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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY SYSTEMS FOR THE SEPARATION OF LOCAL ANAESTHETIC DRUGS WITH APPLICABILITY TO THE ANALYSIS OF ILLICIT COCAINE SAMPLES

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SUMMARY

Two high-performance liquid chromatography (HPLC) systems are presented which are suitable for the separation of local anaesthetic drugs. An octadecyl-silica column is used with aqueous methanolic eluents (15 and 50% respectively) containing *n*-hexylamine–orthophosphoric acid buffers and data for 36 compounds are given. The HPLC systems have application for the identification of unknown drugs in this class while the first eluent (15% methanol, pH 2.5) is particularly useful for the examination of illicit cocaine samples because it gives good separation of common adulterants and impurities.

INTRODUCTION

Samples relating to cocaine abuse are frequently submitted to forensic laboratories so that rapid methods are required for the identification of this drug. Illicit samples of cocaine are rarely pure, often containing other local anaesthetics as adulterants (*e.g.* benzocaine, lignocaine and procaine) along with impurities arising from the manufacturing process (cinnamoylcocaine, benzoylecgonine and benzoic acid). In consequence, chromatographic methods play an important role in the analysis of these samples. Recent reviews[1–4] have considered these methods and include references to the analysis of cocaine and its metabolites (*e.g.* benzoylecgonine) in biological fluids as well as in illicit samples.

High-performance liquid chromatography (HPLC) is particularly suitable for the separation of polar, non-volatile compounds or those which are thermally unstable and this includes many of the local anaesthetics. HPLC methods for the examination of illicit cocaine samples[5–8] and others for the analysis of cocaine and its metabolites in biological fluids[9–12] have been reported. HPLC has also been used to separate the four diastereoisomeric forms of cocaine which can arise by total synthesis[13,14] and groups of local anaesthetics have also been separated[15,16]. Specific HPLC assays for benzocaine[17,18] lignocaine[19–21] and procaine[22] in pharmaceutical products and lignocaine in biological fluids[23–31] have been described.

Recent work in our laboratory has involved the rationalisation of HPLC systems so that only a small range of different packing materials need be maintained in stock. Experience has shown that most HPLC separations can be achieved using either silica or octadecyl-silica (ODS-silica). HPLC eluents for barbiturates[32,33] amphetamines[34], benzodiazepines[35] and tricyclic anti-depressants[36] on a single brand of ODS-silica (5 μm) have been developed. The present paper describes two isocratic HPLC systems using the same ODS-silica column packing material suitable for the chromatography of local anaesthetic drugs. The eluents have been developed to give good peak shapes and retention data have been measured for 36 compounds. The utility of the system is demonstrated for the analysis of illicit cocaine samples.

EXPERIMENTAL

Apparatus

HPLC was performed with a Waters M6000 pump, a Rheodyne 7125 injection valve (fitted with a 20-μl loop) and a Cecil CE272 variable-wavelength UV detector operated at 230 nm. The stainless-steel column (16 cm × 5 mm I.D.) was packed with 5 μm ODS-Hypersil (Shandon, Runcorn, U.K.) by a slurry procedure, dispersing the packing material in isopropanol with hexane as the pressurising solvent.

UV spectra of fractions eluting from the HPLC column were recorded in silica cells of 10 mm pathlengths using an SP8000 spectrophotometer (Pye Unicam, Cambridge, U.K.).

Materials

Methanol (HPLC-grade) was obtained from Rathburn Chemicals (Walkerburn, U.K.). Orthophosphoric acid (85%, Aristar) was obtained from BDH (Poole, U.K.).*n*-Hexylamine was puriss grade from Fluka (Fluorochem, Glossop, U.K.). All other chemicals used were of analytical grade.

All the local anaesthetics were from the drug collection of the Central Research Establishment, Home Office Forensic Science Service except for *trans*-cinnamoylcocaine which was obtained from the Metropolitan Police Forensic Science Laboratory.

Chromatography

Two eluents were necessary to cope with the wide range of polarities encountered for the group of local anaesthetics. The 15% (v/v) methanol eluent (Eluent A) was prepared by mixing methanol (300 ml), water (700 ml), 1% (v/v) phosphoric acid (1000 ml) and *n*-hexylamine (10.71 g; 14 ml). The 50% (v/v) methanol eluent (Eluent B) was prepared by mixing methanol (1000 ml), 1% (v/v) phosphoric acid (1000 ml) and *n*-hexylamine (10.71 g; 14 ml). The 1% (v/v) phosphoric acid was prepared by dissolving concentrated orthophosphoric acid (17 g) in distilled water (1000 ml). The pH values of the two eluents (A and B) were measured and found to be 2.5 and 2.8 respectively.

Eluent flow-rates of 2 ml/min were used in all experiments. Drug samples were dissolved in the appropriate eluent for injection onto the HPLC column. Retention data are expressed as capacity ratios, k', which are defined by $k' = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of the substance under investigation and a

non-retained compound respectively. In the present experiments t_0 was determined by the injection of methanol.

RESULTS AND DISCUSSION

The range of polarities demonstrated by the group of local anaesthetic drugs is too large to allow all compounds to be chromatographed with a single isocratic eluent. Two HPLC eluents were developed to provide good discrimination between compounds to facilitate drug identification. The two eluents (A and B) contain 15 and 50% methanol respectively and both contain an orthophosphoric acid–hexylamine buffer. When a single ODS-silica column was used with repeated changes between the eluents no deleterious effects on the chromatography were observed. Table I contains a list of retention data (k' values) for the more polar compounds using Eluent A, the data being presented to follow the elution order. Table II gives a similar list for the less polar compounds with Eluent B; the long-eluting compounds

TABLE I

HPLC RETENTION DATA FOR LOCAL ANAESTHETICS (ELUENT A)*

Compound	*Capacity ratio (k')*	*Co-eluting compounds*
Procaine	0	
Chloroprocaine	0.24	Butethamine
Butethamine	0.24	Chloroprocaine
Orthocaine	0.33	
Dimethocaine	0.79	Lignocaine
Lignocaine	0.79	Dimethocaine
Tolycaine	1.09	Propoxycaine, octacaine, mepivacaine
Propoxycaine	1.09	Tolycaine, octacaine, mepivacaine
Octacaine	1.09	Propoxycaine, tolycaine, mepivacaine
Mepivacaine	1.09	Octacaine, propoxycaine, tolycaine
Proxymetacaine	1.38	Prilocaine
Prilocaine	1.38	Proxymetacaine
Cocaine	2.68	Tropacocaine
Tropacocaine	2.68	Cocaine
Butanilicaine	4.42	
Piperocaine	4.59	
Leucinocaine	5.07	
Benzoylecgonine	5.68	
cis-Cinnamoylcocaine	6.30	
Amylocaine	7.19	Bupivacaine
Bupivacaine	7.19	Amylocaine
Benzamine	7.86	
Butacaine	8.97	
trans-Cinnamoylcocaine	10.65	
Oxybuprocaine	16.25	Amethocaine, amydricaine
Amydricaine	16.25	Amethocaine, oxybuprocaine
Amethocaine	16.25	Amydricaine, oxybuprocaine
Benzoic Acid	18.57	
Benzocaine	20.06	

* Methanol–water–aqueous phosphoric acid (1%, v/v)–*n*-hexylamine (30:70:100:1.4, v/v/v/v).

TABLE II

HPLC RETENTION DATA FOR LOCAL ANAESTHETICS (ELUENT B)*

Compound	*Capacity ratio (k')*	*Co-eluting compounds*
Benzamine	0.68	Amylocaine
Amylocaine	0.68	Benzamine
Oxybuprocaine	0.86	Bupivacaine
Bupivacaine	0.86	Oxybuprocaine
Amethocaine	1.33	Amydricaine
Amydricaine	1.33	Amethocaine
Benzocaine	1.61	
Diperodon	2.48	Pramoxine
Pramoxine	2.48	Diperodon
Dyclonine	2.78	
Oxethazaine	4.14	
Cinchocaine	5.51	
Cyclomethycaine	10.31	
Dimethisoquin	11.24	

* Methanol–aqueous phosphoric acid (1%, v/v)–*n*-hexylamine (100:100:1.4, v/v/v).

with Eluent A appear in both Tables. The list of compounds consists of local anaesthetic drugs, metabolites, and impurities known to occur in illicit cocaine samples. Table III gives the retention data with both Eluents A and B for an alphabetical listing of the compounds.

Fig. 1(i) shows the separation of a standard mixture using Eluent A consisting of procaine, lignocaine, cocaine, benzoylecgonine, *trans*-cinnamoylcocaine and benzocaine. These compounds which are commonly encountered in illicit cocaine samples show good peak shapes and baseline resolution. The separation of a standard mixture using Eluent B is shown in Fig. 2. This mixture consists of benzamine, oxybuprocaine, amethocaine, benzocaine, diperodon, oxethazaine, cinchocaine and cyclomethycaine. Once again the peak shapes of these non-polar compounds are very satisfactory.

Following the compilation of the retention data for the 36 compounds, those with similar k' values were co-chromatographed to give information on which compounds can not be separated with the two eluents. This information is also included in Tables I and II.

Reversed-phase HPLC systems using an ODS-silica column have been selected for the present work as they are generally robust and experience has shown that the retention data on such systems can be reliably transferred between laboratories. Most of the HPLC methods for local anaesthetics in the literature use similar hydrocarbonaceous bonded packing materials[5–31] and in many cases the chromatograms show poor peak shapes for the drugs. This phenomenon has often been encountered for basic drugs on bonded-phase silicas and has been attributed to the interaction of the compounds with the silica core of the packing material. Such problems can be overcome by the addition of amine modifiers to the mobile phase[37–39]. An orthophosphoric acid–hexylamine buffer was used in the present work as this amine has been shown to be effective at controlling peak shape in a previous study[39]. In preliminary

TABLE III

HPLC RETENTION DATA FOR LOCAL ANAESTHETICS (ALPHABETIC LISTING)

Compound	*Capacity ratios (k')*	
	*Eluent A**	*Eluent B***
Amethocaine	16.25	1.33
Amydricaine	16.25	1.33
Amylocaine	7.19	0.68
Benzamine	7.86	0.68
Benzocaine	20.06	1.61
Benzoic Acid	18.57	
Benzoylecgonine	5.68	
Bupivacaine	7.19	0.86
Butacaine	8.97	
Butanilicaine	4.42	
Butethamine	0.24	
Chloroprocaine	0.24	
Cinchocaine		5.51
cis-Cinnamoylcocaine	6.30	
trans-Cinnamoylcocaine	10.65	
Cocaine	2.68	
Cyclomethycaine		10.31
Dimethisoquin		11.24
Dimethocaine	0.79	
Diperodon		2.48
Dyclonine		2.78
Leucinocaine	5.07	
Lignocaine	0.79	
Mepivacaine	1.09	
Octacaine	1.09	
Orthocaine	0.33	
Oxethazaine		4.14
Oxybuprocaine	16.25	0.86
Piperocaine	4.59	
Pramoxine		2.48
Prilocaine	1.38	
Procaine	0	
Propoxycaine	1.09	
Proxymetacaine	1.38	
Tolycaine	1.09	
Tropacocaine	2.68	

* Methanol–water–aqueous phosphoric acid (1%, v/v)–*n*-hexylamine (30:70:100:1.4, v/v/v/v).
** Methanol–aqueous phosphoric acid (1%, v/v)–*n*-hexylamine (100:100:1.4, v/v/v).

work, a pH titration curve for 1% orthophosphoric acid with hexylamine was constructed (Fig. 3) such that the acceptable pH ranges for this buffer system could be assessed. The present eluents have low pH values (2.5 and 2.8 for Eluents A and B respectively) where the buffer system shows good pH control.

The usefulness of the HPLC system has been demonstrated through the examination of illicit cocaine samples using Eluent A. In all cases, sample preparation involved dissolution of the solid in the eluent followed by immediate injection. Fig. 1(ii) shows a chromatogram typical of an unadulterated, high purity illicit sample of

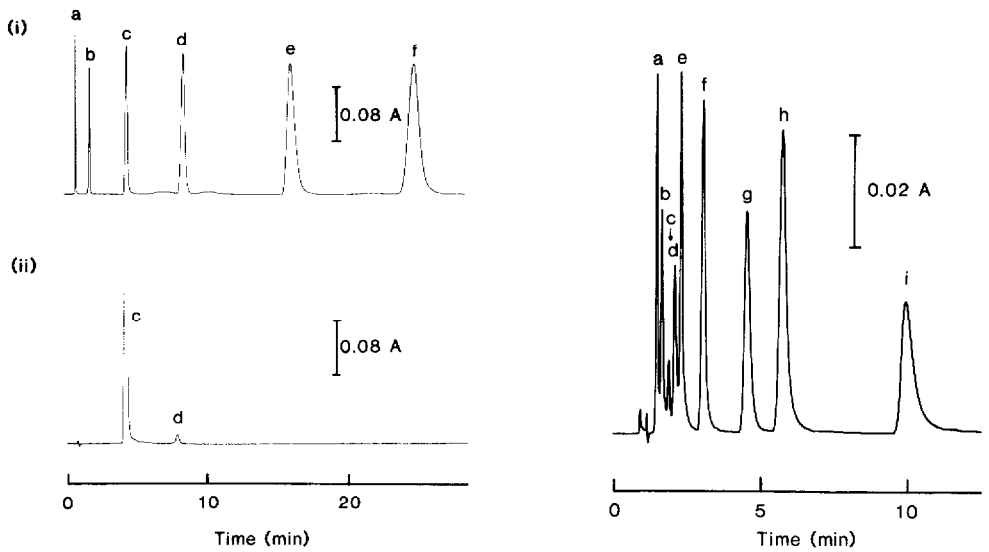

Fig. 1. Chromatography of local anaesthetics on ODS-silica using Eluent A: (i) standard mixture, (ii) relatively pure illicit cocaine sample. Column: ODS-Hypersil, 5 μm (160 × 5 mm I.D.).Eluent: 15% methanol containing hexylamine–orthophosphoric acid buffer (pH 2.5). Flow-rate: 2 ml/min. Detection: 230 nm. Peaks: a = procaine; b = lignocaine; c = cocaine; d = benzoylecgonine; e = *trans*-cinnamoylcocaine; f = benzocaine.

Fig. 2. Chromatography of a standard mixture of local anaesthetics on ODS-silica using Eluent B. Column: ODS-Hypersil, 5 μm (160 × 5 mm I.D.). Eluent: 50% methanol containing hexylamine–orthophosphoric acid buffer (pH 2.8). Flow-rate: 2 ml/min. Detection: 230 nm. Peaks: a = benzamine; b = oxybuprocaine; c = unknown decomposition product of oxethazaine in solution; d = amethocaine; e = benzocaine; f = diperodon; g = oxethazaine; h = cinchocaine; i = cyclomethycaine.

cocaine hydrochloride; this particular sample was a white crystalline solid. In addition to the main cocaine peak, a second minor peak corresponding to benzoylecgonine, was generally observed which may be a true impurity in the sample or arise from hydrolysis of the cocaine in solution before injection. However, the rate of cocaine hydrolysis at the eluent pH (2.5) is very low[40]. Fig. 4(i) shows a chromatogram from

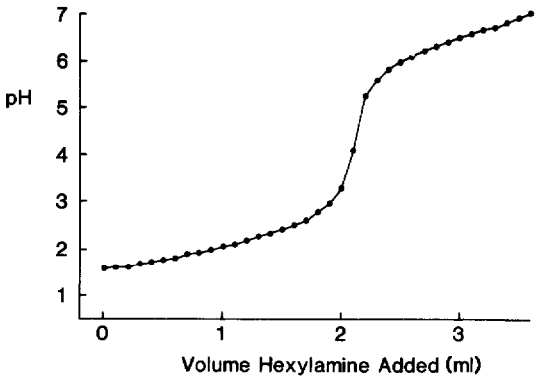

Fig. 3. Titration curve for orthophosphoric acid (1% aqueous solution; 100 ml) with *n*-hexylamine.

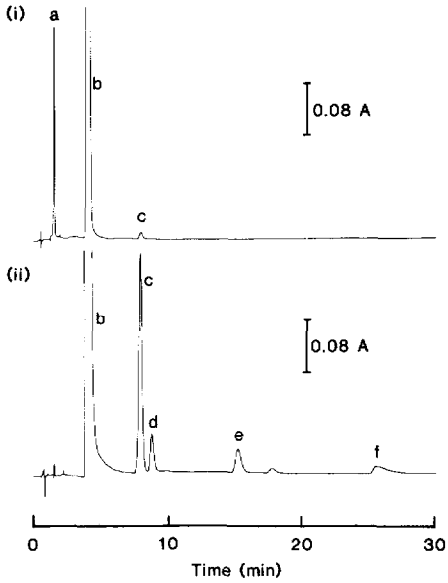

Fig. 4. Chromatography of illicit cocaine on ODS-silica using Eluent A: (i) sample adulterated with lignocaine; (ii) sample containing other coca alkaloids. Other conditions as in Fig. 1. Peaks: a = lignocaine; b = cocaine; c = benzoylecgonine; d = *cis*-cinnamoylcocaine; e = *trans*-cinnamoylcocaine; f = benzoic acid.

a sample of similar appearance to that described above which was found to have been adulterated with lignocaine.

Other samples were found to show complex chromatograms *e.g.* Fig. 4(ii). Generally, these samples were yellowish or brownish-white in colour although some coloured samples showed peaks for cocaine and benzoylecgonine only. Peak e was found to have the same retention time as *trans*-cinnamoylcocaine and this was confirmed by co-injection. Further confirmation of this peak identity was obtained by collecting the HPLC effluent and recording the UV spectrum for comparison with an authentic sample (Fig. 5). The UV spectra have λ_{max} values at 280 nm. A UV spectrum of Peak d (k' = 6.3) was also obtained (Fig. 6) and the compound was found to show a broad absorption peak with λ_{max} at 275 nm. This spectrum is very similar to that shown by Noggle and Clark[6] for *cis*-cinnamoylcocaine isolated from illicit cocaine samples and identified by mass spectrometry. They also report a λ_{max} of 275 nm. No authentic sample of this isomer was available to confirm the retention time of this peak but the presence of both isomers in cocaine samples has been widely reported[6–8,41]. These alkaloids are present in the coca plant from which most illicit cocaine is believed to originate and their presence or absence reflects the methods of extraction and purification employed[41]. Peak c (Fig. 4(ii)) was also collected and the UV spectrum in the eluent is shown in Fig. 6(ii) showing an absorption band with a λ_{max} at 234 nm; this further confirms the identity of the peak to be benzoylecgonine (λ_{max} 234 nm in 0.1 *N* sulphuric acid[42]). Peak f was found to have the same retention time as benzoic acid (k' = 18.6) by co-injection which has also been previously identified in illicit cocaine samples[6,7].

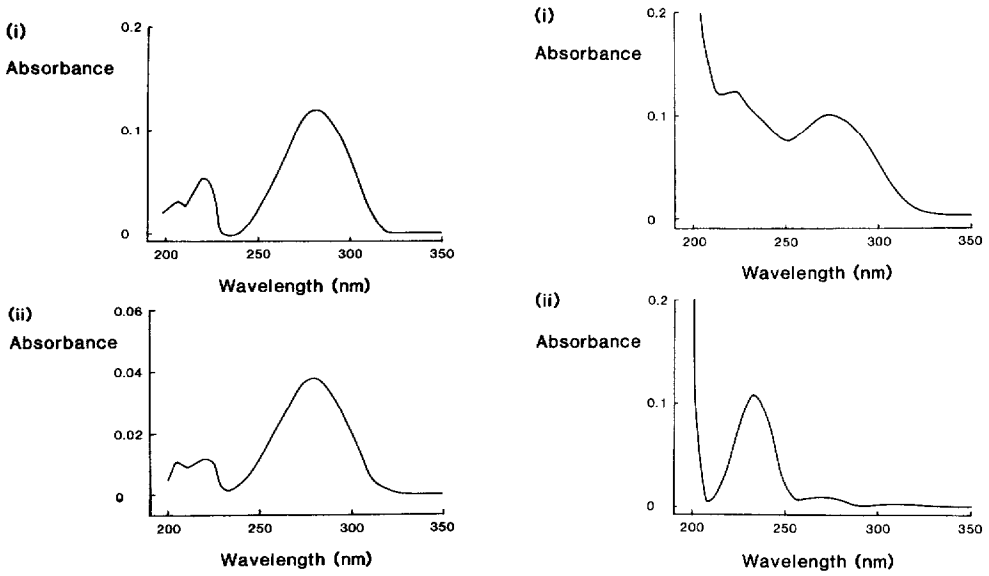

Fig. 5. UV-absorption spectra of *trans*-cinnamoylcocaine in Eluent A following collection of effluent from the HPLC column: (i) authentic sample; (ii) peak e in Fig. 4.

Fig. 6. UV-absorption spectra of impurities in illicit cocaine samples following collection of effluent from the HPLC column [Eluent A; chromatogram shown in Fig. 4(ii)]: (i) peak d = *cis*-cinnamoylcocaine; (ii) peak c = benzoylecgonine.

In conclusion, two HPLC systems using ODS-silica have been developed for the separation of local anaesthetics which are useful for the identification of these drugs. These systems give good peak shapes and hence are suitable for quantitative analysis. Furthermore, the 15% methanolic eluent (Eluent A) gives excellent separation of the components commonly encountered in illicit cocaine samples and should prove to be very valuable for the examination of illicit seizures for intelligence purposes in forensic casework.

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