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Note

The characterization of LSD in illicit preparations by pressure-assisted liquid chromatography and gas chromatography

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The identification of lysergic acid diethylamide (LSD) in drug seizures is an important activity in the forensic field at the present time. Because of the small amounts of drug that constitute an active dose (*ca.* 100 μg), the methods used need to be highly sensitive, and the legal implications of the analysis necessitate that specificity is also a feature of the analytical method.

To assist in the characterization of LSD, a variety of separation techniques has been proposed, including column[1], thin-layer[2], paper[3], gas (GC)[4] and liquid chromatography (LC)[5]. These procedures are generally used in conjunction with spectroscopic techniques such as UV spectrophotometry[1], IR spectroscopy[6], spectrofluorimetry[7] or mass spectrometry[8].

The procedures described in this paper were investigated as part of a programme of improving the speed and sensitivity of the LSD analytical schemes used in the case work of this laboratory. They have been applied to the analysis of a wide range of LSD preparations.

EXPERIMENTAL

Gas chromatography

A variety of column packings was studied and the GC of silylated LSD, prepared by the method of Lerner and Katsiaficas[4], was investigated. The performance of glass, stainless-steel and PTFE columns in achieving a successful separation was also assessed. Subsequently, modifications to the silylation procedure were made by carrying out the derivatization reaction in the injection port of the gas chromatograph using either bis(trimethylsilyl)acetamide (BSA) or N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA). These preliminary experiments led to the selection of the following procedure for the extraction and determination of LSD in tablets.

Half a tablet was crushed in a centrifuge tube with 1 ml of chloroform and the tube flushed with nitrogen. The tube was stoppered and placed for 10 min in an ultrasonic bath to effect solution of the LSD (normally present as the tartrate salt). The solution was then filtered and the filtrate evaporated to dryness in a stream of nitrogen while on a water-bath at 60°. The residue was dissolved in 20 μl of dimethylformamide and 1 μl drawn into a 10-μl syringe containing 2 μl of BSA. The contents of the syringe were flash-injected into the metal injection port of the chro-

matograph (Varian, Model 1400) which was maintained at 280°. The GC separation was effected on a 1.8 m×3 mm I.D. glass column containing 1.5% OV-17 on Chromosorb W (80–100 mesh) AW DMCS. The column was maintained at 270° and the flame ionization detector at 280°. Nitrogen was used as the carrier gas.

Liquid chromatography

In studying the LC characterization of LSD, a gas-pumped solvent system was used similar to that described previously[9]. The eluate was monitored using a UV detector (Varian) and a fluorimetric detector (Laboratory Data Control, Fluoro-Monitor) connected in series. The detector signals were recorded on a dual-pen recorder (Smiths Servoscribe, Model RE 520).

A variety of column packing materials was investigated—Corasil C_{18}, Corasil II, OPN Porasil C, Carbowax 400, Porasil C (all available from Waters Ass., Stockport, Great Britain) and Zipax strong cation exchanger (available from DuPont, Hitchin, Great Britain). Various solvent systems for use with these column packings were studied with the object of resolving LSD from a variety of chemically related ergot alkaloids, which are not legally restricted. The procedure finally adopted for the extraction and determination of LSD in tablets was as follows.

Half a tablet was crushed in 0.5 ml of methanol and 5 μl of the resulting solution, free from suspended matter, was injected on to the LC column. The column used was a 1.2-m length of stainless steel of 3.2 mm O.D. and 2.2 mm I.D. packed with Corasil C_{18}. The solvent was methanol–0.1% aqueous ammonium carbonate (6:4) pumped at 750 p.s.i. to give a flow-rate of 0.65 ml/min. The eluate was monitored with the fluorimetric detector. To maintain moderately isothermal conditions, the column was lagged with polyurethane foam, but to compensate for any variations in retention time a standard LSD solution was always injected before a sample solution.

RESULTS

Gas chromatography

High column temperatures were found to be necessary in order to chromatograph silylated LSD and only two stationary phases (SE-30 and OV-17) were investigated, coated on either glass beads or silanized Chromosorb W. The latter support and the OV-17 stationary phase were found to give the best results, and symmetrical peaks at a retention time of 16 min were obtained when using the conditions described above. The use of glass columns was found to be essential if on-column decomposition was to be avoided.

The flash-injection procedure described was found to work very well and linear, reproducible calibration graphs could be obtained for solutions containing from 0.1 to 5 μg of LSD. The use of MSTFA resulted in lower yields of silylated LSD and led to greater variation in the peak heights of replicate injections.

Although the procedure was effective for detecting LSD in a number of tablets, it was found not to be completely satisfactory. In many cases, for example, the chromatograms were characterized by a multiplicity of peaks that often partially or completely obscured the LSD peak (see Fig. 1). In addition, the use of relatively large amounts of the silylating reagent often led to the development of detector noise due to deposits of silica being formed.

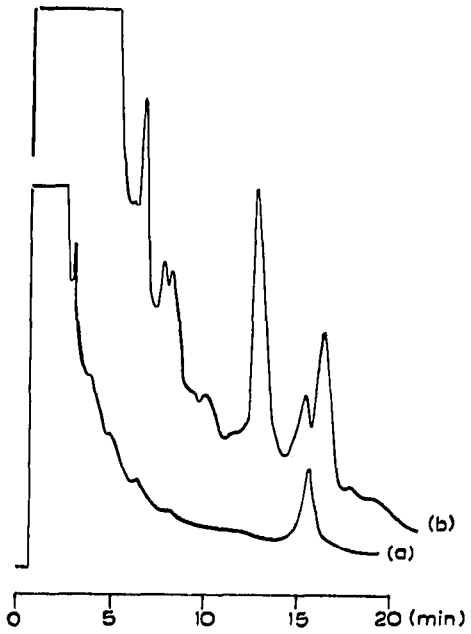

Fig. 1. GC separation of LSD on a 1.8-m column containing 1.5% OV-17 on Chromosorb W at a temperature of 270°: (a) a 2-μg sample of LSD; (b) a tablet extract.

Liquid chromatography

Of the various column and solvent systems examined, the combination detailed above was the only one to give a satisfactory analytical separation of LSD from other ergot alkaloids in an acceptable analysis time. The retention time data are given in Table I and typical chromatograms in Fig. 2. The retention time was found to be temperature dependent, being halved by increasing the temperature from 20 to 40°.

The sensitivities of the two detectors were approximately equal, with a detection limit of about 2–10 ng of LSD. The major limitation to the use of the UV detector was its lack of selectivity. In some tablet samples, for example, the extracts monitored with the UV detector gave large peaks on the chromatograms due to co-extractives derived from tablet excipients. The use of the fluorimetric detector obviated this problem and gave chromatograms displaying a linear relationship between peak height and amount of LSD injected over the range 0–750 ng. The reproducibility of the procedure was high, with the peak height showing a relative standard deviation of 0.86% (10 determinations). The only effect of large amounts of UV absorbing species passing through the detector was to create a slight negative peaking.

About 50 different forms of LSD preparation have now been studied by the described procedure and in all instances a satisfactory analysis has resulted.

DISCUSSION

Gas chromatography

In those cases where the procedure described was successful, the use of in-

TABLE I

RETENTION TIMES OF LSD AND ERGOT ALKALOIDS

The retention time data were obtained under the following operating conditions: column, 1.2 m × 2.2 mm I.D. stainless-steel column packed with Corasil C_{18}; solvent, methanol–0.1% ammonium carbonate solution (6:4); inlet pressure, 750 p.s.i.; flow-rate, 0.65 ml/min; temperature, 27°; detector, Laboratory Data Control FluoroMonitor.

Compound	*Retention time relative to LSD*
LSD	1.00 (retention time = 4.4 min)
Iso-LSD	1.39
Dihydroergocornine base	1.51 *
Dihydroergocristine base *	2.42 *
Dihydroergocryptine methane sulphonate	1.85 *
Ergometrine maleate	0.74
Ergometrinine base	0.78
Ergosine base	1.29
Ergosinine base	1.44
Ergotamine tartrate	1.70
Ergocristinine base	2.98
Ergocryptinine base	2.18
Ergocornine methane sulphonate	1.39
Ergocristine methane sulphonate	2.08
Ergocryptine methane sulphonate	1.71
Ergotoxin ethane sulphonate	1.40, 1.70, 2.09
Lysergic acid monohydrate	0.69
Lysergamide	0.74
Lysergol base	0.89
Methylergometrine maleate	0.71
Methylsergide bimaleate	0.92

* Compounds detected only with the UV detector.

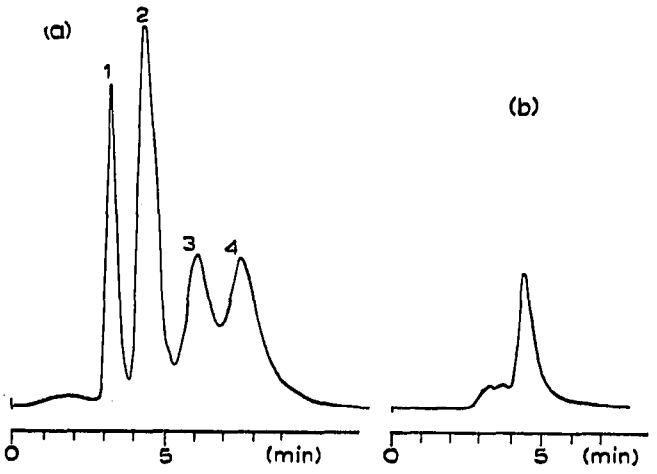

Fig. 2. LC separation of LSD on a 1.2-m column containing Corasil C_{18} with 6:4 methanol–0.1% ammonium carbonate solution as solvent. (a) A synthetic mixture: 1 = ergometrine maleate, 2 = LSD, 3 = iso-LSD, 4 = ergotamine tartrate. (b) A tablet extract.

jection port silylation resulted in significant savings in time compared with previously published methods[4], because the silylation reaction used previously was carried out under reflux. The major drawback to the injection port silylation procedure is that silica is deposited in the detector owing to combustion of the excess of silylating agent. It is unfortunate that the use of MSTFA[10], designed to minimize silica formation, was accompanied by a decrease in the reproducibility of the analysis.

The poor results obtained with a number of illicit tablets could be improved only by using a separation and clean-up stage to isolate LSD from other co-extractives. In the light of the lengthened analysis time that this would introduce, and because of the very satisfactory results obtained by LC, there appeared to be little value in extending our studies in this area.

Liquid chromatography

The LC procedure described appears to be superior to chromatographic methods previously reported for the determination of LSD in terms of both speed and specificity. In this laboratory, for example, two thin-layer separations with a total running time of *ca.* 2 h were necessary to separate LSD from other ergot alkaloids. The LC procedure is also much more readily quantitated than is the thin-layer analysis. The superiority over GC is self-evident from the results reported in this paper.

When compared with the two other pressure-assisted LC separations previously reported for LSD[5,11], the procedure described here has certain advantages. In one of those procedures[5], a preliminary column clean-up was used. It is assumed that this was necessary to remove co-extractives that interfered with the UV detection of LSD. The use of the fluorimetric detector, with its extra specificity, eliminates this type of problem. The other reported procedure[11], although not directly applied to tablet analysis, also uses fluorimetric detection. In that procedure, the separation is based on liquid–solid adsorption and the solvent used was found to cause appreciable fluorescence quenching. The use of a reversed-phase solvent system, which is made possible by using a permanently bonded support such as Corasil C_{18}, obviates this type of problem. The use of a reversed-phase type of system also overcomes one of the major difficulties associated with use of columns based on a liquid–solid adsorption separation. In our experience, it is very difficult to avoid the irreversible adsorption of water on such columns, and this leads to steadily deteriorating separations and poor reproducibility.

The solvent system suggested for the LC separation described in this paper was arrived at experimentally. No systematic study of the effect of using ammonium carbonate solutions in place of water on the ergot alkaloid separation was made, but in general it was found to alter the elution sequence, improve the resolution and shorten the retention time.

The use of LC procedures has the additional advantage over other chromatographic methods of enabling the LSD to be readily collected for examination by a spectroscopic technique. It is our practice to collect the fraction of the eluent ascribed to LSD, dilute it with water and to determine its fluorescence characteristics using a spectrofluorimeter. Although the fluorescence spectrum (418 and 320 nm for the emission and excitation maxima, respectively) is similar to that of the other ergot alkaloids, the combination of this and the unique retention time is sufficient, in our experience, to characterize the material as LSD.

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