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Note

High-performance liquid chromatography system for the separation of ergot alkaloids with applicability to the analysis of illicit lysergide (LSD)

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The alkaloids isolated from ergot, along with their structurally related synthetic and semi-synthetic analogues, include several substances with important pharmacological activity. Forensic science laboratories require reliable methods to discriminate between these ergot alkaloids. Of particular importance are the substances which may arise in cases of illegal abortion (*e.g.* ergometrine) and cases of drug abuse involving the hallucinogenic compound lysergide (lysergic acid diethylamide, LSD).

Most ergot alkaloids are thermally unstable and/or photo-labile and consequently high-performance liquid chromatography (HPLC) is generally the technique of choice to separate mixtures of these compounds[1–13]. Both normal-phase[1–7] and reversed-phase[6–13] systems have been used. Although isocratic conditions have been adopted in most situations, gradient elution has also been used for separating complex mixtures[3,4,8,12].

The high potency of LSD means that illicit preparations contain very small amounts of the drug. Typically, each small tablet ("microdot") or paper square ("blotter") may contain less than 100 μg of the drug and consequently sensitive methods are required for the analysis of such preparations. HPLC systems for the analysis of LSD have been reported in the literature using silica[14–16], octadecyl-silica (ODS-silica)[17–21] and octyl-silica[22] columns. However, reversed-phase systems have been shown to provide better discrimination for the identification of LSD[19]. Although UV detection has been used for the analysis of LSD in illicit preparations, fluorescence detection offers enhanced sensitivity and selectivity.

U.K. Forensic Science Laboratories have recently standardised on the HPLC packing materials used for routine work, namely one silica and one ODS-silica. This decision has arisen from the realisation that packing materials of the same type from different manufacturers can have markedly different properties. By sharing common materials the exchange of HPLC methods is facilitated while the stock of columns required in each laboratory is greatly reduced. We are currently engaged in the development of eluent systems to use with the recommended materials for the separation of specific drug groups. Retention data on the materials have already been published for amphetamines[23], barbiturates[24,25], local anaesthetics[26] and narcotic analgesics[23]. The present note gives retention data for the chromatography of ergot

alkaloids on ODS-Hypersil and is based on original work from this laboratory[19] which used an alternative material. The applicability of the system to the analysis of LSD in illicit preparations is demonstrated.

EXPERIMENTAL

Materials

Methanol (HPLC grade) was obtained from Rathburn (Walkerburn, U.K.) while all other chemicals were AnalaR grade from BDH (Poole, U.K.). All ergot alkaloids were from the drug collection of the Central Research Establishment, Home Office Forensic Science Service.

Chromatography

Chromatography was performed with an Applied Chromatography Systems pump (Model 351), a Rheodyne 7120 injection valve (fitted with a 20-μl loop) and a Kratos UV detector (Model 773) fitted with an 8-μl flow-cell or a Perkin-Elmer fluorescence detector (Model LC1000). The UV detector was operated at 220 nm while the fluorescence detector was operated at 312 nm excitation and 400 nm emission.

The stainless-steel analytical columns (10 or 16 cm $\times$ 5 mm I.D.) were packed with 5-μm ODS-Hypersil (Shandon Southern Products, Runcorn, U.K.) by a slurry procedure, dispersing the packing material in isopropanol and using hexane as the pressurising solvent.

A phosphate buffer was prepared by dissolving sodium dihydrogen phosphate dihydrate (3.43 g; 0.022 moles) and disodium hydrogen phosphate (3.97 g; 0.028 moles) in glass-distilled water (1000 ml). The eluent was prepared by mixing this buffer (400 ml) with methanol (600 ml). The pH of this 60% methanolic eluent was measured to be 8.1. A short column (5 cm $\times$ 4.5 mm I.D.) packed with coarse silica (*ca* 40 μm) was included between the pump and the injection valve to protect the column from the alkaline eluent. In addition the analytical column was never left in contact with static eluent and was washed out with methanol–water (60:40) at the end of each working day. An eluent flow-rate of 2 ml/min was used in all experiments.

Ergot alkaloids were injected onto the column dissolved in methanol. 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 analyte and a non-retained compound, respectively. The injection of sodium nitrate (0.8 mg/ml in eluent) was used to determine t_0.

Extraction of illicit LSD preparations

The procedure of McDonald *et al.*[21] has been used involving the addition of methanol–water (50:50) to the "microdot" or "blotter" with ultrasonic vibration for 20 min. After centrifugation the extract was injected directly onto the column.

RESULTS AND DISCUSSION

The eluent finally selected for the separation of the ergot alkaloids on ODS-Hypersil was 60% methanol buffered at pH 8.1. This represents only a small change from the 65% methanol eluent used by Twitchett *et al.*[19]. Table I lists the k' values

TABLE I

HPLC RETENTION DATA FOR ERGOT ALKALOIDS (ARRANGED IN ORDER OF INCREASING RETENTION)

Compound	*k'*	*Compound*	*k'*
Isolysergide	0	Methysergide	2.33
Lysergic acid	0	Ergosine	7.08
Lysergamide	0.33	Ergotamine	9.58
Ergometrine	0.50	Ergocornine	10.17
Isolysergic acid	0.83	Dihydroergotamine	11.42
Lumi-LSD	0.83	Ergocryptine	15.17
Lysergol	0.83	Dihydroergocryptine	15.90
Methylergometrine	0.83	Ergocristine	17.30
2-Oxo-LSD	0.92	Ergosinine	17.70
Lysergide (LSD)	1.83	Dihydroergocristine	18.25
Lysergic acid methylpropylamide (LAMPA)	1.98	Bromocriptine	44.33

for 22 compounds measured on a 10-cm column and presented in order of elution. It can be seen that the k' values range from 0 to 44.3 with LSD eluting with $k' = 1.83$. Table II presents the same data but arranged in alphabetical order to facilitate the rapid retrieval of information on a specific compound. The good peak shapes which can be achieved on the present system are demonstrated in Fig. 1 for the separation of nine compounds in under 10 min.

The principal consideration in the recommendation of the present system for forensic examination is the separation of LSD from all the other ergot alkaloids examined. Thus, the unique retention time of LSD amongst the other common ergot alkaloids provides a useful parameter in the overall strategy for the identification of this drug. Initial experiments showed that although the ergot alkaloids can be chromatographed using acidic eluents, alkaline conditions (pH 8.1) were essential to maintain complete discrimination for LSD. Methysergide co-eluted with LSD at pH values

TABLE II

HPLC RETENTION DATA FOR ERGOT ALKALOIDS (ARRANGED IN ALPHABETICAL ORDER)

Compound	*k'*	*Compound*	*k'*
Bromocriptine	44.33	Isolysergic acid	0.83
Dihydroergocristine	18.25	Isolysergide	0
Dihydroergocryptine	15.90	Lumi-LSD	0.83
Dihydroergotamine	11.42	Lysergamide	0.33
Ergocornine	10.17	Lysergic acid	0
Ergocristine	17.30	Lysergic acid methylpropylamide (LAMPA)	1.98
Ergocryptine	15.17	Lysergide (LSD)	1.83
Ergometrine	0.50	Lysergol	0.83
Ergosine	7.08	Methylergometrine	0.83
Ergosinine	17.70	Methysergide	2.33
Ergotamine	9.58	2-Oxo-LSD	0.92

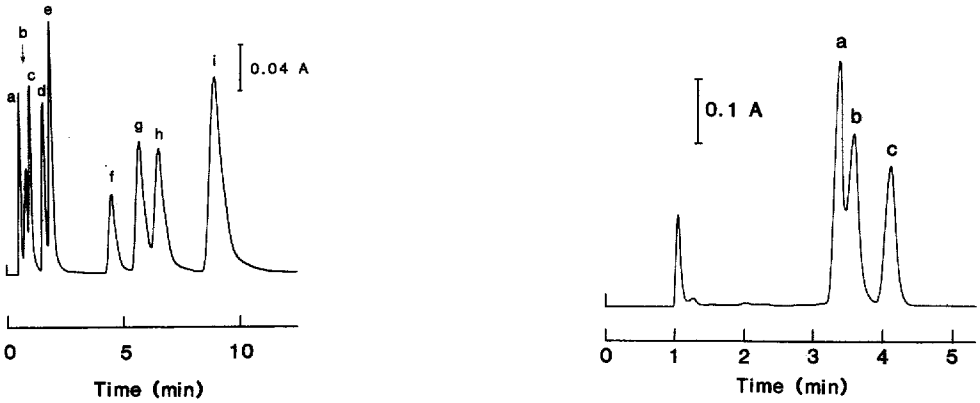

Fig. 1. Chromatography of ergot alkaloids on ODS-silica. Column: ODS-Hypersil, 5 μm (10 cm × 5 mm I.D.). Eluent: 60% methanol containing phosphate buffer (pH 8.1). Flow-rate: 2 ml/min. Detection: UV absorbance at 220 nm. Peaks: a = lysergic acid; b = lysergamide; c = isolysergic acid; d = lysergide (LSD); e = methysergide; f = ergosine; g = ergotamine; h = dihydroergotamine; i = ergocryptine.

Fig. 2. Separation of LSD from LAMPA and methysergide. Column: ODS-Hypersil, 5 μm (16 cm × 5 mm I.D.). Other conditions as in Fig. 1. Peaks: a = lysergide (LSD); b = lysergic acid methylpropylamide (LAMPA); c = methysergide.

of less than 7. Despite the fact that alkaline eluents can be corrosive towards ODS-silica columns, the adoption of a few simple precautions (see Experimental section), in line with our previous experience[27], can ensure that the columns have a long useful life.

The compounds which elute closest to LSD are lysergic acid methylpropylamide (LAMPA) and methysergide. Fig. 2 shows the resolution between these com-

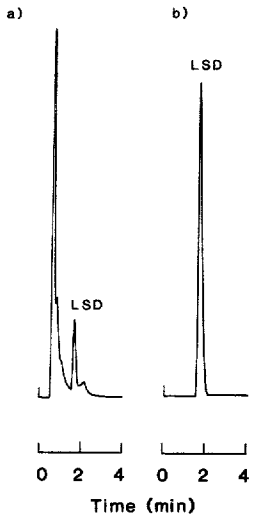

Fig. 3. Analysis of an illicit LSD preparation with (a) UV detection at 220 nm or (b) fluorescence detection with excitation at 312 nm and emission at 400 nm. Other conditions as in Fig. 1.

pounds which was achieved with the present system when using a 16-cm column. Clearly, this separation could be further increased by the use of an even longer column. LSD and LAMPA are isomers, differing only by the nature of the amide substituents, and consequently are generally considered difficult to separate. A previous separation by reversed-phase HPLC has been demonstrated[20] while capillary gas chromatography has also been used[28,29].

The applicability of the present HPLC system to the analysis of illicit LSD preparations has been tested and Fig. 3 shows typical chromatograms arising, in this case, from an aqueous methanolic extract of a "microdot". Fig. 3a shows UV detection at 220 nm while Fig. 3b shows fluorescence detection at 312 nm excitation and 400 nm emission. The selectivity of fluorescence detection is clearly demonstrated, being the detection method of choice for the routine analysis of LSD.

REFERENCES

1 R. A. Heacock, K. R. Langille, J. D. MacNeil and R. W. Frei, *J. Chromatogr.*, 77 (1973) 425.
2 G. Szepesi, M. Gazdag and L. Terdy, *J. Chromatogr.*, 191 (1980) 101.
3 M. Wurst, M. Flieger and Z. Řeháček, *J. Chromatogr.*, 150 (1978) 477.
4 M. Wurst, M. Flieger and Z. Řeháček, *J. Chromatogr.*, 174 (1979) 401.
5 M. Flieger, M. Wurst, J. Stuchlík and Z. Řeháček, *J. Chromatogr.*, 207 (1981) 139.
6 L. Szepesy, I. Fehér, G. Szepesi and M. Gazdag, *J. Chromatogr.*, 149 (1978) 271.
7 C. Eckers, D. E. Games, D. N. B. Mallen and B. P. Swann, *Biomed. Mass Spectrom.*, 9 (1982) 162.
8 F. Erni, R. W. Frei and W. Lindner, *J. Chromatogr.*, 125 (1976) 265.
9 J. Dolinar, *Chromatographia*, 10 (1977) 364.
10 V. Hartmann, M. Rodiger, W. Ableidinger and H. Bethke, *J. Pharm. Sci.*, 67 (1978) 98.
11 A. Yoshida, S. Yamazaki and T. Sakai, *J. Chromatogr.*, 170 (1979) 399.
12 J. C. Young, *J. Environ. Sci. Health*, B16 (1981) 83.
13 B. Herényi and S. Görög, *J. Chromatogr.*, 238 (1982) 250.
14 J. D. Wittwer and J. H. Kluckhohn, *J. Chromatogr. Sci.*, 11 (1973) 1.
15 I. Jane, *J. Chromatogr.*, 111 (1975) 227.
16 J. Christie, M. W. White and J. M. Wiles, *J. Chromatogr.*, 120 (1976) 496.
17 I. Jane and B. B. Wheals, *J. Chromatogr.*, 84 (1973) 181.
18 I. Lurie, *J. Assoc. Off. Anal. Chem.*, 60 (1977) 1035.
19 P. J. Twitchett, S. M. Fletcher, A. T. Sullivan and A. C. Moffat, *J. Chromatogr.*, 150 (1978) 73.
20 G. Megges, *Arch. Kriminol.*, 164 (1979) 25.
21 P. MacDonald, C. F. Martin, D. J. Woods, P. B. Baker and T. A. Gough, *J. Forensic Sci.*, 29 (1984) 120.
22 K. Harzer, *J. Chromatogr.*, 249 (1982) 205.
23 B. Law, R. Gill and A. C. Moffat, *J. Chromatogr.*, 301 (1984) 165.
24 R. Gill, A. H. Stead and A. C. Moffat, *J. Chromatogr.*, 204 (1981) 275.
25 R. Gill, A. A. T. Lopes and A. C. Moffat, *J. Chromatogr.*, 226 (1981) 117.
26 R. Gill, R. W. Abbott and A. C. Moffat, *J. Chromatogr.*, 301 (1984) 155.
27 R. Gill and A. C. Moffat, *J. Chromatogr.*, 233 (1982) 438.
28 H. S. Nichols, W. H. Anderson and D. T. Stafford, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 101.
29 D. T. Stafford, H. S. Nichols and W. H. Anderson, *J. Forensic Sci.*, 29 (1984) 291.