. This pH is close to the pK_a value of scopolamine (7.6) which allows a modification of its ionization degree, and subsequently improves the compound resolution. As shown in Fig. 2, such conditions offer a suitable and rapid method for the simultaneous determination of the three alkaloids. Under acidic conditions, it was not possible to separate these compounds completely because of their structural similarity.

This method was validated according to the ICH guidelines [35] and applied to the determination of selected alkaloids in commercially available aqueous ophthalmic solutions [34]. In all instances, the selected alkaloids were easily determined without any sample pretreatment and no disturbance due to the presence of excipient and preservative in these solutions was observed. As can be seen from Table 1, determination of drug content by the proposed method was in good agreement with the labeled content. In particular, the RSD values attest to the precision of the method despite the fact that, in this case, no internal standard was used.

3.2. Determination of atropine and scopolamine derivatives in pharmaceutical formulations

In a second experiment, a CZE method was developed for the simultaneous analysis of four synthetic atropine and scopolamine derivatives, namely oxitropium, ipratropium, *N*-butylscopolamine and flutropium (Fig. 1).

The following parameters were optimized: the buffer pH, the buffer concentration and the hydroxypropylated-β-cyclodextrin (HP-β-CD) concentration [36]. The studied alkaloids possess different structures and it is thus possible to resolve them at an acidic pH with a low EOF. Under these conditions, tropane alkaloids are more stable, as already published [37]. Using the optimized conditions (80 mM citrate buffer at pH 2.5 in the presence of 2.5 mM HP-β-CD), the four alkaloids were separated (Fig. 3). The presence of HP-β-CD in the buffer solution allowed the manipulation of selectivity, especially between *N*-butylscopolamine and ipratropium, depending on the complexation constant between the alkaloid and the cyclodextrin.

The proposed method was validated and successfully applied to the determination of these alkaloids in different pharmaceutical formulations, namely solutions, tablets, suppositories and aerosols [36]. As reported in Section 2.4., solutions were injected directly, while tablets and suppositories were extracted with water. In the case of aerosols, doses were collected in water and injected directly. Results generated by the developed CZE method were compared with those claimed by the label. Table 2 shows that the results obtained were in good agreement with the labeled content, which demonstrates the good accuracy of the method. It is noteworthy, that in all cases, the recovery was quantitative ($\sim$ 100%) and that the RSD values were low, attesting the performance of the method.

3.3. Determination of selected tropane alkaloids in plant extracts

Because the synthetic production of tropane alkaloids is more expensive than the extraction from plant material, important efforts are made to develop economically feasible in vitro production of these compounds. In this context, Agrobacterium-mediated transformed roots, called hairy roots, may represent a valuable alternative for the production of tropane alkaloids [31].

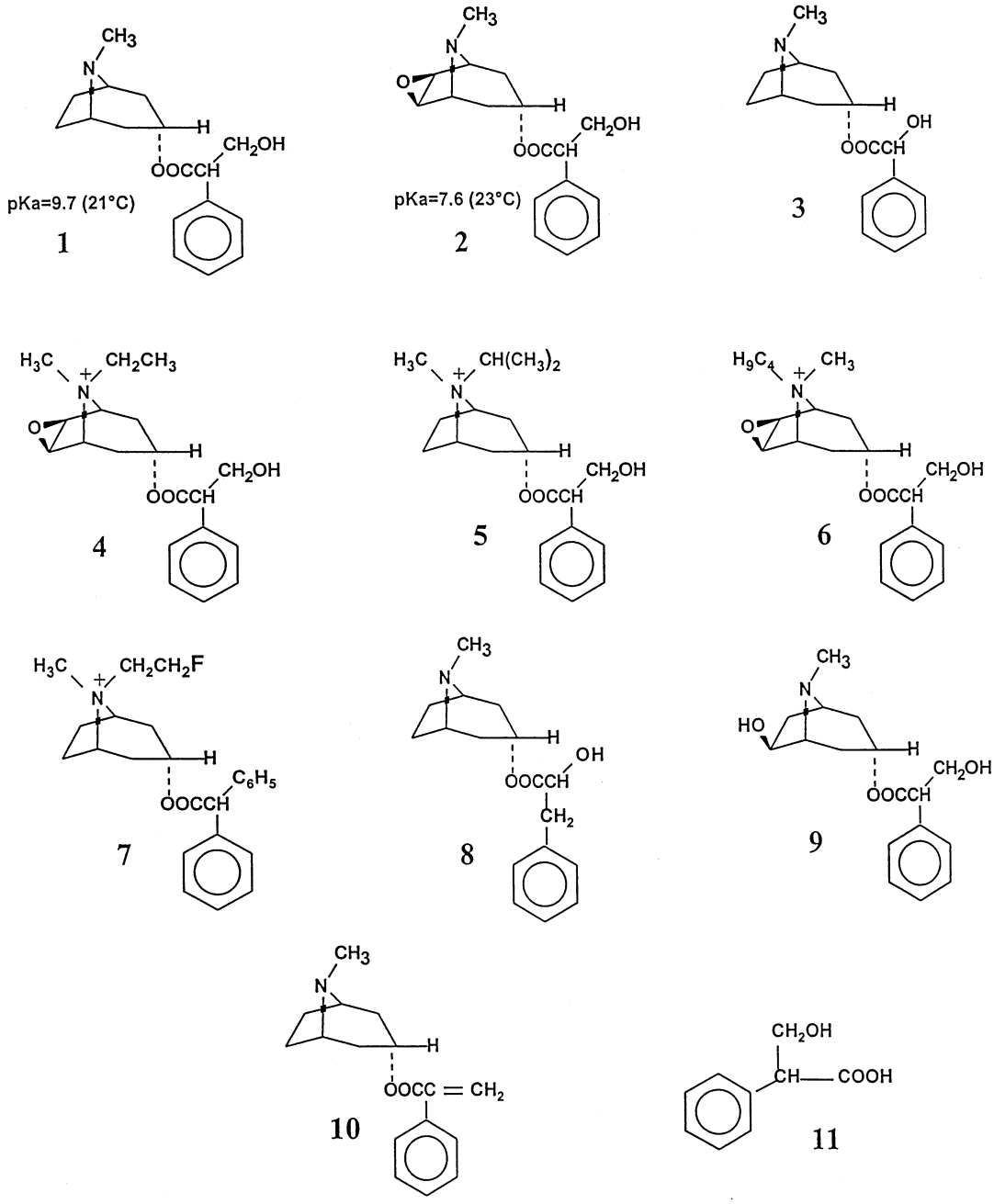

Fig. 1. Structure of the investigated compounds: 1, hyoscyamine; 2, scopolamine; 3, homatropine; 4, oxitropium; 5, ipratropium; 6, *N*-butylscopolamine; 7, flutropium; 8, littorine; 9, 6β-hydroxyhyoscyamine; 10, apoatropine; and 11, tropic acid.

As mentioned previously, due to the complexity of the plant material, a micellar electrokinetic capillary chromatography was selected to separate the tropane alkaloids found in hairy root extracts [38]. MEKC was applied to the simultaneous analysis of hyoscyamine, scopolamine, littorine,

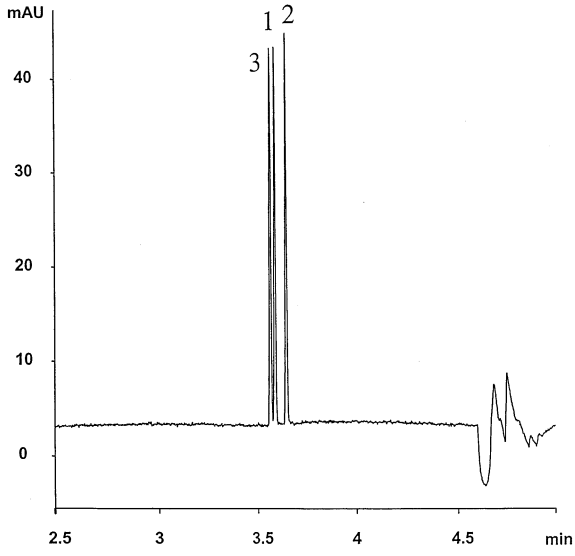

Fig. 2. Typical electropherogram of homatropine (3), atropine (1) and scopolamine (2) obtained by CZE using 100 mM Tris-phosphate buffer at pH 7. Other operating conditions: Capillary: uncoated fused silica, $L = 64.5$ cm, $l = 56$ cm; I.D., 50 μm; applied voltage, 30 kV ($i = 63$ μA); temperature, 25°C. Sample injections (12 nl injection volume) were achieved using the pressure mode for 20 s at 25 mbar.

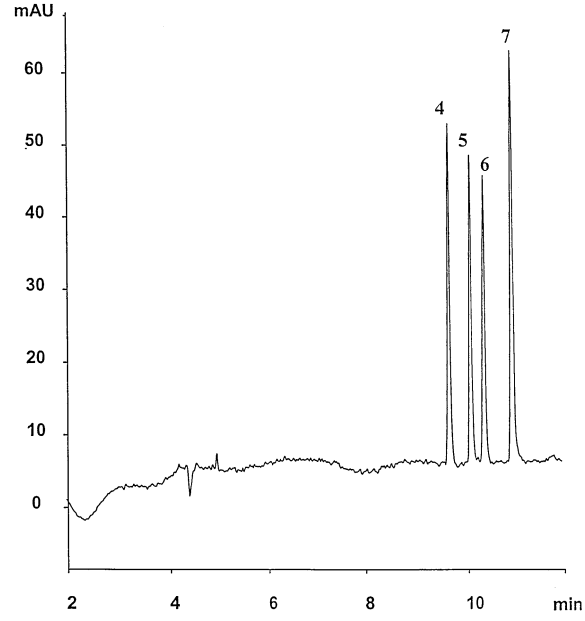

Fig. 3. Typical electropherogram of oxitropium (4), ipratropium (5), *N*-butylscopolamine (6) and flutropium (7) obtained by CZE, using 80 mM citrate buffer pH 2.5 in the presence of 2.5 mM HP-β-CD. Other operating conditions: uncoated fused-silica capillary $L = 64.5$ cm, $l = 56$ cm, I.D., 50 μm; applied voltage, 30 kV ($i = 45$ μA); temperature, 25°C. Pressure injection, 25 mbar for 10 s (6 nl). The detection wavelength was set at 191 nm with a bandwidth of 2 nm.

6β-hydroxyhyoscyamine, apoatropine, homatropine and tropic acid (**11**) (Fig. 1). In this experiment, homatropine was used as an internal standard.

Sodium dodecyl sulfate (SDS) was selected as a micelle agent, because this surfactant has shown to be suitable in a wide range of applications [39]. Operating parameters such as buffer pH and concentration, SDS concentration, type and percentage of organic modifier were investigated in order to optimize the electrophoretic conditions. Successful results were obtained using a 30 mM borate–phosphate buffer at pH 8.5 in the presence of 50 mM SDS. Further-

Table 1
CZE assays of atropine, homatropine and scopolamine in ophthalmic solutions ($n = 3$)

	Dosage formulation		
	Atropine 1% Dispersa Ciba vision (%)	Isopto®-homatropine 1% Alcon (%)	Scopolamine 0.25% Ciba vision (%)
Component	Atropine	Homatropine	Scopolamine
Labeled claim (w/v)	1	1	0.25
Amount found (w/v)	1.04	0.98	0.24
RSD (peak area)	1.82	3.50	2.31
RSD (migration time)	0.18	0.02	0.07

Table 2
CZE assays of ipratropium, *N*-butylscopolamine and flutropium in pharmaceutical preparations

Dosage formulation	Component[a]	Labeled claim	Recovery (%)	RSD (%)
Atrovent® (solution)	Ipratropium	0.25% (w/v)	100.52	1.93
Buscopan® (solution)	*N*-Butylscopolamine	2% (w/v)	100.57	1.64
Buscopan® (tablet)	*N*-Butylscopolamine	10 mg	102.44	3.74
Buscopan® (suppository)	*N*-Butylscopolamine	10 mg	101.22	2.51
Flubron® (aerosol)	Flutropium	30 μg /dose	99.70	1.85

[a] Present as bromide salt in the tested pharmaceutical preparations.

more, it was demonstrated that adding an organic modifier to the buffer is an important parameter to improve selectivity, efficiency and resolution. In particular, the addition of various concentrations of methanol or acetonitrile strongly altered the selectivity.

Due to their structural similarity, the separation of the two positional isomers, littorine and hyoscyamine, is difficult to achieve by GC [40]. The separation of these two compounds was carried out by MEKC in the presence of methanol or acetonitrile. This effect of an organic modifier on the resolution of positional isomers has been attributed to solute desolvation. It is noteworthy that without the addition of an organic modifier, no resolution was obtained. Indeed, in aqueous phase, littorine and hyoscyamine are totally hydrated and therefore have approximately the same size. Fig. 4 depicts the effects of various methanol and acetonitrile percentages on the resolution between hyoscyamine and littorine. Addition of an organic solvent will result in the reduction of the sphere of hydration depending on the isomer. As a consequence, the difference in charge-to-size ratio is increased, resulting in a better compounds resolution. At a given percentage, acetonitrile allows a better resolution than methanol. These results are in good agreement with data reported in the literature [41]. Fig. 5(a–c) shows electropherograms of the alkaloid mixture with and without an organic solvent.

From the above results, the addition of 10% acetonitrile was deemed appropriate for plant extract analysis [36]. The developed method was applied to the qualitative analysis of hyoscyamine and scopolamine in three hairy root extracts. As shown in Fig. 6(a–c), the extracts contain numerous alkaloids among which hyoscyamine, scopolamine, 6-β-hydroxyhyoscyamine and littorine. The method demonstrated that the two positional isomers, littorine and hyoscyamine, can be separated even in a

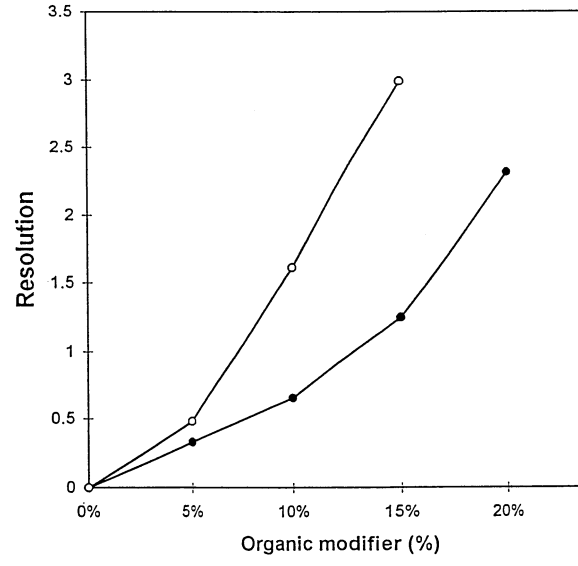

Fig. 4. Effect of methanol (●) and acetonitrile (○) on the resolution of two positional isomers, hyoscyamine and littorine. Conditions: 30 mM phosphate–borate buffer, pH 8.5, 50 mM SDS. Other operating conditions: uncoated fused-silica capillary $L = 64.5$ cm, $l = 56$ cm, I.D., 75 μm; applied voltage, 30 kV; temperature, 25°C. Pressure injection: 25 mbar for 10 s (30 nl).

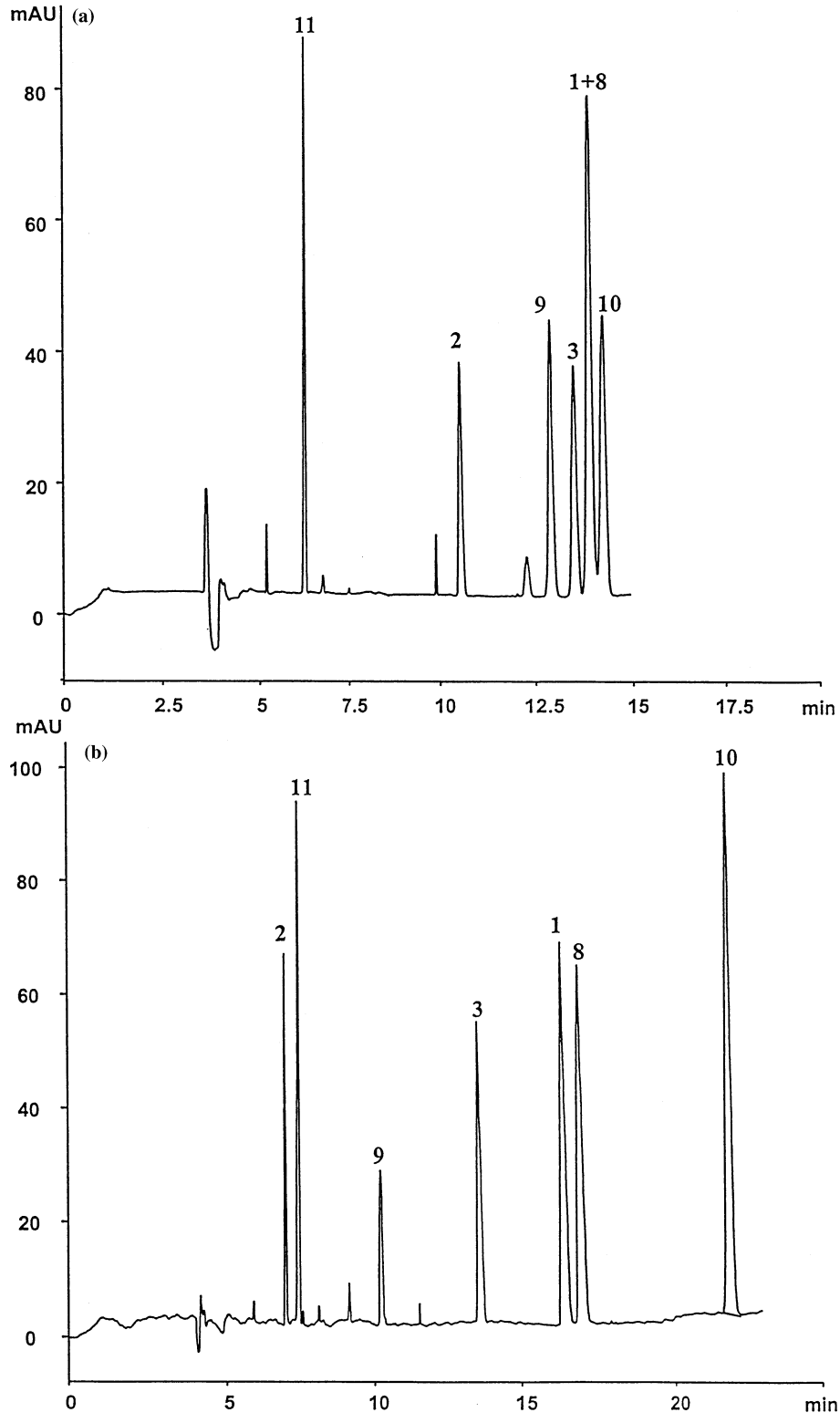

Fig. 5. Electrophoretic separation of selected tropane alkaloid mixture: (a) without organic modifier; (b) 10% acetonitrile and; (c) 20% methanol. Other operating conditions are the same as described in Fig. 4. Peak numbering is the same as for Fig. 1.

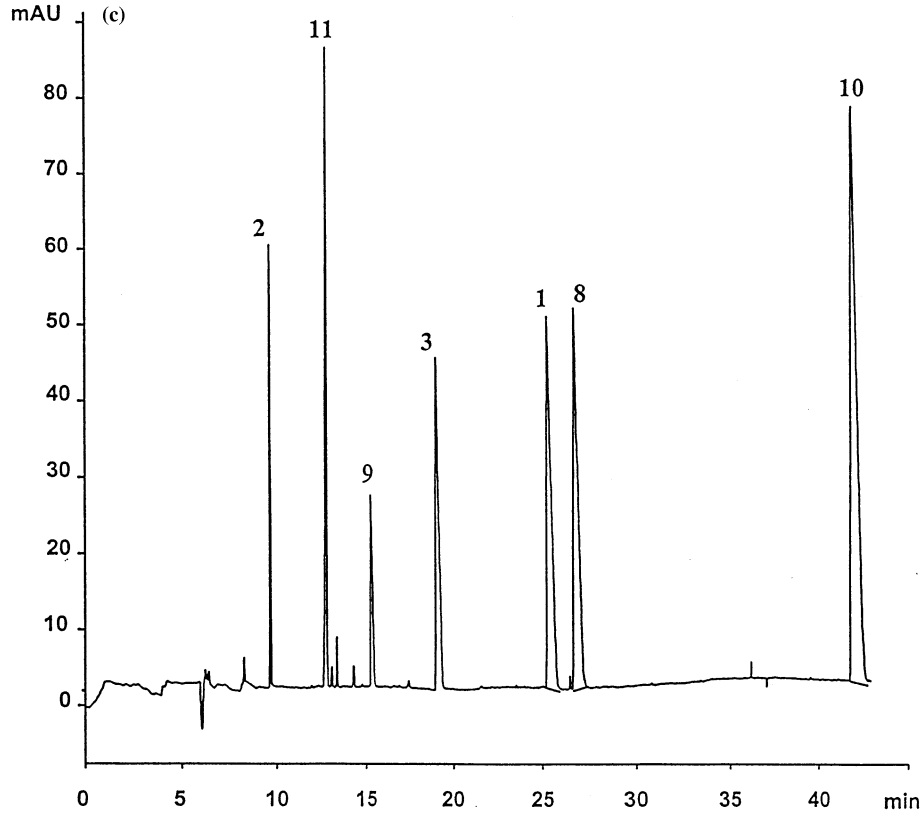

Fig. 5. (*Continued*)

complex alkaloidal mixture in less than 18 min. Several methods, already published, do not allow the separation of littorine and hyoscyamine and thus the content of the latter is often overestimated.

4. Conclusion

The present paper describes the suitability of capillary electrophoresis, in CZE and in MEKC modes, for the analysis of tropane alkaloids in pharmaceutical formulations and in plant extracts. Two validated capillary zone electrophoresis methods were described for the determination of atropine, scopolamine and homatropine in ophthalmic solutions and for atropine and scopolamine derivatives in various pharmaceutical preparations, such as solutions, tablets, suppositories and aerosols. Micellar electrokinetic chromatography was found to be more appropriate for the analysis of hyoscyamine and scopolamine in plant extracts. The separation of hyoscyamine and littorine, two positional isomers, was achieved through the addition of an organic modifier such as methanol or acetonitrile.

Owing to their simplicity and rapidity, the three methods are well appropriate for the assay of tropane alkaloids in various matrices. In comparison to other chromatographic methods, where expensive columns and solvents are needed, the described CE methods are a valuable alternative.

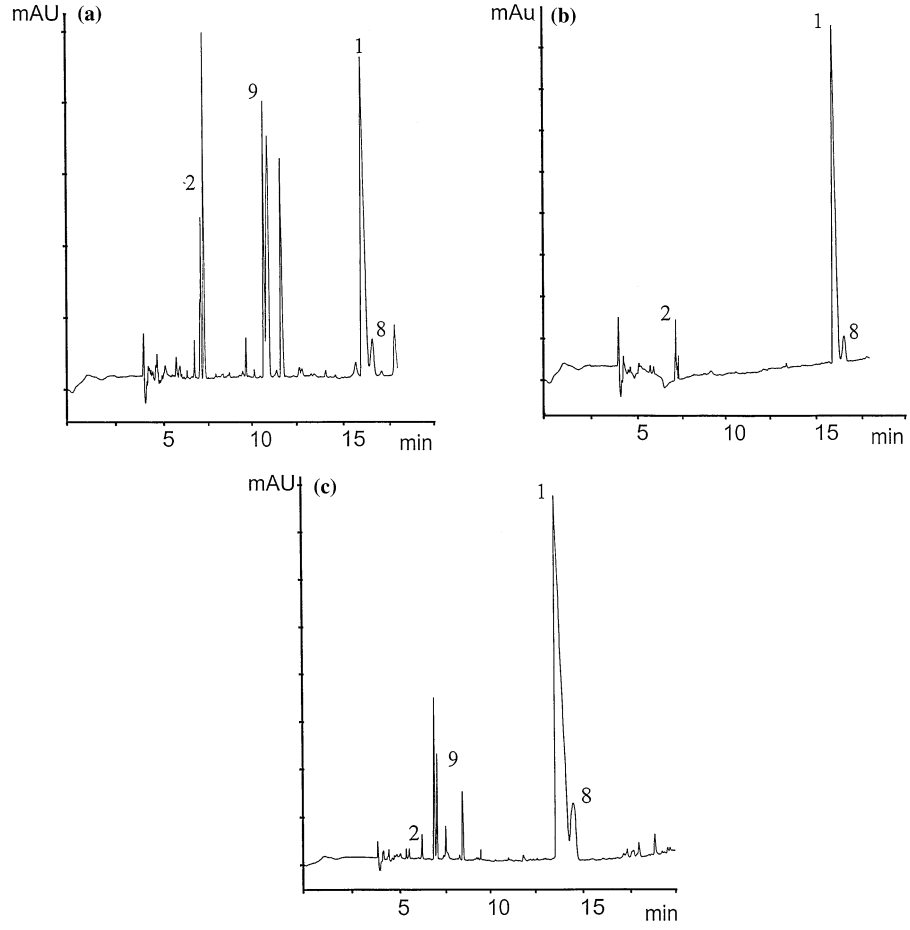

Fig. 6. Electropherogram of hairy root extract from: (a) *Datura candida* x *D. aurea*; (b) *Datura quercifolia*; and (c) *Hyoscyamus albus* analyzed by MECK. Other operating conditions are the same as described in Fig. 4. Peak numbering is the same as for Fig. 1.

References

[1] M. Lounasmaa, T. Tamminen, in: G.A. Cordell (Ed.), The Alkaloids, vol. 44, Academic Press, New York, 1993, pp. 1–114.
[2] A. Romeike, Bot. Notiser 131 (1978) 85.
[3] Goodman and Gilman's, in: J.G. Hardman, L.E. Limbird (Eds.), The Pharmacological Basis of Therapeutics, 9th ed., McGraw-Hill, New York, 1995, pp 141–160.
[4] R.A. De Zeeuw, J.P. Franke, M. van Halem, S. Schaapman, E. Logawa, C.J.P. Siregar, J. Chromatogr. 664 (1994) 263.
[5] P. Majlat, J. Chromatogr. A 241 (1982) 399.
[6] R.L. Ballbach, D.J. Brown, S.M. Walters, J. Pharm. Sci. 71 (1997) 1553.
[7] S.R. El-Shabouri, S.A. Hussin, A.M. Taha, K.M. Imera, Bull. Pharm. Sci. 8 (1985) 120.
[8] T. Aman, A. Hassan, I. Khokhar, A. Rashid, Anal. Lett. 27 (1994) 1833.
[9] J. Pohjola, M. Harpf, J. Chromatogr. A 686 (1994) 350.
[10] C. Van Buuren, J.F. Lawrence, U.A.Th. Brinkman, I.L. Honigberg, R.W. Frei, Anal. Chem. 52 (1980) 700.
[11] U. Lund, S.H. Hansen, J. Chromatogr. 161 (1978) 371.
[12] J.C. Gfeller, G. Frey, J.M. Huen, J.P. Thevenin, J. Chromatogr. 112 (1979) 141.
[13] M. Takahashi, M. Nagashima, S. Shigeoka, M. Nishijima, K. Kamata, J. Chromatogr. A 775 (1997) 137.
[14] O.-W. Lau, C.-S. Mok, J. Chromatogr. A 766 (1997) 270.
[15] M. Popl, J. Fähnrich, V. Tatar, Chromatographic Analysis of Alkaloids, Chromatographic Science Series, vol. 53, Marcel Dekker, New York, 1990, pp. 88–133.
[16] B.A. Bidlingmeyer, J. Chromatogr. Sci. 18 (1980) 525.
[17] E. Cruz, M.R. Euerby, C.M. Johnson, C.A. Hackett, Chromatographia 44 (1997) 151.
[18] R.W. Roos, C.A. Lau-Cam, J. Chromatogr. 370 (1986) 403.

[19] K.-M. Oksman-Caldentey, O. Kivelä, R. Hiltunen, Plant Sci. 78 (1991) 129.
[20] K.-M. Oksman-Caldentey, H. Vuorela, A. Strauss, R. Hiltunen, Planta Med. 53 (1987) 349.
[21] P.D. Grossman, J.C. Colburn (Eds.), Capillary Electrophoresis: Theory and Practice, Academic Press, New York, 1992.
[22] K.D. Altria, J. Bestford, J. Cap. Electrophor. 3 (1996) 13.
[23] A. Shafaati, B.J. Clark, J. Pharm. Biomed. Anal. 14 (1996) 1547.
[24] S. Arrowood, A.M. Hoyt, J. Chromatogr. 586 (1991) 177.
[25] M.G. Quaglia, A. Farina, E. Bossu and, C. Dell'Aquilla, J. Pharm. Biomed. Anal. 13 (1995) 505.
[26] W. Steuer, I. Grant, F. Erni, J. Chromatogr. 507 (1990) 125.
[27] F.A. Tomas-Barberan, Phytochem. Anal. 6 (1995) 177.
[28] I.D. Cruzado, G. Vigh, J. Chromatogr. 608 (1992) 421.
[29] Y.Y. Rawjee, R.L. Williams, G. Vigh, J. Chromatogr. A 680 (1994) 599.
[30] M. Eeva, J-P. Salo, K.-M. Oksman-Caldentey, J. Pharm. Biomed. Anal. 16 (1998) 717.
[31] P. Nussbaumer, I. Kapétanidis, P. Christen, Plant Cell Rep. 17 (1998) 405.
[32] J.M. Dupraz, P. Christen, I. Kapétanidis, Planta Med. 60 (1994) 158.
[33] P. Christen, T. Aoki, K. Shimomura, Plant Cell Rep. 11 (1992) 597.
[34] S. Cherkaoui, L. Mateus, P. Christen, J.-L. Veuthey, J. Chromatogr. B 696 (1997) 283.
[35] ICH: Harmonised Tripartite Guideline. Q2A: Text on Validation of Analytical Procedures (1994). Q2B: Validation of Analytical Procedures: Methodology, 1996.
[36] S. Cherkaoui, L. Mateus, P. Christen, J.-L. Veuthey, J. Pharm. Biomed. Anal. (in press).
[37] G. Blaschke, E. Lamparter, J. Schluter, Chirality 5 (1993) 78.
[38] S. Cherkaoui, L. Mateus, P. Christen, J.-L. Veuthey, Chromatographia 46 (1997) 351.
[39] J. Vindervogel, P. Sandra, Introduction to Micellar Electrokinetic Chromatography, Hüthig Buch Verlag (Ed.), Heidelberg, 1992.
[40] A. Brachet, O. Munoz, M. Gupta, J.-L. Veuthey, P. Christen, Phytochemistry 46 (1997) 1439.
[41] S. Fujiwara, S. Honda, Anal. Chem. 59 (1987) 2773.