

Note

The separation of a wide range of drugs of abuse by high-pressure liquid chromatography

I. JANE

Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London S.E. 1 (Great Britain)

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The large number of illicit drug samples being received in this laboratory necessitates the use of analytical techniques which are rapid, specific, and in many instances, quantitative. Since many of the samples are mixtures or impure, identification or quantitation by spectroscopic methods usually requires preliminary clean-up procedures. The high polarity and low volatility of many of the drugs restrict the use of gas chromatography. Thin-layer chromatography has quantitative limitations. High-pressure liquid chromatography is inherently suitable for dealing with these samples.

Although liquid chromatographic applications to basic drug analysis have been published¹⁻⁵, they would seem to be characterized by their relative inefficiency. Gradient elution is usually required in these methods and each of the column systems suggested is generally restricted to the analysis of one type of drug.

The method described here allows the separation of a wide range of drugs by isocratic elution on a single column. The system has been in routine use for several months and a large number of samples have been successfully examined.

EXPERIMENTAL

A Waters Ass. 6000M reciprocating pump was used to provide solvent flow, and a Cecil Instruments C212 variable wavelength UV detector was used to monitor the eluent. The column was a 25 cm × 4.6 mm I.D. stainless-steel tube, slurry-packed with small-particle silica (Partisil 6 μm, Reeve Angel, Maidstone, Great Britain). A stop-flow injection system was used, with injections being made on to a piece of 400 mesh stainless-steel gauze on top of the column packing. The column and injector design have been described in detail elsewhere⁶.

Each batch of silica was graded by sedimentation to improve the particle-size range. Grading was performed by suspending 10 g of silica in 250 ml of methanol in a measuring cylinder (4 cm I.D.). After 1 h the top 100 ml were removed. This layer contains a high proportion of the smallest silica particles. Separate columns were packed with both the fractions of the silica. Solvent conditions are described in the legends to the figures.

A weighed amount of sample is made up in either water or dilute hydrochloric acid to known concentration, usually 10 mg/ml, and dissolution is assisted by immersion for about 1 min in an ultrasonic bath. An aliquot, 1-5 μl, of this sample is

TABLE I
TYPICAL RESULTS FOR VARIOUS SILICA COLUMNS

Injection of $\frac{1}{2}$ μ l of sample; conditions as in Fig. 1.

	<i>Approx. pressure drop (p.s.i.)</i>	<i>Column efficiency for methylamphetamine</i>
Graded silica	800	9,000
Ungraded silica	1,000	11,000
"Fines"	1,500	18,000

injected on to the chromatographic column. The detector wavelength is adjusted to the UV maximum for the compound of interest.

RESULTS

Typical results for three columns packed with different silica fractions are shown in Table I. Retention data for the phenethylamines, opium alkaloids and

TABLE II
RETENTION OF AMPHETAMINE-TYPE STIMULANTS RELATIVE TO METHYLAMPHETAMINE

Conditions as in Fig. 1.

<i>Stimulant</i>	<i>R_F</i>
Dexamphetamine	0.62
Methylamphetamine	1.00 (12.2 ml)
Ethylamphetamine	0.72
3-Chloropropylamphetamine	0.38
Benzphetamine	0.33
Phentermine	0.58
Mephentermine	1.14
Chlorphentermine	0.57
Hydroxyamphetamine	0.71
Ephedrine	0.92
Pseudoephedrine	0.98
Norephedrine	0.58
Norpseudoephedrine	0.76
N-Methylephedrine	0.84
Diethylpropion	0.40
Fencamfamin	0.52
Fenfluramine	0.62
STP	0.67
Bromo-STP	0.67
Phendimetrazine	0.40
Phenmetrazine	0.45
Pipradol	0.53
Prolintane	0.70
Amitriptyline	0.47
Desipramine	0.98
Imipramine	1.06
Methylphenidate	0.39

TABLE III
RETENTION OF THE OPIUM ALKALOIDS RELATIVE TO MORPHINE
 Conditions as in Fig. 1.

<i>Alkaloid</i>	<i>R_F</i>
Morphine	1.00 (8.9 ml)
Codeine	0.95
Thebaine	0.79
Papaverine	0.47
Narcotine	0.47
Narceine	0.92
Cotarnine	4.10
Dilaudid	1.50
Dicodid	1.32
Dionine	0.87
Paracodeine	1.44
Eucodal	0.60
Protopine	0.61
Laudanosine	0.68
Acedicon	0.74

TABLE IV
RETENTION OF OTHER COMPOUNDS OF FORENSIC INTEREST RELATIVE TO MORPHINE
 Conditions as in Fig. 1.

<i>Compound</i>	<i>R_F</i>	<i>Compound</i>	<i>R_F</i>
Amethocaine	0.58	Methadone	0.74
Atropine	2.35	Methapyrilene	0.59
Antazoline	1.21	Methaqualone	0.45
Benzocaine	0.45	6-Methyldihydromorphine	1.26
Benztropine	2.88	6-Monoacetylisopropylmorphine	0.66
Bromodiphenhydramine	0.65	6-Monoacetylmorphine	0.75
Butacaine	0.58	Morphine	1.00 (8.9 ml)
Caffeine	0.52	Nalorphine	0.55
Chlordiazepoxide	0.48	Nicotine	0.57
Chlorpheniramine	1.02	Nitrazepam	0.46
Chlorpromazine	0.67	Paracetamol	0.46
Cocaine	0.51	Pethidine	0.62
Diethazine	0.64	Phenacetin	0.45
Dextropropoxyphene	0.49	Phenbutrazate	0.45
Diazepam	0.45	Phencyclidine	0.66
Dihydromorphine	1.57	Procaine	0.56
Diphenhydramine	0.64	Quinidine	0.63
Dihydrohydroxymorphinone	0.68	Quinine	0.65
Ethopropazine	0.61	Salicylamide	0.46
Heroin	0.69	Strychnine	1.57
Lignocaine	0.46	Theophylline	0.49
Meclophenoxate	0.57		

other drugs of forensic interest are given in Tables II–IV. Typical chromatograms are shown in Figs. 1 and 2.

Peak heights were used to quantify the components, a relative standard deviation of less than 2% being obtained from replicate injections of a standard solution. The detection limit depends on the UV extinction coefficient of the compound, sensitivity limits for morphine and heroin being approximately 50 ng. A linear calibration is obtained for 0.05–10 μg of morphine injected. The results for replicate analyses of an opium sample for morphine content show a relative standard deviation of less than 5%, and agree with those found by other methods.

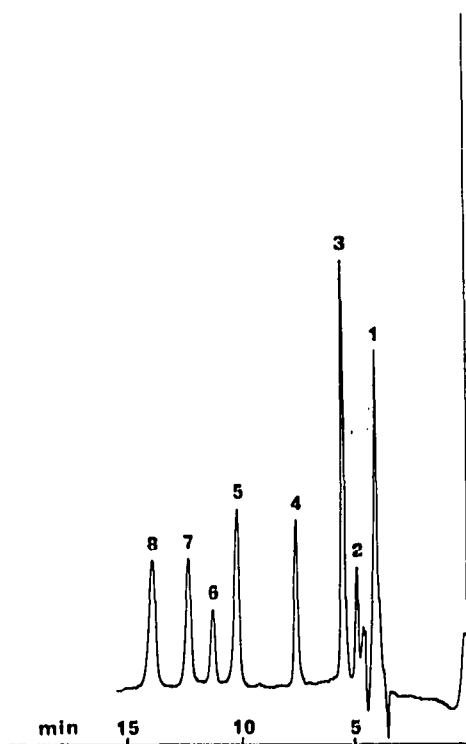


Fig. 1. Separation of 1 μl of a synthetic mixture of phenethylamines, containing: 1, benzphetamine; 2, phendimetrazine; 3, phenmetrazine; 4, dexamphetamine; 5, N-methylephedrine; 6, ephedrine; 7, methylamphetamine; 8, mephentermine. Conditions: column, 25 cm \times 4.6 mm I.D., filled with small particle silica "fines". Solvent, methanol–2 *N* ammonia solution–1 *N* ammonium nitrate solution (27:2:1). Flow-rate, 1 ml/min. Pressure, 1500 p.s.i. Room temperature. Detector wavelength, 254 nm.

Variation of the retention of the compounds on the column can be affected by changes in either the methanol–water ratio, the concentration of ammonia, or the concentration of ammonium nitrate. The variation is not straightforward but in general a less basic, more aqueous, solvent is required for the separation of less basic drugs. The separation of a mixture of ergot alkaloids on the same column but with a modified solvent is shown in Fig. 3.

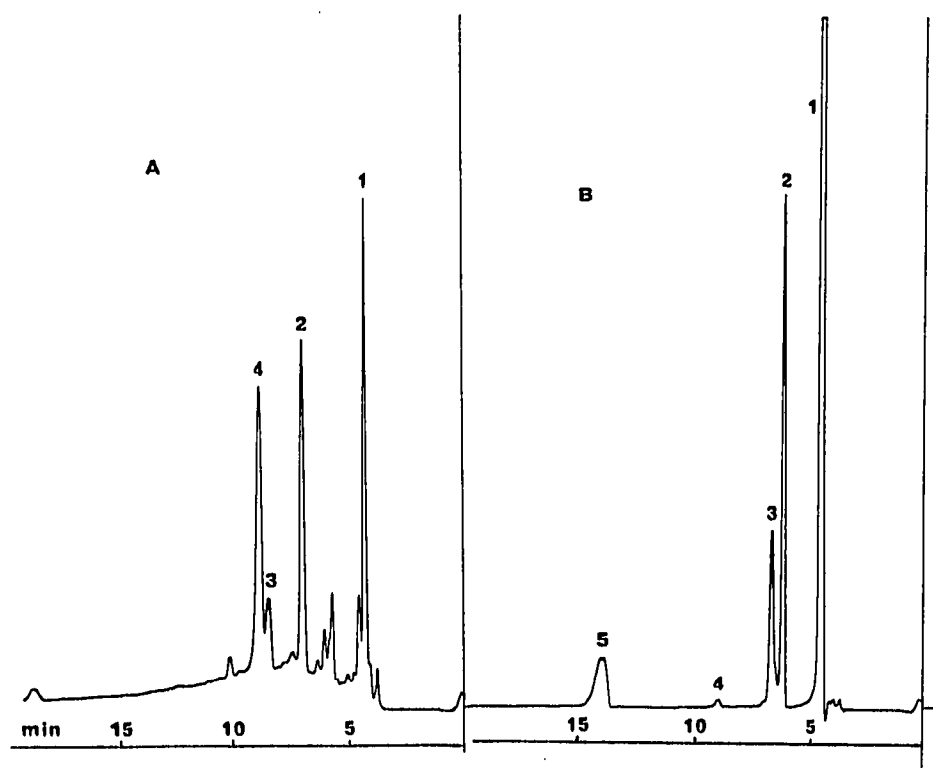


Fig. 2. Separation of (A) an opium sample, the major components being 1, narcotine; 2, thebaine; 3, codeine; 4, morphine. (B) A "Chinese Heroin" sample, the peaks identified being 1, caffeine; 2, heroin; 3, monoacetylmorphine; 4, morphine; 5, strychnine. 1 μ l Injection of each, conditions as in Fig. 1 except the detector wavelength being 278 nm.

DISCUSSION

The procedure described appears to be superior in speed and efficiency to previous liquid chromatographic methods for the analysis of these drugs of abuse. It also has distinct advantages over methods formerly used in this laboratory, especially in the identification and quantitation of mixtures of basic drugs. Since the phenethylamines are frequently encountered as mixtures, especially as white powders with no identifying characteristics, the ability of the system simultaneously to identify and quantitate the components can be of great use.

The system also provides convenient analysis of narcotic analgesics in the various forms that arrive in this laboratory. Although opium is a very complex mixture, separation of the major alkaloids is achieved in less than 10 min, allowing determination of the morphine content. Heroin (diacetylmorphine), which is frequently abused and occurs most commonly adulterated with other compounds as so-called "Chinese Heroin", can also be rapidly determined.

Although the system is primarily designed for analytical work, it can also be used on a micro-preparative scale. Up to 0.1 mg of compound can be injected without

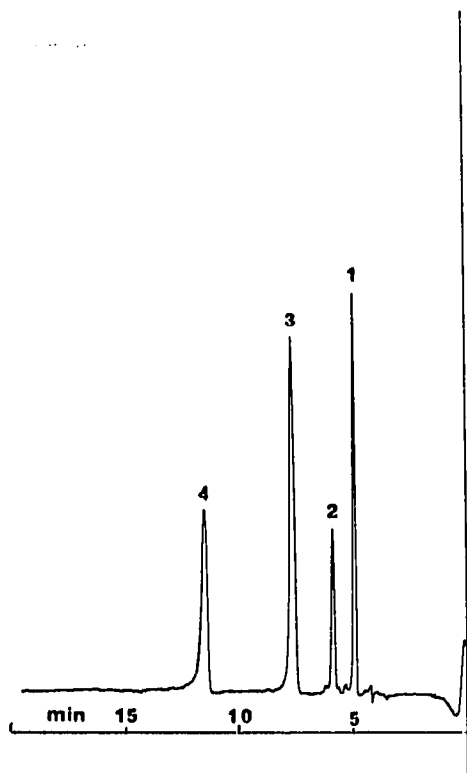


Fig. 3. Separation of 1 μ l of a synthetic mixture of ergot alkaloids, containing: 1, lysergic acid; 2, lysergamide; 3, LSD; 4, iso-LSD. Conditions as in Fig. 1, except detector wavelength being 320 nm; solvent, methanol-0.2 *N* ammonium nitrate solution (3:2); pressure, 2700 p.s.i.

excessive loss of column performance. Since only a single solvent is used, the column does not need regeneration after each injection and thus rapid sample turnover is possible.

Deterioration in performance due to irreversible adsorption of substances on to the column can often be a problem with silica packing materials. However, the use of a very polar eluting solvent appears to have avoided this problem. A system has been in routine use for several months with no noticeable loss in column performance.

The efficiency of the column depends on the particle size of the silica used, smaller particles providing greater efficiency at the cost of a greater pressure drop down the column. Columns packed with the larger particle size silica are generally adequate for most applications.

The silica column with an aqueous methanolic solvent provides a versatile separation mechanism for basic compounds. Although the solvent system described was arrived at by trial and error, consideration of the retention data for the compounds studied makes it possible to predict the solvent composition necessary to perform a certain separation, especially for simple compounds. In general, increase in basicity of the nitrogen atom increases the retention on the column, whereas increase

in steric hindrance, caused by large groups adjacent to the nitrogen, reduces the retention.

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