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An Analytical Study of Illicit Lysergide

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ABSTRACT: A procedure for the analysis of lysergide, applicable to the illicit material dispersed on paper sheets and in tablets is described. Thin-layer and high performance liquid chromatography are used to separate lysergide from related compounds. Attention is drawn to the limitations of infrared spectroscopy for distinguishing between the salt and base forms of lysergide. Ion chromatography is used to separate the anions present in illicit samples. The quantities of any anions present that form stable salts with lysergide are compared with the amount of lysergide in the sample, from which the salt form of the presentation is deduced.

KEYWORDS: toxicology, lysergic acid, chromatographic analysis, spectroscopic analysis

The United Kingdom legislation with respect to the control of drugs of abuse is governed by the Misuse of Drugs Act 1971. Schedule 2 of this Act lists the controlled drugs and included in Part I Paragraph 1 of this list is "lysergide and other *N*-alkyl derivatives of lysergide." Paragraph 4 of the same schedule includes control of "Any salt of a substance for the time being specified in paragraphs 1 to 3 above." It is therefore necessary for the analyst to identify the particular form (that is, salt or base) in which the drug is presented. Failure to specify this could lead to difficulties in a court of law where the defendant is charged with possession (or importation) of a drug in which the form of drug is not specified.

These requirements do not generally present difficulties for most drugs seized at the point of importation as they are relatively pure and available in substantial quantities. Under these circumstances seized drugs can be identified adequately with well-established analytical techniques such as chromatography and infrared spectroscopy (IR), while particular salts may be identified using IR or X-ray diffraction. The position is analytically much more difficult in the case of mixtures of drugs in which different salts may be associated with each of the drugs present. Lysergide (LSD) presents difficulties because most seizures involve only small quantities of material of which lysergide itself forms only a very minor part.

There have been occasions in British courts of law when charges have been dismissed on the grounds that the particular form of the drug was not specified. Two cases in particular refer to lysergide. In *R. v. Leaman and Leaman* (Maidstone Crown Court), the defense argued that it was a legal requirement to state under which paragraph of an Act an indictment is constructed. In the case of the Misuse of Drugs Act 1971, this meant that it was necessary to

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determine whether lysergide was present as the base or a specified salt. The prosecution had not established this and the defendant was charged merely with possessing lysergide. The case was dismissed. In *R. v. Steeper and Parsons* (Inner London Sessions House) it was ruled that "each related chemical analogue is a separate substance." Failure to specify the particular form of a controlled drug must result in the case being dismissed.

It is also helpful to the court if the purity of the drug present, in addition to its weight, can be given in order that a monetary value may be assigned to a seizure. The minimum analytical requirements for forensic science purposes in the United Kingdom are thus identification of the drug, its form (that is, acid, base, or salt) and its quantitation. The present paper is directed towards providing this information for seizures of lysergide.

Discussion

Lysergide is rarely encountered in the form of a powdered drug, the most common forms of presentation being as small tablets, "microdots," and paper sheets, "blotters," in which there is a vast excess of diluent.

Several blotters are depicted in Fig. 1. One dose is generally represented by a 5- by 5-mm square of paper containing about 50 μg of lysergide (Table 1). The blotters range in sophistication from a simple piece of colored paper to multi-layered forms. The latter may consist of a plastic base, a sheet of absorbent paper impregnated with lysergide, and a top paper, or plastic coated paper, bearing a design.

Microdots, Fig. 2, are small tablets, often colored, of varying shapes and containing a dose similar in quantity to that in blotters. Various materials, typically lactose and starch, may be used as excipient.

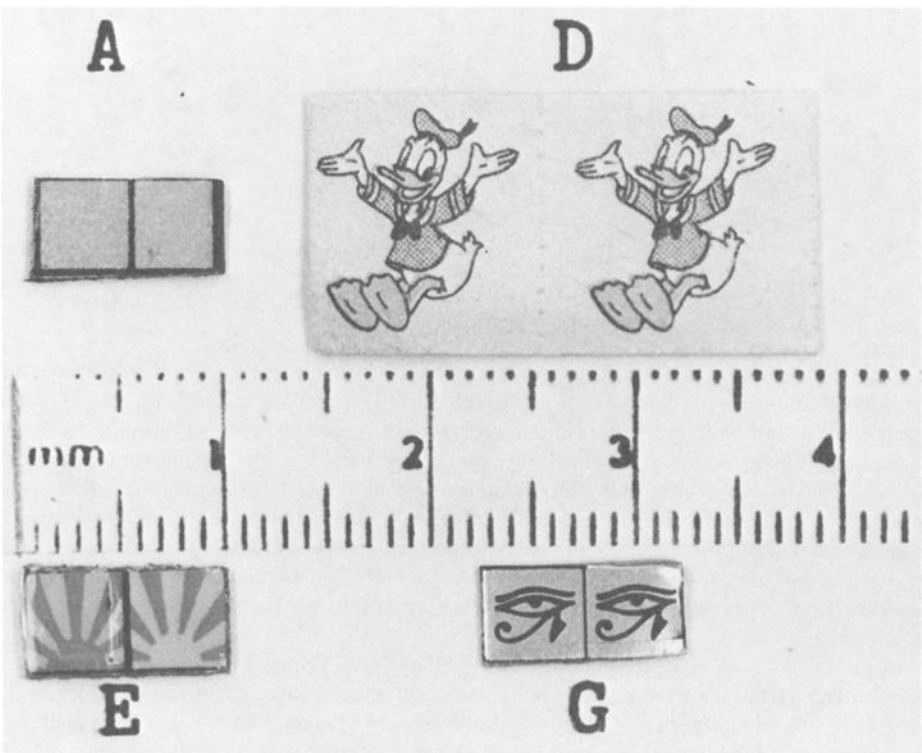

FIG. 1—*Typical lysergide paper presentations.*

TABLE 1—*Quantitative examination of some illicit lysergide presentations.*

Sample Reference	Lysergide/Dose, μg	Tartrate/Dose, μg	
		Found[a]	Expected[b]
A	48.6	ND	...
B	41.6	ND	...
C	40.2	ND	...
D	58.2	11.3	10.9
E	56.0	10.9	10.5
F	35.0	7.2	6.7
G	20.0	ND	...
H	35.0	ND	...
I	50.4	ND	...

[a]ND = not detected.
[b]Based on stoichiometric proportions, that is, $(lysergide)_2$ tartrate.

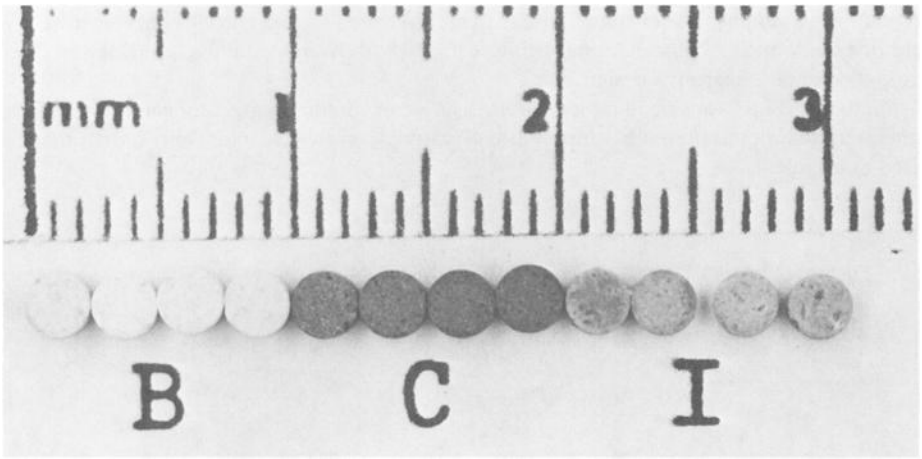

FIG. 2—*Typical lysergide microdot presentations.*

These presentations render the complete analysis of lysergide difficult. Firstly, because the very low dosage, even of a moderately large seizure of lysergide, will yield little drug. For example, a sheet of lysergide paper containing 500 doses would yield in the region of 25 mg of drug. Additionally the concentration of dyes and other additives, such as glues or plasticizers, often is much greater than the concentration of lysergide itself. Naturally occurring ergot alkaloids or reaction intermediates are also present in many seizures, but in themselves present few analytical problems as their concentration is usually less than that of lysergide. Thus, the extraction procedures generally employed yield a complex mixture from which it is not an easy matter to separate sufficient lysergide in a pure form subsequently to analyze by routine methods.

A number of solvent systems have been developed for thin-layer chromatographic (TLC) separation of lysergide from related compounds [*1*]. None of these systems resolves lysergide unequivocally from other ergot alkaloids. Detection of the ergot alkaloids has been achieved by a variety of techniques. For example, their hydrolysis to characteristic amino acids, which can be detected by their reaction with ninhydrin [*2*]. Unfortunately, the hydrolysis product of lysergide is diethylamine and this does not react with ninhydrin. Although some ergot alka-

loids can be detected by their fluorescence under ultraviolet light, many are nonfluorescent and therefore this method is not universally applicable. The most commonly used method of detecting these alkaloids on TLC plates is by a spray reagent containing acidified *p*-aminobenzaldehyde. This reagent reacts with virtually all the ergot compounds of interest, including lysergide, giving a blue or violet coloration.

Although several gas liquid chromatographic (GLC) procedures for the analysis of lysergide have been reported [*1*], they are all limited by the thermal instability of lysergide and other lysergic acid analogues. A number of workers [*3*] have reported satisfactory results by using derivatization techniques, with separation in all-glass chromatographic systems [*4*]. However, there are still indications of thermal decomposition when using these techniques. It has also been observed [*4*] that some samples of illicit lysergide produce several unidentified peaks, some of which interfere with the peak attributed to the derivatised lysergide. Resolution of isomers by GLC, even after derivatization, has not been achieved.

The instability of the ergot alkaloids makes combined GLC and mass spectrometry (GLC/MS) of limited value in establishing their identity. Most published data has been obtained by the direct insertion of pure materials into the ion source [*5,6*]. Mass spectroscopy (MS) is unsuitable for distinguishing between optical isomers [*6*] and therefore the technique cannot distinguish between lysergide and 8-epilysergide (iso-LSD). For illicit samples, MS is only of value after preparative scale TLC or high performance liquid chromatographic (HPLC) separation.

High performance liquid chromatography has proved to be an effective technique for the separation of ergot alkaloids [*7–9*]. Detection is generally by fluorimetry, although this can be supplemented with an ultraviolet (UV) absorbance detector to identify the nonfluorescent ergot alkaloids [*8*]. Thus, HPLC coupled with fluorescence detection provides a technique that is very sensitive and specific and therefore is suitable for the examination of illicit lysergide.

The IR spectra of lysergide tartrate published by Mesley and Evans [*10*] in 1969 match spectra obtained by the present authors on a fresh authentic sample of the tartrate salt. The reference collection of spectra published by Sadtler [*11*] includes a spectrum attributed to lysergide derived from the same source. This spectrum also matches those obtained from the tartrate salt in our own laboratory. However, in the Sadtler collection the compound is referred to as *N,N*-diethyllysergamide, without specifying base or salt. The quoted melting point for the lysergide compound is a literature value corresponding to lysergide base. Cromp and Turney [*12*] published the spectrum of lysergide base and this is in close agreement to the base spectrum of Mesley and Evans [*10*]. The spectrum of lysergide tartrate published by Lerner [*3*], although bearing superficial resemblances to other published spectra of both base and tartrate, exhibits a number of differences. This situation leads to a confusion over lysergide spectra making it difficult to authenticate a given lysergide spectrum.

The technique of attenuated total reflectance IR (ATRIR) is, in principle applicable to samples where the lysergide is present at a surface as on blotters. However, in practice this yields spectra that are difficult to interpret because of the other materials present.

One of the difficulties of handling lysergide lies in its inherent instability, particularly in the form of the base. It is therefore not surprising that the various published spectra show differences some of which, as pointed out by Mesley and Evans [*10*], can be attributed to the use of potassium bromide discs rather than nujol. It is our opinion that a distinction cannot be reliably made between the base and tartrate salt using IR spectroscopy, irrespective of the additional problems associated with co-extracted diluents and dyes.

Ion chromatography in which various anions or cations are separated in the liquid phase may offer a means of establishing whether lysergide is in an ionic form. The detection of a particular anion from a sample does not prove the existence of that salt of lysergide because such ions may be present in the paper or diluent before the addition of lysergide. However, if a quantitative relationship can be shown between lysergide and the anion this would provide strong evidence that the lysergide is present in that particular ionic form. Absence of any anions

commonly associated with lysergide would prove *per se* the presence of lysergide as the free base.

This laboratory has therefore adopted the following analytical procedure for the analysis of LSD from illicit sources: thin-layer chromatography (TLC) for initial identification; high performance liquid chromatography (HPLC) for identification and quantitation based on retention time and fluorescence at a particular wavelength; and ion chromatography to provide evidence for the form of the protonated base.

Methods

Thin-Layer Chromatography

Acetone is used as the mobile phase with two stationary phases, both supported on glass plates. The R_f for lysergide is 0.35 on silica and 0.91 on alumina. The spots are visualized with a spray reagent consisting of *p*-aminobenzaldehyde in orthophosphoric acid and ethanol [*13*].

High Performance Liquid Chromatography

The system is derived from a published method [*9*] and has been shown to separate lysergide from 17 related compounds, including naturally occurring ergot alkaloids. This HPLC system has provided rapid and reliable results for lysergide quantitation and detection in all the illicit preparations encountered in this laboratory.

Ion Chromatography

This technique was first described by Small et al [*14*]. Analyte ions are separated on a pelicular (surface active) column of ion exchange resin of low capacity, using a buffered solution as eluant. A high capacity counter ion resin (the suppressor column) in series with the separator column neutralizes the major conducting ions from the eluant, leaving only those of analytical interest. Detection is by measurement of electrical conductivity.

For the analysis of tartrate and similar organic ions, an eluant of carbonate/bicarbonate buffer (as sodium salts) was found to be satisfactory. The sodium ions are removed from the eluant by a strong cation-exchange column leaving the conducting species in a solution of carbonic acid which is poorly dissociated under the experimental conditions. This therefore gives a background of low conductivity enabling detection of the conducting species to be made.

Materials, Equipment, and Conditions

HPLC

The pump was an Applied Chromatography Systems (Luton, Bedfordshire, U.K.) Model 750/03, the detector a Baird Atomic (Braintree, Essex, U.K.) model FC100 Fluoricord spectrofluorimeter fitted with a flow cell of volume 20 μL. The column was 250- by 4.6-mm internal diameter stainless steel, slurry packed at 41400 kPa (6000 psi) with Spherisorb-5 ODS (Phase Sep, Queensferry, Clwyd, U.K.). The eluant was a mixture of methanol and 0.0025*M* disodium hydrogen phosphate in a ratio of 65:35 by volume. Operating conditions are given in Table 2.

Ion Chromatography

The instrument was a Dionex (Camberley, Surrey, U.K.) Autolon 12, fitted with a standard Dionex S1 anion separator column and cation supressor column. The eluant was a mixture of 0.003*M* sodium bicarbonate and 0.0024*M* sodium carbonate buffer.

TABLE 2—*HPLC and ion chromatography operating conditions.*

HPLC CONDITIONS	
Flow rate	2.0 mL/min
Excitation wavelength	320 nm
Emission wavelength	400 nm
Slit width	10 nm
Injection volume	5 μL (stopped flow technique)
ION CHROMATOGRAPHY CONDITIONS	
Flow rate	3.0 mL/min
Detector sensitivity	10 μS/cm
Injection volume	100 μL

Materials

All reagents were of analytical reagent grade. With the exception of ergocristine and ergocristinine (supplied by Sigma Chemicals, Poole, Dorset, U.K.) the drug standards used in this study were kindly donated by Sandoz Pharmaceuticals (Feltham, Middlesex, U.K.). The samples of blotting paper were from the major U.K. and Dutch manufacturers, obtained through normal retail outlets.

Results

The rates of extraction of lysergide from tablet and paper presentations were measured; the most satisfactory solvent was found to be a 1:1 mixture of methanol and water (Fig. 3). Using ultrasonic vibration extraction was essentially complete after 20 min. Extraction times of up to 1 h did not result in any losses of lysergide by decomposition.

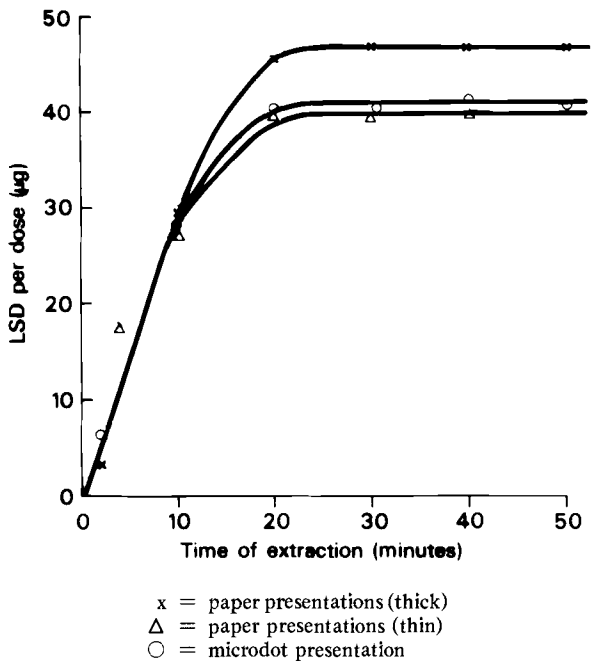

FIG. 3—*Rates of extraction of lysergide from illicit presentations.*

HPLC retention data with respect to lysergide were measured for 17 related compounds (Table 3). Lysergide was shown to be resolved from all other compounds included in this study. The relative retention data compare favorably with those published by Twitchett et al [9] with the exception of iso-LSD and ergometrine. The mass spectra of these compounds used in our laboratory agree with published data [5,6].

The linearity of the detector, over the range of lysergide concentrations encountered in illicit preparations, was confirmed (Fig. 4).

The regression line was $y = 3.016x - 1.067$,
the correlation coefficient = 0.998, and
the detection limit = 1 ng of lysergide on column.

The ion chromatographic system resolved twelve ionic species, the retention data are presented in Table 4. The retention times of these ions were found to be sensibly constant with the exception of nitrate which varied between 13 and 15 min, depending upon concentration.

The linearity of the detector, with respect to tartrate ions, was demonstrated (Fig. 5) over the range of tartrate concentrations encountered in illicit lysergide samples.

The regression line was $y = 4.592x + 0.224$,
the correlation coefficient = 0.999, and
detection limit = 50 ng on column.

A number of illicit preparations known to contain lysergide were analysed along with control samples of blotting paper from various sources (Table 5). All of the samples were found to contain anionic species, the majority of which were inorganic. The inorganic radicals can be discounted as being associated with lysergide (that is, as a salt of lysergide) as it is unstable in the presence of inorganic acids. This instability has been verified experimentally in this laboratory. Aliquots of a methanolic solution of authenticated lysergide tartrate were treated with hydrochloric, sulphuric, and nitric acids, respectively, to give solutions which were ap-

TABLE 3—*HPLC retention data of lysergide and related compounds.*

Standard Substance	Relative Retention[a]
Lysergic acid	0.30
Lysergamide	0.49
Methylergometrine	0.53
Ergometrine	0.56
Ergometrinine	0.57
Lysergic acid monoethylamide	0.62
Lysergol[b]	0.67
Methysergide	0.82
Lysergide (LSD)	1.00
8-Epilysergide (iso-LSD)	1.33
Ergosine	1.45
Ergosinine	1.46
Ergocornine	1.56
Ergotamine	1.87
Ergocryptine	1.93
Ergocryptinine	2.37
Ergocristine	2.38
Ergocristinine	2.97

[a]Retention time relative to lysergide = 1.00; absolute retention of lysergide = 5 min.
[b]10,10α-didehydro-9-hydroxy-7-methylergoline.

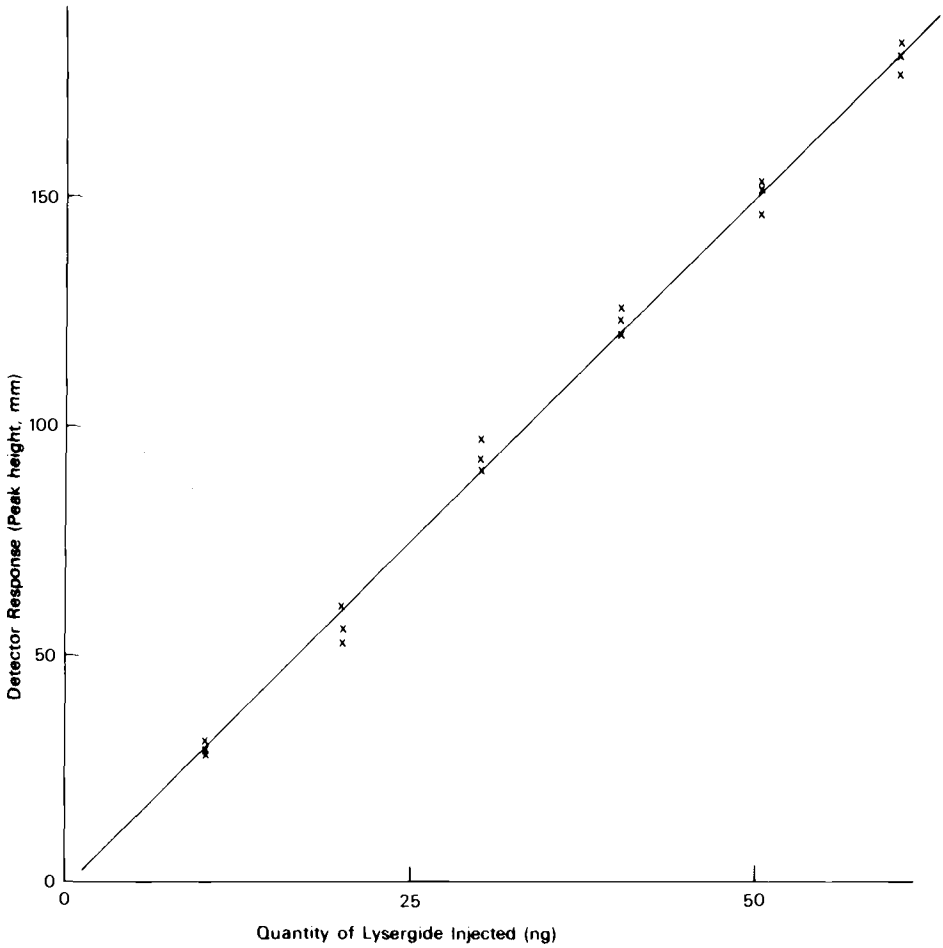

FIG. 4—*Linearity of HPLC fluorescence detector with respect to lysergide.*

TABLE 4—*Ion chromatography retention data of anionic species.*

Anion	Retention time, minutes
Citrate	not eluted
Fluoride	2.0
Formate	2.4
Acetate	2.5
Chloride	4.0
Succinate	11
Phosphate	12
Malonate	13
Nitrate	14 (±1)
Oxalate	16
Tartrate	20
Sulphate	23

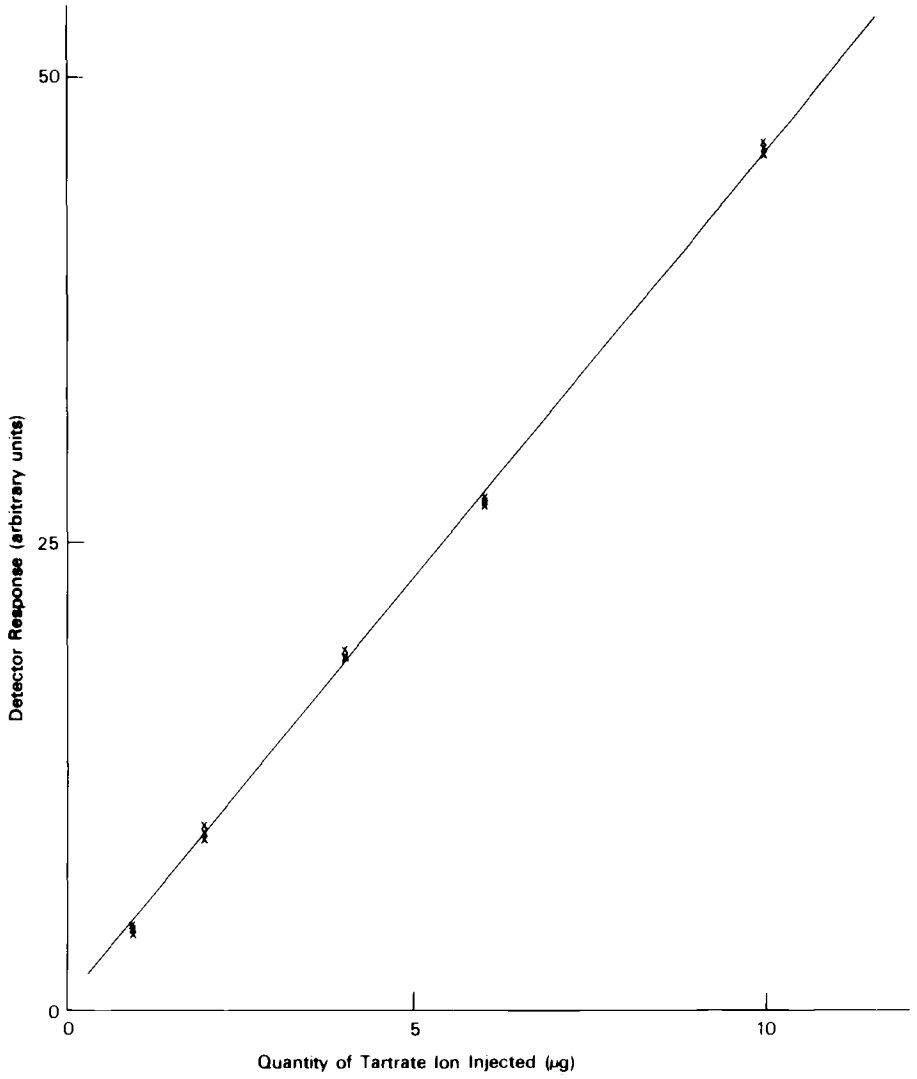

FIG. 5—*Linearity of ion chromatograph conductivity detector with respect to tartrate ions.*

TABLE 5—*List of anions found in illicit lysergide presentations.*

Sample Reference	Fluoride	Chloride	Phosphate	Nitrate	Sulphate	Tartrate
A	x	x	...	...	...	...
B	x	x	x	...	...	...
C	x	x	...	x	x	...
D	x	x	...	...	x	x
E	x	...	x	...	...	x
F	x	x	...	...	x	x
G	x	x	...	...	x	...
H	x	x	...	...	x	...
I	x	x	x	...	x	...

proximately 2*M* with respect to the acid. The reaction was followed by both TLC and HPLC. The TLC pattern in each case showed appreciable decomposition following addition of acid. After 4 h, lysergide was not detectable in any of the solutions. Although the decomposition products were not identified, TLC showed them to be different in each acidic medium. In addition, an experiment was conducted using anhydrous gaseous hydrogen chloride, in place of hydrochloric acid. The gaseous hydrogen chloride was added to a methanolic solution of lysergide tartrate, in a slight molar excess. The result was the same as that obtained using hydrochloric acid. Examination of acidified solutions by HPLC also showed that decomposition was essentially complete within a few hours.

A literature survey revealed few papers which made reference to lysergide in the form of an inorganic salt. Urich et al [*15*] described an extraction procedure for lysergide in which the lysergide was treated with sulphuric acid. It was then immediately extracted into an organic solvent and the use of the mineral acid does not therefore result in any substantial decomposition of the lysergide. No evidence was presented in the paper for the formation of a stable lysergide sulphate and our own experiments indicate that such a salt is unstable. There are two references [*16, 17*] to the use of lysergide hydrochloride, but recent correspondence with these authors has confirmed that lysergide base was in fact used.[2]

All of the inorganic anions found in sheets containing lysergide (Table 5) were also present, at similar levels, in the control samples of ordinary blotting paper. These ions and additionally phosphate anions were found in microdot tablet presentations. The presence of inorganic radicals in lysergide samples is therefore attributed to the paper itself or the tablet excipient, either as a result of manufacturing processes or adventitiously. No organic ions were detected in the control samples of blotting paper, and it must be assumed that the presence of organic radicals is due to their subsequent addition to the paper or tablet. Where no organic anions were detected the lysergide must therefore be present as the free base.

In those samples where tartrate ions were detected, a quantitative relationship was demonstrated between lysergide cation and tartrate anion concentrations (Table 1). This provides strong evidence that the lysergide is present as lysergide tartrate, as the probability of lysergide and tartrate ions being added separately to a substrate in stoichiometric proportions, is very small.

No organic ions, with the exception of tartrate, were found to occur in either paper or tablet presentations of lysergide in any samples examined at this laboratory.

Conclusion

The ionic form of lysergide in illicit preparations can be deduced by using a combination of HPLC and ion chromatography. The only salt of lysergide encountered by this laboratory has been the tartrate. Providing the sample is relatively fresh with little or no degradation of the lysergide, it is possible to obtain an acceptable quantitative relationship between lysergide and tartrate ion concentrations, thus yielding strong evidence for the presence of lysergide tartrate.

Where no tartrate is observed and providing no other organic radicals are detected, the lysergide must be present as the free base as inorganic lysergide salts are unstable. Should another organic radical be detected, in the absence of any other additional organic anions including tartrate, it should, in principle, be possible to characterize this salt by these same procedures.

In the majority of the samples of lysergide encountered in this laboratory, the drug is present as the free base. This would seem to run counter to the belief that the most common forms of lysergide presentations contain lysergide tartrate. This can be explained by examining the common illicit routes to lysergide which end at a stage in which freshly synthesized lysergide base is contained in alcoholic solution. It is an easy matter to produce "blotters"

[2] Philip Seeman, University of Toronto, personal communication.

from this by dipping paper into this solution. Precipitating the lysergide as the tartrate salt and subsequently dissolving this salt in alcohol offers no advantage for "blotters," unlike microdots where it is preferable to add solid active constituent to the excipients.

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