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Reversed-phase high-performance liquid chromatography–photodiode-array analysis of alkaloid drugs of forensic interest

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Abstract

A reversed-phase high-performance liquid chromatography–photodiode-array method for the analysis of 23 drugs of forensic interest is presented. The separation method development was based on mobile-phase optimisation, temperature control and use of three ODS stationary phases. Multiwavelength detection and quantitation was performed at 225, 232, 239, 254, 275 and 289 nm. Absorbance ratioing proved to be very helpful for the identification of these drugs. Recognition of the analysed compounds was achieved by means of correlation of retention time and absorbance ratios. The method was directly applied to the analysis of illicit heroin and cocaine samples and to the analysis of pharmaceutical preparations containing codeine.

1. Introduction

Chromatography combined with a variety of detection modes is extensively used for the analysis of alkaloids of forensic interest [1]. HPLC is being adopted in an increasing number of toxicology laboratories due to its precision, reliability, sensitivity, flexibility and reduced needs for sample preparation. The HPLC analysis of opium alkaloids has been reported since the early 1970s, and the analysis of morphine in biological fluids has been extensively reviewed [2]. Normal-phase HPLC on silica [3–6] gave good results, mainly because of the excellent resolving power for a wide range of drugs [3] which allowed the characterisation of 462 compounds according to their retention and relative response data by UV (254 nm), fluorescence and electrochemical (+ 1.2 V) detection [3,4]. The use of basic eluents with unmodified silica as ion-exchange medium has also been reported [5,6], but many reproducibility problems still remain. Alumina has also been used as ion-exchange material for the analysis of basic drugs [7]. In principle the application of normal-phase methods on the analysis of biological fluids is limited, hence reversed-phase [8–14] and ion-pair HPLC [15,16] are mostly used. Phosphate buffers which are frequently used in the mobile phase in order to overcome peak broadening and tailing, also result in column and instrument deterioration and hamper the HPLC–MS combination. Cocaine and its metabolites are also very frequently analysed by HPLC. The most com-

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monly used mode is the analysis on the ODS column with an acidic eluent (pH 2.7–4.3, usually with addition of phosphate buffers) [17–20]. In the search for a better separation, apart from the typical silica-based C_8 and C_{18} columns, cyano [11], phenyl [13] and polystyrene divinylbenzene columns [14, 21] have also been used.

Detection is usually performed by UV [3–5,8–10,17,19,20], although fluorimetric [11], electrochemical [12–14,18] and chemiluminesence modes [22] offer a higher sensitivity and selectivity. UV detection is preferred, since it is more convenient and reproducible, does not require derivatisation and allows gradient elution. On the other hand, fluorescence detection of opium alkaloids and cocaine can be achieved only after derivatisation, and the simultaneous electrochemical detection of morphine and its metabolites is not possible due to the differences in their redox potentials [23]. Moreover amperometric detection can not be applied in urine, and cocaine shows no inherent activity in the typical HPLC conditions [18]. Combined UV and electrochemical detection allowed the simultaneous quantitation of some opium [13] and cocaine [21] alkaloids in low concentrations. Mass spectrometry detection is maybe the best technique for biotransformation studies, but its application is limited due to its cost and the numerous incompatibilities. The multiwavelength forward optical detector has also been used in the analysis of morphine metabolites, although its reproducibility proved doubtful [23].

Photodiode array detection compared to conventional UV detection offers increased selectivity, multiwavelength detection, automation etc. The identification of the analysed compounds is achieved by the comparison of their spectra or by absorbance ratioing. In a constant chemical system (mobile-phase composition and pH) absorbance ratios of a compound in two or more wavelengths become in fact the ratios of the extinction coefficients, which are characteristic of each compound, especially if more than two wavelengths are used. The recognition of unknown components by dual wavelength ratioing is hampered by instrumental limitations and by the influence of mobile-phase composition in the absorbance ratios [24]. Similar methods have also been applied in toxicological analysis, first by dual wavelength detection with the use of two conventional detectors in series. Using three solvent–column systems, 101 drugs were characterised by their relative retention times and their ratio of absorbance at 254 and 280 nm. Only 9% of these drugs could be distinguished by using only the retention data [25]. The analysis of by-products and impurities in illicit cocaine was achieved by dual detection at 215 and 277 nm, after an ion pair HPLC analysis [26]. Relative retention times and the absorbance ratios A_{220}/A_{260}, A_{230}/A_{260}, A_{280}/A_{260} and A_{320}/A_{260} were used for the identification of 28 common drugs [27]. The identification of diamorphine in illicit heroin samples was achieved by the absorbance ratioing A_{275}/A_{260}, A_{275}/A_{245} and A_{275}/A_{230} after a normal-phase HPLC analysis [28]. A standardised system for toxicological screening of 225 compounds recently reported, employed gradient RP-HPLC–diode-array detection and retention indices on the 1-nitroalkanes scale and absorbance data [29]. In another approach of toxicological screening a computer-assisted system was used for retention prediction and the identification of 65 drugs was achieved with a list of retention time, capacity factor, UV maximum and detection limit data [30]. In all the reported surveys multiwavelength detection is used for qualitative purposes, and quantitation is performed in one selected wavelength.

In this article we present the analysis of 23 drugs of forensic interest by reversed-phase HPLC and photodiode-array detection. The aim of this study was the development of an HPLC method for the analysis of the drugs that are usually employed in toxicological analyses in Greece. The proposed method should also allow the coupling of the HPLC with an MS detector, preferably through a thermospray interface, therefore ammonium acetate was selected as the mobile-phase additive, while phosphate buffers and amine modifiers were not used. The effect of the mobile-phase composition (nature and percent of the organic modifier) and analysis temperature on separation was also studied. Quantitation was performed at 225, 232, 239, 254, 275

and 289 nm. The method was applied to the analysis of illicit heroin and cocaine samples and pharmaceutical preparations containing codeine.

2. Experimental

2.1. Apparatus

The experiments were carried out in a Shimadzu (Kyoto, Japan) quaternary low-pressure gradient system. The solvent lines were mixed in a FCV-9AL mixer and an LC-9A pump was used to deliver the mobile phase to the column which was thermostated in a CTO-6A oven. Introduction of the samples on the column was achieved by a SIL-9A autosampler and detection was performed on a SPDM 6A photodiode-array detector. Chromatograms were stored on the hard disk of a Laptop 286 PC and printed on a Seikosha SP-1900 printer. Degassing of the solvents was achieved by ultrasonication under vacuum and continuous helium sparging in the solvent flasks through a DGU-2A degassing unit.

Separation of the alkaloids was made on the columns: (i) Spherisorb ODS-2, 250 × 4.6 mm I.D., 10 μm, from Spectra Physics (San José, CA, USA); (ii) Lichrosorb RP-18, 250 × 4.6 mm I.D., 10 μm from Alltech Associates (Deerfield, IL, USA); and (iii) Adsorbospher HS C_{18}, 250 × 4.6 mm I.D., 5 μm from Alltech. Data were analysed both on the Laptop 286 PC and on a Vip 386 PC.

UV spectra of the analysed compounds were obtained, in their solutions in the HPLC mobile phase, in a 1-cm cell on a Varian DMS 100S double-beam ultraviolet spectrophotometer (Sugar Land, TX, USA).

2.2. Materials

Morphine, codeine, 6-acetylmorphine, diamorphine, nalorphine, methadone, norcodeine, ethylmorphine, cocaine, benzoylecgonine and ecgonine methyl ester were obtained from Alltech as methanolic solutions with concentrations of 1000 ng/μl. Strychnine and papaverine were obtained from the Forensic Medicine and Toxicology Laboratory of the Aristotle University of Thessaloniki. Quinine sulphate was obtained from BDH Chemicals (Poole, UK). Analytical-grade caffeine, theophylline, theobromine, hyoscyamine, scopolamine hydrochloride and flufenamic acid were obtained from Sigma (St. Louis, MO, USA). Tolfenamic acid was from ELPEN (Athens, Greece). Amphetamine was a kind gift of the Greek State Laboratory. All the stock solutions were prepared by dissolving the appropriate amount in HPLC-grade methanol and kept refrigerated.

Ammonium acetate solutions were prepared by dissolving the appropriate quantity of the analytical-grade compound, which was obtained from Merck (Darmstadt, Germany), in double-deionised water. Glacial acetic acid was purchased from Merck. The aqueous buffers, after their preparation and pH adjustment, were filtered in a glass vacuum solvent filtration apparatus through a 0.2-μm Anodisk 47-mm glass filter (Alltech). All the organic solvents used in this study were of HPLC grade and obtained from Merck.

2.3. Calibration curves

Quantitation was performed separately in each of the chosen different wavelengths (225, 232, 239, 254 and 289 nm). This procedure is directly comparable to the analysis by two UV detectors in series or a multiwavelength UV detector whose signal is manipulated by a dual-channel integrator.

The analysis of opiate alkaloids was performed with flufenamic acid as the internal standard. The working standard solutions were containing morphine, codeine, 6-mono-acetylmorphine and diamorphine, at concentrations from 0.05 to 15 ng/μl. Papaverine concentrations were in the range 0.01 to 3 ng/μl, because this compound has a much higher extinction coefficient due to the many chromophores in its molecule. Flufenamic acid was present in all the solutions in a concentration of 2.5 ng/μl. Each standard mixture was analysed six times and the mean

values were used for the calculations. Quantitation was performed in the four wavelengths 225, 239 254 and 289 nm.

Analysis of coca alkaloids was performed using nalorphine as the internal standard. However, some other compounds that were tested (ethylmorphine, codeine) could also be used for that purpose. The working standard solutions contained cocaine and benzoylecgonine at concentrations from 0.025 to 15 ng/μl. Nalorphine was present in each solution at a concentration of 5 ng/μl. Each standard solution was analysed six times and the mean values were used for the construction of the calibration curves. Ecgonine methyl ester could not be detected, possibly due to the insufficient separation of the compound from the solvent peak. Another reason could be the lack of a chromophore in its molecule, after the elimination of the benzene ring, which resulted in a lower extinction coefficient. Quantitation was performed at 225, 232, 239, 254 and 289 nm.

Samples of heroin and cocaine were accurately weighed and dissolved in the mobile phase. After ultrasonication of the resulting solution, 20 μl of it were injected on column.

The method was also applied in the analysis of pharmaceutical preparations containing codeine phosphate. Feminax tablets were grounded and 0.1089 g of the product were dissolved in 250 ml of methanol–water (1:1). After ultrasonication of the final solution 20 μl of it were injected on column.

A 5-ml volume of Syrup Sival B was diluted to 250 ml with a mixture of methanol–water (1:1). The resulting solution was sonicated and 20 μl of it were injected on column.

3. Results and discussion

3.1. Mobile-phase composition

The three columns were tested with a variety of mobile phases in order to find out the best chromatographic system for the analysis of the alkaloids. The mobile phases were in principle ternary mixtures of an aqueous solution of ammonium acetate with methanol and acetonitrile (ACN). As it was expected, the best results were obtained on the Adsorbospher column, since this consists of a packing material specially treated for the analysis of basic compounds like alkaloids. Lichrosorb RP-18 which has the highest organic loading (22%) of the tested columns, gave also satisfactory results and it could be an alternative for the analysis. Peak shape, column efficiency and therefore resolution improved in the way Spherisorb < Lichrosorb < Adsorbospher. The Adsorbospher column showed the highest efficiency due to the smaller particle size and pore size (60 Å) and the highest area (350 m^2/g). As a rule an increase in the salt concentration in the mobile phase decreased retention and improved peak shape, selectivity and therefore resolution. The increase of the organic content decreased retention in all the columns, as it can bee seen in Fig. 1, where a plot of the retention times of the opium alkaloids versus the acetonitrile content is presented. Application of the same mobile phase in the three columns resulted in higher retention on the Spherisorb column, while retention on the Lichrosorb and the Adsorbospher column was similar. The biggest retention variations were observed for the apolar alkaloids like strychnine, diacetylmorphine, papaverine and quinine, which were strongly retained on the Spherisorb column with weak mobile phases. Polar alkaloids (caffeine, morphine, codeine) did not show such large retention variations on the three columns. Elution order changes were rarely observed with mobile-phase composition variations, either the organic content or the nature of the organic modifier. Relative retention times remained more or less constant in most of the cases and selectivity was not greatly affected by these variations, possibly due to the similarity of the analysed molecules.

The chromatographic system finally chosen was: analysis on the Adsorbospher column, with a mixture of MeOH–ACN–1.2% CH_3COONH_4, (40:15:45) as the mobile phase, at a flow-rate of 0.8 ml/min.

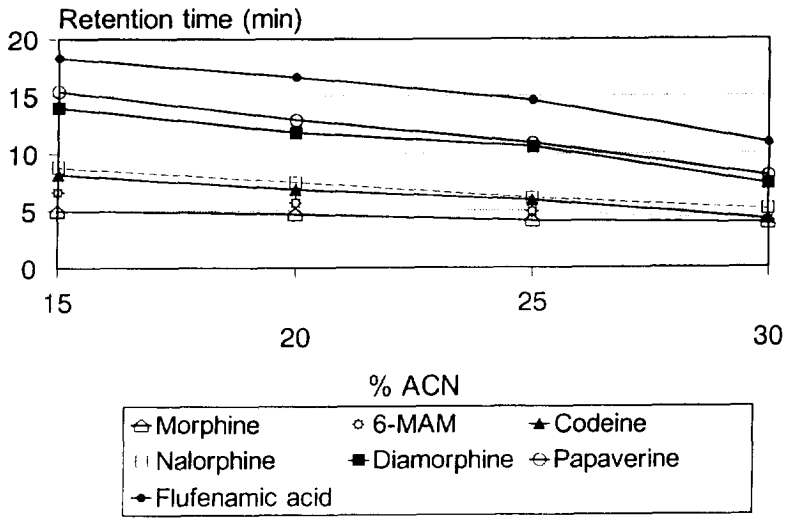

Fig. 1. Plot of the retention time of the alkaloids versus the ACN content in the mobile phase.

3.2. Absorbance ratioing

Absorbance ratioing is usually achieved with computer-assisted methods (mostly via software developed by the authors), that calculate the ratios automatically in the highest point of the chromatographic peak. This method is the most representative and precise of the absolute ratio; however, it has to deal with a diode-array detector combined with powerful software or an experienced analyst. In case of a two- or multi-wavelength detection, through a set of conventional detectors or even through a special multichannel UV detector, this ratioing can be achieved only by the ratioing of the peak areas or the peak heights obtained after integration. In this case, the most precise method for quantitative measurements is the ratioing of peak areas and not peak heights. Another advantage of this approach is that the measurement of peak areas is not so highly affected by the mobile-phase composition or by the flow effects on column as the measurement of the maximum absorbance does. Quantitation was performed at each of the selected wavelengths and the ratios of the peak areas were used as the absorbance ratios.

3.3. Selection of wavelengths

The wavelengths were selected in order to represent the points of maximum, minimum or inversion of the analysed compounds spectra. Detection at 225 nm enhances the sensitivity for all the compounds and represents a maximum for cocaine. At 239 nm the spectra show a turning upwards and there is a maximum for quinine. Undoubtedly, 254 nm is the most used wavelength in HPLC–UV detection and it also represents a minimum in the UV spectra of the compounds. The wavelength 289 nm represents a small maximum in the alkaloids spectra. Differentiation between cocaine and diamorphine is easily achieved in this wavelength where cocaine has a low absorbance. Especially for the analysis of cocaine and its metabolites, quantitation was also performed at 232 nm, since the results obtained at 289 nm were very poor.

3.4. Temperature effect on separation

Temperature affects both column efficiency and selectivity in HPLC. An increase in temperature will generally result in a decrease in

retention and a slight increase in selectivity. We actually observed these variations working in four different temperatures with various mobile phases: 22, 30, 45 and 60°C. A comparison of the resolution obtained after analysis in three different temperatures can be seen in Table 1. The increase in resolution is obvious at the higher temperatures. However, in terms of column protection and life time, analysis at 45°C was finally chosen as a compromise. The increase in analysis temperature resulted also in improved peak shape (reduced peak asymmetry) as can be seen in Table 2.

Typical chromatograms of the analysis of standard solutions of opium and coca alkaloids are given in Figs. 2 and 3, respectively.

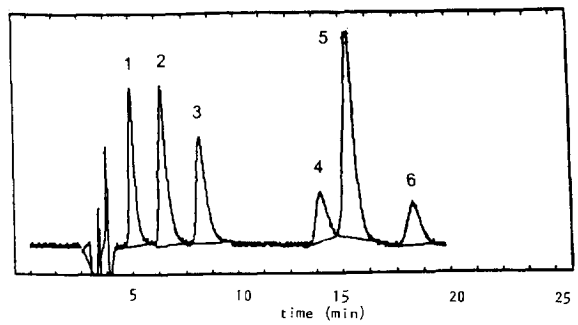

Fig. 2. Chromatographic analysis of the opium alkaloids. Peaks: 1 = morphine (5 ng/μl) = 4.99 min; 2 = 6-MAM (5 ng/μl) = 6.44 min; 3 = codeine (5 ng/μl) = 8.19 min; 4 = diamorphine (5 ng/μl) = 13.97 min; 5 = papaverine (1 ng/μl) = 15.37 min; 6 = flufenamic acid (internal standard) (2.5 ng/μl) = 18.29 min. Quantitation at 239 nm.

3.5. Precision of the identification tools (retention time and absorbance ratios)

The experimental results concerning retention times and absorbance ratios obtained after the analysis of the 23 compounds with the above experimental conditions are given in Table 3. The 23 compounds were identified in all the cases by the use of both the retention and the absorbance ratioing data.

The within-day reproducibility of the retention times was excellent (R.S.D. $<2\%$) for all the

Table 1
Effect of analysis temperature on peak separation

Temperature (°C)	R_s		
	Morphine–6-MAM	6-MAM–codeine	Ethylmorphine–codeine
30	1.249	1.448	0
45	1.477	1.488	0.81
60	1.502	1.763	1.06

Chromatographic conditions: analysis on the Adsorbospher column with a mobile phase of MeOH–ACN–1.2% CH_3COONH_4 (40:15:45, v/v/v); flow-rate 0.8 ml/min.

Table 2
Effect of analysis temperature on peak assymetry A_s

Temperature (°C)	Morphine	6-MAM	Codeine	Diacetylmorphine	Papaverine
30	2.5	3	3	3.1	3.1
45	1.75	2.67	2.33	2.67	2.9
60	1.2	1.33	1.33	1.5	1.5

For experimental conditions see Table 1.

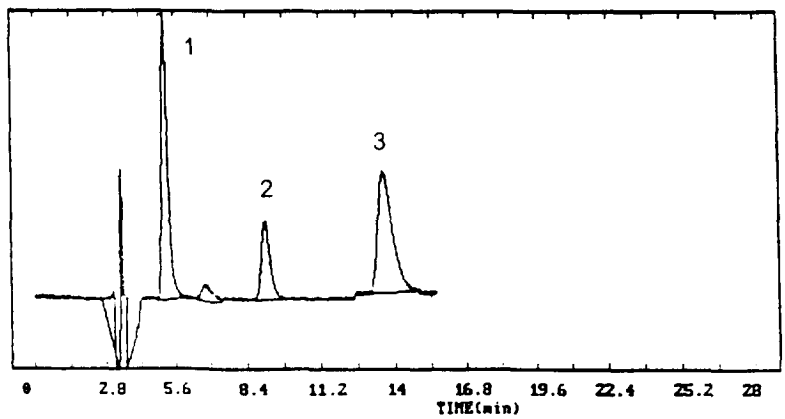

Fig. 3. Chromatographic analysis of (3) cocaine (13.56 min) and its major metabolite (1) benzoylecgonine (5.19 min), with (2) nalorphine (9.14 min) as the internal standard. Concentration of the compounds 5 ng/μl. Quantitation at 239 nm.

Table 3
Retention times and absorbance ratios of the 23 compounds analysed

Analyte	Retention time (min)	A_{225}/A_{239}	A_{225}/A_{254}	A_{225}/A_{289}	A_{239}/A_{254}	A_{239}/A_{289}	A_{254}/A_{289}
Caffeine	1614.50	1.85	2.04	1.17	1.11	0.63	0.94
Theophylline	4.40	1.75	1.93	1.03	1.10	0.59	0.53
Theobromine	4.00	1.73	2.09	1.20	1.21	0.69	0.57
Morphine	4.73	2.49	5.99	6.98	2.42	2.8	1.17
6-MAM	6.14	2.41	7.78	10.15	3.22	4.22	1.31
Ethylmorphine	8.47	1.56	3.12	10.65	2.00	6.81	3.40
Diacetylmorphine	13.27	1.65	8.87	6.02	5.41	3.68	0.69
Codeine	7.72	2.36	2.89	7.41	1.79	3.14	1.76
Norcodeine	4.55	2.07	2.02	2.97	1.05	1.44	1.44
Nalorphine	8.27	2.52	6.07	7.65	2.44	3.07	1.26
Papaverine	14.89	0.58	1.86	5.11	3.19	8.78	2.73
Methadone	18.06	2.96	2.43	2.43	0.82	0.82	1.11
Quinine	16.7	0.83	5.39	8.33	6.53	10.09	1.54
Strychnine	18.43	1.63	1.18	3.87	0.81	2.33	2.76
Cocaine	12.8	0.66	3.85	16.30	5.84	19.30	4.40
Benzoylecgonine	5.37	0.87	4.32	13.50	4.99	19.30	3.18
Ecgonine methyl ester	4.1	2.76	3.03	3.24	1.10	1.20	1.07
Amphetamine	6.51	30.03	5.10	7.51	1.52	6.21	4.05
Bamifylline	13.63	2.95	4.46	0.99	1.51	0.33	0.22
Hyoscyamine	8.69	5.71	6.82	10.12	1.19	1.77	1.48
Scopolamine	5.63	4.95	8.21	10.20	1.66	2.06	1.24
Tolfenamic acid	21.5	2.13	2.61	1.02	1.23	0.47	0.38
Flufenamic acid	18.4	1.91	3.86	0.78	2.41	0.48	0.20

Chromatographic conditions: analysis on the Adsorbospher column with a mobile phase of MeOH–ACN–1.2% CH_3COONH_4 (40:15:45, v/v/v); flow-rate 0.8 ml/min; analysis temperature 45°C.

analysed compounds. A slight increase of the retention times of all the peaks was observed in the day-to-day reproducibility study. Comparing the results obtained after analysis in 12 consecutive days, the highest variation was observed on the flufenamic acid peak (R.S.D. 5.6%), while the lower variations were on the benzoylecgonine (R.S.D. 2.5%) and morphine peaks (R.S.D. 2.89%). These variations were attributed to mobile-phase composition variations and column ageing.

Absorbance ratio values varied more strongly. The best reproducibility was obtained for the analysis of early and sharp peaks like morphine, 6-monoacetylmorphine (6-MAM), codeine and benzoylecgonine. In principle, the ratios between wavelengths where a strong signal is obtained (A_{225}/A_{239}, A_{225}/A_{254}), were much more reproducible than ratios between low-absorbance wavelengths (A_{239}/A_{289}, A_{254}/A_{289}). The R.S.D. of the ratio values were in the range from 2.99% (A_{225}/A_{239} for morphine) to 29.67% (A_{254}/A_{289} for diacetylmorphine).

3.6. Variation of absorbance ratioing with concentration

Variation of the absorbance ratios with concentration should always be considered when a multiwavelength detection is presented. In order to determine these variations, opium alkaloid and cocaine alkaloid solutions of three different concentrations were analysed ten times. In Fig. 4 the variation of the absorbance ratio values for six different standard solutions is plotted versus concentration of the analysed solutions of 6-MAM. Absorbance ratios were affected by concentration variation, but not to a great extent. This phenomenon could be attributed to the way the ratio is calculated. Since the ideal linear relationship between peak area and concentration does not always occur (due to factors affecting peak integration, like the values of peak width and peak threshold, peak tailing etc.), many of these variations were possibly due to the indirect way of absorbance ratioing. The real response time of the spectrophotometer could also be a reason for a non-linear response on higher concentrations. The latter together with flow effects in the detector cell affect all the experimental results and therefore the ratio calculation.

3.7. Quantitative study

The quantitative studies were divided into two sections: the study of the major opium alkaloids and the study of cocaine and its metabolites. The system finally chosen achieved the simultaneous determination of five of the opium alkaloids: morphine, 6-MAM, diacetylmorphine, codeine and papaverine. The great importance of such an

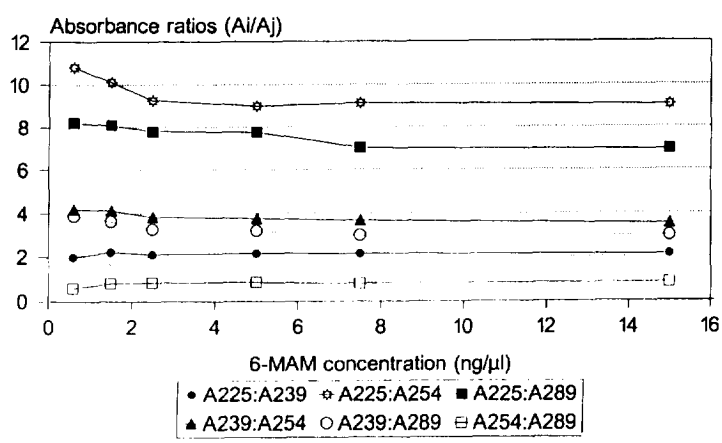

Fig. 4. Plot of the absorbance ratio values versus the concentration of 6-MAM. Conditions in Table 3.

Table 4
Statistical evaluation of the analysis

Alkaloid	Detection limit (ng)	Calibration curve equation[a]	Correlation coefficient (r)
Morphine	2	$y = -0.03292 + 0.292055x$	0.99964
Codeine	2	$y = -0.025765 + 0.31856x$	0.99957
6-MAM	1	$y = 0.21799 + 0.299143x$	0.99777
Diacetylmorphine	3	$y = 0.032825 + 0.157722x$	0.99592
Papaverine	0.2	$y = -1.87 \cdot 10^{-5} + 3.10062x$	0.99889
Benzoylecgonine	1	$y = -0.038656 + 0.54241x$	0.99950
Cocaine	1	$y = -0.010409 + 0.49866x$	0.99952

[a] y = Peak area ratios of alkaloid drugs to internal standard; x = concentration in ng/μl.

analysis is the simultaneous analysis of morphine, diacetylmorphine and its metabolite 6-MAM. The presence of 6-MAM is the clearest indication of heroin abuse and the differentiation from other opium intakes. The results of the statistical analysis are given in Table 4.

The analysis of cocaine and benzoylecgonine was made in the same system with the same procedure and the analysis results are also given in Table 4.

The detection limits were calculated in all cases as the quantities that produced a signal-to-noise ratio of 2.

3.8. Inter- and intra-day precision and accuracy

Three standard opiate mixtures were analysed ten times during a day's time for the evaluation of the within-day precision and accuracy and the results are given in Table 5. The same three

Table 5
Within-day precision and accuracy for the analysis of opium alkaloids

Alkaloid	Added (ng)	Found (ng)	R.S.D. (%)
Morphine	30	50.42	2.22
	50	56.48	2.56
	150	170.7	2.42
6-MAM	30	55.72	4.62
	50	53.49	4.53
	150	149.41	3.17
Codeine	30	56.35	4.32
	50	60.09	4.14
	150	178.81	2.61
Diacetylmorphine	30	60.31	7.04
	50	64.31	6.29
	150	127.01	3.87
Papaverine	6	15.51	4.21
	10	17.16	1.97
	30	49.93	3.53
Benzoylecgonine	20	23.07	8.61
	50	51.74	3.10
	100	100.11	4.85
Cocaine	20	19.27	12.65
	50	53.05	2.70
	100	101.21	5.64

standards were analysed four times each day for a period of 12 days for the day-to-day precision and accuracy evaluation. The results of the study are given in Table 6. The same procedure was used for the analysis of cocaine and benzoylecgonine and the results are given in Tables 5 and 6.

From both tables it comes out that the overall precision and accuracy was satisfactory. Analysis of higher quantities gives more accurate results. The best results are obtained with 239 nm as the quantitation wavelength. For the analysis of cocaine and benzoylecgonine very good results were obtained at 232 nm. These conditions could possibly differ from the ones obtained with the use of conventional UV detectors, where a lower wavelength (e.g. 225 nm) could give better results.

3.9. Analysis of illicit samples and pharmaceutical preparations

Analysis of illicit preparations on an HPLC–photodiode-array offers many advantages for the identification of the compounds present in such samples. The presence and concentration of diluents and the concentration ratios of the opium alkaloids are powerful tools for the search of the origin of such samples. Analysis of illicit preparations was directly accessible with the chosen chromatographic system.

Typical chromatograms of the analysis of illicit samples and pharmaceutical preparations are given if Fig. 5.

Table 6
Day-to-day precision and accuracy for the analysis of opium alkaloids

Alkaloid	Added (ng)	Found (ng)	R.S.D. (%)
Morphine	30	37.16	7.59
	50	50.32	4.56
	150	169.14	7.02
6-MAM	30	35.62	4.32
	50	42.65	4.91
	150	143.71	5.17
Codeine	30	38.38	9.02
	50	54.00	4.67
	150	173.31	4.61
Diacetylmorphine	30	40.21	6.89
	50	57.6	5.52
	150	186.8	6.12
Papaverine	6	5.178	5.23
	10	13.5	8.12
	30	44.9	4.92
Benzoylecgonine	20	20.78	6.36
	50	52.60	3.21
	100	100.14	3.02
Cocaine	20	19.75	6.24
	50	52.33	3.24
	100	94.47	4.02

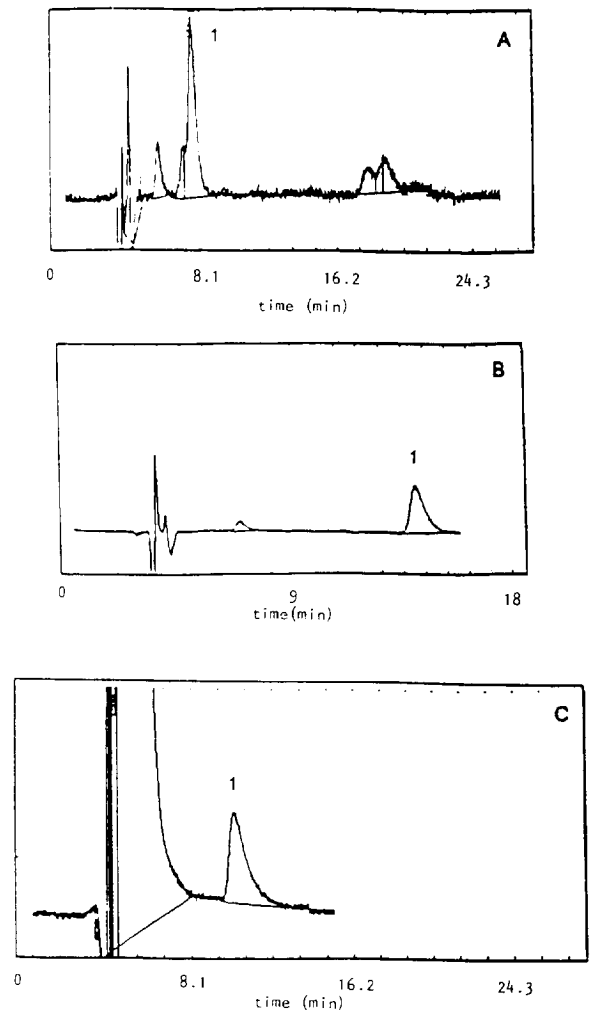

Fig. 5. Typical chromatograms of the analysis of: (A) 1 = illicit heroin, (B) 1 = illicit cocaine, and (C) syrup containing 1 = phosphate codeine. Quantitation at 239 nm. Retention times as in Figs. 2 and 3.

4. Conclusions

The method described achieved the simultaneous determination of the major opium alkaloids and cocaine and its major metabolite benzoylecgonine. The use of a base-deactivated column improved resolution and peak shape. Absorbance ratioing at four selected wavelengths and retention data were used for the identification of the 23 analysed compounds. Detection in the low UV region resulted in lower detection limits, larger linearity and better reproducibility. The HPLC–diode-array system proved to be a very powerful tool for systematic toxicological analysis.

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