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Factors Affecting the Analysis of Illicit Lysergic Acid Diethylamide (LSD) Preparations

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The use of organic solvents for the extraction and spectrophotometric estimation of LSD in illicit preparations is described.

The $E^{1\%}_{1cm}$ values of LSD in eighteen organic solvents were determined. They range from 285 (n-propanol) to 435 (carbon tetrachloride) relative to a value of 280 for LSD in water. Use of an organic solvent therefore increases the sensitivity of the spectrophotometric estimation of LSD by up to 55%, and so 1–2μg of LSD may be quantitatively determined using conventional instrumentation. The use of non-aqueous solvents did not enhance the resolution of the LSD spectrum.

Direct extraction of LSD using an organic solvent was found to be more efficient than processes involving liquid/liquid extraction. The losses of LSD that occur during liquid/liquid extractions and back-extractions were found to be high, particularly where back-extraction from chloroform to hydrochloric acid was carried out.

The losses of LSD due to acid or alkaline reagents, illumination and high temperatures were briefly investigated and found to be small under the conditions likely to be employed in routine analysis. The recovery of LSD after thin-layer chromatography was found to be high.

Introduction

The LSD content of illicit preparations varies from several to several hundred micrognammes (μg) per dosage unit. A successful analysis scheme should ensure maximum extraction of adulterant-free LSD and unequivocal identification of LSD at concentrations down to the microgramme level. In the majority of cases, routine analysis by conventional methods gives successful results, but difficulties may be encountered in cases where a single dosage unit containing only a few micrognammes of LSD is submitted for analysis.

In this paper, the use of organic solvents for the extraction and spectrophotometric estimation of small quantities of LSD is described. The aims of the investigation were:

(a) To measure the $E^{1\%}_{1cm}$ values of LSD in organic solvents in order to find the best solvent for quantitative ultraviolet (*UV*) spectrophotometry.

(b) To compare the effects of aqueous and organic solvents on the fine structure of the *UV* spectrum of LSD.

(c) To find the optimum conditions for the extraction of LSD from illicit preparations.

A homogeneous mixture of LSD base and starch (approximately 170μg LSD/100mg starch) was used in the experiments to simulate an illicit LSD preparation. This was prepared by evaporating an ether solution of LSD in the presence of starch and thoroughly mixing the dried residue.

$E^{1\%}_{1cm}$ values of LSD in organic solvents

The $E^{1\%}_{1cm}$ values of LSD in methanol and ethanol are higher than that of LSD in aqueous solution (Bowd et al., 1970; Canaff and DeZan, 1970) and so the spectrophotometric estimation of LSD is more sensitive if alcohol is used as the solvent instead of water. This simple method of increasing the sensitivity of a routine procedure is obviously applicable to forensic analysis.

The $E^{1\%}_{1cm}$ values of LSD in a number of organic solvents were measured. Equal volumes of a solution of LSD in ether were evaporated to dryness under identical conditions. Measured aliquots of the organic solvents were immediately added to the evaporation tubes and the *UV* spectra of the solutions were run against pure solvent blanks on a Unicam SP800 double beam spectrophotometer. In each series of measurements an aqueous solution of LSD was prepared in the same way to act as a reference standard. The results were calculated assuming a value of 280 for the $E^{1\%}_{1cm}$ of LSD in water. The results, given in Table 1, are the averages of duplicates and their accuracy is estimated to be ±5%. Bowd et al. (1970) found the extinction coefficients (Σ_{max}) of LSD in water and ethanol to be 16,000 and 19,500 respectively. Conversion to $E^{1\%}_{1cm}$ values by simple proportion (assuming a value of 280 for LSD in water) gives a value of 341 for LSD in ethanol. This compares well with the value of 335 obtained in the present study.

The $E^{1\%}_{1cm}$ values found in this study range from 285 for n-propanol to 435 for carbon tetrachloride, thus the use of an organic solvent can increase the sensitivity of the spectrophotometric determination of LSD by up to 55%. Theoretically, 1μg of LSD in 1ml of cyclohexane would have an optical density of 0·04 at a wavelength of 305nm when the path length is 1cm. This is equivalent to 20% of the full-scale deflection on a spectrophotometer (such as the Unicam SP8000) with a 0–0·2 optical density range. In practice it was found that an acceptable spectrum could be obtained from 1–2μg of LSD although the instrumental noise was somewhat high.

The UV spectra of LSD in organic solvents

A polar solvent such as water tends to diminish the resolution of vibrational structure in the *UV* spectra of organic compounds (Rao, 1967). The *UV* spectra of LSD in organic solvents were therefore compared with the spectrum of aqueous LSD to ascertain if enhanced resolution occurred with organic solvents.

The solvents used and the λ_{max} values are given in Table 1. The spectra closely resembled the aqueous LSD spectrum in shape. The only indication of enhanced resolution occurred with 1,1,1-trichloroethane, hexane and cyclohexane, the absorption maxima being slightly broadened.

Organic solvents therefore offer no advantage over aqueous solvents in resolving LSD spectral structure.

The extraction of LSD from illicit preparations

Direct solvent extraction

Direct solvent extraction of LSD from illicit preparations requires a solvent in which LSD is readily soluble. Dyestuffs and other excipients should preferably be insoluble to minimize interference in the analysis, and the boiling point of the solvent should be low to allow easy recovery of the extracted LSD.

Various organic and aqueous solvents were screened to determine their suitability by extracting 100mg portions of LSD/starch mixture with two 3ml aliquots of each solvent. The LSD/starch mixture was supported on a base of glass wool in a Pasteur pipette and eluted with solvent at a rate of approximately 1ml/min. Long-wave *UV* light was used to check whether LSD remained on the starch after each extraction. Percentage extractions of LSD were measured spectrophotometrically and the results are given in Table 1.

Twelve of the solvents extracted 100% of the LSD in the first 3ml aliquot, and so (with the exception of benzene which is toxic) these are suitable solvents for LSD extraction. Two of these, dichloromethane and diethyl ether, combine high $E^{1\%}_{1cm}$ values for LSD with low boiling points and are thus suitable for the spectrophotometric estimation and subsequent recovery of small quantities of LSD.

TABLE 1

$E^{1\%}_{1cm}$ AND λ_{max} VALUES OF LSD IN VARIOUS SOLVENTS AND THE PERCENTAGE EXTRACTION OF LSD BY THE SOLVENTS

Solvent	$E^{1\%}_{1cm}$	λ_{max} (*nm*)	*Boiling point of solvent* (°C)	*Percentage of LSD extracted by: 1st aliquot of solvent*	*2nd aliquot of solvent*	*Remarks*
Distilled water	280	310	100	59	29	Incomplete extraction
0·5N HCl	—	—	—	96	4	
0·5N NH_4OH	—	—	—	97	3	
Methanol	340	310	65	100	0	
Ethanol	335	312	78	100	0	
n-Propanol	285	312	97	100	0	
iso-Propanol	320	311	82	83	17	
n-Butanol	305	311	117	91	9	
iso-Butanol	305	312	107	100	0	
sec-Butanol	315	312	99	100	0	
t-Butanol	310	312	82	90	10	
n-Amyl alcohol	345	311	137	73	27	
Dichloromethane	405	309	40	100	0	
Chloroform	380	309	61	100	0	
Carbon tetrachloride	435	307	77	100	0	
1,1,1-Trichloroethane	405	308	74	100	0	
Hexane	400	299	68	26	19	Incomplete extraction
Cyclohexane	400	300-307 (flat)	81	16	13	
Benzene	385	310	80	100	0	
Diethyl ether	370	309	35	100	0	
Ethyl acetate	365	309	77	100	0	

TABLE 2

LOSSES OF LSD UNDER VARIOUS CONDITIONS

	Temperature (°C)	*Illumination*	*Change in optical density*	*Change in* λ_{max} (*nm*)	*Duration of experiment* (*hours*)
Neutral solution	18 (approx).	Daylight	0	0	6
	100	Darkness	2% drop	0	1·5
Acidic solution*	18 (approx.)	Daylight	16% drop	310→305	3
	100	Darkness	5% drop	310→308	1·5
Alkaline solution†	18 (approx.)	Daylight	4% increase	0	6
	100	Darkness	6% increase	308→305	1·5
Neutral, acidic and alkaline solutions	18 (approx.)	UV‡ 310nm	0	0	0·2

* One drop conc. HCl added to neutral solution (3ml).
† One drop 50% NaOH added to neutral solution (3ml)
‡ Unicam SP800 UV spectrophotometer in constant-wavelength scanning mode.

LSD may be present as the salt form in illicit preparations. It can be converted to the free base by crushing the preparation with a small quantity of concentrated ammonium hydroxide. The resulting paste can be extracted by stirring with the organic solvent which is then decanted off and centrifuged if necessary. LSD is efficiently extracted by this process. It is particularly useful if methylcellulose is present as an excipient since it avoids the difficulties that occur if the usual aqueous/organic extraction procedures are applied.

Liquid/liquid extraction

In common with numerous other drugs, LSD can be extracted from an aqueous base by organic solvents and back-extracted into acid. Variations of this general method which have been published include:

1. Extraction with ethyl acetate from sodium bicarbonate solution followed by back-extraction into tartaric acid solution. The acid is then made alkaline with sodium bicarbonate and the extraction is repeated (Stoll et al., 1955).
2. Extraction with ethylene dichloride from potassium carbonate solution and back-extraction into citric acid solution (Goldenberg and Goldenberg, 1956).
3. Extraction with heptane containing 2% of iso-amyl alcohol from dilute sodium hydroxide solution saturated with sodium chloride, followed by back-extraction into 0·004N hydrochloric acid (Axelrod et al., 1957). This method has also been used without the addition of sodium chloride (Aghajanian and Bing, 1964).
4. Extraction with three aliquots of chloroform from a solution of pH 8·5–9·0 saturated with sodium chloride, followed by back-extraction into 0·01N hydrochloric acid. The acid is then made alkaline and the extraction is repeated (Dal Cortivo et al., 1966).
5. Extraction with three aliquots of chloroform from sodium bicarbonate solution followed by back-extraction into 0.01N hydrochloric acid. The acid is then made alkaline with sodium bicarbonate and extracted twice with chloroform (Cromp and Turney, 1967).
6. Extraction with four aliquots of chloroform from ammonium hydroxide (Look, 1968).

Quantitative recovery data for the above procedures are not given, although Cromp and Turney (1967) suggest that the completeness of extraction be checked under long-wave *UV* light, and Axelrod et al. (1957) give distribution ratios of LSD between water at different pH values and heptane as an assay of purity.

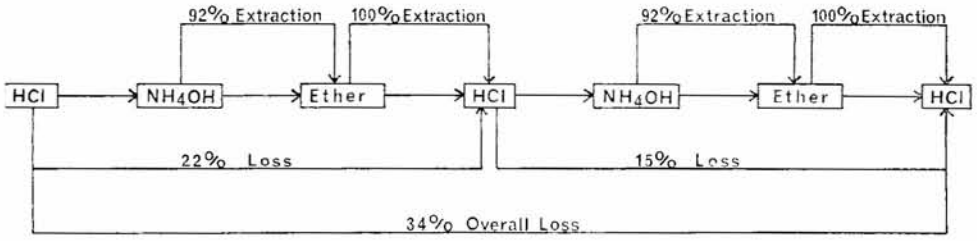
Experiments were therefore carried out to determine the losses that occur during routine solvent extraction and back-extraction of LSD. In each experiment, a solution of LSD in 10ml of 1N hydrochloric acid (approximately 35μg LSD/ml HCl) was made just alkaline and extracted with an equal volume of organic solvent. The LSD was then back-extracted into an equal volume of 1N hydrochloric acid and the process was repeated. The LSD concentrations in the various extracts were measured spectrophotometrically. The percentage extractions and losses of LSD are given diagrammatically in Figure 1.

The loss of LSD in each case was somewhat greater than could be accounted for by the incomplete extractions. In addition to small manipulative losses, some of the unexplained losses may perhaps be accounted for by adsorption on to the surface of the glassware used (Brodie et al., 1947).

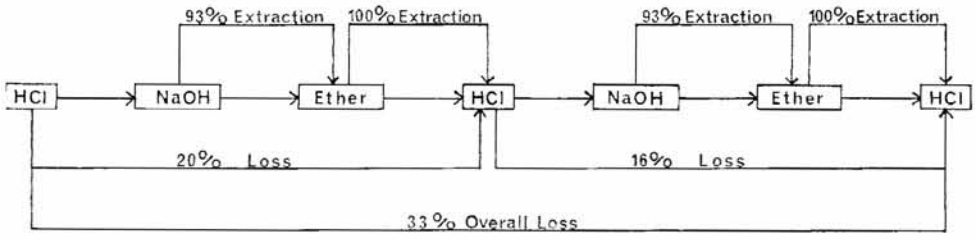
Despite the unexplained losses, however, the results show that although chloroform extraction of LSD from basic solution is more efficient than ether extraction, back-extraction from chloroform to hydrochloric acid is only half as efficient as back extraction from ether to hydrochloric acid. A further experiment showed that back-extraction from chloroform to 1N sulphuric acid was more than 95% efficient, therefore sulphuric acid is preferable to hydrochloric acid when chloroform is used as the organic phase.

When saturated sodium bicarbonate solution was used as the basic phase,

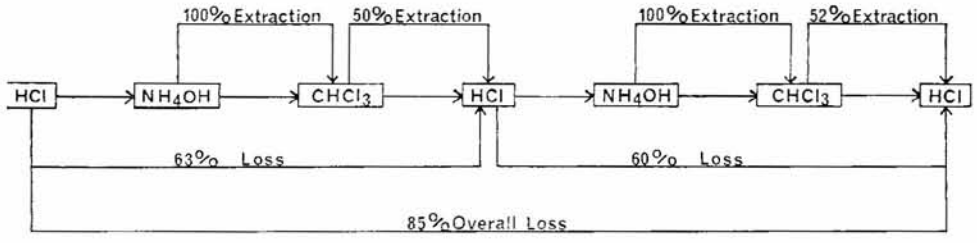
1. HCl – NH_4OH – Diethyl Ether



2. HCl – NaOH – Diethyl Ether



3. HCl – NH_4OH – $CHCl_3$



4. HCl – NaOH – $CHCl_3$

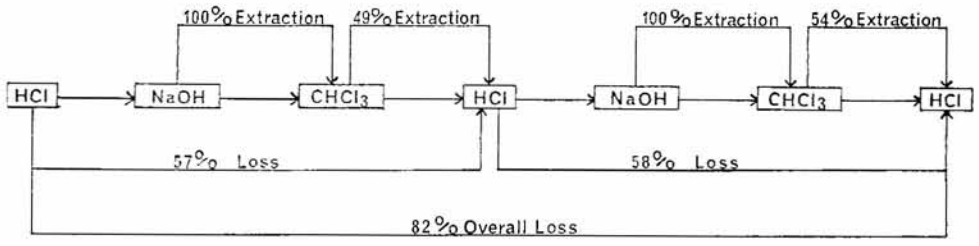


Fig. 1. **Extraction of LSD by aqueous/organic solvent systems.**

the extraction of LSD into ether or chloroform was found to be 100% efficient. Saturated sodium bicarbonate solution is therefore a useful basic phase for the extraction of LSD with the additional advantage that strongly alkaline conditions are avoided, thus eliminating the risk of converting LSD to iso-LSD (Look, 1968).

Comparison of extraction procedures

The results of the preceding sections show that direct solvent extraction is a preferable alternative to liquid/liquid extraction. This conclusion was confirmed in the following experiments in which extraction procedures were compared. 50mg aliquots of the LSD/starch mixture were used and the LSD concentrations were measured spectrophotometrically.

1. 50mg of LSD/starch mixture were stirred for 1 minute in 2ml of ethanol. The suspension was centrifuged and the ethanol was decanted. The extraction was repeated with a further 2ml of ethanol.

1st extract contained 82μg LSD
2nd „ „ 2·5μg LSD
Total 84·5μg LSD

2. 50mg of LSD/starch mixture were ground in a small mortar with one drop of concentrated ammonium hydroxide and 5ml of diethyl ether. The ether was decanted off, evaporated on a water bath at 60°C and the residual LSD was dissolved in 2ml of ethanol. The extraction was then repeated.

1st extract contained 80μg LSD
2nd „ „ 5μg LSD
Total 85μg LSD

3. 50mg of LSD/starch mixture were stirred in 5ml of distilled water for 1 minute and the suspension was centrifuged. The aqueous layer was decanted, made alkaline with 4 drops of concentrated ammonium hydroxide and extracted with 5ml of diethyl ether. The ether was back-extracted with 2ml of 0·1N hydrochloric acid and the LSD concentration in the acid layer was measured. A further 5ml of distilled water were added to the LSD/starch mixture and the process was repeated.

1st extract contained 62μg LSD
2nd „ „ 6μg LSD
Total 68μg LSD

4. Procedure 3 was repeated using 5ml aliquots of 0·2N ammonium hydroxide instead of distilled water.

1st extract contained 69μg LSD
2nd „ „ 6μg LSD
Total 75μg LSD

5. Procedure 3 was repeated using 5ml aliquots of 0·1N hydrochloric acid instead of distilled water.

1st extract contained 78μg LSD
2nd extract—spectrum masked by co-extractant from starch.
Total 78μg LSD

As expected, the procedures involving liquid/liquid extractions were subject to greater losses than those in which direct solvent extraction was used.

Loss of LSD due to conditions of analysis

Experiments were carried out to determine losses of LSD due to the effects of reagents, temperature and illumination. 3ml aliquots of LSD solution (12·5μg/ml) were used and LSD concentrations were measured spectrophotometrically. The results are given in Table 2 and show that relatively small losses of LSD are to be expected as a result of the conditions employed during routine analysis, at least so far as the spectral characteristics are concerned. There is, however, the risk that contact with concentrated reagents will modify the LSD molecule without affecting its spectrum to any extent, e.g., the alkaline isomerization of LSD to iso-LSD (Look, 1968).

Recovery of LSD after thin-layer chromatography (TLC)

Occasionally it is necessary to use preparative *TLC* to remove LSD from interfering excipients such as dyes. In the following experiment, the recovery of LSD after *TLC* was measured.

Approximately 250μg of LSD were dissolved in diethyl ether and the concentration of LSD was determined by *UV* spectrophotometry. The solution was concentrated to small volume and applied as a band to a *TLC* plate coated with silica gel. The plate was developed in acetone/chloroform (4 : 1), the LSD band was located under long-wave *UV* light, scraped off the plate and shaken with 5ml of ethanol. After centrifugation, the concentration of LSD in the ethanol was measured and the recovery of LSD was calculated to be 95%.

Conclusions

The experiments described in this paper show that organic solvents may usefully be employed for the extraction and estimation of LSD in illicit preparations. The losses of LSD are less than those in procedures involving aqueous/organic solvent extractions.

The $E^{1\%}_{1cm}$ values of LSD in a number of organic solvents were measured and found to be considerably higher than the $E^{1\%}_{1cm}$ value of LSD in water. Therefore, by using appropriate instrumentation and an organic rather than an aqueous solvent, it is possible to determine 1–2μg of LSD by quantitative *UV* spectrophotometry. This paper is concerned only with the use of organic solvents for minimizing losses of LSD during extraction and for increasing the sensitivity of routine spectrophotometric estimations of LSD without lengthening the analysis time. For this reason, no mention is made of alternative techniques such as spectrophotofluorimetry and high-pressure liquid chromatography which offer greater sensitivity but are less routinely available.

The *UV* spectra of LSD in aqueous and organic solvents were compared and found to be similar. The use of organic solvents did not enhance the spectral resolution.

The losses of LSD due to alkaline and acid reagents, high temperatures and varying illumination were briefly investigated and found to be small under the conditions likely to be employed in routine analysis. The recovery of LSD after *TLC* was found to be high.

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