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The Separation of Lysergide (LSD) from Related Ergot Alkaloids and Its Identification in Forensic Science Casework Samples

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ABSTRACT: The separation of lysergide (LSD) from related ergot alkaloids, and its isomer lysergic acid methylpropylamide (LAMPA), has been investigated using the techniques of capillary gas chromatography and high performance liquid chromatography (HPLC). Capillary gas chromatography using fused silica nonpolar bonded phase columns gave good discrimination, and retention indices for several ergot alkaloids have been measured. The applicability of this technique to the analysis of illicit LSD preparations has been demonstrated by preparing extracts from LSD microdot tablets and card and paper squares. The HPLC systems examined in the present study were unable to achieve baseline resolution of LSD and LAMPA.

KEYWORDS: toxicology, lysergic acid, chromatographic analysis

Methods are required by forensic science laboratories for the identification of lysergide (lysergic acid diethylamide, LSD) from related ergot alkaloids as cases involving this substance currently form up to 5% of all drug cases in the United Kingdom. LSD is rarely encountered in forensic science casework as a powdered drug, but appears as impregnated card or paper squares and microdot tablets, which typically contain 100 μg of the drug. Analysis of these illicit preparations is therefore difficult because of the low dosage and the nature of the dosage form.

High performance liquid chromatography (HPLC) using ODS-silica has been used to separate ergot alkaloids [*1,2*], and coupled with fluorescence detection it offers the necessary sensitivity and selectivity for LSD analysis. The retention properties of 22 ergot alkaloids on this HPLC system have been measured [*2*], and all compounds are well separated from LSD with the exception of lysergic acid methylpropylamide (LAMPA). Baseline resolution of LSD and LAMPA was not obtained with this system; however, other workers [*3,4*] have reported satisfactory resolution of these compounds using silica HPLC columns or reversed phase ion pair chromatography. LSD and LAMPA are isomeric compounds which differ only in the nature of the alkyl substituents (Fig. 1). LAMPA, like LSD, is therefore a controlled substance and is listed in Part I of Schedule 2 to the Misuse of Drugs Act 1971.

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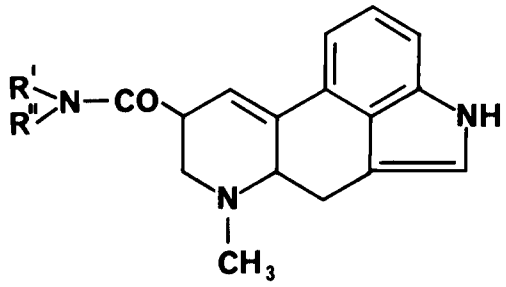

FIG. 1—*LSD:* R′ = R″ = C_2H_5
LAMPA: R′ = CH_3; R″ = n-C_3H_7

The introduction of fused silica bonded phase capillary columns has facilitated the gas chromatographic (GC) analysis of labile ergot alkaloids. The separation of LSD from LAMPA by capillary gas chromatography has been demonstrated in several publications [5,6].[2] Using a methylsilicone phase, separations have been achieved under a variety of instrumental conditions (both isothermal and temperature programmed) and using either split or on-column injection.

The purpose of the present work is to consider the identification of LSD from related ergot alkaloids with particular reference to the discrimination of LSD and LAMPA in forensic science casework. The role of HPLC and GC in such analyses has been considered and extraction methods for illicit LSD preparations have been examined.

Experimental Procedure

Materials

Methanol, methyl-tert-butyl ether, isopropanol, and acetonitrile (S grade) were all HPLC grade and obtained from Rathburn Chemicals (Walkerburn, Great Britain). Other chemicals were AnalaR grade from BDH (Poole, Great Britain). All ergot alkaloids were from the drug collection of the Central Research Establishment, Home Office Forensic Science Service.

Straight chain alkane hydrocarbons from C-22 and C-32 (except C-31) were obtained from SGE (Milton Keynes, Great Britain) and Sigma (Poole, Great Britain).

Capillary Gas Chromatography

Chromatography was performed using a Perkin Elmer Sigma 3B gas chromatograph fitted with a capillary injector operated in the split mode at a split ratio of 20:1. The oven was operated isothermally at 275°C with an injector temperature of 300°C and a detector temperature of 325°C. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. Flame ionization detection was used.

The column was a 25-m BP1 fused silica bonded phase column, 0.22-mm inner diameter (ID) with a film thickness of 0.25 μm (SGE, Milton Keynes, Great Britain).

One-microlitre samples of LSD, LAMPA, and related ergot alkaloids, present as free bases in *n*-butyl acetate, were injected onto the column using the "hot needle" technique [7,8]. Kovats retention indices [9] were calculated for each ergot alkaloid, the isothermal retention index being a logarithmic interpolation between the two aliphatic hydrocarbons

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eluting immediately before and after the component. The appropriate hydrocarbons were injected onto the capillary column as solutions in pentane. All retention index measurements were carried out in duplicate.

High Performance Liquid Chromatography

Chromatography was performed with an Applied Chromatography Systems pump (Model 351), a Rheodyne 7125 injection valve (fitted with a 5-μL loop), and a Kratos ultraviolet (UV) detector (Model 773) operated at 220 nm.

Experiments were carried out with two HPLC systems:

1. A 16-cm by 5-mm ID stainless steel column was packed with 3-μm ODS-Hypersil (Shandon Southern Products, Runcorn) by a slurry procedure, dispersing the packing material in isopropanol and using hexane as the pressurizing solvent. The eluent was that described by Gill and Key [2], that is, 60% methanol containing phosphate buffer (pH 8.1). The 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). An eluent flow rate of 1.5 mL/min was used. A series of 60% methanolic eluents at different pH values (7.0, 7.5, 8.7, 9.1, 10.0, and 11.0) were also prepared by the addition of phosphoric acid or sodium hydroxide to the initial eluent.

A short column (5-cm by 4.5-mm ID) packed with coarse silica (about 40 μm) was included between the pump and the injection valve to protect the column from these alkaline eluents. In addition, the analytical column was never left in contact with static eluent, and was washed out with methanol/water (60:40 v/v) at the end of each working day.

For all of the eluents examined, LSD and LAMPA were injected on to the column dissolved in methanol.

2. A 10-cm by 5-mm ID stainless steel column was packed with 5-μm APS-Hypersil (Shandon Southern Products, Runcorn) by a slurry procedure, dispersing the packing material in isopropanol and using hexane as the pressurizing solvent. An eluent of methyl-tert-butyl ether/isopropanol (95:5 v/v) was prepared, and solutions of LSD and LAMPA present as free bases in methyl-tert-butyl ether were injected on to the column.

Extraction of Illicit LSD Preparations

Extracts for analysis by capillary GC were prepared from the three types of illicit LSD samples (card squares, paper squares, and microdot tablets) commonly encountered in forensic science casework. Before extraction, the multiple layers of the card squares were separated before being cut into small pieces. Paper squares were also cut into pieces before extraction while microdot tablets were crushed.

Two approaches to the extraction of LSD were tried:

1. To an LSD square or microdot tablet, 500 μL of methanol/water (50:50 v/v) was added and the sample vortex mixed for 30 s. Concentrated ammonia (about two drops) and 1 mL of methyl-tert-butyl ether were then added and the mixture placed in an ultrasonic bath for 20 min in the dark. The ether layer was removed and evaporated to a small volume (about 20 μL) under a stream of nitrogen gas. A 1-μL injection of this fraction was analyzed by capillary GC.

2. To an LSD square or microdot tablet, 500 μL of methanol was added and the sample vortex mixed for 30 s. Concentrated ammonia (about two drops) was then added and the mixture placed in an ultrasonic bath for 20 min in the dark. The methanol layer was removed from the card/paper debris and evaporated to a small volume (about 20 μL) under a stream of nitrogen gas. A 1-μL injection of this fraction was analyzed by capillary GC.

Results and Discussion

The success of previous workers in achieving good resolution between LSD and LAMPA [5,6][2] on nonpolar bonded phase fused silica columns prompted the use of a similar column in the present study. A survey of capillary columns in routine use within the Drugs and Toxicology Divisions of U.K. Forensic Science Laboratories has shown that a particular bonded methylsilicone column (BP1, manufactured by SGE) is the most widely used. Consequently a 25-m column of this type was selected for the present work.

Table 1 gives a list of retention indices for ten ergot and related alkaloids in order of elution from the capillary column at 275°C. The ten alkaloids chosen for analysis were those more likely to be encountered and those for which samples were readily available. The retention index of LSD was found to be 3130, and under these isothermal conditions it was well resolved from all related ergot alkaloids examined, including LAMPA (retention index 3175). Several of the ergot alkaloids gave multiple peaks in the chromatogram, showing the presence of minor impurities, probably breakdown products. Lysergol, iso-lysergic acid, and 2-oxo-LSD gave slightly tailing peaks, whereas the other compounds gave good peak shapes.

For the present work, isothermal GC conditions have been used. This is because of the temperature dependence of retention indices. The retention index concept was developed for use under isothermal conditions where a logarithmic relation between retention time and carbon number is observed [9]. Retention indices have been shown to vary under different temperature programmed conditions [10,11], and this is highlighted by the retention index values reported for LSD on nonpolar capillary columns with different programs (3153[2] and 3081 [5,6]).

If gas chromatography is used for the identification of LSD in forensic science casework, it is always desirable to check the performance of the column for the separation of this drug from related ergot alkaloids. Since LAMPA elutes close to LSD, a convenient check on chromatographic resolution would be achieved by injecting a mixture of these two compounds. The retention time of the "unknown" could then be compared with the retention times of LSD and LAMPA for identification purposes. However, because of the instability of solutions containing ergot alkaloids, fresh standards must be prepared regularly. This is particularly costly for LSD and LAMPA since pure drug samples are not readily available. An alternative approach has been investigated whereby a drug (noscapine or etodroxizine) which elutes between LSD and LAMPA acts as a retention marker. A simple coinjection test to determine the retention time of the "unknown" relative to one of the retention markers can clearly discriminate between LSD and LAMPA. The separations obtained between LSD and LAMPA and the marker compounds noscapine and etodroxizine are shown in Figs. 2 and 3, respectively. The retention indices of noscapine and etodroxizine are 3155 and 3148, respec-

TABLE 1—*GC retention data for LSD and related ergot alkaloids arranged in order of increasing retention (minor peaks are shown in brackets).*

Compound	Retention Index
Ergotamine	2410 (2373)
Lysergol	2686
Iso-lysergic acid	2947 (3013)
Dihydroergotamine	2953 (2318, 2342)
Ergometrine	2999
Lysergide (LSD)	3130 (2819, 2839)
Lysergic acid methylpropylamide (LAMPA)	3175 (3090)
Lumi-LSD	3200
Methysergide	3300
2-Oxo-LSD	3415 (3210, 3325)

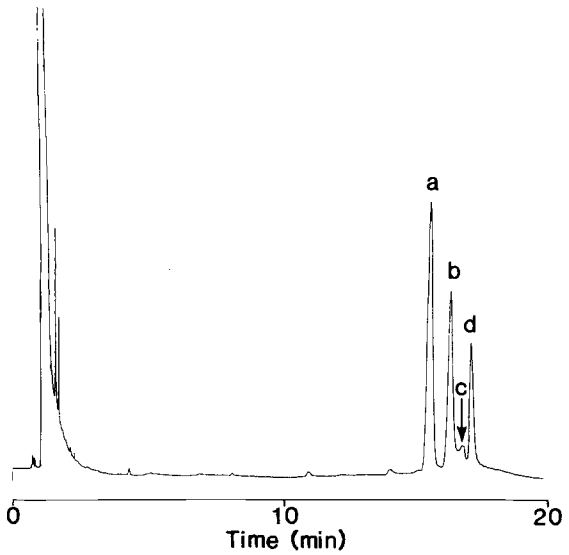

FIG. 2—*Chromatogram of LSD* (a), *noscapine* (b), *noscapine impurity* (c), *and LAMPA* (d). *Chromatographic conditions: 25-m BP1 fused silica bonded phase column, 0.22-mm ID, 0.25-μm film thickness. Isothermal oven conditions 275°C, injector temperature 300°C, detector temperature 325°C (FID). Nitrogen carrier gas flow rate of 1 mL/min. Split injection: 20:1 split ratio.*

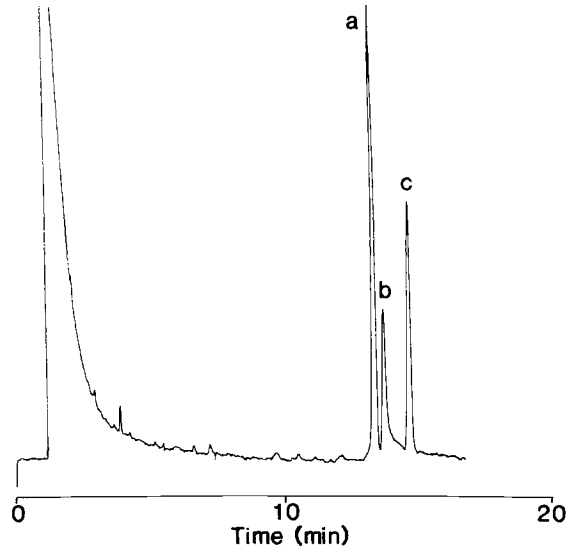

FIG. 3—*Chromatogram of LSD* (a), *etodroxizine* (b), *and LAMPA* (c). *Chromatographic conditions as in Fig. 2.*

tively. Noscapine gave a minor peak at retention index 3163 which by mass spectrometry was found to give the same base peak ($m/z = 220$) as the parent drug.

In addition to the work with capillary GC, attempts have been made to improve the resolution of LSD and LAMPA using HPLC since the 5-μm ODS-silica system described by Gill and Key incompletely resolves these two compounds [*2*]. A column packed with 3-μm ODS-silica was investigated, but this did not significantly improve resolution. Further experiments using the 3-μm column in combination with a series of 60% methanolic eluents of increasing pH (from 7.0 to 11.0) showed that the separation of LSD and LAMPA improved with increasing alkalinity, to such a point that with an eluent of pH 11.0, baseline resolution was almost achieved. However, because alkaline eluents are corrosive towards ODS-silica, leading to reductions in column lifetime, this approach is not recommended.

Further attempts to resolve LSD and LAMPA involved a normal phase HPLC system using an amino-propyl bonded phase (APS-Hypersil) and an eluent containing methyl-tert-butyl ether/isopropanol (95:5 v/v). Similar systems have been employed previously for the separation of ergot alkaloids [*12–14*]. LSD and LAMPA gave capacity factors of 3.36 on this system but showed no resolution.

The extraction of illicit LSD preparations for analysis by HPLC is straightforward involving the addition of methanol/water (50:50 v/v) with ultrasonic vibration [*15*]. Sensitive and specific detection for LSD can be achieved with a fluorescence detector, providing a good method of quantification in forensic science casework [*1,2,15*]. The preparation of extracts suitable for analysis by capillary GC has been investigated here. It was important to obtain an efficient extraction procedure because of the small amounts of LSD present in illicit preparations. Further, the extracts must be clean to provide sufficient selectivity when using flame ionization detection (FID). The extraction method selected for casework samples involved the addition of methanol/water, liberating the free base by the addition of concentrated ammonia, followed by extraction with methyl-tert-butyl ether. Chromatograms obtained for extracts prepared in this way for three illicit LSD preparations [Fig. 4(*a*), (*b*), and (*c*)] show LSD to be well separated from coextracted material and that the technique has sufficient sensitivity.

Extractions using diethyl ether gave comparable results to those obtained with methyl-tert-butyl ether, but because of its lower volatility, the latter is preferred to avoid excessive solvent evaporation during ultrasonic vibration. Methyl-tert-butyl ether was also preferable to *n*-butyl acetate because cleaner extracts were obtained. The addition of sodium chloride during the extraction procedure was investigated to observe whether extraction efficiency increased but no improvement occurred. Similarly, the use of sodium bicarbonate rather than concentrated ammonia was investigated but gave poorer extraction efficiencies. The simple addition of methanol to an illicit LSD preparation with no subsequent extraction into a second solvent showed LSD to be present but the level of coextracted components was high and interfered with analysis.

Conclusions

For the analysis of LSD in forensic science casework, the techniques of HPLC and capillary GC can complement each other. HPLC, using ODS-silica columns coupled with fluorescence detection, is recommended for quantification [*1,2,15*]. Using HPLC, LSD is differentiated from most ergot alkaloids, but baseline resolution of LSD and LAMPA was not obtained with the reversed phase system used [*2*]. However, if a LAMPA reference sample is available and the column resolution can be tested, then the separation of these compounds by HPLC is satisfactory. Capillary GC using fused silica bonded phase columns gave excellent separation of ergot alkaloids including LSD and LAMPA, making the technique ideal for the identification of these compounds. Furthermore, retention markers (noscapine or etodroxizine) can be used to assist GC identification if LAMPA samples are not readily avail-

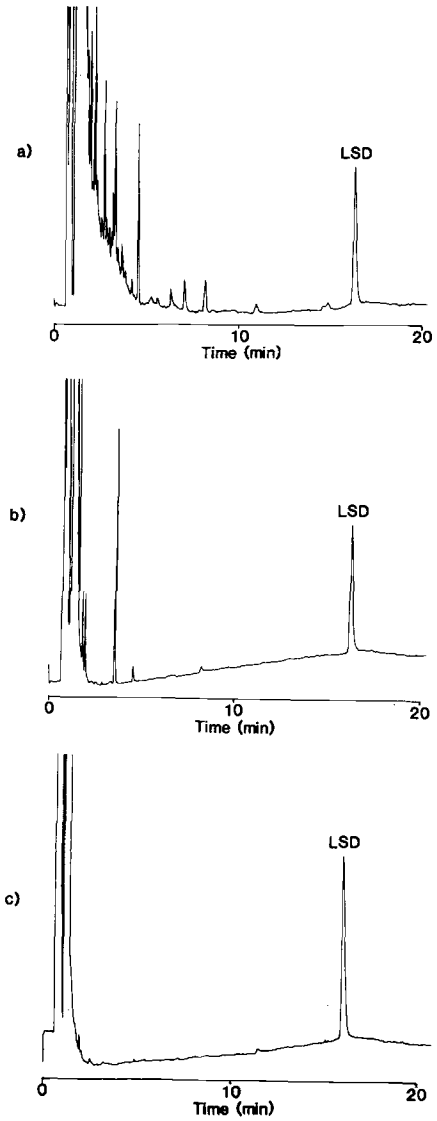

FIG. 4—*Analysis of three illicit LSD preparations:* (a) *card square,* (b) *paper square, and* (c) *microdot tablet. Chromatographic conditions as in Fig. 2.*

able. The extraction of illicit LSD preparations for analysis by either HPLC or capillary GC is simple and straightforward.

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