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Note

Analysis of illicit diamorphine preparations by high-pressure liquid chromatography

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Methods for the identification of illicit diamorphine samples have employed a variety of techniques[1-6], alone, or in combination, and the presence of monoacetyl-morphine, morphine, caffeine, quinine, cocaine, barbitone, codeine, paracetamol, ephedrine and strychnine in samples has been reported[7,8]. For the purpose of comparison of samples, however, the method of choice should allow rapid qualitative identification of all likely components together with a capability for quantitation of at least the morphine-derived compounds. High-pressure liquid chromatography (HPLC) offers obvious advantages by combining a separative power superior to that of thin-layer chromatography with a means of accurate quantitation and easy trapping of eluted material for subsequent confirmation of structure. The purpose of the present work has been to investigate the possible application of HPLC in this field and to devise a method for the rapid and total analysis of illicit diamorphine samples.

Several modes of HPLC have been applied to the separation of morphine alkaloids[9-13], but not to the diverse and complex mixtures encountered as illicit diamorphine seizures. Ion-exchange chromatography of morphine alkaloids has been extensively investigated by Knox and Jurand[12,13], and the present work may be regarded as an extension of their investigation adapted to the requirements of forensic analysis.

In this paper a procedure is described for the qualitative and quantitave analysis of illicit diamorphine seizures by means of high-pressure cation exchange chromatography. No extraction or derivatisation is required, and the chromatographic separation of diamorphine from thirteen likely contaminants is achieved in twelve minutes.

EXPERIMENTAL

Equipment

Chromatographic columns (120 cm × 2.1 mm I.D.) were dry-packed with Zipax SCX strong cation-exchange resin (DuPont, Wilmington, Del., U.S.A.) and eluent was delivered from two constant-flow pumps (Waters M-6000) controlled by a Waters' M-660 solvent programmer. A variable wavelength UV photometer (Cecil Instruments) fitted with an 8-μl flow cell was coupled to the chromatography column by means of capillary-bore PTFE tubing.

Confirmation of the structure of eluted components was achieved by mass spectrometry (VG Micromass 12). Eluent solutions were made up of analytical grade reagents (BDH, Poole, Great Britain, "AnalaR") with the exception of acetonitrile (Fison's, Loughborough, Great Britain, "Spectrosol").

Method

Illicit diamorphine samples were homogenised by grinding in a mortar and 20–25 mg, accurately weighed, were dissolved in 50% aqueous methanol. The solution was made up to 2 ml in a volumetric flask and 2 μl were taken for an initial qualitative analysis using the following chromatographic conditions: column, 120 cm $\times$ 2.1 mm Zipax SCX; eluents: (a) H_3BO_3 (0.2 M, aqueous), adjusted to pH 9.3 with 40% NaOH, (b) H_3BO_3 (0.2 M, aqueous)–acetonitrile–n-propanol (86:12:2) adjusted to pH 9.8 with NaOH (40% aqueous); flow-rate, 2 ml·min^{-1}; linear gradient, 0–100% (b) in 6 min; UV absorbance detector, 270 nm, 0.2 absorption units full scale.

The separation of eight compounds of a reference mixture is shown in Fig. 1.
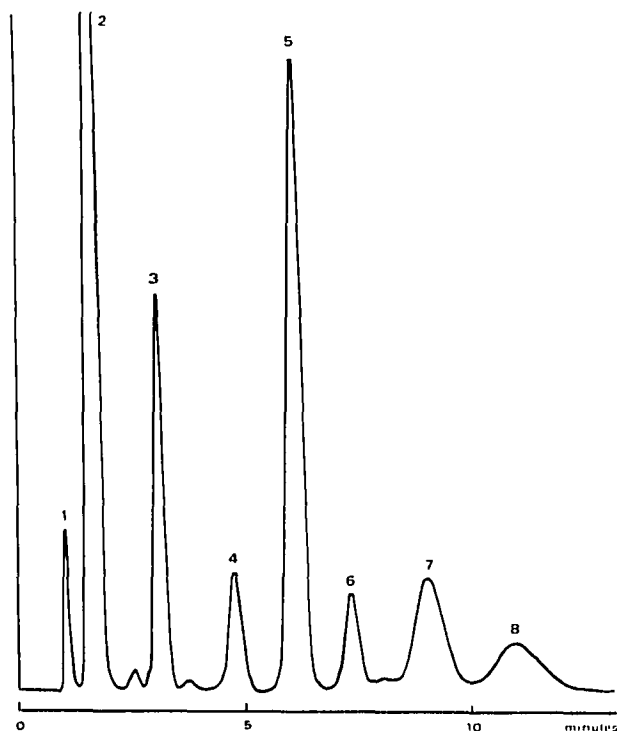

Fig. 1. HPLC of some constituents of illicit diamorphine seizures. For column conditions, see text. 1 = Barbitone; 2 = caffeine; 3 = morphine; 4 = monoacetylmorphine; 5 = strychnine; 6 = diamorphine; 7 = quinine; 8 = cocaine.

For a quantitative analysis, diphenylamine (0.2 ml of a 2 mg·ml^{-1} methanolic solution) was added as an internal standard to the 2-ml diamorphine solution and the sample was re-chromatographed. The peak heights for the morphine alkaloid components were measured and related to that for the internal standard. Quantitation
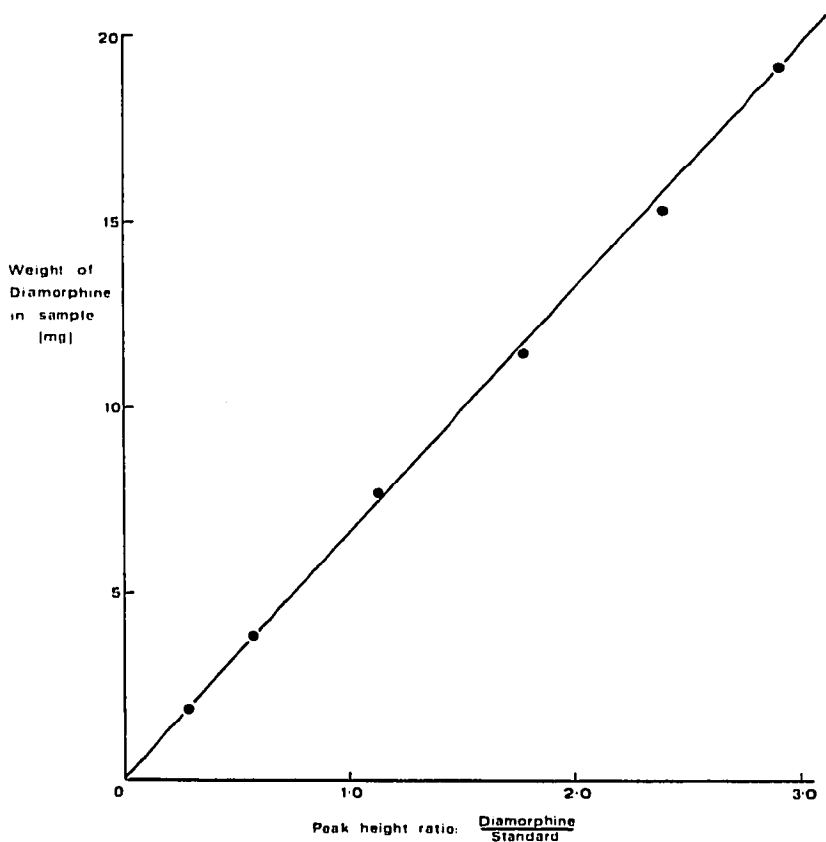
Fig. 2. Calibration plot for diamorphine (λ = 270 nm).

of each component was determined by reference to calibration graphs and the excellent linearity of response is illustrated by the plot for diamorphine (Fig. 2).

The mass spectra of eluted components were obtained after extraction of the eluent fractions with diethyl ether or with chloroform.

RESULTS AND DISCUSSION

No single isocratic solvent system was found adequate for analysis of the complex drug mixtures encountered in illicit diamorphine seizures. However, elution with a pH and organic component gradient gave excellent resolution of fourteen components in 12 min. The linear gradient profile described was found to yield optimum conditions for resolution and time of analysis in the presence of the internal standard.

The sensitivity of detection for any component depends not only on the UV absorption characteristics of the compound, but also on the wavelength at which the detector operates. In the present work, the detection wavelength of choice was found to be 270 nm. Detection at 235 and 254 nm was also investigated, and although greater sensitivity was achieved at 235 nm, baseline drift during solvent programming was more pronounced at shorter wavelengths (Fig. 3).
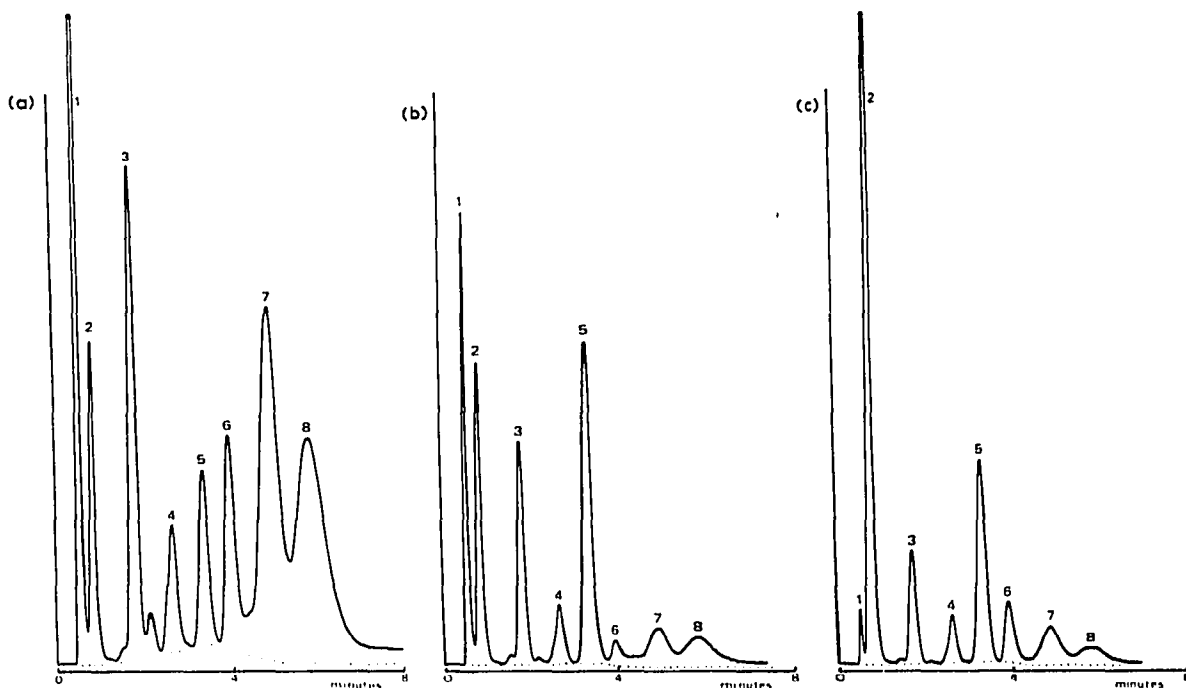
Fig. 3. Effect of UV detector wavelength on response to a standard drug mixture. (a) 235 nm; (b) 254 nm; (c) 270 nm. For column conditions, see text, except column length 60 cm, gradient time 3 min and detection sensitivity 1.0 absorption units full scale. Peak identity, as in Fig. 1. Gradient blank shown as dotted line.

TABLE I

RESULTS OF ANALYSIS OF TWENTY ILLICIT DIAMORPHINE SAMPLES

Sample	Morphine (%)	O^6-Monoacetyl-morphine (%)	Diamorphine (%)	Total	Other components
1	1.4	40.0	14.9	56.3	caffeine
2	—	7.6	38.7	46.3	caffeine, strychnine (0.2%)
3	—	7.2	34.3	41.5	caffeine, strychnine (1.4%)
4	2.0	42.0	9.2	53.2	caffeine
5	—	9.0	36.2	45.2	caffeine, strychnine (0.4%), quinine
6	—	4.9	30.8	35.7	caffeine, strychnine (0.4%), quinine
7	1.1	6.4	42.0	49.5	caffeine, strychnine (0.2%)
8	—	8.5	46.2	54.7	caffeine, strychnine (0.3%), quinine
9	1.0	29.4	20.3	50.7	caffeine
10	0.7	12.7	34.1	47.5	caffeine, strychnine (0.6%)
11	1.5	18.2	30.1	49.8	caffeine, strychnine (0.6%)
12	1.9	43.2	20.2	65.1	caffeine
13	0.9	11.2	37.1	49.2	caffeine, strychnine (0.4%), quinine
14	—	4.8	47.5	52.3	caffeine, strychnine (0.5%)
15	1.6	32.9	17.1	51.6	caffeine
16	1.7	37.2	12.2	51.1	caffeine
17	—	2.0	43.2	45.2	caffeine, strychnine (0.85%), quinine
18	—	7.9	32.1	40.0	caffeine, strychnine (1.1%), quinine
19	—	9.5	37.5	47.0	caffeine, strychnine (0.5%), quinine
20	—	5.4	36.7	42.1	caffeine, strychnine (0.7%)

TABLE II

RETENTION TIMES AND VOLUMES ON ZIPAX SCX COLUMN

	Time (min)	Volume (ml)
Barbitone	1.1	2.2
Paracetamol	1.1	2.2
Caffeine	1.8	3.6
Morphine	3.4	6.8
O^6-Monoacetylmorphine	4.7	9.4
Codeine	5.7	11.4
Strychnine	6.3	12.8
Dihydrocodeine	6.8	13.6
Lignocaine	7.15	14.3
Diamorphine	7.4	14.8
Procaine	7.55	15.1
Ephedrine	8.3	16.6
Quinine	9.3	18.6
Cocaine	11.5	23.0

The results of analysis of twenty illicit diamorphine samples are given in Table I, and Table II gives the retention characteristics of fourteen possible components. It is interesting to note that in many samples strychnine was present in addition to the major diluent, caffeine, and the diamorphine hydrolysis products. A chromatogram of a typical illicit sample is shown in Fig. 4.

Use has been made of retention time as an index for the tentative identification of drugs, but for this to be valid, the reproducibility of the system must be high. If retention times (and not retention volumes) are to be measured, then accurate constant-flow pumps are indicated, and if gradient elution is employed, a precise control of the solvent gradient is especially necessary. The reproducibility of retention
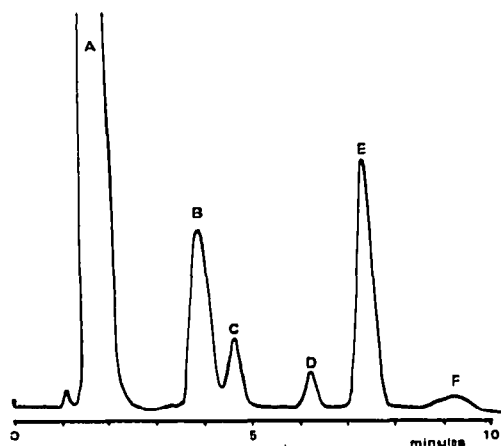

Fig. 4. Chromatogram of an illicit diamorphine sample. For chromatographic conditions, see text. A = Caffeine; B = diphenylamine (internal standard); C = monoacetylmorphine; D = strychnine; E = diamorphine; F = quinine.

time for diamorphine has been determined for thirty samples chromatographed over several days. The standard deviation in retention time was calculated to be 0.09 min, giving a coefficient of variation of 1.2%.

For a quantitative analytical method the reproducibility is again of prime importance, and this has been assessed by performing replicate analyses on ten weighed aliquots of the same carefully homogenised "Chinese Heroin" sample. The content of morphine alkaloids was determined from peak height measurements and the standard deviation for the analysis of diamorphine and mono-acetylmorphine was calculated (Table III). The variation for monoacetylmorphine is higher than that for diamorphine as the former is incompletely resolved from the internal standard.

TABLE III

REPLICATE ANALYSES OF AN ILLICIT DIAMORPHINE SAMPLE (TEN ANALYSES)

	Diamorphine (%)	Monoacetylmorphine (%)
Mean content	36.7	5.4
Standard deviation	0.8	0.25
Coefficient of variation	2.3	4.5

The analysis of illicit diamorphine described in this paper exemplifies the use of cation-exchange gradient elution chromatography for the separation of a number of basic drugs. Although ion-exchange is a less predictable mode than liquid–solid or liquid–liquid chromatography, the versatility may be enhanced by employing a pH or ionic strength gradient. In contrast to the methanol–water solvent gradients used in reversed-phase liquid–liquid chromatography, the effect of a pH gradient upon the UV detector baseline is minimal, a particular advantage where the ultimate in sensitivity is required.

REFERENCES

1 A. S. Curry and D. A. Patterson, *J. Pharm. Pharmacol.*, 22 (1970) 198.
2 P. De Zan and J. Fasanello, *J. Chromatogr. Sci.*, 10 (1972) 333.
3 J. M. Moore and F. E. Bena, *Anal. Chem.*, 44 (1972) 385.
4 G. R. Nakamura, T. T. Noguchi, D. Jackson and D. Banks, *Anal. Chem.*, 44 (1972) 408.
5 G. R. Nakamura and H. J. Meuron, *Anal. Chem.*, 41 (1969) 1124.
6 R. C. Shaler and J. H. Jerpe, *J. Forensic Sci.*, 17 (1972) 668.
7 D. W. Johnson and J. W. Gunn, *J. Forensic Sci.*, 17 (1972) 629.
8 R. W. Jenkins and D. A. Patterson, unpublished results.
9 C.-Y. Wu, S. Siggia, T. Robinson and R. D. Waskiewicz, *Anal. Chim. Acta*, 63 (1973) 393.
10 P. J. Cashman and J. I. Thornton, *J. Forensic Sci. Soc.*, 12 (1972) 417.
11 J. D. Wittwer, *J. Forensic Sci.*, 18 (1973) 138.
12 J. H. Knox and J. Jurand, *J. Chromatogr.*, 82 (1973) 398.
13 J. H. Knox and J. Jurand, *J. Chromatogr.*, 87 (1973) 95.