Chapter 3
The Analysis of LSD

Learning Objectives

- To have an appreciation of LSD as a controlled substance.
- To be aware of the sampling procedures and descriptions required for LSD analysis.
- To understand the extraction of LSD from blotter acids for analysis.
- To be aware of the chemical and fluorescence testing procedures for LSD.
- To understand the principles of TLC analysis of LSD.
- To be aware of the confirmatory techniques available for the analysis of LSD.

3.1 Introduction

While a large number of drugs are known which are of plant origin, or have plant products as starting materials for the synthesis of the drugs, there are, equally, a number of drugs of fungal origin. Of these, perhaps lysergic acid diethylamide (LSD) (1) is the most ‘famous’, i.e. well known, and it is on this drug that this chapter focuses. Interestingly, the drug is an indole alkaloid which presents special difficulties and opportunities in terms of drugs analysis.

Lysergic acid diethylamide (LSD) is one of the most potent hallucinogens known to man. It was first synthesized in 1938 and was discovered to be psychoactive in 1943. It was initially used, experimentally, in the treatment of mental disorders but has not been used in this way for some 30 years. LSD encountered in the illicit drugs market of today is produced in clandestine laboratories. These are rarely detected because they make a large quantity of LSD, which lasts for an extremely long period of time, since only very small doses are administered and subsequent syntheses are not required [1]. LSD is, in the main, prepared from
lysergic acid, via a series of complex reactions which require careful monitoring and control. The forensic scientist will see the resulting drug in a number of differing dosage forms. The materials may be added to inert substrates or to sugar cubes, or mixed with molten gelatin which is then cooled and cut into small pieces containing the appropriate dose. These latter are known as ‘window panes’. However, these dose forms suffer from great inhomogeneity and the vast majority of LSD observed in the forensic science laboratory today is encountered in the form of ‘blotter acid’. In this form, an absorbant paper is dipped into a solution of LSD, and then dried. Such a procedure allows an even distribution of the drug through the paper. A typical blotter acid dose contains between 30 and 150 µg of LSD per dose. Blotter papers are frequently decorated, with some examples being shown on Plates 3.1 and 3.2, and represent the dose form on which our discussion will centre.

LSD doses vary between users and with the desired effect. Commonly, the doses lie in the range between 30 and 150 µg of LSD per dose, although stronger doses of 150 to 400 µg of LSD per dose (or even higher) are sometimes encountered. The onset of effect takes between 30 and 120 min and lasts between 6 and 14 h. After-effects can last for up to 24 h.

In terms of legislative control, in the United Kingdom lysergamide, lysergide and any N-alkyl derivatives of lysergamide are controlled as Class A drugs. In the United States, LSD is controlled as a Schedule I drug. As part of the forensic science process, therefore, it is necessary to prove the presence of the drug in any sample thought to contain LSD.

### 3.2 Qualitative Identification of LSD

The process of analysis of LSD blotter acid follows the same general principles and sequence as for other controlled substances, namely physical description, presumptive testing, TLC and confirmatory analysis. These processes are discussed below.
Plate 3.1 Bird of paradise pattern on LSD blotter acid, covering the whole sheet of the paper. Copyright Michael D. Cole, Anglia Polytechnic University, Cambridge, UK, and reproduced with permission.

3.2.1 Sampling and Physical Description of LSD Blotter Acid

As with all forensic science analyses, the first stage in the process is a full physical description of the material under investigation. In the case of blotter acids, this includes a count of the number of dose units, the size of each of the dose units (length × breadth), whether they fit together, the number and depth of
Plate 3.2 Illustration of a ‘ghost’ on LSD blotter acid, with each image covering a few dose units. Copyright Michael D. Cole, Anglia Polytechnic University, Cambridge, UK, and reproduced with permission.

the perforations of the dose units and a note of the pattern and whether it covers all, some, or single dose units (see Plates 3.1 and 3.2).

DQ 3.1
Why is it necessary to record in such detail the physical information relating to an LSD seizure?

Answer
By recording such information, it may be possible to relate one or more samples to each other. This may be especially important when seizures from different occasions are being compared, or comparison between seizures is being made in different laboratories.

Having carried out a full physical description of the seizure, items must be chosen for analysis. While it might be assumed that the dose units are all identical,
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This might not be the case and it is therefore necessary to sample a number of them from the seizure. The United Nations Drug Control Programme (UNDCP) recommends the following procedure. For sample sizes up to 10 dose units, all should be analysed, while for sample sizes between 11 and 27 dose units, three quarters of the items should be selected at random (the number being rounded up to the next highest integer). For sample sizes in excess of 28 dose units, 50% should be selected at random, with a minimum of 21 dose units up to a maximum of 50 units [2].

DQ 3.2
How might random samples be chosen?

Answer
A random sample means that every item in a population has an equal chance of being chosen. Simply choosing materials by eye does not satisfy this criterion. Each of the dose units should be assigned a number, starting at 1 and ending with the last number (i.e. the number of items in the sample). The materials to be chosen should then be picked by using either a computerized random-number generator or random-number tables. Whichever method is used, it should be documented.

3.2.2 Extraction of LSD Prior to Analysis
Since the drug is impregnated onto a paper substrate, it is necessary to extract the material prior to analysis. In order to do this for presumptive testing or qualitative analysis, the extraction can simply be achieved by mixing the test sample for 30 s with sufficient methanol to achieve a sample concentration of 1 mg LSD ml⁻¹ [2]. Alternatively, a methanol/water (1:1) mixture has been reported to extract the LSD more efficiently [3, 4]. It should be remembered that any solid material should be removed from the extract prior to any chromatographic analysis being carried out. This can be achieved either by centrifugation or by passing the extract through a 5 μm filter.

SAQ 3.1
Why should the solid material be removed prior to analysis?

If quantitative analyses are to be carried out, it is necessary to completely extract the LSD from the paper. This can be achieved by suspending the material in a large volume (15 ml is suggested) of 1% tartaric acid solution in a separatory funnel. The mixture is extracted three times with an equal volume of chloroform and then the aqueous layer is basified with 1 M NaHCO₃. The resulting mixture should be extracted, three times, with an equal volume of chloroform, and the
chloroform extracts combined, filtered or centrifuged, and evaporated under a stream of nitrogen. The residue should then be reconstituted in a known volume of solvent [2]. Other acids have been used (summarized in Veress [3]), although the physico-chemical principles remain the same.

**SAQ 3.2**
What are the physico-chemical principles behind using an acid to extract the LSD from the paper and the subsequent processing?

### 3.2.3 Presumptive Testing for LSD

The presumptive tests for LSD involve a fluorescence test and a chemical test.

#### 3.2.3.1 Fluorescence Testing for LSD

One of the properties of LSD that can be exploited during the identification process is its fluorescence. In such a test, the original dosage form, or a drop of the methanolic extract from the dose form, is placed on a filter paper and allowed to dry. The material is then observed under long-wavelength UV light (360 nm). If LSD is present, blue fluorescence will be observed.

**DQ 3.3**

If a methanol extract solution is used, what are the appropriate positive and negative controls and what do they show?

**Answer**

*If methanol is used, the appropriate negative control is methanol alone (not exposed to sample or drug). The relevant positive control is a methanolic solution of LSD at approximately the same concentration as the sample provides.*

The negative control demonstrates that the fluorescence, if observed, is due to the drug extracted into the methanol. The positive control provides a reference colour reaction and gives an idea of the intensity of the fluorescence that might be observed.

#### 3.2.3.2 Chemical Testing for LSD

The classical presumptive test for LSD is the Ehrlich’s reagent test. In this, 1 g of the reagent ($p$-dimethylaminobenzaldehyde) is dissolved in 10 ml of orthophosphoric acid. A small amount of the reagent solution is then added either directly to the test substrate or to the extract to be tested and any colour change observed.
If a blue/purple colour develops, then the presence of LSD may be suspected and confirmatory tests should be carried out.

**DQ 3.4**

Why is a confirmatory test required?

*Answer*

*Ehrlich’s reagent reacts with a wide range of controlled substances and other indole alkaloids. A positive reaction does not therefore prove the presence of a ‘specific’ drug. This is why it is necessary to carry out an additional confirmatory test.*

### 3.2.4 Thin Layer Chromatography of Samples Containing LSD

Following the possible identification of the presence of LSD, the next stage in the analysis is the use of thin layer chromatography (TLC). This is employed because although it cannot be used to *prove* the identity of LSD, it can be used as a rapid, cost-effective method to eliminate those samples which gave a positive colour reaction in the presumptive tests but which do not contain this drug. These will be rarer when blotter acids are suspected, but may be more common where other substrates have been used as the carrier medium for the LSD itself.

The extracts are prepared as described above (see Section 3.2.2). Activated silica gel plates containing a fluorescent dye (which fluoresces at 254 nm) are used. The materials to be tested, plus positive and negative controls, are spotted onto the plate and the chromatograms developed in the normal way. The solvent systems which can be used include chloroform/methanol (9:1, by volume) and chloroform/acetone (1:4, by volume). Following chromatogram development, the plates are removed from the chromatographic tank, the solvent fronts marked, and the plates air-dried and observed under short- (254 nm) and long- (360 nm) wavelength UV light. Under the former lighting conditions, LSD will appear as a dark spot on a light background, while under the latter conditions it will appear as a bright spot on a dark background. The chromatogram should then be developed with Ehrlich’s reagent, with which indole alkaloids (including LSD) react to give a purple product. If a product gives the same results (retardation factor, $R_f$, and colour reaction) as LSD under all of the conditions described, then further confirmatory tests should be carried out. However, if the materials do not yield the same physico-chemical responses as LSD under all conditions, then an exclusion has been achieved.

### 3.2.5 Confirmatory Tests for the Presence of LSD

Since there are a large number of materials which will produce the same result as LSD in one or more of the screening tests, it is necessary to confirm the presence of this drug by using an instrumental technique. Of the methods available,
HPLC with fluorescence detection is the most commonly used, although GC techniques have been reported [4, 5]. HPLC is also used as the means of quantifying LSD.

### 3.2.5.1 HPLC Analysis of LSD

A number of HPLC methods are available for the analysis of LSD. Two are presented here, namely (i) an ion-suppression technique, and (ii) an ion-paired method. The majority of techniques that are used exploit the same physico-chemical characteristics of the systems as those described here.

The first method is a reversed-phase method, using the HPLC system conditions described in Table 3.1. LSD is particularly amenable to fluorescence detection, which also has the advantage of providing both selectivity and sensitivity.

#### SAQ 3.3

How is selectivity of detection achieved when using a fluorescence detector to detect analytes in an HPLC eluant?

#### DQ 3.5

How does the ion-suppression work in this system?

**Answer**

*At pH 8.0, the LSD will be present in the free base form. It will not be highly charged and hence will exhibit good chromatographic properties.*

The second of the methods [3] uses an ion-pairing system, the conditions of which are given in Table 3.2. This system is not as specific as that employing fluorescence detection, although it is included here to illustrate that other separation mechanisms can be employed.

#### SAQ 3.4

Why is this detection system not as specific as the fluorescence detection one?

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### Table 3.1 Typical HPLC (ion-suppression) operating conditions and parameters used for the identification and quantification of LSD [2]

<table>
<thead>
<tr>
<th>System/parameter</th>
<th>Description/conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>ODS&lt;sup&gt;a&lt;/sup&gt; silica: 10 cm × 4.6 mm i.d.; 5 µm particle size</td>
</tr>
<tr>
<td>Solvent</td>
<td>Methanol (65%): 25 mM Na&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;, pH 8.0 (35%)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 ml min&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Detection</td>
<td>Fluorescence: excitation λ, 320 nm; emission λ, 400 nm</td>
</tr>
</tbody>
</table>

<sup>a</sup>ODS, octadecasyl.
**Table 3.2** Typical HPLC (ion-pairing) operating conditions and parameters used for the identification and quantification of LSD [3]

<table>
<thead>
<tr>
<th>System/parameter</th>
<th>Description/conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>ODS(^a) silica: 25 cm × 4.6 mm i.d.; 0.8 (\mu)m particle size</td>
</tr>
<tr>
<td>Solvent</td>
<td>Acetonitrile (45%): 50 mM KH(_2)PO(_4)/5 mM C(<em>8)H(</em>{17})SO(_3)Na, pH 3.5 (55%)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.9 ml min(^{-1})</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>220 nm</td>
</tr>
</tbody>
</table>

\(^a\)ODS, octadecasyl.

**SAQ 3.5**
What is the chromatographic mechanism operating in the solvent system described in Table 3.2?

**3.2.5.2 GC Analysis of LSD**
Gas chromatographic analysis of LSD is problematic because of the relatively limited volatility of this (controlled) substance [3]. However, GC-based methods have been applied, e.g. GC–MS [5]. The conditions reported for such analysis are shown in Table 3.3.

**SAQ 3.6**
What are the potential difficulties in analysing LSD by using isothermal gas chromatography?

**3.2.5.3 Identification of LSD by Microscope FTIR Spectroscopy\(^\dagger\)**
More recently, it has become possible to identify LSD in situ, without the need for extraction of the drug [6]. In this technique, LSD blotter acid is ‘described’ (see

<table>
<thead>
<tr>
<th>System/parameter</th>
<th>Description/conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>BP-5: 25 m × 0.2 mm i.d.; (d_l), 0.33 (\mu)m</td>
</tr>
<tr>
<td>Injection temperature</td>
<td>270°C</td>
</tr>
<tr>
<td>Column oven temperature programme</td>
<td>100°C; rising at 24°C min(^{-1}) to 270°C; isothermal for 35 min</td>
</tr>
<tr>
<td>Detection</td>
<td>Mass spectrometric</td>
</tr>
<tr>
<td>Split ratio</td>
<td>—(^a)</td>
</tr>
</tbody>
</table>

\(^a\)Not reported.

\(^\dagger\)This is a technique used to measure the IR adsorption or reflection spectra of very small samples. In this method, a sample is placed on a KBr disc and a microscope is then used to focus the IR beam onto the material.
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Section 3.2.1) and then subjected to a simple extraction procedure, followed by microscope FTIR spectroscopy directly on the extract. In this approach, the blotter acid is first soaked in water for 1 s, which swells the fibres of the papers and was found to facilitate the extraction of the drug for further spectroscopic analysis. The excess water was then removed from the blotter, which was subsequently placed on a KBr disc and heated to 120°C for 1 min. Dichloromethane/ammonia (100:1) was added to the blotter, which dissolved the LSD. The solution was then removed by using a microsyringe, placed on a KBr disc, and its spectrum recorded.

**DQ 3.6**

Why is ammonia added to the dichloromethane?

**Answer**

LSD is a basic drug. The ammonia will convert any LSD from the salt form to the free base state. In this form, the LSD is more soluble in organic solvents such as dichloromethane and thus the extraction process will be more efficient.

The IR spectrum was obtained by scanning in the wavenumber range 700–4000 cm⁻¹, with 30 scans at a resolution of 4 cm⁻¹ being collected. From such data it was possible to identify LSD directly.

**SAQ 3.7**

Why were 30 scans taken and what is the advantage of using the scan range from 700 to 4000 cm⁻¹?

**Summary**

LSD is one of the most potent hallucinogens known to man. This drug can occur in the form of either tiny tablets or as gelatin blocks, but is most commonly found as ‘blotter paper’ – small pieces of highly decorated paper into which the LSD has been impregnated. The analysis of blotter acids includes a physical description of the item, followed by drug extraction from the paper. The extract is then used for fluorescence and colour tests to determine if LSD may be present. Following this, TLC and HPLC with fluorescence detection can be used to confirm the presence of the LSD, although GC methods are available for such analysis. An interesting new technique, using microscope FTIR spectroscopic analysis of the drug in situ on the paper, has also been developed.
References

1. Huizer, H., personal communication.
Plate 3.1  Bird of paradise pattern on LSD blotter acid, covering the whole sheet of the paper. Copyright Michael D. Cole, Anglia Polytechnic University, Cambridge, UK, and reproduced with permission.
Plate 3.2  Illustration of a ‘ghost’ on LSD blotter acid, with each image covering a few dose units. Copyright Michael D. Cole, Anglia Polytechnic University, Cambridge, UK, and reproduced with permission.