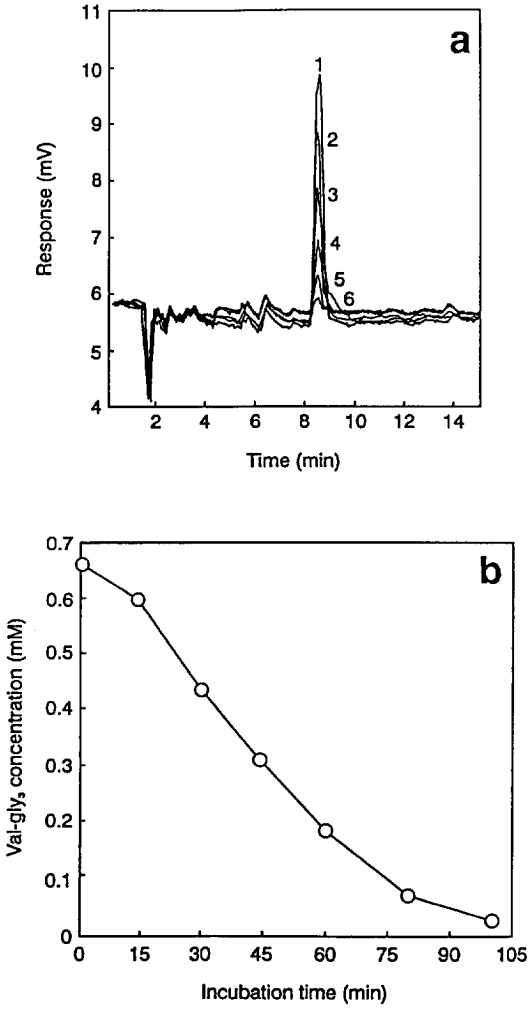

Fig. 4. Time-course of val-gly$_3$ uptake by bacterial cells. Uptake experiments and CE were performed as described in the Experimental section. Samples in (a) were taken at times: 1 = 15 min, 2 = 30 min, 3 = 45 min, 4 = 60 min, 5 = 80 min and 6 = 100 min. Val-gly$_3$ concentrations (b) were calculated from the peak areas and the initial val-gly$_3$ concentration.

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JOURNAL OF CHROMATOGRAPHY A

Determination of morphine and related alkaloids in crude morphine, poppy straw and opium preparations by micellar electrokinetic capillary chromatography

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Abstract

A rapid method for the determination of morphine and related alkaloids in crude morphine, poppy straw and opium preparations by micellar electrokinetic capillary chromatography (MEKC) has been developed. Morphine, codeine, thebaine, oripavine, papaverine, narcotine, narceine, cryptopine and salutaridine were separated in less than 10 min using a 70 cm × 50 μm I.D. uncoated fused-silica capillary column with a buffer consisting of 10% dimethylformamide, 90% 0.05 *M* cetyltrimethylammonium bromide, 0.01 *M* potassium dihydrogen orthophosphate, 0.01 *M* sodium tetraborate, pH 8.6. An applied voltage of −25 kV and a temperature of 28°C gave the best separation of the alkaloids. Pholcodine was used as the internal standard. The compounds were detected by UV at 254 nm. The levels of morphine and related alkaloids determined by MEKC were in good agreement with those determined by high-performance liquid chromatography (HPLC). The coefficients of variation for area calculation (%C.V.) for multiple sample and standard injections by MEKC were slightly greater than for HPLC but were still acceptable (morphine content of poppy straw: %C.V. MEKC 1.7%, %C.V. HPLC 0.3%).

1. Introduction

Analytical procedures based on the relatively new technique of micellar electrokinetic capillary chromatography (MEKC) are rapidly gaining acceptance as rugged analytical methods that are suitable for use in analytical laboratories with high sample throughput [1–6]. In MEKC, the electrophoretic buffer is modified with an ionic surfactant to provide a phase for chromatographic separation [7]. MEKC separations exhibit superior resolution to high-performance liquid chromatography (HPLC) separations, have the same order of repeatability and are faster and less costly to operate than HPLC methods [8]. Both anionic and cationic surfactants have been used as micelle modifiers to separate mixtures that cannot be easily separated using traditional capillary zone electrophoresis (CZE) [8]. Sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide (CTAB) are often used as surfactants for

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MEKC. The addition of an organic solvent (e.g. methanol, acetonitrile, dimethylsulphoxide) to the buffer can also affect the separation of complex mixtures [8].

Weinberger and Lurie [9] separated a wide range of illicit substances by MEKC with a phosphate–borate buffer modified with acetonitrile and SDS. This separation showed the excellent separating potential and resolving power of MEKC. We recently reported the separation and quantitation of diacetylmorphine (heroin), acetylcodeine, O^6-monoacetylmorphine, morphine, codeine, papaverine and narcotine and a number of other compounds found in illicit heroin seizures by MEKC using a phosphate–borate buffer modified with CTAB and acetonitrile. The compounds were separated in less than 13 min and the method was far superior to the isocratic HPLC method that was currently used in our laboratory for the routine testing of illicit heroin seizures [1]. We extended this work to the separation of a number of components of illicit cocaine seizures [2]. Both illicit heroin and cocaine seizures could be analysed with the same electrophoretic conditions: the settings on the UV detector were all that needed to be altered. A small change in the acetonitrile content of the buffer gave complete separation of other naturally occurring alkaloids as well as additives/adulterants of the illicit cocaine seizures.

The success of our work with heroin and cocaine prompted us to extend this work to the analysis of crude morphine, poppy straw and opium preparations. High-performance liquid chromatography (HPLC) with UV–Vis detection as the determinative step is the method of choice for the quantitative determination of morphine and related alkaloids found in crude morphine preparations, poppy straw and opium [10–12]. However, the run times are reasonably long. It was of interest to see if MEKC would give a superior separation of the alkaloids found in crude morphine, poppy straw and opium preparations and provide a faster and less costly analysis with comparable quantitative results to HPLC [10].

2. Experimental

2.1. Reagents

Morphine, codeine, papaverine hydrochloride, narceine, cryptopine, salutaridine, thebaine, oripavine and pholcodine were obtained from the Curator of Standards, Australian Government Analytical Laboratories (N.S.W., Australia). Cetyltrimethylammonium bromide was obtained from Sigma Chemical (St. Louis, MO, USA). The poppy straw was a gift from Glaxo Australia (Port Fairy, Vic., Australia). All other chemicals and solvents were AR grade or HPLC grade and used without further purification.

2.2. MEKC buffer

Amounts of 0.05 *M* CTAB buffers were prepared by dissolving 0.92 g CTAB in 50 ml of a 1:1 mixture of 0.01 *M* sodium tetraborate and 0.01 *M* potassium dihydrogen orthophosphate. The pH of the solution was 8.6. Then 2.5 ml of dimethylformamide was added to 22.5 ml of the buffer and the solution filtered through a 0.8-μm PTFE filter disc before use.

2.3. Apparatus

MEKC

The samples were analysed with an uncoated fused-silica capillary column (70 cm × 50 μm I.D.) with an effective length to the detector of 45 cm (Polymicro Technologies, AZ, USA), using an Isco Model 3140 Electropherograph (Isco, Lincoln, NE, USA) operating at −25 kV and at 28°C. The sample solutions were loaded under vacuum (vacuum level 2, 10 kPa·s) and the alkaloids were detected at 254 nm at 0.005 AUFS (absorbance units full scale). The detector response was linear to 1 mg/ml for morphine and codeine, and 0.2 mg/ml for oripavine, thebaine, papaverine and narcotine. The capillary was flushed with running buffer for 2 min between analyses. Also, the running buffer was replaced after 20 analyses. The capillary was cleaned on a weekly basis by washing with 0.1 *M*

sodium hydroxide for 10 min followed by de-ionised water for 10 min before filling with running buffer. Electropherograms were recorded with either the ICE Data Management and Control Software supplied with the Electropherograph or a HP 3350 Laboratory Data System (Hewlett-Packard, Palo Alto, CA, USA). Peak areas were used in the calculations.

HPLC

The analyses were performed with a Model 501 HPLC pump, Model 712 WISP and a Model 484 tunable absorbance UV detector using a 250 × 4.6 mm stainless-steel column filled with 10-μm Spherisorb CN packing material equipped with a CN pre-column. A mobile phase consisting of a 100:10:5 mixture of 1% w/v aqueous ammonium acetate (adjusted to pH 5.8 with 10% acetic acid), acetonitrile and dioxane at a flow-rate of 1.5 ml/min was used for the analyses. The compounds were detected at 254 nm at 0.05 AUFS.

Peak areas obtained from a HP 3350 Laboratory Data System (Hewlett-Packard) were used in the calculations.

2.4. Samples and standards for MEKC and HPLC

Samples

The crude morphine preparations were dissolved in 0.01 *M* HCl. For MEKC analysis, pholcodine was added as an internal standard at a final concentration of 0.4 mg/ml.

Opium and opium dross was extracted using the procedure of Srivastava and Maheshawari [13]. Essentially, 1 g of opium was extracted with 20 ml of 2.5% acetic acid and then made up to volume (100 ml) with water. For the opium dross, 1 g of sample was extracted with 2.5% acetic acid (5 × 20 ml) and the solutions combined. The solutions were filtered and 20 ml added to 60 ml of water. The pH of the solution was adjusted to 9.2 with concentrated ammonia solution and the solution was then extracted with chloroform (6 × 50 ml). The chloroform was dried over sodium sulphate and the solvent removed in vacuo with a rotary evaporator. The residue was dissolved in 5 ml of 0.05 *M* HCl. Then 1 ml of this solution was diluted to 5 ml with de-ionised water for MEKC and HPLC analysis. For MEKC analysis, pholcodine was added as an internal standard at a final concentration of 0.4 mg/ml.

The poppy straw was extracted with (a) lime-water, (b) 5% acetic acid solution and (c) soxhlet extraction with 30% ethanol in chloroform.

(a) Lime-water extraction [14]. An amount of 100 ml of de-ionised water was added to 8 g of finely milled poppy straw and 2 g of calcium hydroxide and the mixture shaken vigorously for 25 min. The mixture was filtered and 20 ml was diluted with 60 ml of de-ionised water. The pH of the solution was adjusted to 9.2 with 10% acetic acid and then extracted with 25% ethanol in chloroform (5 × 50 ml). Care had to be exercised to avoid the formation of emulsions. The combined extracts were washed with de-ionised water (2 × 30 ml) and then dried with sodium sulphate. The solvent was removed in vacuo with a rotary evaporator. The residue was dissolved in 25 ml of 0.05 *M* HCl. For MEKC analysis, 9 ml of the solution was diluted with 1 ml of internal standard solution (4 mg/ml) and filtered before analysis. For HPLC analysis, 1 ml of the solution was diluted to 5 ml with de-ionised water.

(b) Acetic acid (5%) extraction [13]. An amount of 100 ml of 5% acetic acid was added to 8 g of finely milled poppy straw and the mixture shaken vigorously for 25 min. The mixture was filtered and 20 ml was diluted with 60 ml of de-ionised water. The pH of the solution was adjusted to 9.2 with concentrated ammonia solution and then extracted with chloroform (5 × 50 ml). The combined extracts were washed with de-ionised water (2 × 30 ml) and then dried with sodium sulphate. The solvent was removed in vacuo with a rotary evaporator. The residue was dissolved in 25 ml of 0.05 *M* HCl. Then 1 ml of this stock solution was diluted to 5 ml with de-ionised water for HPLC analysis. For MEKC analysis, 9 ml of the stock solution was diluted with 1 ml of

internal standard solution (4 mg/ml) and filtered before analysis.

(c) ethanol in chloroform (30%) extraction. An amount of 8 g of finely milled poppy straw was extracted with 120 ml of 30% ethanol in chloroform in a soxhlet extractor for 6 h. Then 25 ml was removed and taken to dryness with a rotary evaporator. The residue was dissolved in 10 ml 0.05 *M* HCl. Then 2 ml of this stock solution was diluted to 5 ml with de-ionised water for HPLC analysis. For MEKC analysis, 0.5 ml of internal standard solution (4 mg/ml) was added to 2 ml of the stock solution and the volume made to 5 ml with de-ionised water and filtered before analysis.

Standards

Standard solutions were prepared in 0.01 *M* HCl for both MEKC and HPLC. The solutions were filtered before analysis. Pholcodine was added as the internal standard at a final concentration of 0.4 mg/ml for MEKC analyses. No internal standard was used for the HPLC analyses.

3. Results and discussion

The separation and quantitation of the components of illicit heroin seizures (heroin, acetylcodene, O^6-monoacetylmorphine, morphine, codeine, papaverine and narcotine) by MEKC was accomplished using a 75 cm × 75 μm I.D. uncoated fused-silica capillary column with a buffer consisting of 10% acetonitrile–90% 0.05 *M* CTAB, 0.01 *M* potassium dihydrogen orthophosphate, 0.01 *M* sodium tetraborate, pH 8.6 [1]. Morphine and codeine, the major alkaloids found in *Papaver somniferium* poppies, were reasonably well separated, as were the alkaloids papaverine and narcotine. Thebaine and oripavine are two other important alkaloids found in various amounts in poppy straw, opium and crude morphine preparations [12,14]; however, they are not found in illicit heroin seizures as they rearrange when the crude morphine/opium is converted to heroin with hot acetic anhydride [15]. Narceine, cryptopine and salutaridine are also present in various amounts in *Papaver somniferium* poppies. Initial attempts to separate the alkaloids on a 50-μm uncoated fused-silica column with the buffer used for the analyses of illicit heroin seizures were encouraging. Increasing the amount of acetonitrile to 12.5% resulted in near-baseline separation of the alkaloids (Fig. 1C). The separation of the alkaloids, except for oripavine and cryptopine, was maintained over twenty repetitive injections. Pholcodine was well separated from the other alkaloids and was therefore suitable for use as the internal standard. Other organic modifiers were then tried in an attempt to achieve baseline separation of the alkaloids. Optimum separation was achieved when the acetonitrile was replaced with 27.5% methanol as the modifier (Fig. 1B). The separation was maintained over twenty repetitions. With this buffer, codeine migrated before morphine, however, the separation of oripavine and thebaine was not as pronounced. The run time for the separation increased from 10 to 15 min with this buffer. Dioxane and tetrahydrofuran were unsuitable as organic modifiers as morphine and codeine could not be completely separated. The buffer containing tetrahydrofuran was particularly unsuitable as the migration times of the alkaloids changed dramatically over repeated injections of the standard solution.

One of the features of CZE is that the buffers are transparent in the low UV range (210–220 nm). Detection at these wavelengths allows for increased sensitivity and has been exploited on a number of occasions [8]. Acetonitrile, methanol, dioxane and tetrahydrafuran are often the first choices as organic modifiers as buffers containing these solvents are UV transparent at 210–220 nm. Dimethylformamide, dimethylsulphoxide and hexamethylphosphoramide can also be used as organic modifiers but absorb UV light in the low UV range (<240 nm) and so have limited use when optimum sensitivity is required. In this work, the alkaloids were detected at 254 nm and so these compounds could be used as organic modifiers. Various amounts of these solvents with the CTAB buffer were tried. Optimum separation of the alkaloids was obtained with buffers

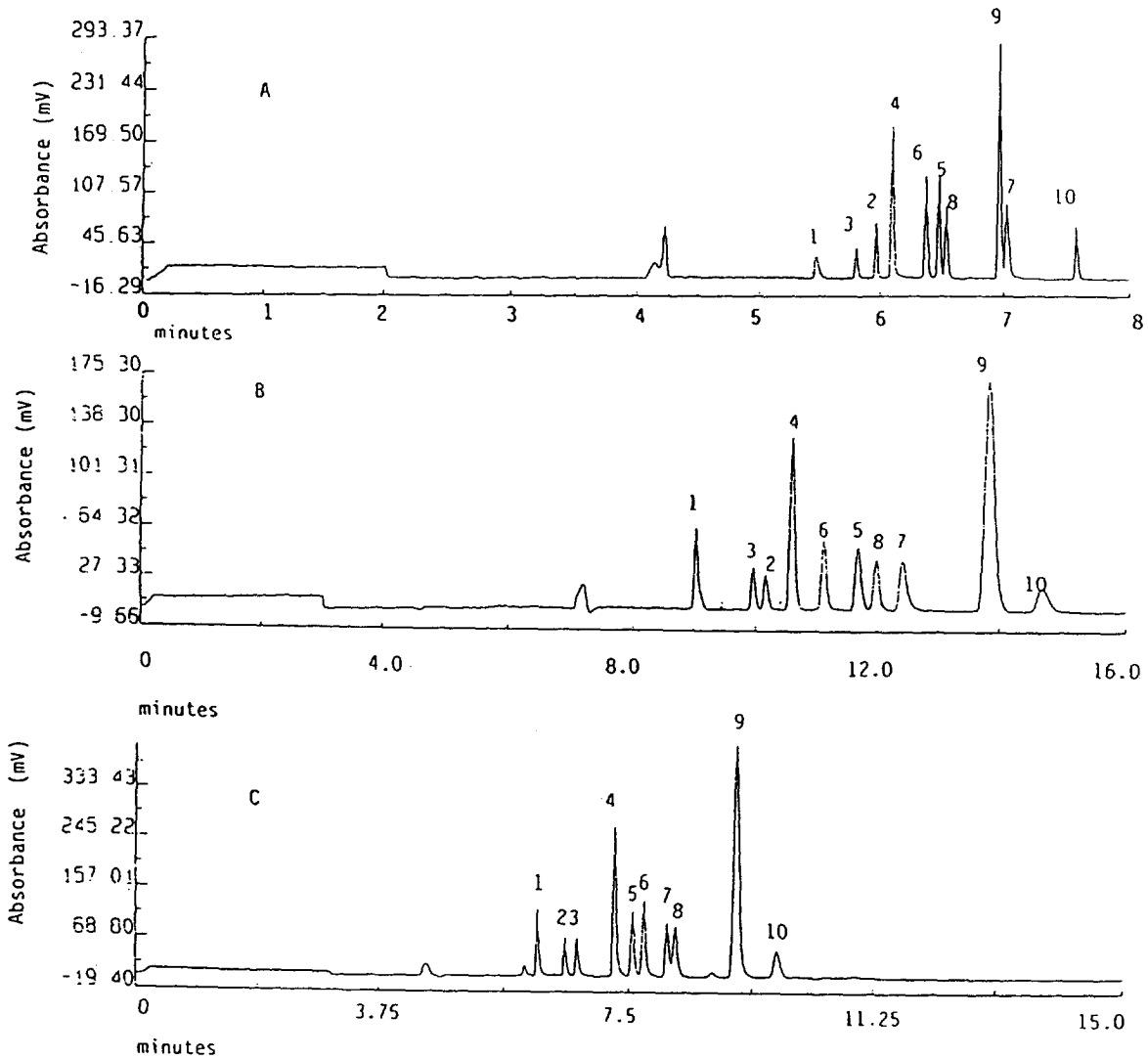

Fig. 1. Separation of (1) pholcodine, (2) morphine, (3) codeine, (4) salutaridine, (5) oripavine, (6) cryptopine, (7) narceine, (8) thebaine, (9) papaverine and (10) narcotine using a mixture of (A) 10% dimethylformamide (DMF) and 90% of a buffer consisting of 0.05 *M* CTAB and a 1:1 mixture of 0.01 *M* potassium dihydrogen orthophosphate and 0.01 *M* sodium tetraborate, pH 8.6, (B) 27.5% CH_3OH and 72.5% of a buffer consisting of 0.05 *M* CTAB and a 1:1 mixture of 0.01 *M* potassium dihydrogen orthophosphate and 0.01 *M* sodium tetraborate, pH 8.6, and (C) 12.5% CH_3CN and 87.5% of a buffer consisting of 0.05 *M* CTAB and a 1:1 mixture of 0.01 *M* potassium dihydrogen orthophosphate and 0.01 *M* sodium tetraborate, pH 8.6.

containing 20% dimethylsulphoxide, 5% hexamethylphosphoramide and 10% dimethylformamide. The buffer containing 10% dimethylformamide as the organic modifier gave the best separation of the ten alkaloids (Fig. 1A). This separation was extremely reproducible over twenty repetitive injections of a standard solution. Also, the separation of the alkaloids was quite consistent over the pH range 8.4 to 8.8, except for narceine, which migrated before papaverine below pH 8.5, and oripavine, which comigrated with cryptopine at pH 8.5 (Fig. 2). Minimal separation was seen when both dimethylformamide and CTAB were absent from the buffer. The addition of 0.05 *M* CTAB gave partial separation of the alkaloids, but complete

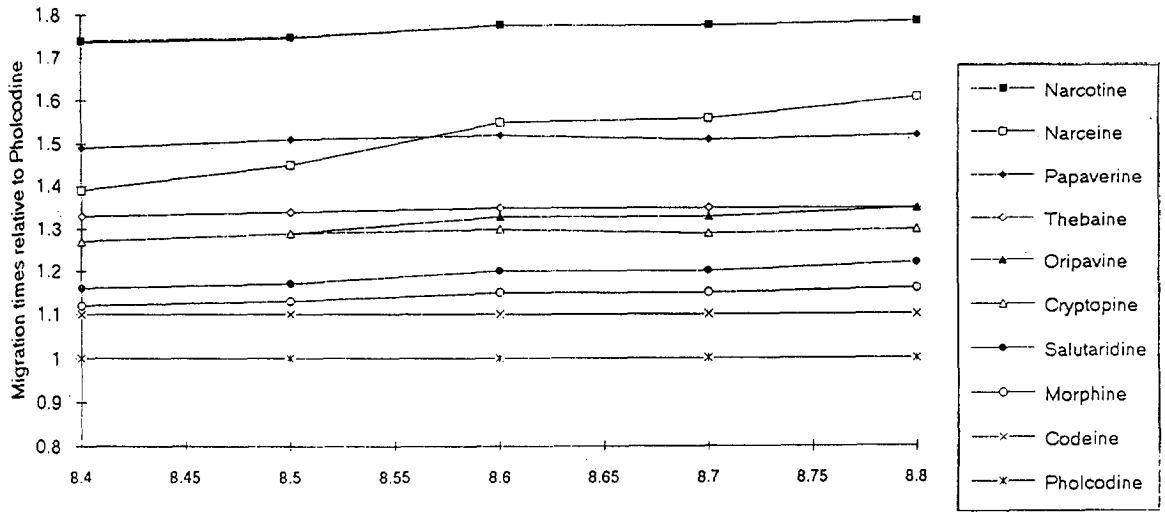

Fig. 2. Migration times of opium alkaloids relative to the internal standard using a buffer consisting of 10% DMF and 90% of 0.05 *M* CTAB and a 1:1 mixture of 0.01 *M* potassium dihydrogen orthophosphate and 0.01 *M* sodium tetraborate, pH 8.6. Results show change in migration times with variations of buffer pH.

separation was not achieved until the buffer contained 10% dimethylformamide.

The instrument repeatability data for area calculation (%C.V.) for seven consecutive injections of a number of standards of different concentrations were acceptable and are displayed in Table 1.

A number of crude morphine preparations that were available from a previous study [15] were analysed by MEKC and by HPLC [10]. The levels of the various alkaloids in the samples and the instrument repeatability for area calculation (%C.V.) data for seven repetitive injections for one sample are listed in Table 2. In general, there was a good agreement for the levels of the alkaloids determined by MEKC and HPLC in the samples. The MEKC run time was much faster than the HPLC run time (12 compared to 25 min). The amounts of the minor components of crude morphine preparations and the instrument repeatability data for area calculation (%C.V.) were more accurately determined by analysing more-concentrated solutions. The excellent separation of the alkaloids was main-

Table 1
Coefficient of variation for area calculation (%C.V.) for MEKC for standard solutions of varying concentrations

Concentration (mg/ml)	C.V. (%)					
	Codeine	Morphine	Oripavine	Thebaine	Papaverine	Narcotine
0.01	–	–	10	12	4.3	–
0.02	10	8.0	3.6	6.1	4.4	7.8
0.05	4.8	4.3	2.4	2.1	1.5	3.5
0.1	2.4	1.7	2.4	1.7	1.8	1.8
0.2	–	–	1.4	3.2	0.9	2.7
0.5	3.8	2.5	–	–	–	–
1.0	2.4	2.9	–	–	–	–

Buffer consisting of 10% DMF and 90% 0.05 *M* CTAB and a 1:1 mixture of 0.01 *M* potassium dihydrogen orthophosphate and 0.01 *M* sodium tetraborate, pH 8.6. %C.V. data obtained from seven replicate injections.

Table 2
Comparison of the quantitative results (%) and the %C.V. for area calculation for MEKC and HPLC for samples of crude morphine, poppy straw, opium and opium dross using a buffer consisting of 10% DMF and 90% 0.05 *M* CTAB and a 1:1 mixture of 0.01 *M* potassium dihydrogen orthophosphate and 0.01 *M* sodium tetraborate, pH 8.6. %C.V. data obtained from seven replicate injections of standard solutions

Sample	Codeine (%)		Morphine (%)		Oripavine (%)		Thebaine (%)		Papaverine (%)		Narcotine (%)	
	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC
1-70	3.9	3.6	85.4	81.6	1.6	1.4	2.0	2.0	–	–	–	–
%C.V.[1]	9.0	0.6	4.3	0.3	4.1	0.8	6.4	0.9				
%C.V.[2]	1.8	–	–	–	0.9	–	1.0	–				
1-44C	5.5	5.9	62.4	58.2	1.6	1.9	1.4	1.4	–	–	–	–
1-49	1.0	0.8	67.3	61.9	–	–	–	–	–	–	–	–
1-54E	1.5	1.4	95.5	93.2	0.4	0.4	–	–	–	–	–	–
Poppy straw[3]	0.1	0.1	0.9	0.9	0.01	0.01	0.05	0.05	–	–	–	–
Poppy straw[4]	0.1	0.1	1.2	1.2	0.01	0.01	0.02	0.02	–	–	–	–
Opium[5]	5.3	4.7	11.5	9.9	–	–	2.0	1.7	0.5	0.4	0.4	0.6
Dross[5]	1.7	1.6	2.9	2.9	–	–	0.2	0.2	0.6	0.6	0.9	0.9

[1] 11.4 mg/10ml 0.01 *M* HCl.
[2] 51.5 mg/10ml 0.01 *M* HCl.
[3] Soxhlet extraction.
[4] Lime-water extraction.
[5] Acetic acid extraction.