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Short communication

Determination of opiate alkaloids in process liquors using capillary electrophoresis

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Abstract

This paper describes the determination of opiate alkaloids (morphine, codeine, oripavine and thebaine) in industrial process liquors using capillary zone electrophoresis with UV-absorption detection at 214 nm. A study of cyclodextrin type and concentration revealed that the addition of 30 mM hydroxypropyl-β-cyclodextrin to the electrolyte solution (100 mM Tris adjusted to pH 2.8) was suitable to resolve the four analytes of interest. Typical analysis time was 12 min and the limit of detection for each alkaloid was 2.5×10^{-6} M. The results for the proposed methodology were in good agreement with those of a conventional HPLC procedure. Under the same conditions, short-end injection was used to reduce the effective separation length from 41.5 to 8.5 cm, which allowed the determination of morphine and thebaine in process liquors within 2.5 min.

Keywords: *Papaver somniferum* alkaloids; Morphine; Codeine; Cyclodextrins

1. Introduction

The use of alkaloids from *Papaver somniferum* (opium poppies) has had a dichotomous impact on society. Although opiate alkaloids have an important place in medicine, the illegal trafficking and abuse of heroin (the diacetyl derivative of morphine) has become a widespread problem [1]. Therefore, there is a need to accurately determine opiate alkaloids and their derivatives in a diverse range of samples [1–19] (Table 1). Due to the complex nature of these matrices, separation techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC) or capillary electrophoresis (CE) are normally required [1]. Previous authors have described CE as an attractive option for the separation of opiate alkaloids, particularly as it offers greater resolution and faster separation than HPLC, and does not require analyte derivatisation [2,11,15]. Published CE methods for the determination of opiate alkaloids mainly focus on forensic science applications, but the pharmaceutical industry will also benefit from analytical methodologies that will improve the efficiency of the extraction process. We have already reported several analytical methods for monitoring opiate alkaloid processing [6,20–22].

Opiate alkaloids, including codeine, thebaine, morphine, oripavine and pseudomorphine (Fig. 1), are extracted and purified from poppy straw at a GlaxoSmithKline facility using a series of alkaline, acidic and solvent extractions. Codeine is also produced by methylation of morphine. To optimize extraction yield, alkaloid concentrations must be monitored at numerous stages of this industrial process. HPLC with UV-absorbance detection has been routinely used for this purpose [23]. The samples have high ionic strengths and contain numerous structurally related opiates due to the *P. somniferum* biosynthetic pathway and the extraction process itself. We have previously examined capillary electrophoresis with chemiluminescence detection for the determination of opiate alkaloids in process liquors [6,24]. Complete separation of all analytes was not required due to the highly selective mode of detection, but two different chemiluminescence reagents were needed to determine all compounds of interest.

The addition of a suitable cyclodextrin to the electrolyte solution has been shown in a number of studies to improve

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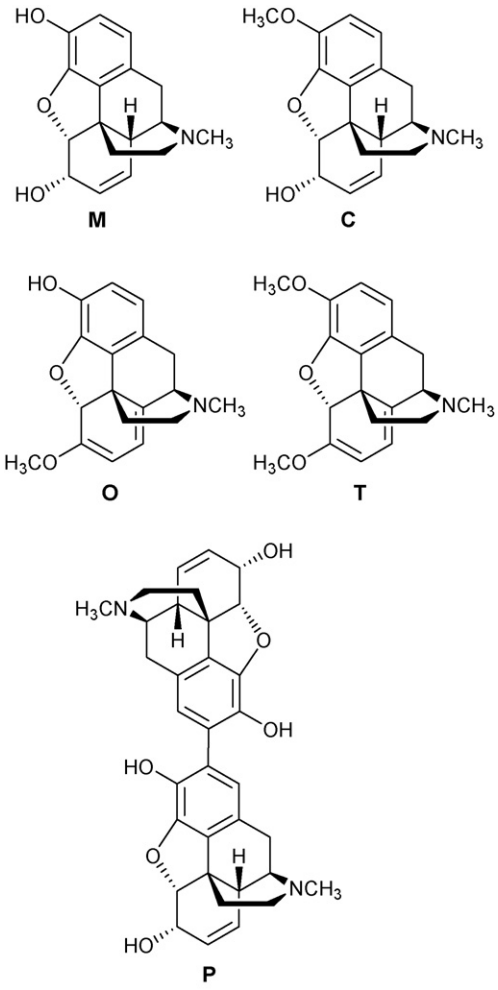

Fig. 1. Chemical structures of: (M) morphine; (C) codeine; (O) oripavine; (T) thebaine; (P) pseudomorphine.

the electrophoretic separation of certain alkaloids (Table 1), due to the formation of 'guest–host' complexes between the solute and buffer additive that change the migration rate of the solute molecules [4,15]. For example, we have found that α-cyclodextrin provided sufficient separation of morphine, oripavine and pseudomorphine in process liquors when coupled with selective chemiluminescence detection [6]. Proksa found that γ-cyclodextrin gave the highest resolution of epimers of morphine and codeine *N*-oxides, but heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (dimethyl-β-cyclodextrin) was superior for the separation of the mono- and di-*N*-oxides of pseudomorphine and 2,2′-biscodeine [25]. Bjørnsdottir and Hansen examined the addition of surfactants (above the critical micelle concentration) and cyclodextrins to establish the optimum conditions for their capillary electrophoresis determination of morphine, codeine, papaverine, thebaine and noscapine in crude opium [4]. They found that dimethyl-β-cyclodextrin provided better resolution than other cyclodextrins and the micellar electrokinetic chromatography approach. Lurie et al. [2] reported that a mixture of dimethyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin imparted excellent selectivity for opiate alkaloids in opium gum and latex samples using dynamically coated capillaries. Other authors have shown that the addition of β-cyclodextrin to the run buffer was suitable for the determination of heroin metabolites in urine extracts [15] and the components of clandestine heroin preparations [10]. We have, therefore, investigated the influence of different cyclodextrins to achieve the optimum selectivity for the determination of morphine, codeine, oripavine and thebaine in process liquors.

2. Experimental

Samples were separated using a HP ^{3D}CE (Agilent Technologies; Forest Hill, Victoria, Australia) with fused silica capillaries (50 μm i.d., 365 μm o.d., 50 cm in length; Polymicro Technologies, Phoenix, Arizona, USA) and UV-absorbance detection at 214 nm (bandpass of 20 nm, no reference wavelength). Samples were injected using a pressure of 50 mbar for 2 s; 25 kV was applied for separation. The length to the detection window was 41.5 cm. The electrolyte chosen for this study was based upon the work of Proksa [25] and consisted of tris(hydroxymethyl)aminomethane (Tris, 100 mM) adjusted to pH 2.8 with phosphoric acid (3 M). The capillary was preconditioned prior to each run by flushing it with the separation buffer for 2 min. Rich extract (RE) and partially spent extract (PSE) liquors were diluted 10-fold and rich solvent (RS) liquors were diluted 50-fold, in each case using an acetic acid solution (10%, v/v). Peaks were initially identified by spiking standard mixtures with a single alkaloid; migration time was used thereafter.

All reagents were analytical grade and solutions were prepared with deionised water (MilliQ system; Millipore, Bedford, MA, USA). Tris(hydroxymethyl)aminomethane and phosphoric acid were obtained from Ajax (Sydney, NSW, Australia). Hydroxypropyl-β-cyclodextrin was obtained from Aldrich (Milwaukee, WI, USA) and the α-, β- and γ-cyclodextrin were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). All buffer and sample solutions were filtered through a 0.45 μm nylon membrane (Advantec MFS; Pleasanton, CA, USA) prior to each analysis. Alkaloids and process samples were obtained from GlaxoSmithKline (Port Fairy, Victoria, Australia).

3. Results and discussion

The alkaloids of interest have very similar size-to-charge ratios under acidic conditions and are difficult to separate by capillary zone electrophoresis. Numerous workers have successfully separated alkaloids by adding a suitable cyclodextrin to the electrolyte (Table 1). We examined the effect of cyclodextrin type on the separation of a standard mixture (1×10^{-4} M) of five alkaloids and the results are shown in Fig. 2. Only α-cyclodextrin and hydroxypropyl-β-cyclodextrin were capable of resolving the five alkaloids whereby migration order correlated with decreasing polarity of the analytes. β-Cyclodextrin and γ-cyclodextrin afforded different selectivities, which were attributed to a change in the stability of their alkaloid inclusion complexes. The best separation was achieved with hydroxypropyl-β-cyclodextrin, which was used in all subsequent experiments.

Table 1
Some capillary electrophoresis methods for the determination of opiate alkaloids and their derivatives

Sample	Analyte(s)	Preparation	CE method	Run time (min)	Detection	Reference
Opium gum and latex	Morphine, codeine, thebaine, heroin, papaverine, noscapine	Dilution	CZE (coated capillary; 75 mM DM-β-CD and 25 mM HP-β-CD)	12	UV-DAD	[2]
Gum opium	Morphine, codeine, thebaine, papaverine, narcotine	LLE	CZE	23	UV	[3]
Crude opium and pharmaceuticals	Morphine, codeine, thebaine, papaverine, noscapine	LLE	CZE (30 mM DM-β-CD)	12	UV-DAD	[4]
Crude morphine, opium and poppy straw	Morphine, codeine, oripavine, thebaine, papaverine, narcotine	LLE	MEKC (50 mM CTAB)	10	UV	[5]
Process liquors	Morphine, oripavine, pseudomorphine	Dilution	CZE (80 mM α-CD)	5	CL	[6]
Pharmaceuticals	Morphine	Dilution	CZE (non-aqueous)	12.5	UV-DAD	[7]
Pharmaceuticals	Codeine, 6-methylcodeine, thebaine, dichlorovinylmorphine, biscodeine	Dilution	CZE	12	UV	[8]
Seized drugs	Heroin	Dilution	MEKC (25 mM SDS), short-end injection	1.5	UV-DAD	[9]
Illicit heroin	Morphine, monoacetylmorphine, heroin, codeine, acetylcodeine, papaverine, narcotine	LLE	CZE (20 mM β-CD)	10	UV	[10]
Heroin	Heroin, O^3- and O^6-acetylmorphine, morphine, codeine, acetylcodeine, papaverine, noscapine	Dilution	MEKC (103 mM SDS)/CZE (100 mM DM-β-CD) (coated capillaries)	15	UV-DAD	[11]
Stored morphine	Morphine-*N*-oxide, pseudomorphine, 10-*S*-hydroxymorphine	Dilution	CZE (5 mM DM-β-CD)	12	UV-DAD	[12]
Plasma	Morphine	Direct injection	CZE/MEKC (60 mM SDS)	6	UV	[13]
Urine	Codeine, morphine	Dilution	CZE	14	EC	[14]
Urine	Morphine, normorphine, 6-acetylmorphine codeine	SPE	CZE (15–20 mM β-CD)	15	UV, LIF	[15,16]
Urine	Morphine, codeine, norcodeine, nordihydrocodeine, dihydrocodeine and alkaloid glucuronides	Dilution, SPE or LLE	CZE	9	Multiple MS	[17,18]
Human hair	Morphine	LLE	CZE	10	UV	[19]

CZE: capillary zone electrophoresis; MEKC: micellar electrokinetic chromatography; LLE: liquid–liquid extraction; SPE: solid-phase extraction; DM-β-CD: dimethyl-β-cyclodextrin (heptakis(2,6-di-*O*-methyl)-β-cyclodextrin); HP-β-CD: hydroxypropyl-β-cyclodextrin; CTAB: cetyltrimethylammonium bromide; SDS: sodium dodecylsulfate; UV-DAD: ultraviolet absorbance-diode array detector; CL: chemiluminescence; EC: electrochemical; LIF: laser-induced fluorescence.

Cyclodextrin concentration affects the resolution of alkaloids by CE [4,10,12,15]. In the studies shown in Table 1, a wide range of cyclodextrin concentrations have been found to be optimum or sufficient to achieve the desired separation (from 5 mM [12,26] to 100 mM [11]), which is not surprising due the diverse range of conditions and applications. We, therefore, examined the influence of hydroxypropyl-β-cyclodextrin concentration (between 10 mM and 30 mM) on the separation of pseudomorphine, morphine, codeine, oripavine and thebaine. The greatest resolution was obtained with 30 mM hydroxypropyl-β-cyclodextrin.

The calibration functions for morphine, codeine, oripavine and thebaine approximated linearity ($R^2 \geq 0.9998$) from 1×10^{-5} M to 1×10^{-3} M and the detection limit ($3 \times$ S/N) for each of these analytes was 2.5×10^{-6} M. Under these conditions, the precision of the migration time and peak area, expressed as the percentage relative standard deviation, for six replicate injections of a thebaine standard (1×10^{-4} M) were 0.6% and 1.5%, respectively.

Nine process samples, including rich extract (RE), partially spent extract (PSE) and rich solvent (RS) liquors, were obtained from GlaxoSmithKline and analysed for morphine, codeine, oripavine and thebaine with the capillary electrophoresis methodology outlined above (Fig. 3) and reversed-phase ion-pairing HPLC with gradient elution and UV-absorption detection [23]. As shown in Table 2, the alkaloid concentrations varied significantly in the different process liquors. The concentration of thebaine ranged from approximately 0.32 mM to 33 mM, but in spite of this high variation there was good correlation between the results of the two techniques. Morphine was detected in two of the three process liquor types, where it was present at relatively high concentrations (between 5.6 mM and 6.7 mM) and reasonable agreement between the two techniques was obtained. The concentration of codeine was considerably lower than that of thebaine and morphine in all of the process liquors, ranging from 0.07 mM and 1.17 mM. For six of the nine samples, the results compared favourably. However, for

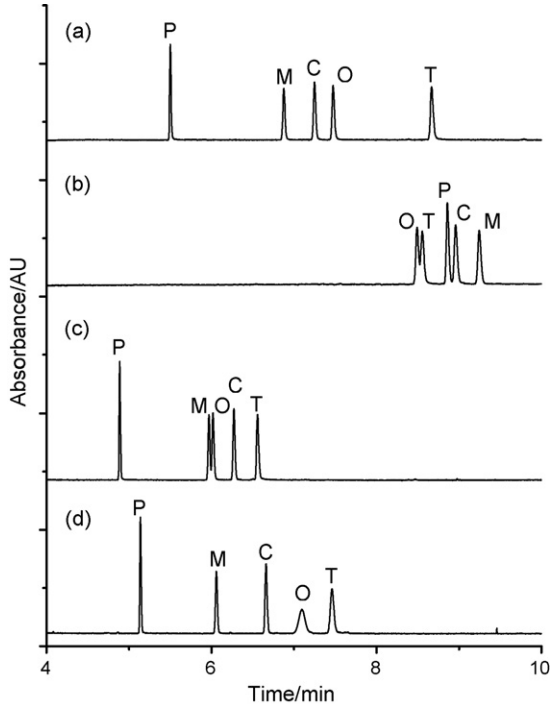

Fig. 2. Influence of (a) hydroxypropyl-β-cyclodextrin; (b) γ-cyclodextrin; (c) β-cyclodextrin; (d) α-cyclodextrin on the separation of (P) pseudomorphine; (M) morphine; (C) codeine; (O) oripavine; (T) thebaine at 1×10^{-4} M. Conditions: 100 mM Tris–phosphate, pH 2.8; cyclodextrin concentration of 30 mM except for β-cyclodextrin (10 mM due to limited solubility); applied voltage of 25 kV; 2 s injection at 50 mbar; 20 °C; capillary 50 cm × 50 μm i.d.; UV-absorption detection at 214 nm.

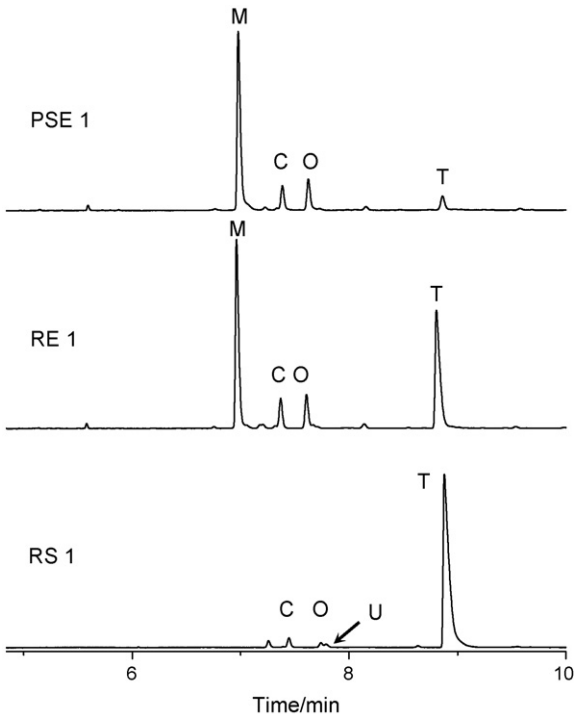

Fig. 3. Electropherograms of three process liquors obtained using capillary electrophoresis with UV-absorption detection at 214 nm. Peak identities were: (M) morphine; (C) codeine; (O) oripavine; (T) thebaine; (U) unknown impurity.

samples PSE1, PSE3 and RE1, the HPLC results were 16%, 67% and 92% lower than those obtained with CE. It is possible that the CE methodology provided a closer estimate of the actual concentration in these samples, as only small variations in the concentration of each alkaloid occurred within each process sample type, with the exception of the HPLC results for codeine in the PSE and RE liquors. With regard to oripavine, the relatively large differences between the results for the RS samples were attributed to co-migration of a related alkaloid, as several additional small peaks were observed in the HPLC chromatograms.

The proposed CE methodology had a typical analysis time of 12 min, which was four times faster than the HPLC procedure; a significant improvement in terms of process monitoring. Nevertheless, short-end injection was evaluated to further reduce analysis time. As the minimum capillary length on commercial instruments is fixed (approximately 32 cm for the HP ^{3D}CE), the shortest effective length to the detector using conventional injection is approximately 23.5 cm. However, as demonstrated by Altria et al. [27], the effective length can be further reduced by injecting the sample at the capillary end nearest to the detector and reversing the polarity of applied voltage. This 'short-end injection' was used by Bjørnsdottir and Hansen [28] for the rapid separation of illicit drug substances in a non-aqueous medium, and by

Table 2
Comparison of alkaloid concentrations in nine process liquor samples, determined by standard HPLC, CE, and CE with short-end injection

	Thebaine (mM)			Morphine (mM)			Codeine (mM)		Oripavine (mM)	
	HPLC	CE	CE[a]	HPLC	CE	CE[a]	HPLC	CE	HPLC	CE
PSE 1	0.32	0.35	0.90	6.3	6.0	6.7	0.53	0.63	1.08	0.94
PSE 2	0.35	0.32	0.58	6.3	5.6	6.7	0.67	0.63	1.18	0.94
PSE 3	0.32	0.32	0.48	6.0	5.6	6.3	0.20	0.60	1.04	0.98
RE 1	3.5	3.5	3.8	6.0	6.0	7.0	0.07	0.80	1.24	0.98
RE 2	3.5	3.5	4.2	6.7	6.0	7.0	0.77	0.80	1.18	1.01
RE 3	3.2	3.5	3.8	6.0	5.6	6.7	0.67	0.70	1.14	0.98
RS 1	33.4	31.8	30.8	0	0	0	1.07	1.10	0.03	0.61
RS 2	28.6	30.2	30.8	0	0	0	1.14	1.14	0.03	0.64
RS 3	31.8	30.2	29.6	0	0	0	1.17	1.07	0.03	0.57

[a] Short-end injection.

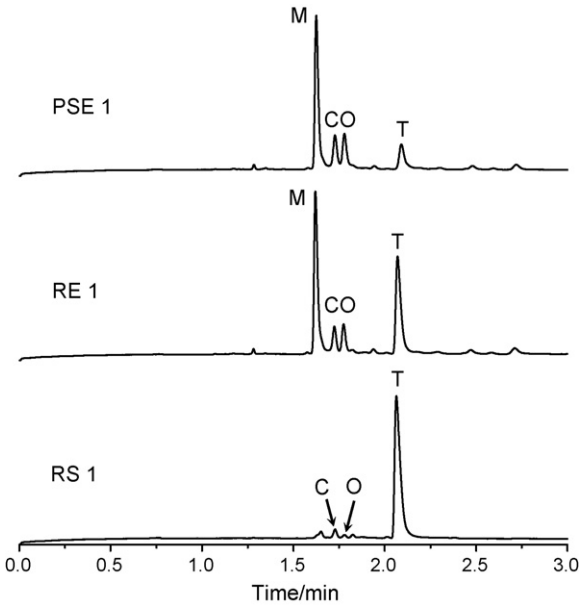

Fig. 4. Electropherograms of three process liquors obtained using capillary electrophoresis with short-end injection and UV-absorption detection at 214 nm. Peak identities were: (M) morphine; (C) codeine; (O) oripavine; (T) thebaine. Conditions: 100 mM Tris–phosphate, pH 2.8 with hydroxypropyl-β-cyclodextrin (30 mM); 20 °C; 2 s injection at −50 mbar; UV-absorbance detection at 214 nm, applied voltage of −25 kV; capillary 50 cm × 50 μm i.d. with an effective length of 8.5 cm.

Anastos et al. [9] for the analysis of heroin drug seizures with MEKC.

Using our instrumentation, short-end injection reduced the effective capillary length to 8.5 cm, which reduced the separation efficiency by approximately 85% and the resolution by 75%. However, a standard mixture of five alkaloids was separated in less than 2.5 min. The calibration functions for the short-end injection method were very similar to those of the normal approach: approximately linear ($R^2 \geq 0.9992$) from 1×10^{-5} M to 1×10^{-3} M, but the reduced separation length minimised analyte diffusion, which improved the limit of detection for the four alkaloids by a factor of 2.5. The relative standard deviation of the migration time and peak area for six injections of a thebaine standard (1×10^{-4} M) was 0.1% and 1.7%, respectively.

When this approach was applied to the analysis of industrial process liquors (Fig. 4), morphine, thebaine and pseudomorphine were sufficiently separated from endogenous compounds. The results for the determination of morphine and thebaine were compared to those obtained with HPLC (Table 2). Reasonable agreement was observed for thebaine concentrations in RE and RS liquors, but in PSE samples the results of the short-end injection capillary electrophoresis procedure were significantly higher than those obtained with HPLC. This discrepancy was attributed to incomplete separation of thebaine from an unknown compound, due to the shorter effective capillary length. For all samples, there was good agreement between the two methodologies for the concentration of morphine, which is the primary analyte of interest in this extraction process.

4. Conclusions

The proposed capillary electrophoresis procedure is simple and robust, and is, therefore, suitable for the determination of opiate alkaloids in an industrial process setting. It is four times faster than the standard HPLC procedure, which is a significant advantage in terms of process monitoring. Furthermore, the same conditions could be used with short-end injection to determine morphine and thebaine (and provide semi-quantitative information on other alkaloids) on a near-continuous basis.

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