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Use of dynamically coated capillaries with added cyclodextrins for the analysis of opium using capillary electrophoresis

Ira S. Lurie*, Sini Panicker, Patrick A. Hays, Agnes D. Garcia, Bryan L. Geer
US Drug Enforcement Administration, Special Testing and Research Laboratory, 22624 Dulles Summit Court, Dulles, VA 20166, USA

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Abstract

A rapid, precise, accurate, and robust method using capillary electrophoresis (CE) with dynamically coated capillaries for the analysis of the major opium alkaloids in opium is presented. Dynamic coating of the capillary surface is accomplished using a commercially available reagent kit (polycation coating followed by polyanion coating). The addition of dual cyclodextrins (hydroxypropyl-β-cyclodextrin and dimethyl-β-cyclodextrin) to the run buffer imparts excellent selectivity for the opium alkaloids. For the determination of morphine, papaverine, codeine, noscapine and thebaine in opium gum and opium latex samples (using tetracaine as an internal standard) good agreement with values obtained by gradient high-performance liquid chromatography is obtained. Compared to the latter technique, CE affords better resolution with significantly faster analysis time (12 min versus 29 min). Dynamically coated capillaries, which give rise to a relatively high and robust electroosmotic flow (EOF) at the background electrolyte pH of 2.5, allow for rapid analysis and excellent migration time and peak area precision (RSDs≤0.12% and ≤1.2%, respectively). Reproducible separations (relative migration times) for over 500 samples have been obtained on a single capillary. The nature of the injection solvent, the injection time and the contents of the waste vials have a profound effect on the pressure injection precision of the relatively hydrophobic solutes. The CE conditions reported in this study are also applicable to the analysis of lysergic acid diethylamide (LSD) exhibits.
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Keywords: Forensic analysis; *Papaver somniferum*; Cyclodextrins; Alkaloids; LSD; Lysergic acid diethylamide

1. Introduction

Mature capsules of opium poppy (*Papaver somniferum*) contain opium. In Asian countries opium is harvested by lancing the capsules and allowing the sap to air-dry on the capsule to form a sticky gummy material (opium gum). In South American countries, the sap is collected when it is still in the liquid form, which is referred to as opium latex. The analysis of opium gum and opium latex is important for legal and intelligence purposes since it contains the major alkaloids morphine, codeine, thebaine, noscapine and papaverine all of which are of significant interest to the forensic community. The quantitation of these alkaloids in opium is typically performed by separation techniques such as gas chromatography (GC) [1], high-performance liquid chromatography

*Corresponding author. Fax: +1-703-668-3320.
E-mail address: llurie@erols.com (I.S. Lurie).

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(HPLC) [2–5], and capillary electrophoresis (CE) [6–11].

Although capillary GC affords high resolution, derivatization of morphine is required and thebaine and noscapine exhibit poor chromatographic performance [11]. Furthermore, HPLC, which allows for the direct analysis of these compounds, inherently lacks resolution and can exhibit poor peak shapes. The use of extensive sample clean-up can reduce the occurrence of overlapping peaks [4,5].

CE, which has significantly greater resolving power than HPLC and gives good electrophoretic performance for the opium alkaloids without derivatization, is an excellent technique for opium analysis. Due to similarities in charge to size ratios of these solutes, electrokinetic and non-aqueous techniques have been employed, including run buffers with micelles and/or cyclodextrins [6–11]. Trenerry et al. [6] used micellar electrokinetic capillary chromatography (MECC) with a positive micelle, cetyltrimethylammonium bromide (CTAB), for the determination of opium alkaloids in crude morphine, poppy straw and opium preparations. Although an excellent separation of standard compounds (major opium alkaloids) was obtained in under 8 min, there was a significant peak overlapping with noscapine in an actual opium sample. The use of dimethylformamide and CTAB (bromide ion) in the run buffer precluded the use of wavelengths $<$240 nm which significantly reduced detector sensitivity, and made the determination of some of the solutes present at low levels difficult. Although still reduced in sensitivity, some improvement in sensitivity was obtained by replacing dimethylformamide with acetonitrile (allowable wavelengths $\geq$220 nm) [9]. Lurie [11], using a negatively charged micelle sodium dodecylsulfate in combination with a charged cyclodextrin β-cyclodextrin sulfobutyl ether IV, was able for an opium sample to obtain baseline resolution for the five major opium alkaloids (run time 35 min). Although high resolving power is obtainable with MECC, limitations exist in terms of robustness. The slightly basic pH employed in the run buffers (range 8.6–9.0) is near the pK_a values of many of the solutes and therefore careful pH control is important. Evaporation of organic solvent in the run buffer during lengthy overnight runs could cause significant selectivity changes. In addition, the high currents generated by MECC significantly reduce capillary lifetimes.

Bjornsdottir and Hansen [7] fully resolved in under 11 min the five major opium alkaloids in a crude opium sample using a pH 4 run buffer (considerably below the pK_a of solutes) containing dimethyl-β-cyclodextrin. Additionally, a single concentration of individual neutral and charged cyclodextrins were also investigated for the separation of the opium alkaloids. For this study only limited validation data were presented. Accuracy data were presented for morphine only in two Pectyl cough mixtures with no short term migration time precision or long term migration time reproducibility studies. Jelinek et al. [10] using β-cyclodextrin and carboxy-β-cyclodextrin at pH 3.7 were unable to resolve the major alkaloids in an opium preparation.

Although Bjornsdottir and Hanesen [8] obtained an excellent separation of the five major opium alkaloids in a crude opium sample using non-aqueous CE, no validation data were presented. Similar to MECC, evaporation of organic solvent in the run buffer and changes in run buffer pH (apparent pH near the apparent pK_a of solutes) could be a concern. Compared to electrokinetic techniques non-aqueous CE employs no buffer additives.

The use of dynamically coated capillaries allows for the rapid, precise, and reproducible separation of moderate to strong basic solutes (p$K_a \geq 5$) at pH 2.5 [12–17]. Compared to uncoated capillaries, with run buffers at the same pH, separation times are up to two times faster with similar resolution. In addition, a significantly lower migration time and relative area precision are obtained [13]. The coating procedure, developed by Chevigne and Janssens [18], is a two-step process in which between every run the capillary (after base hydrolysis) is first coated with a polycation (an initiator) then coated with a polyanion (an accelerator). The run buffer is the latter coating reagent. For the analysis of basic drugs both the proprietary polycation and proprietary polyanion are dissolved in 75 m*M* phosphate buffer pH 2.5. This process gives rise to a highly reproducible electroosmotic flow (EOF) at a low pH and to a capillary surface with more favorable kinetics. Additionally, highly reproducible relative migration times are obtained for both day-to-day and capillary-to-capillary usage. Ciccone [12], using the above coating system for the separation of a mixture of 10 basic solutes (including compounds of forensic interest ephedrine and nortryptiline), reported for most of the compounds excellent migration time run-to-run precision (RSDs<0.2%) and very good migration time day-to-day and capillary-to-capillary reproducibility (RSDs<1.2%). Compared to a conventional buffer system, shorter migration times, higher plate counts, and better peak shapes were obtained. Lurie et al. [13] employed a very similar dynamically coated capillary protocol for the analysis of seized drugs such as phenethylamines and cocaine. Excellent migration time run-to-run precision (RSDs<0.3%) and excellent relative migration time day-to-day and capillary-to-capillary reproducibility (RSDs<0.2% and <0.06%, respectively) were obtained. In addition, compared to uncoated capillaries, coated capillaries significantly improved precision with run times lowered by at least a factor of two with accurate quantitation obtained. Boone et al. [14] used the same coating reagents for the forensic toxicological analysis of basic drugs and obtained similar improvement in precision and separation speed over uncoated capillaries. Clohs and Winstanley [15] analyzed propranolol in serum using dynamically coated capillaries with the same coating reagents and also reported improvement in precision, separation speed and plate count over uncoated capillaries. Weinberger [16] reported on the highly reproducible separation of the enantiomers of epinephrine using the same reagent kit with added dimethyl-β-cyclodextrin. Bendahl et al. [17] obtained excellent intracapillary migration time precision and good intercapillary migration time reproducibility for basic solutes at pH 2–4 using a modification of a dynamically coating procedure reported by Katayama [19]. The coating reagents were Polybrene and poly(vinylsulfonate) with the latter reagent contained in smaller amounts in the run buffer. For standard injections stable coatings were obtained without recoating both layers for 54 injections. For this report the analysis of opium gum and opium latex using dynamically coated capillaries is described. As in previous studies [12–16] a commercially available kit is the basis for coating, while in this study cyclodextrins (CDs) are added to the accelerator for added selectivity. The CE system developed for opiates is also applicable for LSD.

2. Experimental

2.1. Reagents

All drug standards used were obtained from the reference collection of the Drug Enforcement Administration Special Testing and Research Laboratory (Dulles, VA, USA). CElixir Reagent A, CElixir Reagent B (pH 2.5) and 75 m*M* phosphate (monobasic) buffer (pH 2.5) were obtained from MicroSolv Technology (Eatontown, NJ, USA). Hydroxypropyl-β-cyclodextrin (HP-β-CD) and dimethyl-β-cyclodextrin (DM-β-CD) were acquired from Sigma (St. Louis, MO, USA). Sodium phosphate (monobasic), phosphoric acid, and sodium hydroxide were reagent grade. HPLC-grade methanol was obtained from Burdick and Jackson (Muskegon, MI, USA). High purity and deionized water was obtained from a Millipore Milli-Q-Gradient A10 water system (Bedford, MA, USA).

2.2. CE run buffer

The run buffer for CE analysis consisted of 25 m*M* HP-β-CD and 75 m*M* DM-β-CD in CElixir Reagent B (pH 2.5). This reagent was prepared by weighing 394 mg of HP-β-CD plus 998 mg of DM-β-CD into a 25-ml Erlenmeyer flask and pipetting 10.0 ml of CElixir Reagent B (pH 2.5) followed by vigorous shaking. This solution as well as other reagents used for CE analyses were filtered through an SRI 0.5 μm nylon filter (Eatontown, NJ, USA).

2.3. HPLC eluents

The eluents consisted of a sodium phosphate buffer, pH 2.1 with added hexylamine (solvent A) and methanol (solvent B). The sodium phosphate buffer is prepared by mixing 3480 ml of water, 120 ml of 2 *M* sodium hydroxide, and 40 ml of phosphoric acid into a 4-l Erlenmeyer flask. After filtering the buffer through an SRI 0.5 μm nylon filter, hexylamine is added (pipette 3.0 ml of hexylamine for 870 ml of buffer) and solution mixed.

2.4. Apparatus

2.4.1. CE

An Agilent Model HP^{3D}CE capillary electrophoresis system equipped with a diode array detector (Waldbronn, Germany) was used for CE experiments. All experiments were carried out with fused-silica 32 cm (23.5 cm to detector window)×50 μm I.D. capillaries obtained from Polymicro Technologies (Phoenix, AZ). For conditioning new dynamically coated capillaries, the columns were first flushed with 0.1 *M* sodium hydroxide for 5 min, then water for 2 min, followed by CElixir Reagent A (positive coating reagent) for 1 min and finally CElixir Reagent B (pH 2.5) (negative coating reagent) (with or without added CD(s)) for 2 min. Between injections, a similar conditioning program is used except the 0.1 *M* sodium hydroxide and water flush step are 1 min each, respectively. For overnight or prolonged storage the capillary is flushed with water for 10 min and stored with the inlet and outlet dipped in water.

2.4.2. HPLC

An Agilent Model 1100 HPLC system equipped with a quaternary pumping system and a 1100 series diode array detector was used for the HPLC analysis of opium gum and opium latex. A Whatman 5 ODS-3 column (12.5 cm×3.2 mm) (Clifton, NJ, USA) was used at ambient temperature. For gradient analysis the initial mixture was methanol–(phosphate-hexylamine buffer pH 2.1) (2:98) for 3 min (flow 0.76 ml/min.); linear gradient to (35:65) in 9.5 min, hold for 4.5 min (flow 0.76 ml/min.); linear gradient to 100% methanol in 1 min; linear flow to 1.5 ml/min in 1 min, hold for 3 min; linear gradient to initial conditions in 3 min (flow 1.5 ml/min); linear flow to 0.76 ml/min in 4 min, hold for 5 min.

2.5. Samples and standards for CE and HPLC

2.5.1. Samples

Approximately 200 mg of opium gum sample are weighed into a 50-ml volumetric flask. After diluting the flask to volume with methanol, the sample is sonicated at an elevated temperature (50–60 °C) for 30 min. For CE, pipette 25 ml of the stock solution into a 50-ml volumetric and dilute to volume with 3.75 m*M* phosphate buffer (pH 3.2). (This buffer is prepared by weighing 1034 mg of sodium phosphate monobasic into a 100-ml volumetric flask and diluting to volume with water. The pH is adjusted to 2.6 using phosphoric acid (85%, w/w). The contents are transferred into a 2000-ml flask and diluted to volume with water (final pH 3.2).) Into a 2.0-ml HPLC auto sampler vial are pipetted 200 μl of sample and 1.0 ml of 3.75 m*M* phosphate buffer (pH 3.2) containing internal standard (0.03 mg/ml tetracaine·HCl). Approximately 500 μl of filtered solution (0.45 μm regenerated cellulose Titan filter) are transferred to a 1.0-ml polypropylene CE injection vial. For HPLC, pipette 20 ml of the opium stock solution into a 100-ml volumetric flask and dilute to volume with sodium phosphate buffer, pH 2.1 (HPLC eluent). Into a 50-ml Erlenmeyer flask are pipetted 5.0 ml of sample, 3 ml of internal standard solution (0.5 mg/ml procaine·HCl) and 22.0 ml of sodium phosphate buffer, pH 2.1. Approximately 1.0 ml of filtered solution (0.45 μm regenerated cellulose Titan filter) is transferred to a 2.0-ml HPLC autosampler injection vial.

Approximately 1 g of opium latex sample (after thoroughly mixing) is weighed into a 50-ml volumetric flask, diluted to volume with methanol and vortexed for 1 min. For CE, 15.0 ml of the stock solution are pipetted into a 100-ml volumetric flask and after the addition of 35 ml of methanol the sample is diluted to volume with 3.75 m*M* phosphate buffer (pH 3.2). Into a 2.0-ml HPLC auto sampler vial are pipetted 200 μl of sample and 1.0 ml of 3.75 m*M* phosphate buffer (pH 3.2) containing internal standard (0.03 mg/ml tetracaine·HCl). Approximately 500 μl of filtered solution (0.45 μm regenerated cellulose Titan filter) are transferred to a 1.0-ml polypropylene CE injection vial. For HPLC, 15.0 ml of the stock solution are pipetted into a 100-ml volumetric flask and diluted to volume with sodium phosphate buffer, pH 2.1. Into a 50-ml Erlenmeyer flask are pipetted 5.0 ml of sample, 3 ml of internal standard solution (0.5 mg/ml procaine·HCl) and 22.0 ml of sodium phosphate buffer, pH 2.1. Approximately 1.0 ml of filtered solution (0.45 μm regenerated cellulose Titan filter) is transferred to a 2.0-ml HPLC autosampler injection vial.

2.5.2. Standards

For CE weigh appropriate amounts of morphine (base or HCl), codeine (base or HCl), thebaine base, noscapine base and papaverine (base or HCl) into a 100-ml volumetric flask in order to obtain an approximate final concentration of 0.025 mg/ml. Pipette 10.0 ml of internal standard [0.25 mg/ml tetracaine HCl (1:11 mixture of methanol and 3.75 m*M* phosphate buffer (pH 3.2)] into the above volumetric flask and dilute to volume with a 1:11 mixture of methanol and 3.75 m*M* phosphate buffer (pH 3.2). Approximately 500 μl of filtered solution (0.45 μm regenerated cellulose Titan filter) are transferred to a 1.0-ml polypropylene CE injection vial. For HPLC, weigh appropriate amounts of morphine (base or HCl), codeine (base or HCl), thebaine base, noscapine base, and papaverine (base or HCl) into a 100-ml volumetric flask in order to obtain an approximate final concentration of morphine of 0.050 mg/ml and a final concentration for the additional alkaloids of 0.020 mg/ml.

3. Results and discussion

The dynamically coated capillary approach used for the analysis of phenethylamines [13] gave poor resolution of the opium alkaloids. For this reason, the effect of the addition of various concentrations of

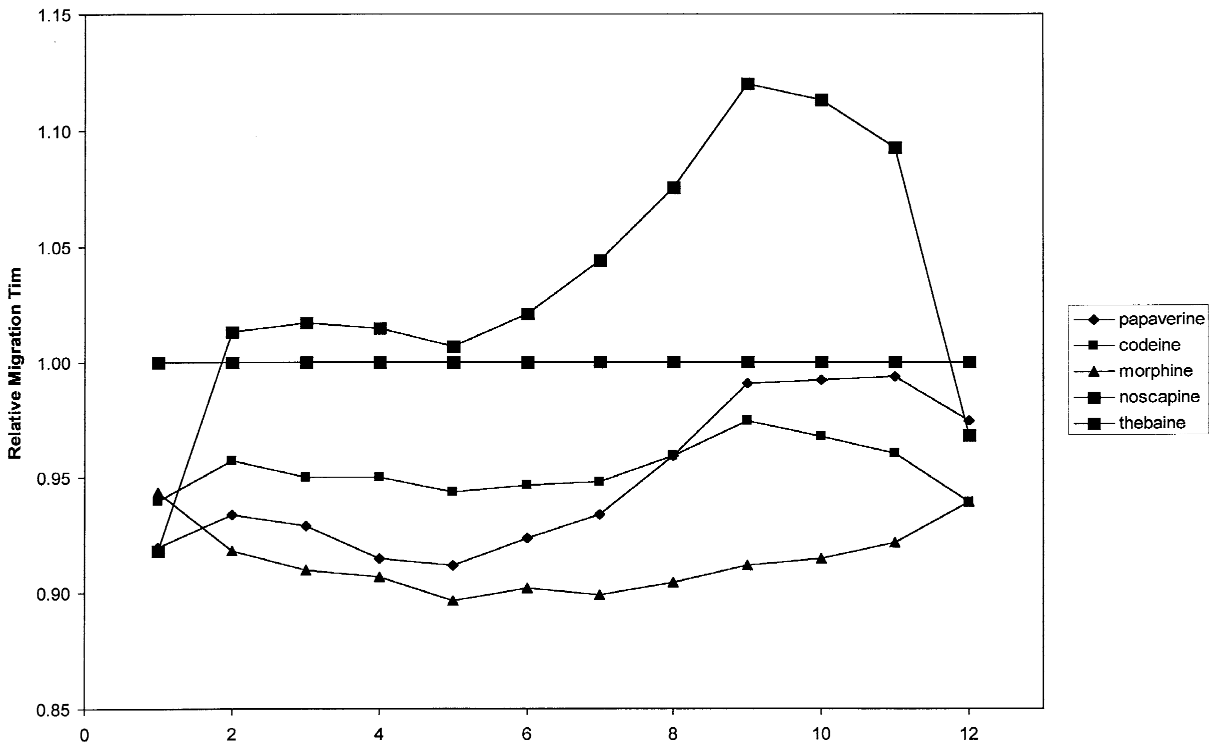

Fig. 1. The effect of the addition of various concentrations of DM-β-CD and HP-β-CD added to CElixir Reagent B (pH 2.5) on the separation of morphine, codeine, thebaine, and papaverine relative to noscapine. A 32 cm (23.5 cm to detector window)×50 μm I.D. fused-silica capillary operating at 15 °C and 20 kV with UV detection at 205 nm was used. The solvent systems consisted of: (1) CElixir Reagent B (pH 2.5); (2) CElixir Reagent B (pH 2.5)+25 m*M* DM-β-CD; (3) CElixir Reagent B (pH 2.5)+50 m*M* DM-β-CD; (4) CElixir Reagent B (pH 2.5)+75 m*M* DM-β-CD; (5) CElixir Reagent B (pH 2.5)+100 m*M* DM-β-CD; (6) CElixir Reagent B (pH 2.5)+25 m*M* HP-β-CD+75 m*M* DM-β-CD; (7) CElixir Reagent B (pH 2.5)+50 m*M* HP-β-CD+50 m*M* DM-β-CD; (8) CElixir Reagent B (pH 2.5)+75 m*M* HP-β-CD+25 m*M* DM-β-CD; (9) CElixir Reagent B (pH 2.5)+100 m*M* HP-β-CD; (10) CElixir Reagent B (pH 2.5)+75 m*M* HP-β-CD; (11) CElixir Reagent B (pH 2.5)+50 m*M* HP-β-CD; (12) CElixir Reagent B (pH 2.5)+25 m*M* HP-β-CD.

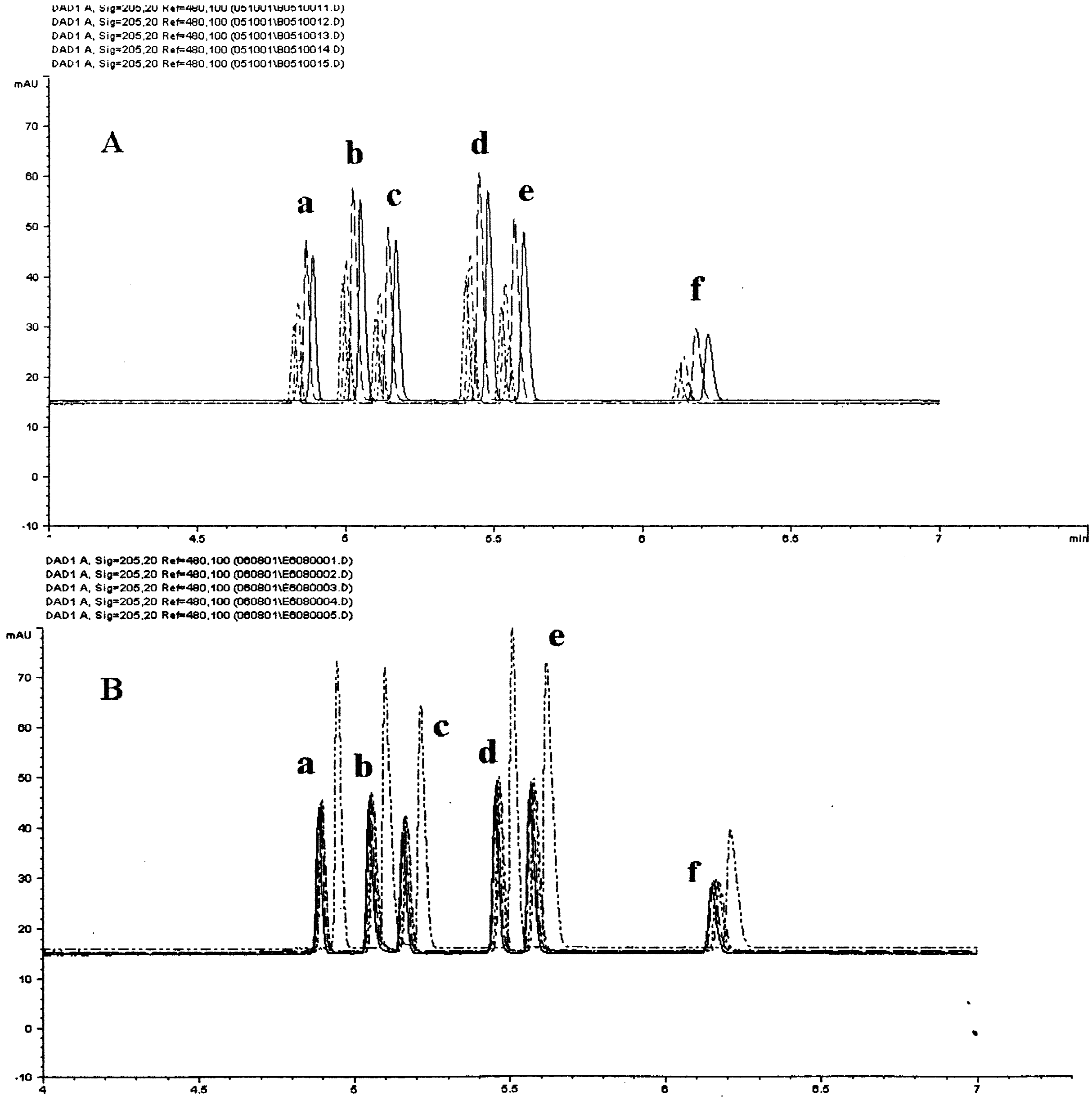

Fig. 2. Effect of solute concentration and injection solvent on peak area precision. (A) Overlay of five electropherograms depicting the separation of a standard mixture of (a) morphine, (b) papaverine, (c) codeine, (d) noscapine, (e) thebaine, and (f) tetracaine (internal standard). Solute concentration was 0.15 mg/ml with an injection solvent of methanol–3.75 m*M* phosphate buffer, pH 3.2 (50:50) with 100 mbar s injections and initially empty waste vials (injection and conditioning). A 32 cm (23.5 cm to detector window)×50 μm I.D. fused-silica capillary operating at 15 °C and 20 kV with UV detection at 205 nm was used with a run buffer consisting of CElixir Reagent B (pH 2.5)+25 m*M* HP-β-CD+75 m*M* DM-β-CD. (B) Same as (A) except waste vials contain 250 μl of water.

DM-β-CD and HP-β-CD added to CElixir Reagent B (pH 2.5) (alone or in combination) was investigated. Migration times for morphine, codeine, thebaine and papaverine relative to noscapine are shown in Fig. 1. Without the addition of cyclodextrin, papaverine and thebaine as well as morphine and codeine co-migrated. As reported previously [8], major changes in selectivity occurred upon the addition of cyclodextrin(s) to the run buffer due to the effect of secondary equilibria. As shown in Fig. 1 the addition of either DM-β-CD or HP-β-CD also significantly alters the migration order of the various opium alkaloids. Compared to DM-β-CD the relative migration time of thebaine relative to noscapine and morphine relative to papaverine is significantly greater for HP-β-CD. Conversely the relative migration time of papaverine relative to noscapine for DM-β-CD is significantly greater than for HP-β-CD. Therefore it is not surprising that a 25:75 mixture of HP-β-CD and DM-β-CD gives the best separation of the opium alkaloids.

Although an excellent separation was obtained for the five major opium alkaloids and an internal standard tetracaine, run-to-run peak area precision was poor using an injection solvent of methanol–3.75 m*M* phosphate buffer, pH 3.2 (50:50) with 100 mbar s injections and initially empty waste vials (injection and conditioning) (see Fig. 2A). It was believed that a variable resistance from initially empty waste vials during pressure conditioning and injection was causing differences in both dynamic coating and hydrodynamic injection. As shown in

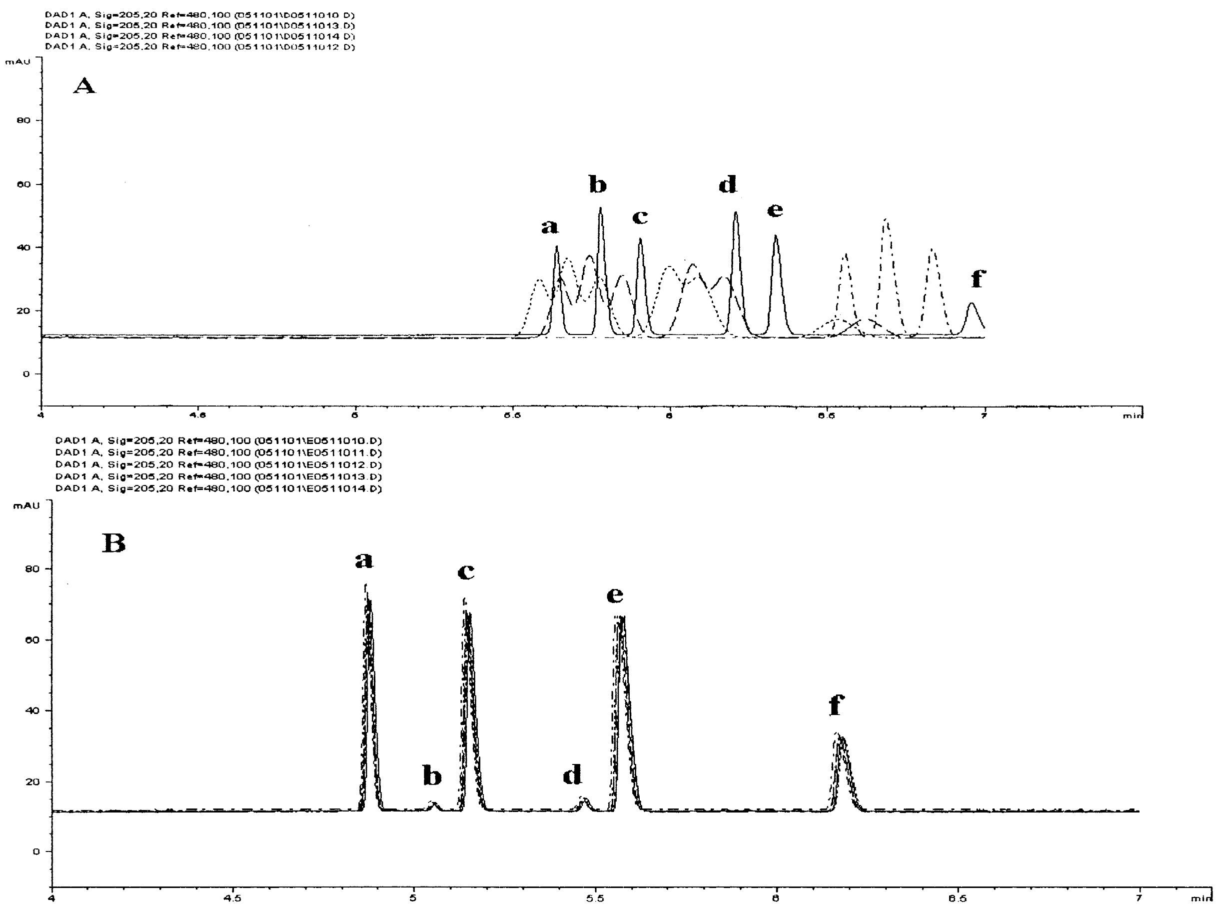

Fig. 3. Same as Fig. 2B except (A) solute concentration was 0.025 mg/ml with 500 mbar s injections and (B) injection solvent consisted of methanol–3.75 m*M* phosphate buffer, pH 3.2 (9:91).

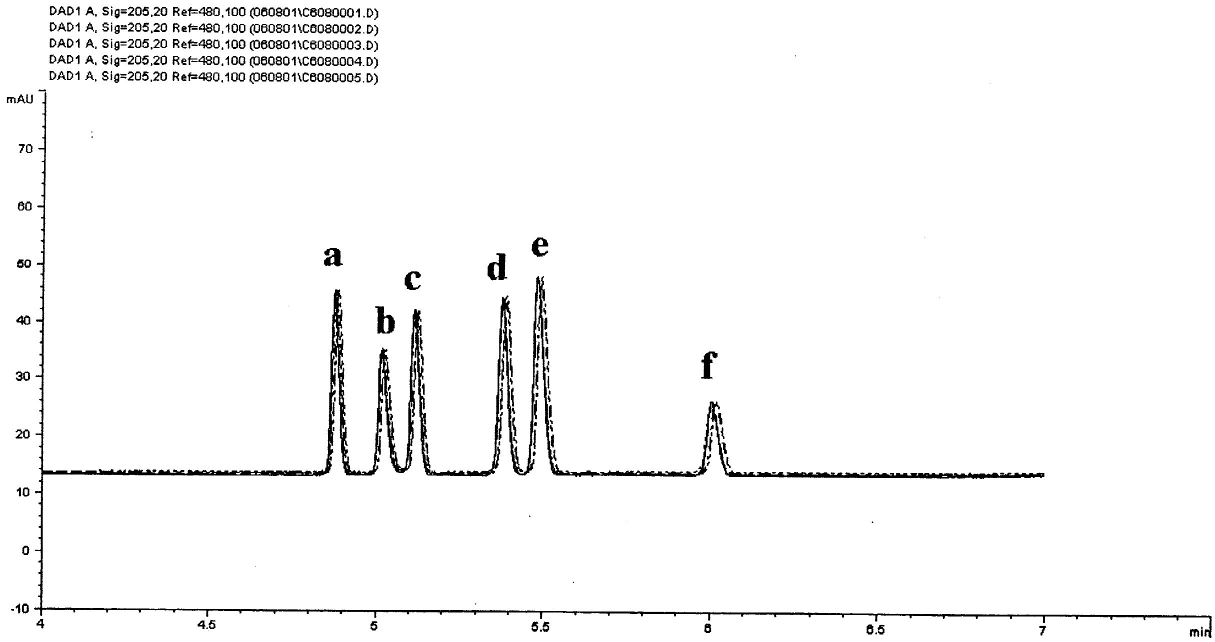

Fig. 4. Same as Fig. 3A except injection solvent consisted of methanol–3.75 m*M* phosphate buffer, pH 3.2 (9:91).

Fig. 2B some improvement in peak area and migration time precision was obtained using waste vials initially filled with 250 μl of water; however, peak area precision is still poor (both experiments were repeated with the same result). It was hypothesized that a combination of a highly viscous injection plug over a relatively small injection time was responsible for this anomaly. For the same injection solvent with 1/6th the solute concentrations, 500 mbar s injections resulted in poor peak area and migration time precision as well as loss in resolution (see Fig. 3A). Although the injection solvent contains a stacking buffer (background electrolyte diluted 1:20 with water), the high organic content in the run buffer does not allow for a large injection plug probably due to the absence of methanol in the run buffer. The reason for this effect is not clear. As shown in Fig. 3B, dissolving the original standard mixture in a lower viscosity injection solvent [methanol–3.75 m*M* phosphate buffer, pH 3.2 (10:90)] with 100 mbar s injections does improve precision; however, noscapine and papaverine have limited solubility in the lower organic content injection solvent. For the original standard mixture diluted 1:6 with methanol [injection solvent methanol–3.75 m*M* phosphate buffer, pH 3.2 (8.3:91.7)] excellent peak area and

Table 1
Run-to-run precision (%RSD) $n=5$ using a dynamically coated capillary system. Data relative to tetracaine

Solute	Concentration (mg/ml)	Migration time	Relative migration time	Area solute	Area solute/area internal standard (IS)
Morphine	0.0267	0.10	0.05	1.04	0.83
Papaverine	0.0243	0.10	0.03	0.89	0.65
Codeine	0.0247	0.10	0.04	1.20	1.02
Noscapine	0.0277	0.11	0.02	1.17	0.95
Thebaine	0.0240	0.11	0.02	0.88	0.70
Tetracaine (IS)	0.0253	0.12		0.41	

migration time precision was obtained (see Fig. 4 and Table 1). Peak area run-to-run RSDs≤1.20% and run-to-run migration time RSDs≤0.12% were obtained. Also shown in Table 1 was that precision was improved when using relative area (RSDs≤1.02%) and relative migration times (RSDs≤0.05%). The latter injection conditions were used for all further studies.

The results for the linearity study for the opium alkaloids are shown in Table 2. Excellent linearity was obtained for each of these solutes (0.99999≥R^2≥0.99998), with plots of area standard/area internal standard versus concentration passing through the origin.

Good agreement was obtained for the analysis of opium gum and five opium latex samples analyzed by CE and HPLC (see Table 3). For both methods methanol is the initial extraction solvent (see Section 2), with sonication at elevated temperature required for the dried preparation (opium gum). Re-extraction of both opium gum and opium latex with methanol yielded only negligible quantities of alkaloids (≤2%). An electropherogram and chromatogram of an opium gum sample for both CE and HPLC are shown in Figs. 5 and 6, respectively. Inspection of data depicted in Figs. 5 and 6 shows that CE provides a significantly improved separation over HPLC in terms of resolution of the major alkaloids and speed of analysis (7 min versus 22 min[1]). It is of interest to note the considerably larger number of peaks generated by HPLC versus CE. Unlike the gradient HPLC method which can detect all classes of solutes, the CE technique will only detect moderate and strongly basic compounds. Both methods use multiple wavelength detection for increased selectivi-

Table 2
Results for linearity study

Solute	Linearity range (mg/ml)	Correlation coefficient R^2
Morphine	0.00116–0.0745	0.99999
Papaverine	0.00111–0.0713	0.99999
Codeine	0.00118–0.0756	0.99999
Noscapine	0.000938–0.0601	0.99998
Thebaine	0.00110–0.0709	0.99999

[1]Does not include 5 min of flushes between CE injections or HPLC gradient re-equilibration time of 7 min.

Table 3
Comparison of quantitation of opium gum and opium latex samples (calculated as the base) using CE and HPLC

Sample	Solute	CE	HPLC
Opium gum 1	Morphine	12.9	12.6
	Papaverine	2.37	2.40
	Codeine	2.76	2.64
	Noscapine	7.97	8.06
	Thebaine	1.15	0.94
Opium gum 2	Morphine	17.9	18.4
	Papaverine	3.32	3.34
	Codeine	1.22	1.23
	Noscapine	11.8	12.0
	Thebaine	0.49	0.38
Opium gum 3	Morphine	16.6	16.3
	Papaverine	2.17	2.16
	Codeine	2.84	2.79
	Noscapine	8.14	8.03
	Thebaine	1.26	1.16
Opium gum 4	Morphine	14.0	14.4
	Papaverine	2.81	2.90
	Codeine	1.64	1.69
	Noscapine	8.57	8.88
	Thebaine	0.41	0.34
Opium gum 5	Morphine	8.56	8.70
	Papaverine	2.36	2.29
	Codeine	1.25	1.39
	Noscapine	8.35	8.79
	Thebaine	0.26	0.20
Opium latex 1	Morphine	3.53	3.25
	Papaverine	1.36	1.23
	Codeine	0.38	0.29
	Noscapine	2.68	2.47
	Thebaine	0.11	0.07
Opium latex 2	Morphine	4.69	4.56
	Papaverine	1.39	1.32
	Codeine	0.46	0.37
	Noscapine	3.44	3.22
	Thebaine	0.15	0.12
Opium latex 3	Morphine	5.52	5.43
	Papaverine	1.21	1.12
	Codeine	0.41	0.35
	Noscapine	3.64	3.37
	Thebaine	0.19	0.17
Opium latex 4	Morphine	6.78	6.72
	Papaverine	1.38	1.30
	Codeine	0.44	0.36
	Noscapine	4.14	3.66
	Thebaine	0.28	0.28
Opium latex 5	Morphine	7.05	6.83
	Papaverine	2.72	2.58
	Codeine	0.22	0.19
	Noscapine	4.96	4.82
	Thebaine	–[a]	–[a]

[a] Not detected.

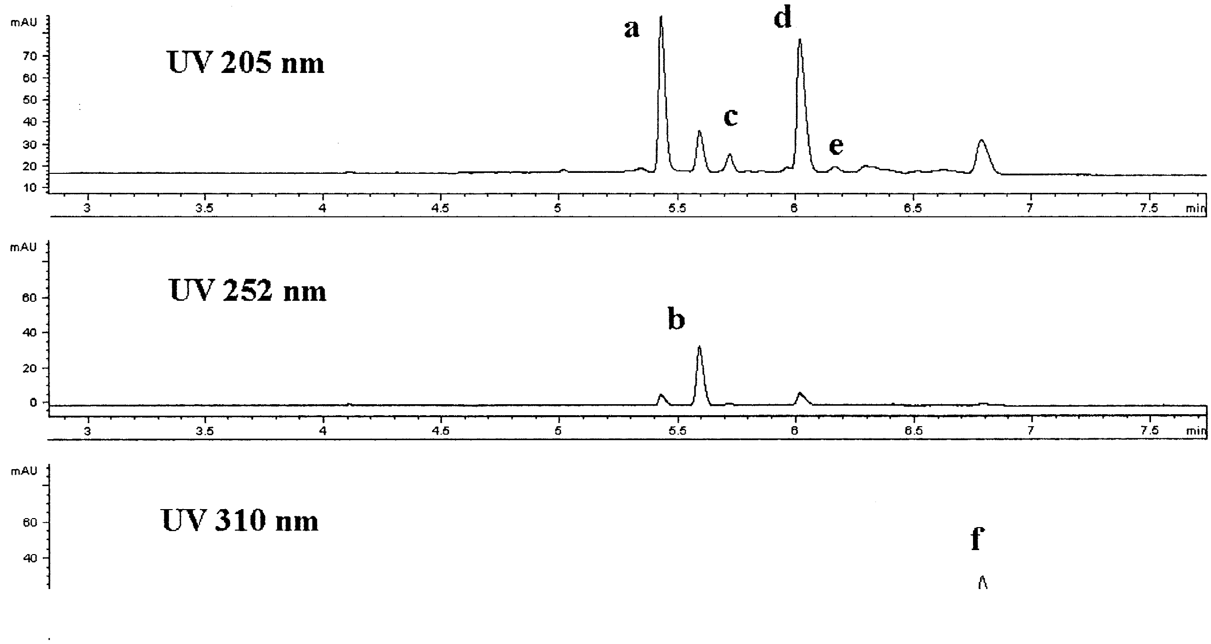

Fig. 5. Multi-wavelength electropherograms of an opium gum sample. CE conditions and identity of peaks are the same as Fig. 4.

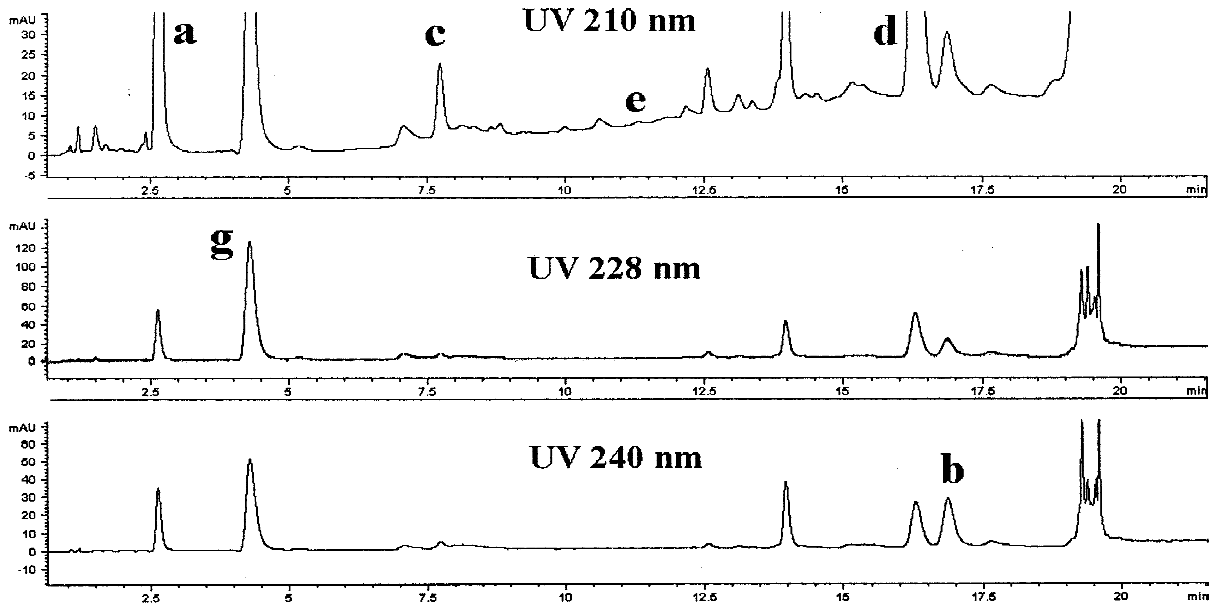

Fig. 6. Multi-wavelength chromatograms of an opium gum sample. A Whatman 5 ODS-3 column (12.5 cm×3.2 mm) was used at ambient temperature with 10 μl injections. For gradient analysis the initial mixture was methanol–(phosphate-hexylamine buffer pH 2.1) (2:98) for 3 min (flow 0.76 ml/min); linear gradient to (35:65) in 9.5 min, hold for 4.5 min (flow 0.76 ml/min.); linear gradient to 100% methanol in 1 min; linear flow to 1.5 ml/min in 1 min, hold for 3 min; linear gradient to initial conditions in 3 min (flow 1.5 ml/min); linear flow to 0.76 ml/min in 4 min, hold for 5 min. Identity of peaks are the same as Fig. 5 except for (g) procaine (internal standard).

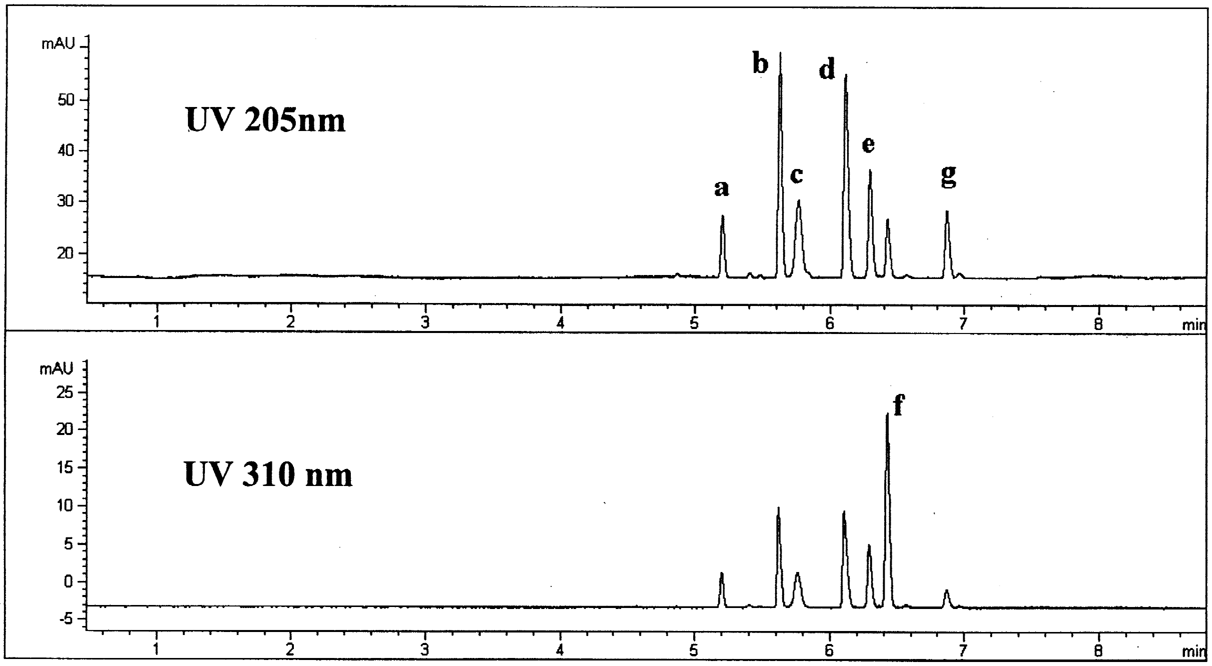

Fig. 8. Multi-wavelength electropherograms of a standard mixture of (a) lysergic acid amide, (b) lysergic acid diethylamide (LSD), (c) lysergic acid methyl-propylamide (LAMPA), (d) iso-LSD, (e) lysergic acid, (f) tetracaine (internal standard) and (g) ergotamine. CE conditions (except for wavelengths) identical to Fig. 4.

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References

[1] H. Huizer, in: T. Gough (Ed.), The Analysis of Drugs of Abuse, Wiley, Chichester, 1991, p. 23.
[2] J.D. Wittwer, J. Forensic Sci. 18 (1973) 138.
[3] I. Lurie, J. Assoc. Off. Anal. Chem. 60 (1977) 1035.
[4] L. Krenn, S. Glantschnig, U. Sorgner, Chomatographia 47 (1998) 21.
[5] L. Krenn, B. Boros, R. Ohmacht, L. Jelinek, Chomatographia 51 (2000) S175.
[6] V.C. Trenerry, R.J. Wells, J. Robertson, J. Chromatogr. A 718 (1995) 217.
[7] I. Bjornsdottir, S.H. Hansen, J. Pharm. Biomed. Anal. 13 (1995) 687.
[8] I. Bjornsdottir, S.H. Hansen, J. Pharm. Biomed. Anal. 13 (1995) 1473.
[9] J. Vandepeer, V.C. Trenerry, G. Keogh, Electrophoresis 17 (1996) 1361.
[10] I. Jelinek, B. Gas, I. Zuskova, E. Niklickova, I. Buben, Chem. Listy 91 (1997) 487.
[11] I.S. Lurie, J. Chromatogr. A 780 (1997) 265.
[12] B. Ciccone, Am. Lab. 33 (2001) 30.
[13] I.S. Lurie, M.J. Bethea, T.D. McKibben, P.A. Hays, P. Pellegrini, R. Sahai, A.D. Garcia, R. Weinberger, J. Forensic Sci. 46 (2001) 1025.
[14] C.M. Boone, E.Z. Jonkers, J.P. Franke, R.A. de Zeeuw, K. Ensing, J. Chromatogr. A 930 (2001) 203.
[15] L. Clohs, A.K. Winstanley, LC–GC Eur. (2002) 288.
[16] R. Weinberger, Am. Lab. 31 (1999) 59.
[17] L. Bendahl, S.H. Hansen, B. Gammelgaard, Electrophoresis 22 (2001) 2565.
[18] J. Janssens, R. Chevigne, P. Louis, US patent #5,611,909, 3/18/97.
[19] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254.