The Analyst

The Forensic Analysis of Drugs of Abuse

A Review

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Introduction and Scope

The principal aim of this review is to aid forensic drug analysts by collating information on different analytical methods under the heading of the drug or group of drugs to which such techniques have been usefully applied. A secondary purpose is to bring together background information on drug analysis that the scientist may find helpful when called upon to present evidence in Courts-of-Law or Courts-Martial. The reviewers do not propose to recommend specific methods of analysis for particular drugs, but to suggest approaches that may help the scientist to select a technique appropriate to the problem in hand.

The scope of this review has deliberately not been extended to cover the analytical techniques that particularly apply to the analysis of drugs from physiological specimens. The reviewers consider simple separation and unequivocal identification of the very small amounts of drugs found in such samples to be a specialised analytical field, which merits discussion as a separate topic. The reviewers have drawn on the experience of their colleagues, past and present, at the Laboratory of the Government Chemist and other forensic science laboratories in the UK and overseas in their selection of the drugs and drug classes covered in this review; it is therefore restricted to those substances that a practising forensic drug analyst is likely to encounter, albeit rarely in some instances. Few details of actual methods are presented.
as the reader is referred to the original literature throughout this review. No topic as wide as forensic drug analysis can be comprehensively covered in one review; however, about 1300 original papers, books and other documents have been read by the reviewers to provide the background information that has been selected and, where necessary, critically reviewed. Literature available to the reviewers up to the end of 1981 has been surveyed.

Screening Techniques

Introduction

There is not only a wide range, but also many different presentations of drugs that may be submitted to the forensic analyst for identification. Many thousands of drugs are currently available licitly, either over-the-counter or upon presentation of a prescription from a medical practitioner. Considerable numbers of drugs with potential for abuse or misuse are further controlled by law. New products appear continually, often in a variety of presentations. The analyst may be asked to examine many products ranging from tablets and capsules bearing easily identifiable manufacturers' markings to illicit products of poor quality and unknown composition. Amounts may vary from traces in clothing, motor vehicles, syringes or smuggling concealments to seizures of many tons. Samples may consist of synthetic drugs or vegetable matter, or a mixture of the two. Purity may vary from pharmaceutical-grade drug substance to fractions of a per cent. in samples found at “street” level, which may be adulterated (“cut”) with physiologically inactive materials or mixed with other drugs. Drugs in solution may be encountered in or from ampoules or concealed within licit media such as wines and spirits. They may be present either as a salt or the free base, or acid. It may be necessary for the analyst to consider the stereoisomeric composition of the material. However, although the list of drugs for analysis may be potentially large, only a relatively small number of drugs are commonly encountered. The experienced analyst may swiftly be able tentatively to identify many drugs from their physical appearance, but in laboratories where the caseload is low or where analysts are lacking in experience, identification must begin with simple screening tests.

Physical Characteristics of Drug Samples

Solid drug samples submitted to the analyst may either be licit preparations (tablets, capsules or ampoules) or illicitly manufactured materials. The latter may be in raw (undivided) form or as an illicit preparation that is tabletted or otherwise divided into crude dosage forms. The majority of legally manufactured tablets, most of which do not contain drugs controlled by the Misuse of Drugs Act 1971, may be tentatively identified from readily available guides covering the UK and many other parts of the world (e.g., references). A new tablet and capsule identification system (Tablident) has recently been proposed to cover UK preparations; the system is based on measurement of size and the colour of the sample, as in current guides, but uses a computer for storing background data.

Confirmation of the active constituents of a licit preparation may then be carried out by a simple technique such as ultraviolet (UV) or infrared (IR) spectrometry or by chromatography. Many forensic science laboratories have large collections of licit drug presentations, which are useful for comparison with submitted samples. In addition, the reviewers have invariably found drug manufacturers to be extremely helpful in identification of licit presentations bearing a maker's mark or code, but which are unfamiliar to the laboratory. Further confirmatory tests on materials identified in this way are mandatory in view of the number of forged presentations on the illicit market, many of which may be of high quality and bear manufacturers’ markings, perhaps from stolen or forged tablet punches. Where marks are absent on tablets or capsules, screening is, of course, necessary and identification of the actual drug present is made by an appropriate analytical technique. Considerable useful evidence concerning manufacture may be deduced from a detailed physical examination of illicit tablets; this has been discussed by Gomm et al. Simulations of licit tablets have been encountered that contain drugs not present in authentic material. The reviewers advise confirmation of the identity of a supposedly licit preparation by comparison of its properties with an authentic sample. Further studies on the analysis of dyes used in illicit tablets have also been made. Individual illicit drugs are discussed under appropriate headings in this review.
Few botanical species are controlled by the Misuse of Drugs Act 1971\textsuperscript{1}: those which are so controlled will be discussed under the appropriate drug class heading. Der Marderosian and Chao\textsuperscript{13} presented a brief review of the problems involved in the identification of botanical material. Jolliffe and Jolliffe\textsuperscript{14} described a computer-aided identification programme for powdered vegetable drugs, which they considered would enable a relatively inexperienced microscopist to identify such materials. However, in the reviewers' opinion, the recognition of botanical material solely by microscopy should not be undertaken without professional training in botany.

**Chemical Spot Tests**

Many reagents have been suggested for use in spot tests for particular drugs; for example, more than 50 have been put forward for qualitative and quantitative reaction with opium alkaloids,\textsuperscript{15} and it is beyond the scope of this review to discuss their uses and limitations more than briefly.

Before the advent of more sophisticated and readily available equipment, colour tests were often employed for identification; even as recently as 1971, Hider\textsuperscript{16} described a system for the rapid identification of unknown drugs using colour and microcrystal tests. Despite this author's emphasis on the use of authentic standards at all stages, the reviewers assert that the use of simple tests alone to identify drugs is an unsafe procedure; the wide range of mixtures encountered, for example in illicit narcotics, and the numerous closely related isomers of stimulants make this an inherently unreliable method of identification. Gupta \textit{et al.}\textsuperscript{17} considered that identification using only colour tests was likely to lead to mis-identification in many instances. Velapoldi and Wicks\textsuperscript{15} and Johns \textit{et al.}\textsuperscript{18} have published valuable reports on the use of colour tests for analysing drugs of abuse. Both these groups considered that the primary purpose of colour tests was to narrow the list of substances possibly present in an unknown sample. The former group considered the main problems associated with colour tests in general and spot test kits in particular were the interpretation of the colours and the lack of specificity of the tests. Both groups discussed multiple reagent testing schemes using up to seven reagents and numerical codes for possible identification. Velapoldi and Wicks\textsuperscript{15} emphasised that final identification should always be made by skilled laboratory personnel and that colour tests should never be used as the sole evidence of identification. The reviewers agree strongly with both these opinions. Considerable data on colour test results are given in the publications discussed above and by Masoud\textsuperscript{19}; Stevens\textsuperscript{20} has discussed many colour tests and Clarke has presented a comprehensive list of the more important results.\textsuperscript{21,22} Colour tests for individual groups of compounds (such as narcotics) and individual drugs are discussed in the relevant sections of the review. Simple precipitation tests such as addition of silver nitrate or barium chloride (to detect chloride or sulphate ions) may be used to indicate whether a drug is present as a salt or a free base. This may be confirmed by more sophisticated techniques such as IR or nuclear magnetic resonance (NMR) spectrometry. The application of these techniques to salt identification is discussed in more detail under the appropriate drug class.

**Field Tests**

Police and Customs investigators frequently need a rapid sorting test for the detection of controlled drugs at the time and place of seizure. Such a test should, where negative, minimise the risk of unnecessary detention of a person or goods and, where positive, justify professional analysis. An account has been given\textsuperscript{23} of the development of a composite field test kit, difficulties encountered with packaging and storage under various climatic conditions and the philosophy of testing. In the reviewers' experience, the introduction of field kits for investigators can dramatically increase the proportion of positively identified drugs of abuse.\textsuperscript{24} In some instances the demonstration of a successful field test in the presence of a suspect may elicit an admission of guilt. These tests, and others requiring greater discrimination or involving simple chromatographic procedures, can assist scientists attending the scene of crime, or screening substances discovered in clandestine laboratories, and generally when working in accommodation without regular analytical equipment.

There are numerous schemes wherein selected spot tests are adapted as preliminary colour tests undertaken with minimum equipment remote from laboratory facilities. In this review
particular examples are quoted under specific classes of drugs. A United Nations working group collected information available up to 1974 on field tests for drugs of abuse commonly found in illicit traffic; their selection was based on the relative merits of stability, specificity, simplicity and safety. A range of colour tests convenient for field use for major classes of controlled drugs, as well as the use of portable UV lamps and thin-layer chromatography (TLC) kits, were described by Phillips. Commercial pre-coated polyester, glass and aluminium sheets with a propellant cartridge and chromogen reservoirs provide a better facility for a TLC field kit than hand-coated microscope slides. Odour tests generally are at best only grounds for suspicion [e.g., acetic acid vapour associated with degraded heroin (or aspirin or vinegar)]. Even a more distinctive field test, such as the transesterification of cocaine to yield the characteristic odour of methyl benzoate (see under Cocaine), is better left to scientific evaluation.

For searching vehicles, furniture and clothing for traces of drugs, a modified vacuum cleaner attachment that collects debris into a Soxhlet thimble has been recommended. However, while field tests may be useful for screening small amounts, caution should be exercised in not consuming a significant part, or contaminating the remainder, of the visual evidence. For trace amounts of drug material, the reviewers' strongly recommend removing all debris thus recovered.

Many kits are commercially available for field use either by trained analysts or by lay personnel. Although almost all such tests lack specificity, the proportion of misleading results in preliminary sorting tests can be markedly reduced by the use of a "logical tree" sequence. One such procedure has been patented and used in a commercially available kit. However, the warning already given in connection with colour tests intended for laboratory use applies a fortiori; field tests taken alone should not be relied upon to be more than presumptive evidence requiring further action and appropriate confirmation.

**Ultraviolet Spectrometry**

The use of UV spectrometry as a screening test suffers from similar limitations to colour tests. Rarely is it possible to identify a drug more than tentatively by this technique, but useful indications as to the structure of an unknown may be obtained from absorption maxima and minima or from the effects of varying the pH of the solution under examination. It is of very limited value for quantitation except for pure drug substances. Useful discussions on the use of UV spectrometry for tentatively identifying drugs have been published and considerable data have been assembled by Clarke. Major collections of UV data covering a wide range of compounds have been published (for example, The Sadtler Catalogue). UV data on specific compounds and groups are discussed in this review. Extinction coefficients may vary by several orders of magnitude and therefore a small proportion of a strongly absorbing impurity could totally mask the spectrum of a weakly absorbing major component, giving rise to erroneous identification and quantitation. Illicitly prepared drugs containing many structurally related impurities with similar or identical spectra may also be wrongly identified and quantified. The technique is useful in confirming the identity of the active ingredient in licit preparations, but even with these apparently simple samples, it is often necessary to confirm identity by using an alternative technique, which may involve chromatographic separation.

**Infrared Spectrometry**

In contrast to UV spectrometry, which can only rarely be applied to the identification of a particular compound, IR spectrometry finds wide application because, with few exceptions, every compound produces a different spectrum. Even when samples are very impure, it is often possible to identify at least one component from an IR spectrum. The recognition of characteristic spectra is a routine matter for experienced forensic analysts, particularly as many laboratories maintain collections of spectra of commonly encountered drug presentations. However, when drugs may be present in small amounts relative to other materials present, some form of initial clean-up procedure must be employed prior to identification of any drug in the sample. Particular problems are encountered both with tablets, whether licit or illicit, as large amounts of excipient may be present, and in diluted samples of drugs encountered at "street" level. Many different methods of sample clean-up have been
described\textsuperscript{35}; simple and rapid clean-up on Celite columns has been commonly used\textsuperscript{36,37} and minor variations may be used to separate, for example, neutral and basic drugs. Simple acid-base extraction may also achieve similar ends and many procedures have been described by de Faubert Maunder.\textsuperscript{38}

Identification of an unknown drug by IR spectrometry relies on comparison of the spectrum of the unknown with that of an authentic specimen; spectra of many of the more common drugs were illustrated by Clarke,\textsuperscript{21,22} but the reproductions, though clear, are very small. De Faubert Maunder\textsuperscript{38} discussed Clarke’s system in detail, drawing attention to the problems of polymorphism and sample matrix interactions, especially ion exchange. Major collections of IR spectra are also available (e.g., the Sadtler Catalogue\textsuperscript{34}) and many of these are updated annually; these are of particular use when totally unknown samples are submitted. The identity of a purported medicinal substance may be checked if it is one of 358 for which British Pharmacopoeia Infrared Reference Spectra have been published.\textsuperscript{39} Computer-based systems are also becoming available and these have been listed by Moss \textit{et al.}\textsuperscript{40}; these authors also discussed the use of IR spectra for classification of drugs into groups.

The reviewers consider that IR spectrometry is a most useful screening technique, which can often lead to the identification of an actual drug substance; in their own laboratory they have found that one of the most useful spectra collections is that of drug presentations encountered in the course of routine analysis. For rapid and accurate identification of drugs during screening, it is most helpful if both the unknown spectrum and any reference collection spectra are on chart paper of the same size and preferably run on the same instrument, enabling comparison to be made by direct superimposition using a light box. De Faubert Maunder\textsuperscript{38} gave many useful practical hints on the use of IR spectrometry by forensic drug analysts.

\section*{Microcrystal Tests}

Crystal or microcrystal tests find little current application in the forensic analysis of drugs of abuse, at least in the UK. Clarke\textsuperscript{41} considered that although useful for supporting a provisional diagnosis, being simple, rapid and specific, such tests were unsuitable as a primary means of identification as they did not lend themselves to forming the basis of a scheme of identification. Der Marderosian and Chao\textsuperscript{13} recognised that although some tests were very specific, others were more general in application, and that there was a tendency for related compounds to give similar crystals with the same reagent. Short discussions of the technique have been published with useful references.\textsuperscript{13,42} Clarke\textsuperscript{41} has discussed the topic more fully and described tests on many individual drugs. Fulton\textsuperscript{43} has discussed the whole range of microcrystal tests for drugs in considerable detail.

In view of the lack of data on closely related compounds (for example, amphetamine isomers) and the lack of a systematic identification system, the reviewers do not recommend microcrystal tests as a means of identification of illicit drugs without confirmation by a more specific technique. However, microcrystal tests may still have a role to play in drug screening, but considerable time and skill is necessary to acquire the relevant expertise.\textsuperscript{13}

\section*{Chromatographic Techniques}

The application of chromatography in the forensic analysis of drugs has been reviewed in full up to July 1982 by Gough and Baker.\textsuperscript{44}

\section*{Nuclear Magnetic Resonance Spectrometry}

Until recently, NMR spectrometry has found only limited application in the forensic analysis of drugs. However, the increasing popularity of \textsuperscript{13}C NMR spectrometry, particularly in pharmaceutical analysis, suggests that this technique may play a significant role in the future. Examples are cited under individual drugs.

\section*{Mass Spectrometry}

Lawson\textsuperscript{45} considered that the high cost and complexity of mass spectrometry (MS) equipment was a considerable drawback to its routine use. Very few laboratories have the resources to use MS as a screening tool and the reviewers believe that the principal use of
the technique, at least in the analysis of drugs above trace levels, is in the confirmation of the identity of a substance tentatively identified by some other technique. Klein has reviewed the development of MS as a tool in forensic drug analysis. Finkle and co-workers have published data on both electron impact (EI) and chemical ionisation (CI) MS of drugs; these authors emphasised the importance of matching all data and analytical information prior to the acceptance of a positive identification. Mass spectral data collections, which include drugs, have been published. The use of MS in drug analysis has been discussed by Scaplehorn.

The reviewers consider that trace amounts of drugs found, for example, in motor vehicles or clothing, and tentatively identified perhaps by chromatographic techniques, should always be confirmed by MS where this provides unequivocal identification.

Miscellaneous Techniques

Folen presented X-ray powder diffraction (XRD) data for some drugs, excipients and adulterants in illicit samples and considered that this technique could be applied to the identification of drugs in illicit samples without prior clean-up. The reviewers have not found this technique to be widely applied in routine drug analysis; however, in their experience it has proved useful for the identification of particular salts in complex mixtures of illicit narcotics. Bowen et al. considered the use of circular dichroism as an alternative method of drug analysis. However, in the reviewers' opinion the technique is limited to samples containing a single active ingredient in a pure state; the numerous optically active impurities in illicit preparations of lysergide (LSD), for example, are likely to lead to erroneous conclusions.

Cannabis Products

Introduction

In the UK, products from plants of the genus Cannabis are listed in separate parts of Schedule 2 of the Misuse of Drugs Act 1971; cannabinol (CBN) and cannabinol derivatives (i.e., chemical substances) are included with many other drugs in Part I (Class A drugs), whereas cannabis and cannabis resin are listed in Part II (Class B drugs). Cannabis derivatives are defined as "the following substances, except where contained in cannabis or cannabis resin, namely tetrahydro derivatives of cannabinol and 3-alkyl homologues of cannabinol or of its tetrahydro derivatives." Thus are controlled tetrahydrocannabinol (THC), and synthetic variants thereof in which a larger 3-alkyl group is included (for example, "synhexyl"). Cannabis was redefined in the Criminal Law Act 1977 as "(except in the expression 'cannabis resin') any plant of the genus Cannabis or any part of any such plant (by whatever name designated) except that it does not include cannabis resin or any of the following products after separation from the rest of the plant, namely—(a) mature stalk of any such plant, (b) fibre produced from mature stalk of any such plant and (c) seed of any such plant." Cannabis resin is explicitly defined as "the separated resin, whether crude or purified, obtained from any plant of the genus Cannabis." Thus, in the UK no question need arise as to the species of Cannabis, a problem not infrequently encountered by analysts in the USA. Recent case law (R. vs. Best and Others) has determined on appeal that alternative charging of cannabis and cannabis resin does not constitute "duplicity" for a single act of possession, but it is still important to differentiate between these substances if there is more than one exhibit. Phillips has discussed difficulties arising under previous legislation, including traces identified in smoking residues or clothing. Liquid cannabis (hash oil) is no longer explicitly controlled by UK legislation and although Extract and Tincture of Cannabis were both named specifically in succeeding Dangerous Drugs Acts, they were not specified as such in the Act of 1971. Consequently, it falls to the analyst to demonstrate the precise legal status of this substance in order that correct charges may be laid.

Forensic analysis of products from plants of the genus Cannabis may be subdivided into four (overlapping) operations: screening for the presence of a Cannabis product in the sample, identification of the product, quantitation of that product and, rarely, precise quantitation of a specific cannabinoid in the sample. The products of the Cannabis plant differ considerably in their macroscopic appearance, from simple plant material, through resins to
In view of the number of samples submitted to forensic science laboratories, and their varied presentations, identification procedures for Cannabis products have been very widely studied and, in general, are simple and rapid techniques. Procedures fall into three groups: microscopic and macroscopic appearance, colorimetric (field) tests and chromatographic methods, primarily TLC.

Microscopy

The microscopic features of Cannabis have been fully described; where these are present in a sample, unequivocal identification of the presence of cannabis may be made by a trained microscopist, but where distinctive morphology is missing or damaged other supporting techniques must be employed. In the reviewers’ opinion, this limits the general application of microscopy to plant material alone because in the majority of cannabis resin samples many characteristic features have been destroyed and in liquids are absent. Residues or traces, such as in smoking utensils or clothing, are also rarely amenable to simple microscopic examination, owing either to combustion or gross contamination by other materials. Numerous authors have discussed the microscopic appearance of Cannabis and the criteria necessary for unequivocal identification by this technique. Although some authors in the forensic science field describe identification of Cannabis products by microscopy, the majority of reports rely on a combination of tests that may include microscopy. Mitosinka et al. examined Cannabis and other plant materials using the scanning electron microscope and the use of this instrument in the preparation of evidence for presentation in Courts-of-Law has been discussed.

Gross Morphology

The gross morphological appearance of different illicit Cannabis products has been described; it is clear from both of the publications cited that there is a very wide variation in the appearance of Cannabis products from different geographical origins.

Colour (Field) Tests

Prior to the elucidation of the structures of the major cannabinoids and the development of chromatography, chemical identification of Cannabis products was made by simple colour tests. Of the many tests for presumptive identification, those which have found widest discussion in the literature are those based on an original test described by Duquenois and Mustapha. Generally described as the Duquenois or Duquenois-Negm test, there is considerable confusion in the literature over the many variations of the original test, and it is rarely clear which test has been used. However, the modification that is most widely used is that generally referred to as the Duquenois-Levine test. Thornton and Nakamura and Pitt et al. have discussed the mechanism of this test in detail in the light of more recent discoveries of the structures of the major cannabinoids. The latter authors, in a discussion of the specificity of the Duquenois-Levine test, concluded that if the criteria for a positive test were rigorously adhered to and if botanical evidence was also available, then this test was a reliable screen for cannabinoids. However (they say), if botanical evidence is not available, then the test must be supplemented with chromatographic evidence. This conclusion is substantiated by a report that certain brands of coffee give a positive Duquenois-Levine test. Bailey discussed the value of the Duquenois test in detail and found that the Duquenois-Levine test was the most specific of all the modifications of the original test. The Beam test and a modification have also been widely used but, although reported to be more specific, these were found less sensitive than the Duquenois-Levine test, and have also been shown to be unreliable. It has been suggested that this reagent reacts with cannabidiol (CBD) and cannabigerol or their acids but not with THC. Consequently, it is not surprising that the test has proved unreliable in view of the absence of CBD from some samples of cannabis. This test does not appear to be in wide current use.

The rapid field test (and the improved procedure) described by de Faubert Mauder as a reliable indicator of the absence of Cannabis products within samples has been used in the Laboratory of the Government Chemist for over 14 years and has been incorporated in a commercially available Drug Test Kit. A positive test is presumptive evidence for the
presence of a *Cannabis* product within the sample; only nutmeg and mace are reported to give false positive reactions.58

Many other colour tests have been described; these include the Ghamravy test,91 the furfural test92,93 and a number of others94–99; these tests do not appear to have found widespread usage. There is little evidence that many laboratories are identifying *Cannabis* products on the basis of chemical or colour testing alone; Mechoulam et al., in a major review (1976),97 considered that “by their nature, colour tests are non-specific and should be confined to field work and screening while other techniques should be used for identification.” The reviewers concur with this opinion and emphasise that such screening tests should be limited to bulk seizures and should not be applied to trace amounts or smoking residues where evidence may be destroyed by such testing. Further, it is also the reviewers’ opinion that criminal prosecutions relating to *Cannabis* products where the only evidence is a positive field test are inherently unsafe and should not be pursued. There seems to be little to choose between the use of the Duquenois – Levine test97 for the presumptive identification of *Cannabis* products and the rapid field test for their presumptive absence described by de Faubert Maunder98; however, in the reviewers’ experience, the latter is more convenient to use in the field. The reviewers consider that the identification of cannabis and cannabis resin can be achieved unequivocally in most instances by combining one of the above two colour tests, correctly carried out, with a careful consideration of the morphology of the sample.

Where both macro- and microscopic features are absent or damaged, as in some cannabis resins or extracts and in trace amounts or smoking residues, colour tests alone cannot be relied upon, and the identification of the presence of a product from *Cannabis* must be made by a second technique. Most commonly used is TLC and the many systems in use have been discussed by Gough and Baker.44 Mechoulam et al.,97 although of the opinion that gas - liquid chromatography (GLC) has superseded TLC for many routine analyses, felt that the use of TLC would persist where requirements are not stringent and where speed and convenience are of greater importance. In an important paper,98 Hughes and Warner noted that no mixture of components previously reported had the same TLC characteristics as *Cannabis* products. The possible confusion between nutmeg or mace and cannabis that might arise using the rapid field test58 may be resolved using TLC.99 In 1973, Mechoulam100 reported that cannabinoids had not been isolated from any plant or animal except *Cannabis* and the reviewers are not aware of any more recent reports which indicate otherwise. Fenselau et al.,101 examined hop extracts (genus *Humulus*) by MS and found that cannabinoids were not present (detection level less than 10 ng g$^{-1}$). *Humulus* and *Cannabis* are the only genera in the family Cannabinaceae. In a valuable study, Coutts and Jones81 suggested that only if the “three-parameter approach” (morphology, colour tests and TLC) could be shown to be in error, should further tests be considered a necessary part of the identification of *Cannabis* products. These authors re-examined 100 seizures using GLC combined with MS (GLC - MS). Agreement was found with all but two analyses; these two errors arose from the Duquenois - Levine test97 giving erroneous negative results on smoking residues. TLC indicated the presence of THC and CBN. These authors concluded that TLC identification of THC and one other cannabinoid in an extract was sufficient to show that the source of the extract was a *Cannabis* product and that the “three-parameter approach” was unequivocal in identifying *Cannabis* products. It is the reviewers’ opinion that authentic standards of cannabinoids, whether pure or in a standard tincture, should always be used when TLC is applied to the identification of cannabinoids.

Some authors have suggested that GLC should be used for the identification of a *Cannabis* product102–104 but in the reviewers’ opinion TLC is sufficient. Macro- and microscopic sorting may be helpful in determining the proportion of the *Cannabis* product in the sample. Normally, all that a Court requires in the United Kingdom is the mass of *Cannabis* product. With trace amounts and smoking residues, it may be necessary to take into account the detection limit of the analytical technique used. Gough and Baker44 have discussed TLC detection limits of several chromogens used for cannabinoids. It is always necessary to identify the actual drug present,57 whether it is (or was) cannabis or cannabis resin.58 Samples of cannabis resin have always been found to contain CBD,60 as do some samples of cannabis from the “resin belt,”59 whereas many samples of cannabis are devoid of CBD.59,60,87 Consequently, if TLC indicates the absence of CBD in a sample where no morphology is apparent, the analyst can decide with a considerable degree of certainty that the cannabinoids present...
derive from cannabis rather than from cannabis resin. The converse is, of course, not so. Similar considerations apply to samples of liquid cannabis; those devoid of CBD may be considered to have been made by extraction of cannabis whereas those containing CBD could have been prepared from either cannabis resin or from Cannabis grown in a resin-producing area.

Although Cannabis is grown in many parts of the world, there are only a small number of countries from which illicit Cannabis products reach the UK. It has been considered\textsuperscript{104,105} that a means of identifying the geographical origin of illicit Cannabis products would be extremely useful in criminal investigation and in international control. Baker et al.\textsuperscript{60} have summarised available data on cannabinoid content in relation to country of origin and have studied the physical and chemical features of Cannabis products illicitly imported into the UK from known geographical origins. On the basis of their study of the many thousands of specimens of Cannabis products from all over the world maintained as a reference collection at the Laboratory of the Government Chemist, these workers\textsuperscript{60} consider that a combination of careful visual inspection of a sample of unknown provenance and comparative TLC enables an analyst to offer an opinion as to its geographical origin. In order to validate further such an opinion, these same workers made studies of the physical and chromatographic properties of Cannabis plants grown in the UK from seeds of known origin.\textsuperscript{106,107} The gross physical appearance and cannabinoid patterns of many of the UK-produced specimens were, in general, closely related to those of their parents but some exceptions were recorded. In comparison with imported material,\textsuperscript{108} cannabis produced in the UK had higher tetrahydrocannabinolic acid (THCA) to THC ratios than imported material. In the first study,\textsuperscript{106} the authors also reviewed previously published data on Cannabis plants grown under controlled conditions (although not necessarily from seeds of known provenance).

It is often necessary to compare two samples of a Cannabis product in order to establish or refute a common origin. Methods for comparison are primarily chromatographic and have been reviewed by Gough and Baker.\textsuperscript{44}

It is rarely necessary to quantify a particular cannabinoid for forensic purposes, although it may be necessary to measure levels in particular samples, for example, the THC content of Thai cannabis\textsuperscript{109} or that of oils or extracts when so required by Courts-of-Law. Determination of THC content is normally made by GLC.\textsuperscript{44} Such determinations give the total THC content, \textit{i.e.}, THC + THCA, the latter being decarboxylated on injection into the chromatograph.

**Opium and Poppy Straw**

In the UK the Misuse of Drugs Act 1971\textsuperscript{1} controls three natural products of the opium poppy, \textit{Papaver somniferum}, under Part I of Schedule 2: opium (whether raw, prepared or medicinal), poppy straw and concentrate of poppy straw. Opium, otherwise known as raw opium, is the latex obtained by incision of the unripe seed capsule of \textit{Papaver somniferum} L., dried or partly dried by heat or spontaneous evaporation.\textsuperscript{110} Part IV of Schedule 2 of the Act includes powdered or granulated opium within this definition. Prepared opium is defined in Paragraph 37 of the Act as “opium prepared for smoking and includes dross or any other residues remaining after opium has been smoked.” Medicinal opium is defined in Part IV of Schedule 2 as “raw opium which has undergone the process necessary to adapt it for medicinal use in accordance with the requirements of the British Pharmacopoeia, whether it is in the form of powder or is granulated or is in any other form and whether it is or is not mixed with neutral substances.” Certain low dosages of medicinal opium are excluded from control under the Act by the Misuse of Drugs Regulations 1973.\textsuperscript{111} Poppy straw and concentrate of poppy straw are defined in Part IV of Schedule 2 as “all parts, except the seeds, of the opium poppy after mowing” and “the material produced when poppy straw has entered into a process for the concentration of its alkaloids,” respectively. It is of interest that whereas cultivation of Cannabis is specifically prohibited by the Act,\textsuperscript{1} cultivation of \textit{Papaver somniferum} is not so controlled. In contrast, opium smoking equipment intended for use is controlled, even if unused, whereas no such control is exercised over unused equipment specifically designed for the smoking of Cannabis products.
Opium

Opium contains 25–30 alkaloids, of which morphine, averaging 10% by mass in Indian opium, codeine, thebaine, noscapine, papaverine and narceine are the most important. In a detailed and valuable paper, de Faubert Maunder described field and laboratory tests for raw and prepared opium; a comprehensive account was given of the reactions of opium to common colour tests and the author presented a general analytical scheme for these materials. Lim and Kwok discussed opium usage in Singapore and based part of their analytical procedure on de Faubert Maunder’s scheme. Unless samples were very small or in a dry powdered form, they found little difficulty in successfully identifying different types of opium. Final confirmation of the identity of an opium sample may be made by TLC using authentic opium as a standard. Smith recommended GC-MS for the forensic identification of opium, but the reviewers consider that this is unnecessary unless only very small samples are available for analysis. Antipyrine, a synthetic drug, has been reported in samples of Iranian opium and mixtures of opium with cannabis resin have been seized, but no other mixtures of illicit opium are known to the reviewers. The Misuse of Drugs Act 1971 does not specify any particular alkaloid levels in opium and this review does not therefore cover analytical techniques for such determinations as they are not forensically necessary.

Poppy Straw

Samples may be identified by the presence of opium within the seed capsules using appropriate techniques. Although Fairbairn considered morphine and codeine to be of very limited occurrence in other species of Papaver, Bentley reported the occurrence of morphine in Fructus papaveris (blue poppy). The reviewers therefore consider that, in addition to chemical evidence for the presence of opium alkaloids, botanical evidence should always be obtained prior to final identification of poppy straw.

Concentrate of poppy straw may be identified from its alkaloidal constituents and any botanical debris present. The reviewers consider the borderline between concentrate of poppy straw and crude or impure morphine to be ill-defined. Concentrate of poppy straw is not clearly defined in the Explanatory Notes to the Customs’ Co-operation Council, although it is listed for tariff purposes. Such cases, should they arise, may have to rely on the expert opinion of the analyst as to the appropriate charge in Law. In such cases, the reviewers consider that a morphine content determination is imperative in order that the Courts may gauge the seriousness of any charge.

Narcotics

Introduction

Part 1 of Schedule 2 of the Misuse of Drugs Act 1971 consists mainly of a list of narcotic analgesics. Most of these drugs have been synthesised for experimental therapeutic purposes and few have any current use. Only a small number are misused to any considerable extent. The major misused narcotics are diamorphine (heroin; 3,6-diacetylmorphine) and morphine. A small number, including codeine (controlled by Part II of Schedule 2), dipipanone, pethidine and methadone, are occasionally encountered and the rest scarcely at all. Stereoisomers, esters, ethers, salts and preparations or products containing these substances are also controlled. Certain preparations containing low levels of morphine and codeine are excluded from control under the Misuse of Drugs Act Regulations 1973, but all their preparations designed for administration by injection are controlled (codeine preparations designed for injection are controlled under Part 1 of the Schedule). In view of the predominance of heroin and morphine in the field of abused narcotics, the reviewers do not propose to discuss in any detail other narcotics, but mention will be made of them where relevant.

Although small amounts of licitly manufactured preparations containing narcotics are occasionally seized, most narcotics analysed in forensic science laboratories are illicitly manufactured. Large seizures have been made of licitly manufactured morphine tablets (15 or 30 mg of active ingredient) after illegal importation from the Indian sub-continent, but this is the sole major exception to this generalisation.
No licit preparations of heroin are known to the reviewers to be widely available or misused, although small amounts reach "street level," mainly resulting from thefts from pharmacists' premises or hospitals. Such preparations may be tentatively identified from codes or manufacturers' markings, but such occurrences are rare. Licit preparations of some other controlled narcotics are encountered at street level and therefore guides and charts may find application to their tentative identification.

Illicit preparations vary from essentially pure heroin hydrochloride (intended for injection) to crude and impure materials, often containing heroin base and probably intended for smoking. Johnson and Gunn\textsuperscript{122} discussed in detail the diluents and adulterants in "street level" heroin seizures and Baker and Gough\textsuperscript{123} listed many of the compounds found in heroin at importation. Important diluents include sugars and quinine. Heroin is manufactured by acetylation of morphine and the major impurity is 6-acetylmorphine, as well as acetylmorphine and other opium alkaloids. Major adulterants with physiological effects include caffeine and strychnine (in products from South-East Asia) and procaine (from South-West Asia). The appearance of illicit heroin varies from pure white crystalline samples of heroin hydrochloride, through amorphous beige and brown powders, to dark brown lumpy material. It is the reviewers' experience that there is very little correlation between colour and heroin (as hydrochloride or base) content. Clark and Miller\textsuperscript{124} studied dyes added to illicit heroin samples and found that their use was widespread. The appearance of Chinese No. 3 heroin, which contains caffeine, is characteristically granular and beige to pale brown in colour, often with a distinct odour of acetic acid. Some samples of Middle-Eastern heroin have an odour of opium and may be prepared by acetylation of opium extracts rather than from crude morphine.

Two useful studies of the specificity of analytical techniques for the identification of heroin have been presented\textsuperscript{125,126} and these will be discussed in detail below.

**Colour and Microcrystal Tests**

Until the advent of routine and relatively cheap analytical instrumentation, identification of heroin was based on colour and microcrystal tests.\textsuperscript{127} The Marquis reagent\textsuperscript{128} (formaldehyde and concentrated sulphuric acid) gives a characteristic purple colour with heroin, morphine and structurally related compounds\textsuperscript{129} and is widely used as a field test. Lerner\textsuperscript{129} found the test to be sensitive to as little as 1\mu g of pure heroin. He used concentrated nitric acid as a second colour test for heroin, the colour change from yellow to pale green being, in his opinion, specific for heroin but considerably less sensitive. However, Manura et al.\textsuperscript{126} showed in a study of the colour reactions of heroin and 56 structurally related compounds that neither the Marquis nor the nitric acid test,\textsuperscript{129} nor the two tests in combination, were in any way specific for heroin. Clark\textsuperscript{125} showed that 17 compounds structurally related to heroin gave similar colours with the Marquis\textsuperscript{126} and Mecke reagents.\textsuperscript{130} Engelke and Vincent\textsuperscript{131} found some improvement in the specificity of colour reagents if colour reference charts were used, but data were only presented on pure alkaloids. Clarke\textsuperscript{21,22} listed the colours given by the principal narcotics with the Marquis reagent\textsuperscript{129} and some other common colour tests.

The reviewers consider that, although the Marquis test\textsuperscript{128} is non-specific, it is still useful for screening at point-of-seizure, particularly if part of a "logical tree" scheme,\textsuperscript{29,96} and it is currently included in many commercial field test kits. Tetracycline, a widely used antibiotic, may be confused with illicit narcotics as it gives an instant purple with this reagent, but the colour changes rapidly to yellow-brown, which distinguishes it from the narcotics, where the purple colour is stable for many minutes. In addition, tetracycline preparations are yellow, an unusual colour for illicit narcotics.

Fulton\textsuperscript{132} described in detail several microcrystal tests for heroin and related compounds, but only pure drugs were used. Lerner and Mills\textsuperscript{133} found problems in microcrystal formation with illicit heroin samples, which they attributed to the presence of 6-acetylmorphine. Clark\textsuperscript{125} found no correlation between molecular structure and crystal shape in a study of heroin and 17 related compounds and, in addition, found difficulty in describing crystal shapes. Manura et al.\textsuperscript{135} in a study of heroin and 56 related compounds, found that microcrystal descriptions were subjective and encountered considerable problems with impure samples. Clarke\textsuperscript{21,22} has described common microcrystal tests for the principal narcotics. The reviewers consider that microcrystal tests have little value in modern forensic analysis of narcotics.
Nuclear Magnetic Resonance Spectrometry

NMR spectrometry is little used in the forensic analysis of narcotics. Clark, in a study of heroin and structurally related compounds, found that although each of their NMR spectra were different, fairly pure samples were needed for unambiguous identification.

Ultraviolet Spectrometry

UV spectrometry has been found to be far from specific in the analysis of illicit narcotics. Although the technique may be useful for the screening of licitly manufactured materials, the reviewers consider that the use of this technique is very limited either for identification or for quantitation of illicitly manufactured material. UV spectrometric absorption at selected wavelengths is, however, very widely and satisfactorily used as a detection system in the analysis of narcotics after their separation by high-performance liquid chromatography (HPLC).

Infrared Spectrometry

Curry and Patterson used IR spectrometry as the first technique in a procedure for the analysis of illicit heroin samples. In a study of the IR spectra of many samples, all were found to have at least six major absorption maxima in their IR spectra. Caffeine was also identified in many samples. These authors considered that the presence of heroin in illicit samples could be virtually confirmed on the basis of their IR spectra. They also consider that although heroin might appear to be absent from suspect samples, this absence should be confirmed by chromatography. Clark, in a study of heroin and structurally related compounds, and Manura et al., in a larger study, found that all the individual compounds and their salts were each distinguishable by their IR spectra. The latter authors considered IR spectrometry to be the most specific technique for the identification of heroin. Shaler and Jere studied illicit heroin samples by GLC coupled with IR spectrometry and considered this a useful and very specific technique for the unambiguous identification of heroin. In the reviewers' experience, IR spectra identify the presence of heroin in illicit heroin samples with little difficulty and it is their opinion that, as an identification technique, it is of great value. The spectra of some samples of illicit heroin containing appreciable levels of 6-acetymorphine and/or morphine are, however, difficult to interpret. Morphine, codeine and other narcotics may also be identified from their IR spectra.

Chromatographic Techniques

The identification and quantitation of heroin may be achieved by the principal chromatographic techniques widely used in forensic science laboratories. Most common illicit narcotics may be analysed by GLC or HPLC, often after screening of the sample by TLC. Chromatographic techniques for the analysis of major narcotics have been reviewed by Gough and Baker. The reviewers consider that identification should always be based on two different quantitative chromatographic techniques if IR spectrometry is unavailable or an ambiguous spectrum is obtained.

Mass Spectrometry

Saferstein et al. presented CI MS data on morphine, codeine, heroin, the two acetylmorphines and acetylcodine. Nakamura et al. used GC - MS to identify heroin in “street” samples at the 5-20% level. Herman and Kan found β-chloromorphide in a sample of partially acetylated morphine and presented mass spectral data to support this identification. Clark studied GC - MS data from heroin and related compounds and found that each was unambiguously identifiable. This author considered that this was a particularly useful identification technique, as preliminary clean-up was not required. Jere et al. used single ion monitoring as a quantitative technique for the identification and assay of heroin and found it valuable, particularly in the quantitation of heroin in small seizures.

MS has been little used in the identification of heroin and other major narcotics in illicit seizures as identification can usually be made by IR spectrometry. However, we believe its use to confirm the presence of narcotics in trace amounts, particularly from syringes and from scenes-of-crime, to be mandatory. In addition, it is valuable in identifying minor constituents of larger seizures, particularly when combined with prior GLC analysis.
ANALYSIS OF DRUGS OF ABUSE

Coca Leaf

Introduction

In the UK coca leaf is controlled under Part I of Schedule 2 of the Misuse of Drugs Act 1971 and is defined in Part IV of the same Schedule to that Act as "the leaf of any plant of the genus Erythroxylon from whose leaves cocaine can be extracted either directly or by chemical transformation." Archer and Hawkes described other alkaloids structurally related to cocaine that occur in the leaves of plants of the genus Erythroxylon. Aynilian et al. briefly discussed the distribution of cocaine within the different species of that genus. Seizures of coca leaf in the UK are rare and usually small. Most of the Coca species are native to South America, mainly from Peru, Bolivia and Columbia.

Identification

The dry uncurled coca leaf can be recognised by its tea-like odour and by two longitudinal indentations on either side of the midrib. These are more conspicuous on the grey-green underside than on the dark green upper side. Powdered coca leaf may be identified by microscopic examination. Because the control of coca leaf under the Misuse of Drugs Act 1971 places emphasis on the extractability of cocaine from the leaf, the reviewers consider that identification of this material in the forensic laboratory should always be confirmed by a chemical analysis, which demonstrates the presence of cocaine in the leaf material presented for identification. After extraction of the alkaloids from the leaf, detection of cocaine together with other related alkaloids is usually made by chromatographic techniques. This aspect of coca leaf analysis has been reviewed by Gough and Baker.

Cocaine

Introduction

Cocaine has been used throughout recorded history for its local anaesthetic activity. However, the stimulant and euphoric properties of cocaine induce a high level of psychological dependence and hence its widespread illicit use. A detailed discussion of the historical aspects of cocaine use and abuse has been presented by Aldrich and Barker. As a result of the potential for abuse, control of cocaine is now almost worldwide. In the UK cocaine, together with its stereoisomers, esters, ethers, salts and any preparation or product containing cocaine or any of these derivatives, are controlled under Part I of Schedule 2 of the Misuse of Drugs Act 1971. Certain preparations of cocaine containing very small dosages are excluded from control by the Misuse of Drugs Regulations 1973. The same Part of the Schedule also controls ecgonine and any derivative of ecgonine which is convertible to ecgonine or cocaine. This latter group includes acyl esters of the 2-hydroxyl (e.g., benzoylecgonine) and alkyl esters of the 3-carboxyl such as methyl ecgonine; both series can be converted into cocaine by an esterification step. A third, important derivative of ecgonine is cinnamoylcocaine (that is, cinnamoylecgonine methyl ester), a compound naturally occurring in coca leaf, which can be converted into ecgonine or cocaine using simple techniques. The significance of the occurrence of this compound in seizures of cocaine has been discussed by Moore. The physical and chemical properties of cocaine and its principal derivatives have been described.

Seizures of cocaine made in the UK by Officers of Her Majesty's Customs and Excise originate principally from Bolivia, Columbia and Peru. Such seizures vary from being essentially pure to highly adulterated, often with non-controlled topical anaesthetics. It is not uncommon in the reviewers' laboratory to analyse a sample that consists wholly of one or more of the latter compounds. The adulteration of cocaine samples has been discussed by Baker and Gough.

Colour Tests

The majority of colour tests for cocaine depend on the production of a blue colour when cocaine reacts with cobalt(II) thiocyanate in acidic media. Alliston et al. examined several cobalt thiocyanate-based reagents as part of a preliminary sorting sequence prior to full laboratory analysis. Although the specificity of any single reagent was not good, most
reagents also reacting in a positive fashion to methadone, methaqualone, pethidine and some local anaesthetics, these workers achieved some measure of discrimination by disregarding responses not apparent in less than $5 \text{s}$. When used as an integral part of a logical test kit$^{29,90}$ and in particular in conjunction with a test reagent based on 4-dimethylamino-benzaldehyde (DMAB)$^{158,154}$ some improvement in specificity was obtained. This sequence of testing was used by Baker and Gough$^{150}$ in preliminary tests on mixtures of cocaine with local anaesthetics. These authors warn that a positive response to these tests is by no means specific and reiterate that field tests are only for preliminary sorting. Scott$^{155}$ developed a cobalt thiocyanate-based reagent that was described as sensitive, specific and difficult to misinterpret. Only tropacocaine, a local anaesthetic, rarely encountered in seizures gave a false positive reaction to this reagent. Phencyclidine and a number of local anaesthetics gave negative reactions. Winek and Eastly$^{156}$ evaluated the test and considered it useful for pure materials suspected of being cocaine, but found false positive reactions with some drug mixtures that did not contain cocaine. These authors recommended that confirmation of drug identity should always be sought by an alternative (chromatographic) technique. Grant et al.$^{157}$ considered specific cobalt thiocyanate-based test reagents were unlikely to be developed and suggested that the characteristic odour of methyl benzoate, generated when cocaine is transesterified by heating with methanolic alkali, was both more sensitive and more specific than the more commonly used field test reagents. Most drugs were found to give no odour under the conditions of this test and further the smell was persistent and could be distinguished from, for example, methyl acetate arising from the testing of aspirin (acetylsalicyclic acid). Bastos and Hoffman,$^{42}$ in a brief review of field testing, emphasised that all field tests should be confirmed in the laboratory, a point which the reviewers have emphasised.

Microcrystal Tests

Bastos and Hoffman$^{42}$ consider that microcrystal tests were the best physico-chemical techniques for the identification and confirmation of small amounts of cocaine. These authors have briefly reviewed this topic.$^{42,149}$ They recommend gold chloride in phosphoric acid as particularly useful because local anaesthetics are poorly precipitated by this medium. Fulton$^{158}$ suggested that gold bromide in a mixture of acetic and sulphuric acids was also useful when local anaesthetics were likely to be present. Valanju et al.$^{159}$ described microcrystal tests for ecegonine and benzoylecgonine. The reviewers consider that microcrystal tests are of little value in the identification of cocaine.

Ultraviolet Spectrometry

Bastos and Hoffman$^{42,149}$ in reviewing UV and colorimetric methods for the analysis of cocaine and related compounds, concluded that although cocaine, benzoylecgonine and ecegonine could be detected and quantified by UV spectrometry,$^{160}$ this method was neither specific nor sufficiently sensitive for use in the analysis of drugs of abuse. Moore$^{148}$ observed that cinnamoylcocaine could be detected in cocaine seizures by UV spectrometry and that the presence or absence of this compound might be a useful pointer to the method of manufacture of the cocaine. This author emphasised that the presence of cinnamoylcocaine should be confirmed.

Infrared Spectrometry

O’Brien and Sullivan$^{161}$ found that IR spectrometry could distinguish between cocaine and local anaesthetics. Bastos and Hoffman$^{149}$ considered that the technique was very useful in the identification of cocaine. Moore$^{148}$ established the presence of cinnamoylcocaine in illicit cocaine seizures using IR spectrometry. Trinler and Reuland$^{162}$ considered that IR spectrometry was the best method of unequivocally identifying cocaine in mixtures in view of the high cost of routine MS. These authors used a preliminary HPLC separation of cocaine from local anaesthetics prior to identification by IR spectrometry.

Chromatography

The forensic analyst not only must unequivocally identify the drug (or drugs) present in a
sample, but also the amount of that drug must be quantified accurately. The most common technique used is GLC. Chromatographic methods for the analysis of cocaine and related compounds have been reviewed by Gough and Baker.44

Mass Spectrometry

Mass spectrometric data for cocaine and some derivatives, mainly after GLC, have been presented.147,148,165–166 The reviewers do not consider that routine MS of cocaine in bulk seizures is necessary to confirm identity as chromatographic and IR spectrometric techniques provide sufficient evidence. However, when sufficient sample is not available for structural confirmation by the usual methods or when the sample is highly contaminated, such as in the examination of trace amounts from clothing or vehicles, confirmation of identity by MS is, in the reviewers’ opinion, mandatory. In a valuable paper, Clark166 discussed in detail the identification and quantitation (by addition of deuterium-labelled cocaine) of cocaine by MS. No difference in accuracy (at the 95% confidence level) was observed in quantitative results from this method as compared with gas chromatography.167

Optical Isomers and Stereoisomers

It is probable that all currently available illicit cocaine is prepared from coca leaves and therefore consists of the natural isomer, (-)-cocaine.168 However, as the occurrence of synthetic (±)-cocaine or (+)-cocaine is possible, it may be necessary for the forensic analyst to distinguish between the optical isomers and the racemic mixture. In the USA, Federal and most state laws subsume cocaine within the following definition: “coca leaves, any salt, compound, derivative or preparation of coca leaves, and any salt, compound, derivative or preparation thereof which is chemically equivalent or identical with any of these substances,” wording that Siegel and Cormier169 consider imprecise. Indeed, some authorities assert that, in the USA, only (-)-cocaine is controlled, the other optical isomers of cocaine and its stereoisomers, pseudococaine, allococaine and pseudoallococaine, being not controlled. Useful discussions of the legal position in the USA have been presented in several papers.169–172 In the UK, all stereoisomeric forms of cocaine are subsumed by Paragraph 2 of Part I of Schedule 2 of the Misuse of Drugs Act 1971; thus the question as to whether a substance identified as “cocaine” may not be a controlled drug cannot arise under UK legislation. However, it may be of forensic importance for the analyst to determine which isomer (or isomers) is present in a sample. Such information may be useful in determining the method of manufacture of the sample. Chromatographic techniques for the separation of optical and stereoisomers have been reviewed by Gough and Baker.44 Siegel and Cormier169 described the preparation of (+)-pseudococaine from (-)-cocaine; the former was characterised by commonly used chromatographic and physical techniques. The authors concluded that the use of these (routine) tests would confirm or refute pseudococaine as a substance purporting to be cocaine. Allen et al.,172 in an important paper, described a logical sequence for the identification of the stereoisomer present (by IR, NMR and MS) and then determination of chirality by crystal tests, IR spectrometry, melting-points or optical rotation. Kroll170 determined the enantiomorph composition of cocaine using the chiral lanthanide shift reagent europium tris-d-trifluoroacetyl camphorate; 2 mg of cocaine sample could be analysed (less if Fourier transform NMR spectrometry was used) with an isomer detection limit of 5%.

Other Forensic Aspects

In illicit seizures of cocaine, the drug is more commonly present as the hydrochloride than as other salts or the free base. Anions may usually be confirmed with common precipitation reagents, but greater certainty is possible using IR spectrometry161 or X-ray diffraction.173 Moore148 has suggested that cocaine samples may be compared on the basis of impurities and gave spectral data on cinnamoylcocaine. Lukaszewski and Jeffery164 suggested that NMR spectroscopy could be used for the comparison of cocaine samples by studying the presence and amount of cinnamoylcocaine. These authors further suggested that the presence of this compound in a cocaine sample indicated that the material was derived from Coca rather than prepared synthetically. The converse is, however, not true as the sample could have been prepared by hydrolysis of all ecgonine esters to ecgonine and re-esterification.
Samples that do not contain cinnamoylcocaine cannot therefore be unequivocally identified as synthetic.

**LSD and Indolic Hallucinogens**

**Introduction**

In the UK, the Misuse of Drugs Act 1971, as originally promulgated, controlled in Part I of Schedule 2 only the limited number of hallucinogens specified in previous legislation. These include bufotenine, lysergamide (lysergic acid amide), lysergide (lysergic acid diethylamide, LSD) and other N-alkyl derivatives of lysergamide, psilocin, NN-dimethyltryptamine (DMT) and NN-diethyltryptamine (DET). In addition, stereoisomers, esters, ethers, salts and preparations or other products containing these substances are controlled. Psilocybin, the phosphate ester of psilocin, therefore falls into this category. A subsequent Modification Order added a further group of compounds derived from tryptamine to Part I of Schedule 2 (where not controlled under the 1971 Act), describing them generically as "any compound structurally derived from tryptamine or from a ring-hydroxy tryptamine by substitution at the nitrogen atom of the side-chain with one or more alkyl substituents, but no other substituent."

LSD is not, as far as is known, a naturally occurring product, but many hallucinogens are substances naturally occurring in plants (and, for bufotenine, also in a species of toad). For many centuries, people in whose area these plants grew exploited their hallucinogenic nature; several discussions and reviews of the occurrence of hallucinogenic plants have been presented. The history of the use of these materials and their occurrence has been described in detail by Emboden. The occurrence of hallucinogens in *Psilocybe* species, Argyreia nervosa (Hawaiian Baby Wood Rose), Virola perviana, *Rivea corymbosa* and Ipomoea violacea (Morning Glory) and in ergot fungus have been described.

Whereas cannabis, coca leaf and poppy straw are controlled by the Misuse of Drugs Act 1971, no plants containing controlled hallucinogens are explicitly named. Indeed, it is implicit in the House of Lords' ruling in the case of DPP vs Goodchild that drugs while still contained within a plant (or animal) (i.e., in their natural state) do not fall within the scope of the Act. However, in many countries, control of botanicals is explicit. Despite lack of control, it may be necessary to identify such materials in the UK, for example in relation to military prosecutions and in cases of poisoning.

Despite the occurrence of numerous hallucinogenic plants from which a large number of chemicals could be extracted, the chief source of hallucinogens for misuse is illicit laboratories. Although there have been occasional reports, principally from the USA, of misuse of DMT and DET, and an upsurge of interest in *Psilocybe* mushrooms in some countries, LSD is the most commonly abused hallucinogen and almost all analytical data on hallucinogens concern this compound. LSD is active in man at doses as low as 1 µg kg⁻¹ body mass. Illicit dosage units contain very variable amounts of LSD and impurities and excipients are often present in an excess. Precursors of LSD are present in many plants, one of the most important being lysergamide in Morning Glory seeds. Brief reviews of identification methods for hallucinogens have been published.

**Physical Characteristics**

Many of the more obscure hallucinogenic plants are not described microscopically in readily available literature and the reviewers recommend that any identification is made by expert botanists. Descriptions of the gross morphology of many plants containing hallucinogens are given by Emboden. Heim *et al.* have described the microscopic appearance of *Psilocybe* genus. Lophophora williamsii (peyote) was described by Kapadia and Fayez. DMT and other tryptamines are inactive orally and are usually inhaled as snuff or smoked and thus may be encountered as finely powdered material or mixed with tobacco.

Few of the hallucinogens, either within natural products, extracted or synthetic, have been encountered in large amounts with the single exception of LSD. LSD is encountered in many presentations: in solution, on paper, often with elaborate patterns, added (in solution) to other tablets, on sugar lumps, in refilled capsules, as illicitly prepared tablets ("microdots" or "domes") and occasionally mixed with other drugs, although in the reviewers' experience
this is rare. Once encountered, many of these presentations are easily recognisable by the forensic analyst, but the occurrence of outright fakes is higher than with many drugs and the analyst should be on his guard. Many forensic drug laboratories maintain collections of LSD presentations and the matching of punch marks and tablet sizes may be indicative of a common origin of supply.10 Dyestuffs in tablets may also indicate to the analyst a common origin of samples.11,12

**Colour Tests**

Although some presentations of LSD and related indolic hallucinogens may be detected by observing their fluorescence under UV light,203 caffeine and quinine, which are common adulterants in drugs,128 and certain detergents153 may respond in like manner.86 Dimethyl-aminobenzaldehyde (DMAB) reacts with many a- or b-unsubstituted indoles in the presence of air and strong acid to form violet or blue compounds. This reaction has been utilised universally as a field test for LSD and other hallucinogens.204 Look205 prepared test papers by saturating filter-paper in DMAB solution and air drying them. A portion of the suspect material was placed on this prepared paper followed by one drop of concentrated hydrochloric acid; a positive response, indicated by a violet - red or violet - blue colour, indicated that LSD or an indolic hallucinogen might be present. The test was sensitive to 1 pg of LDS. This test was subjected to collaborative study175 and found to be valuable in the preliminary examination of suspected hallucinogens. Alliston et al.153 devised an improved test, avoiding the prepared test papers, whereby one drop of a mixture of DMAB, methanol and concentrated hydrochloric acid was added to the sample on a filter-paper. Violet or purple striations on the filter-paper indicated the possible presence of an indolic hallucinogen. This test was found to be more specific and more sensitive than fluorescence. In a further improvement,159 orthophosphoric acid was substituted for hydrochloric acid, resulting in a stronger final response and a sensitivity of 10 ng for LSD. This reagent is used as part of a logical tree sequence field test kit196 issued to Officers of HM Customs and Excise and has been found to remain satisfactory after prolonged storage. In earlier reagents, the vapour of concentrated hydrochloric acid tended to destroy labels and instructions and acidify other test reagents in the kit. Some local anaesthetics give orange colours with this reagent, which may be helpful in the preliminary screening of suspect cocaine samples, where these are common adulterants.150 The reviewers also consider that the careful use of this test reagent may considerably reduce the hazards inherent in the investigation of illicit laboratories. Such laboratories may contain large amounts of prepared drugs, precursors and impure specimens and preliminary screening of all suspect materials found may prevent accidental ingestion of drugs during scenes-of-crime investigations such as fingerprint searches. This reagent has been found by the reviewers to give a positive response to many natural products containing tryptamines, which may help in the sorting of botanicals. Again, we emphasise the importance of laboratory confirmation of all field test results.

**Ultraviolet and Fluorescence Spectrometry**

Although field tests may be carried out with advantage on raw botanicals and crude preparations containing hallucinogens, the small dosages used makes clean-up by solvent extraction206,207 or column chromatography176,206 a necessary preliminary to the use of either UV or fluorescence spectrometry. Although LSD in the pure state may be accurately quantified by UV spectrometry,175,196,206 the impurities likely to be present in illicit preparations make this a technique of limited value and sensitivity.196 Bailey et al.208 showed that UV spectrometry did not distinguish lysergic and isoylseric acid derivatives.

DeZan et al.209 have compiled fluorescence data for a number of hallucinogens including LSD, bufotenine, DMT (and DET and NN-dipropyltryptamine), psilocin and psilocybin. The inherent fluorescence of many hallucinogens has made fluorescence spectrometry a method of choice for the detection and quantitation of LSD.193,209,206,207,210 The technique is not specific and is of limited use with impure specimens. However, when fluorescence detection is coupled with a separative technique such as TLC or HPLC it is both sensitive and specific and is now widely used for identification and quantitation of LSD and related compounds.44
Infrared Spectrometry

Although identification of LSD by IR spectrometry may be difficult in view of the small amounts available in individual doses, the current generation of instruments has considerably reduced this problem. Samples of pure LSD or its salts can be identified by direct IR spectrometry, but the presence of impurities may make interpretation of IR data difficult. Mesley and Evans made a detailed study of the IR spectra of LSD and its tartrate salts and drew attention to the variability of the spectra and the appreciable changes observed when using potassium bromide discs. The LSD spectra were, however, distinctive and useful for identification. The same authors also published a detailed survey of the IR spectra of tryptamines and their salts and noted the occurrence of polymorphism in many salts and some bases. Detailed discussion was presented on the characteristic absorptions of these compounds, which, the authors considered, would permit recognition of compounds of this type even in the absence of reference materials or spectra. Cromp and Turner studied the IR spectra of LSD and related compounds and considered that specific identification of these compounds was possible by this technique. Martin and Alexander reported that they could distinguish LSD and iso-LSD but no IR spectra were published. Although the spectra were similar, pure samples of ten lysergic and isolysergic acid dialkylamides were reported by Bailey et al. to be distinguishable by IR spectrometry.

Chromatographic Techniques

TLC and HPLC are widely used for both preliminary and final identification and quantification of LSD and other hallucinogens. LSD is degraded by UV light to lumino-derivatives and this property has been used to confirm the identity of LSD by TLC. The chromatography of hallucinogens has been reviewed by Gough and Baker.

Mass Spectrometry

GLC analysis of many hallucinogens is unsatisfactory as many of these compounds are thermally labile. Consequently, most mass spectral data have been obtained using direct insertion, by either EI or CI techniques. Ardrey and Moffat presented both EI and CI MS data on 19 hallucinogens and concluded that the identity of specific ergot alkaloids and LSD could be unambiguously confirmed (usually after TLC) by this technique. Psilocin and psilocybin cannot be distinguished by MS and preliminary identification by chromatographic techniques must be initially made. Trimethylsilyl (TMS) derivatives have been prepared from some tryptamines prior to MS. Psilocin and psilocybin may be distinguished by MS and preliminary identification by chromatographic techniques must be initially made. Trimethylsilyl (TMS) derivatives have been prepared from some tryptamines prior to MS. Psilocin and psilocybin may be distinguished by MS if TMS derivatives are first prepared. Repke et al. identified and distinguished between these compounds after extraction from a sample of Psilocybe cubensis. Mass spectral data on LSD and related derivatives have been presented and White postulated from mass spectral evidence that a third hallucinogen, 4-phosphoryl-N-methyltryptamine (baeocystin), originally isolated from P. baeocystis, may be present in P. semilanceata.

Amphetamines and Structurally Related Drugs

Introduction

Numerous drugs structurally related to amphetamine (d,l- \( \alpha \)-methylphenethylamine) are controlled by the Misuse of Drugs Act 1971 or by subsequent Modification Orders. Such drugs may be stimulants and anorectics structurally derived from amphetamine, or may be hallucinogens of little medical use, derived by ring substitution. Three substances of the latter type named explicitly in Part I of Schedule 2 of the Act are mescaline, 2,5-dimethoxy-\( \alpha \),4-dimethylphenethylamine ("STP") and 4-bromo-2,5-dimethoxy-\( \alpha \)-methylphenethylamine ("bromo-STP"). Further compounds of this type are controlled generically as follows: "any compound [not being methoxyphenamine or a compound for the time being specified in sub-paragraph (a) above] (this being Part I of Schedule 2 of the Act and compounds added by Modification Orders) structurally derived from phenethylamine, any \( N \)-alkylphenethylamine, \( \alpha \)-methylphenethylamine, any \( N \)-alkyl-\( \alpha \)-methylphenethylamine, \( \alpha \)-ethylphenethylamine or any \( N \)-alkyl-\( \alpha \)-ethylphenethylamine by substitution in the ring to any extent with alkyl, alkoxy, alkylenedioxy or halide substituents, whether or not further substituted in the ring by one or more univalent substituents." Although numerous
compounds are subsumed under this paragraph, few have been encountered on the illicit market. The following drugs have, however, been seized by law enforcement officers either in the UK or in the USA: 4-methylamphetamine, 4-methoxyamphetamine (PMA), 2,5-dimethoxyamphetamine (DMA), 2,4,5-trimethoxyamphetamine (TMA), 3,4-methylene-dioxyamphetamine (MDA), 2- and 5-methoxy-3,4-methylenedioxymethylamphetamine (MMDAs) and 3,4-methylenedioxy-N-methylamphetamine (MDMA). The stimulant drugs explicitly controlled in Schedule 2 include amphetamine, dexamphetamine, methylamphetamine, methylphenidate and phenmetrazine (in Part II) and benzphetamine, chlorphentermine, mephentermine and phenmetrazine (in Part III). Stereoisomers, salts and products containing the above drugs are also controlled by the Act, as are ethers and esters of those drugs specified in Part I of the Schedule.

As dexamphetamine and amphetamine are listed separately by the Act, it is necessary for the forensic analyst to identify which optical isomer is present in seized material. In view of the greater stimulatory effect of dexamphetamine on the central nervous system\(^{220}\) there may also be medical reasons for discrimination between these two substances in overdose situations. In the UK, amphetamine, dexamphetamine and methylamphetamine are the most commonly encountered members of the group; the others are rarely found.

Amphetamine, dexamphetamine and methylamphetamine, although widely used in the past to treat a variety of medical conditions, have been controlled in the UK since 1964\(^{221}\) when their misuse had become prevalent.\(^{222}\) These drugs have a high potential for abuse and are now more widely used as stimulants than as appetite suppressants,\(^{223}\) originally a principal therapeutic use. The hallucinogenic and stimulatory activity\(^{224}\) and the structure-activity relationships\(^{225}\) of the drugs controlled generically have been discussed. Although some licitly manufactured preparations of amphetamine and structurally related drugs are occasionally examined in the reviewers’ laboratory, a result of almost world-wide control is that the majority of amphetamine seizures are manufactured in illicit laboratories.\(^{226-228}\)

**Colour Tests**

A preliminary identification of licitly manufactured tablets may be made by reference to charts and guides\(^{2-9}\) or to tablet collections held within laboratories. However, the occurrence of such presentations is decreasing as manufacturers withdraw these materials from the market.

Preliminary classification of a sample into this class may be made using commonly available colour tests, but the close structural relationships between many of these compounds limits the ability of simple reagents to discriminate between them in many instances. Although Masoud\(^{19}\) described amphetamine and methylamphetamine as giving a similar colour with the Marquis reagent to the opiates (i.e., purple), it is the reviewers’ experience that a yellow-orange colour is produced. Clarke\(^{21,22}\) listed the colours obtained when the principal amphetamines were tested with the Marquis reagent.

**Microcrystal Tests**

Auerbach\(^{229}\) distinguished \(d\)- and \(d,l\)-methylamphetamine and dexamphetamine and amphetamine by volatilisation of the sample into a hanging drop of gold chloride. By simple direct examination of crystals formed between amphetamine optical isomers on a microscope slide, Clark\(^{230}\) was able to distinguish \(d\)- and \(l\)-isomers. Julian and Plein\(^{231}\) formed the 5-nitrobarbituric acid derivatives of the amine drugs found in illicit preparations in the USA ("mini-bennies" or "white cross" tablets); these authors used the same descriptions of morphological forms as Clarke\(^{41}\) and presented microcrystallographic data on optical isomers of amphetamine, methylamphetamine and some related drugs. Caffeine and theophylline, which may be present in many illicit drug presentations, were found not to interfere with this test. Genest and Lowry\(^{232}\) have described microcrystalloptic tests for STP. Bastos and Hoffman\(^{42}\) considered that microcrystal tests were the simplest technique for the differentiation of optical isomers of amphetamine and methylamphetamine. These authors also summarised the important reagents used for microcrystal tests. The reviewers consider that great care should be taken in the interpretation of results from these tests in view of the close structural relationships and the lack of data on microcrystalline derivatives of many amphetamines.
Fluorescence, Ultraviolet and Visible Spectrometry

The majority of illicit amphetamine presentations, especially tablets, contain only a small proportion of stimulant, often mixed with other drugs such as barbiturates. Prior to analysis by any spectrometric method, most of these presentations will require a simple clean-up, by either column chromatography or solvent extraction to remove excipients and any neutral or acidic drugs.

Unreacted amphetamine is only weakly fluorescent. Bastos and Hoffman briefly reviewed the many fluorescent derivatives that may be prepared from amphetamine. Most are derived from aldehydes or polycarbonyl compounds, the resulting fluorophores often being of unknown structure. Clark considered that such derivatives were tedious to prepare and non-specific. It is the reviewers’ opinion that although the sensitivity of fluorescence may be helpful in the analysis of trace samples, the lack of specificity of the technique, taken with the unknown nature of the derivatives, renders this unsafe in the forensic analysis of amphetamines.

UV spectrometry is widely used in amphetamine analysis, but tedious clean-up is needed, as samples are often dilute and low in drug content and amphetamine has low specific absorptivity. The UV spectra of common non-ring-substituted amphetamines and structurally related compounds are generally similar, rendering the technique of little value in identification, but it is useful for screening purposes. In view of the low absorptivity of amphetamines and the occurrence of considerable levels of impurities in many amphetamine samples, UV spectrometry is not, in the reviewers’ opinion, a reliable method of quantitation of amphetamines.

Fontani and Morandini described a colorimetric method for the determination of amphetamine in solid dosage forms. Derivatisation was with DMAB after heating in 2,5-dimethoxytetrahydrofuran; secondary and tertiary amines did not interfere with quantitative measurements. Clark considered colorimetric determinations of amphetamine to be inherently non-specific, a point with which the reviewers agree and consequently do not recommend for forensic analysis.

Infrared Spectrometry

IR spectrometry is an extremely valuable technique for the identification of this class of compound, but must almost invariably be preceded by a simple clean-up. In an important paper, Mesley and Evans reviewed the published spectra of many amphetamines and assigned characteristic absorptions. These authors discussed the occurrence of polymorphism and found it rare in this class of compound, although more frequent in salts than bases. Dexamphetamine sulphate and levamphetamine sulphate were found to have identical IR spectra, which were different from that of (racemic) amphetamine sulphate. The same was found for the hydrochloride salts. The optical isomers of the free bases were not distinguishable from the racemic base, all being liquids. d-Methylamphetamine hydrochloride and d,l-methylamphetamine hydrochloride were not distinguishable by IR spectrometry. Warren et al. confirmed this finding. Heagy distinguished all three optical forms of amphetamine by reaction with d-mandelic acid followed by IR spectrometry of the mandelates. This method has been examined at the reviewers’ laboratory using authentic standards of the three optical forms (checked by polarimetry); it was found that the spectra identifying the optical isomers in the published paper were mis-captioned, the spectrum identified as that of the l-amphetamine d-mandelate in fact being that of d-amphetamine, d-mandelate and vice versa. Once this had been ascertained, the reviewers found it to be a useful technique. Clark has presented the spectra of the principal stimulants subject to control.

Chromatographic Techniques

TLC has been widely used for the preliminary identification of amphetamines. These drugs may be quantified by GLC. HPLC has found little application in this field. Optical isomers of amphetamines may also be separated and quantified by GLC. The chromatography of these drugs has been reviewed by Gough and Baker.

Nuclear Magnetic Resonance Spectrometry

Warren et al. have presented NMR spectral data on amphetamine and methyl-
amphetamine. Wainer et al.\textsuperscript{237} differentiated between and quantified \textit{d}-, \textit{d},\textit{l}- and \textit{l}-amphetamine by proton NMR in the presence of a europium chiral NMR shift reagent; a plot of molar fraction of \textit{d}-isomer against molar fraction of \textit{l}-isomer in synthetic mixtures was linear over the range 0–100\% with a minimum detection limit of 5\% of either isomer. Liu \textit{et al.}\textsuperscript{238} used a similar technique to determine the ratios of methylamphetamine enantiomers in mixtures. The reviewers consider that these are valuable techniques as special derivative preparation is not required, a problem with GLC quantitation of isomer mixtures.\textsuperscript{44} Bailey and Legault\textsuperscript{239} studied the $^{13}$C NMR spectrum of amphetamine and found it to be distinctive and suitable for identification. This technique also distinguished salts from bases and the authors considered this an excellent means of distinction, identification and confirmation of structural authenticity. Proton NMR has been little used for amphetamine identification as IR spectrometry provides a simpler unequivocal means of identification.

### Mass Spectrometry

MS has been little used with the amphetamine group as the majority of the compounds give weak spectra with little or no molecular ion. Reisch \textit{et al.}\textsuperscript{246} gave data on the mass spectra of 18 phenethylamines including amphetamine, methylamphetamine, chlorphentermine and methylphenidate. Bellman\textsuperscript{188} reported that both mescaline and STP gave weak molecular ions. Coutts \textit{et al.}\textsuperscript{241} prepared $N$-trimethylsilyl derivatives of amphetamine prior to mass spectral analysis and found that characterisation of this compound using this derivative was possible.

### Hallucinogenic Amphetamines

Although numerous hallucinogenic\textsuperscript{225} drugs structurally related to amphetamine are controlled by the Misuse of Drugs Act 1971\textsuperscript{1} and its subsequent modifications,\textsuperscript{174,219} few have been encountered in the UK although rather more have been seized in the USA. 2,5-Dimethoxy-$\alpha$-4-dimethylphenethylamine (STP), a hallucinogenic amphetamine,\textsuperscript{242} was studied by Phillips and Mesley,\textsuperscript{243} who used NMR, UV, IR, XRD and MS; these techniques unequivocally identified the drug. XRD and IR spectrometry showed the base to be polymorphic.

Warren \textit{et al.}\textsuperscript{233} distinguished 3- and 4-methoxyamphetamine by IR and NMR spectrometry. Bailey \textit{et al.}\textsuperscript{32} studied ring-monomethylated and ring-monomethoxylated amphetamines and showed that UV, NMR and MS data distinguished the two series, but that IR spectrometry was necessary to differentiate between the isomers and distinguish salts from bases. Only weak molecular ions were recorded by MS and the authors considered this technique to be useful only in providing corroborative evidence. The UV spectra of the methoxy compounds closely resembled those of the dimethoxy compounds. Coutts \textit{et al.}\textsuperscript{241} showed that it was possible to characterise the monomethoxyamphetamine from the mass spectra of $N$-trimethylsilyl derivatives. Direct insertion was necessary as these compounds were thermally labile and therefore could not be subjected to GLC. Bailey and Legault\textsuperscript{239} showed that the $^{13}$C NMR spectra of individual ring-monomethoxyamphetamine were distinguishable and useful for identification and authentication. Salts were also distinguishable from bases by this technique.

Bailey \textit{et al.}\textsuperscript{244} in a study of the six isomeric ring-substituted dimethylamphetamines, found that MS analysis gave weak molecular ions and was of little value in distinguishing isomers. NMR spectrometry was found to identify individual compounds but only with difficulty. However, IR spectrometry allowed the bases to be distinguished, provided that samples were free from impurities and excipients.

Shaler and Padden\textsuperscript{245} identified 2,5-dimethoxyamphetamine from NMR, MS and IR data. Bailey\textsuperscript{246} showed, by studying the mass spectra of the dimethoxyamphetamine, that although the spectra were weak, they were distinguishable and characteristic, but required careful assessment. He considered that mass spectra alone were probably not reliable for the unequivocal identification of individual isomers, especially if small amounts of other substances with strong mass spectra were present. In a subsequent study, Bailey \textit{et al.}\textsuperscript{247} used proton NMR, IR and MS and showed that positive identification of each isomer could be made. IR and NMR spectrometry were shown to distinguish and identify each isomer and also to distinguish the monomethoxyamphetamine from dimethoxyamphetamines. Bailey
and Legault found the $^{13}$C NMR spectra of these six compounds were distinctive, suitable for identification and authentication purposes, and would distinguish salts from bases. The same authors also found that this technique would identify each of the six trimethoxy-ampetamines.

Bailey et al. identified 2-, 3- and 4-methoxy-N-methylamphetamine, 3-methoxy-4,5-methylenedioxyamphetamine and 3,4-methylenedioxy-N-methylamphetamine by proton NMR and IR spectrometry. Proton NMR spectrometry also distinguished the salt of each compound from the base and from the corresponding non-$N$-methylated compound.

Other Forensic Aspects

Although *Lophophora williamsii* (peyote), the cactus containing mescaline, is not itself controlled by the Misuse of Drugs Act 1971, it may be necessary, for example under military law, to identify this botanical. Chao has discussed the legal status of peyote in the USA. Kapadia and Faye have described the physical appearance of the plant and the former authors have discussed its active constituents.

Barron et al. discussed the significance of impurities in illicit methamphetamine samples. A knowledge of contaminants and by-products in such samples may help the forensic analyst to decide whether the drug was manufactured licitly or illicitly and upon its method of preparation. Comparison of different seizures may be possible using fingerprint patterns of impurities and knowledge of actual impurities may help to reduce interferences in quantitative analysis. Many of the contaminants in illicitly manufactured materials are more toxic than the intended drug and knowledge of these is therefore important from a toxicity standpoint. Impurities in amphetamines have been studied by a variety of techniques including UV, IR, NMR and MS. Lukasiewski studied the UV, IR, NMR and mass spectra of a number of possible precursors, intermediates and impurities in samples of MDA that might interfere with analyses. A review with 40 references on the comparison of illicit drugs has been published.

Phencyclidine and Analogues

Introduction

1-(1-Phenylcyclohexyl)piperidine (phencyclidine, PCP) is controlled in the UK by a Modification Order made under the Misuse of Drugs Act 1971. Although illicit importation and misuse of this drug has not been widely reported in the UK, it is widely abused in the USA and Canada. The ready availability of this drug is due largely to its comparatively simple synthesis from cheap and readily available starting materials. It was, at one time, available as an anaesthetic, for both human and veterinary purposes, but these applications were discontinued because of adverse effects on patients. Further, the therapeutic dose was found to be close to the toxic dose and many deaths have resulted from the misuse of this drug.

Two principal problems confront the forensic analyst when examining samples of suspected PCP: the presence of contaminants resulting from careless illicit synthesis and the large number of structurally related analogues that have been manufactured in illicit laboratories. The toxic precursor, 1-piperidinocyclohexanecarbonitrile (PCC), is controlled in the USA and a number of analogues controlled in both the USA and Canada are candidates for international control. These include the thienyl analogue tenocyclidine (known as TCP), rolicyclidine (PHP or PCPy, in which pyrrolidine replaces piperidine) and eticyclidine (PCE, where ethylamine has replaced piperidine). PCC has been found to be present in many samples of illicit PCP.

PCP and related preparations may be taken orally or smoked when mixed with spices or cannabis; forensic analysts should therefore be alert to this when analysing apparently innocuous botanical materials. A major problem, at least in the UK, is the general unavailability of authentic standards of PCP and related substances; the identification of PCP should therefore be made by the most rigorous techniques.

Colour Tests

PCP reacts positively to the cobalt(II) thiocyanate field test for cocaine and in the improved cocaine test of Alliston et al. in a field test kit. PCE has been reported to
react similarly.\textsuperscript{269} PCP and PCE give no colour with the Marquis reagent.\textsuperscript{269} Although data are lacking, the reviewers would expect the majority of PCP analogues to give similar results with these field tests.

**Ultraviolet and Infrared Spectrometry**

Column chromatographic\textsuperscript{270} or solvent extraction\textsuperscript{264} techniques have been described for the clean-up of illicit samples of PCP; in view of the probability of adulterants and impurities in such samples, the reviewers consider that this is an essential first step prior to spectrometric analysis. UV spectra have been found to differentiate between groups of structurally related PCP analogues\textsuperscript{271} but were found not to be useful for identifying particular compounds.\textsuperscript{267} For which IR spectrometry is recommended. Bailey et al.\textsuperscript{271} in a study of PCP and five analogues, found that the IR spectra of both the free bases and the hydrochloride salts were clearly distinguishable. In a related study of PCE and its analogues, Bailey and Legault\textsuperscript{267} found the IR spectra of the bases were similar to, but distinguishable from, those of the PCP analogues, whereas the IR spectra of the hydrochloride salts were suitable for identification purposes.

**Nuclear Magnetic Resonance Spectrometry**

NMR spectrometry has been found to be useful for distinguishing PCP and related compounds. Proton NMR spectra have been used to distinguish PCP from TCP, its thienyl analogue,\textsuperscript{271} from PCE and its analogues\textsuperscript{267} and in the identification of PCE,\textsuperscript{269} \textsuperscript{13}C NMR spectra have been reported to be suitable for the identification of PCP, PCE and their analogues.\textsuperscript{272} Brine et al.\textsuperscript{273} studied the hydrochloride salts of PCP and 16 related substances by \textsuperscript{13}C NMR and considered that they could be easily differentiated from each other using this technique, but that differentiation by proton NMR was more difficult. Bailey and Legault\textsuperscript{274} studied the \textsuperscript{13}C NMR spectra of 20 possible aminocyclohexanecarbonitrile precursors of PCP and related compounds and found that such spectra could be used to identify these compounds when reference materials were available and would provide a preliminary identification of unknown materials.

**Chromatographic Techniques**

Gough and Baker\textsuperscript{44} have discussed in detail the TLC, GLC and HPLC analyses of PCP and related compounds. These authors drew attention to the instability of PCC and the possibility of mis-identification of this compound when GLC was used for its analysis.

**Mass Spectrometry**

Mass spectral data on PCP have been presented by Lindgren et al.\textsuperscript{270} Bailey et al.\textsuperscript{271} analysed PCP and some analogues by MS and found it a valuable technique which not only would distinguish the compounds used in this particular study, but would also help to elucidate the structure of uncharacterised compounds structurally related to PCP. Molecular ions were found to be variable in intensity but to be assignable. Freeman and Martin\textsuperscript{268} found that MS, using single ion monitoring, would identify phenylcyclohex-1-ene (a product resulting from the smoking of PCP) in the presence of PCP. Bailey and Legault\textsuperscript{267} found MS (with prior GLC) suitable for the identification of PCP, PCE and their analogues. In two studies, Cone and co-workers\textsuperscript{275,276} analysed PCP precursors, analogues and metabolites by CI MS; these authors considered this technique to be the most specific means of identifying these compounds.

In view of the structural similarities between PCP and its analogues and the rarity of their occurrence (at least in the UK), the reviewers consider that both IR and MS data should be used before a conclusion is reached as to the identity of a suspected phencyclidine sample.

**Methaqualone**

Methaqualone is at present controlled in the UK by being specified in Part III of Schedule 2 of the Misuse of Drugs Act 1971.\textsuperscript{1} Preliminary identification of licit preparations may be made by reference to charts and guides.\textsuperscript{2-9} However, the recent large numbers of well manufactured fake preparations, some containing methaqualone and many more containing
other drugs, encountered in North America and Europe make it imperative to confirm the presence of methaqualone by rigorous analysis. Methaqualone reacts positively to the cocaine field test of Alliston et al.,152 which, although by no means specific, is useful as a sorting test because no licit or illicit tabletted preparation of cocaine has been encountered, in the reviewers' recent experience.

The relatively large dose of methaqualone (150 mg or more) in many licit preparations allows the characteristic UV spectrum of methaqualone277 to be used for identification,278 particularly as its absorption maxima vary with pH. However, in view of the occurrence of fakes and structurally related, usually illicitly manufactured, compounds such as mecloqualone, more specific techniques should, in the reviewers' opinion, be applied. Identification by IR spectrometry is possible and has been described.279 Chromatographic techniques for the analysis of methaqualone are included in reviews by Gough and Baker.44 MS data on methaqualone have been presented.45,46 Dal Cason et al.280 presented the IR, NMR and MS data on methaqualone and 15 analogues and isomers including mecloqualone; using data from these techniques, these authors could differentiate between all the compounds studied. Dal Cason et al.281 have discussed the illicit synthesis of mecloqualone. This drug is a candidate for international control and its forensic identification may become more important.

Barbiturates

Introduction

Barbiturates have a high dependence and addiction potential even in "normal" clinical usage.282 Barbiturates are not currently controlled in the UK by the Misuse of Drugs Act 1971 but many countries do exercise control over this class of drugs. A number of these substances are specifically listed in the Schedules to the Convention on Psychotropic Substances, 1971,283 to which the UK is not, currently, a signatory. Schedule III includes amylobarbitone, cyclobarbitone, pentobarbitone and quinalbarbitone, whereas barbitone, methylphenobarbitone and phenobarbitone appear in Schedule IV. Over 2500 barbiturates have been synthesised and about 50 have found clinical acceptance284; 11 barbiturates (including thiopentone sodium) are currently available in 23 preparations on prescription in the UK285 compared with 61 preparations of 14 barbiturates in December 1979.

Three possible systems of control in the UK have been publically discussed: (a) all 5,5-disubstituted barbituric acid derivatives, with or without addition of methylphenobarbitone; or (b) all 5,5-disubstituted barbituric acid derivatives excluding phenobarbitone; or (c) five explicit medium acting barbiturates that are frequently abused, namely amylobarbitone, butobarbitone, cyclobarbitone, pentobarbitone and quinalbarbitone. Stead et al.284 have discussed these proposals in detail. Analytical schemes for the analysis of suspect barbiturate-containing samples will vary, depending on the form of control finally adopted. Whatever that might be, the reviewers believe that the forensic analyst will still be required to identify the particular barbiturate present. The case of Muir vs. Smith,57 although concerning Cannabis products, implies that the Courts require explicit identification of controlled drugs. In addition, the identification of barbiturate preparations, specifically in overdose situations, is perhaps of greater importance as the physiological and toxicological properties of these drugs vary considerably.286

Colour Tests

Whilst licit barbiturate preparations remain widely available, many samples of tablets or capsules may be tentatively identified by reference to the appropriate guides.2–9 Such identification must be confirmed by laboratory tests.

The classic chemical field test for barbiturates is the modification by Dille and Koppányi287 of the Zwikker test288; colours are developed by the addition of an amine to an anhydrous cobalt(II) acetate solution of a barbiturate. Many commercial field test kits contain reagents based on this reaction. De Faubert Maunder289 considered that the original test lacked sensitivity and specificity and developed an improved version. A single solution of cobalt(II) thiocyanate in methanol containing 2,6-dimethylmorpholine is added to a small amount of the suspect sample on a filter-paper: a violet or purple colour on the paper is interpreted as a positive reaction. Detection limits of 0.2–0.5 µg were obtained with five common barbiturates; similar colours were given by some hydantoin hypnotics. The author advised31 that
the test be used as part of a logical tree field test procedure and such a test is included in a commercial field test kit. Following a positive field test, the presence of a barbiturate must be confirmed in the laboratory.

Ultraviolet Spectrometry

This technique has been very widely used for the determination of barbiturates using methods devised by Broughton.\textsuperscript{290} 5,5-Disubstituted barbituric acid derivatives are weak acids with two \( pK_a \) values; initial ionisation is at pH 7–8 and the second at pH 12–13. The mono-ions exhibit UV absorption at around 240 nm and the di-ions absorb at about 255 nm. Stead \textit{et al.}\textsuperscript{284} found that UV spectrometry alone would reliably establish the presence of a 5,5-disubstituted barbiturate in a sample. These authors also presented a useful discussion on the use of UV spectra in barbiturate analysis; Daglish\textsuperscript{291} and Higgins and Leach\textsuperscript{292} have also described the applications of UV spectrometry to barbiturate analysis.

However, UV spectrometry does not identify the actual drug present. If only specific barbiturates [control system (c)] were to become controlled in the UK, or if phenobarbitone is excluded [system (b)], application of UV spectrometry to barbiturate analysis becomes more limited. Problems may also occur when two barbiturates are mixed, as occurs even in some licit preparations. Synthesis of barbiturates from readily obtainable chemicals is a simple process and, should barbiturates become controlled, there seems little reason why their manufacture in illicit laboratories should not become common (as is so with amphetamines). Further, there is no reason why such laboratories should limit their operations to currently common barbiturates. If such illicit operations become widespread, impurities and uncommon preparations may limit even further the use of UV spectrometry in the screening of samples for 5,5-disubstituted barbiturates. Post-column ionisation techniques, utilising the increased UV absorption of ionised barbiturates over the free acids, have been used to detect these compounds after separation by HPLC.\textsuperscript{293} Nevertheless, the reviewers consider that, in spite of the drawbacks outlined above, UV spectroscopy of barbiturates could play a useful part in a fuller analytical scheme for the identification of these compounds as a class, whichever method of control is finally adopted in the UK.

Infrared Spectrometry

Most IR studies of barbiturates have been made on pure compounds; in view of their structural similarities, preliminary clean-up of samples is likely to be necessary prior to use of IR spectrometry. Mesley and Clements\textsuperscript{294} discussed the IR spectra of barbiturates in considerable detail in a study of 12 such compounds and drew attention to the widespread occurrence of polymorphism in this class of compounds (up to 13 forms of phenobarbitone).\textsuperscript{295} However, they found that although different forms of the same material might not always be distinguishable from their IR spectra, they could be differentiated from other barbiturates. Stead \textit{et al.}\textsuperscript{284} reported that only cyclobarbitone and heptabarbitone could not be distinguished from 28 other barbiturates. Heptabarbitone was not studied by Mesley and Clements.\textsuperscript{294} Discussions of the IR spectra of barbiturates have also been presented by Chapman and Moss.\textsuperscript{35,296}

Nuclear Magnetic Resonance Spectrometry

Although this technique has not been widely used in barbiturate analysis, Stead \textit{et al.}\textsuperscript{284} considered it most promising and reported that all the major barbiturates could be distinguished. However, samples of good purity were considered necessary and these authors reported problems in interpretation of the NMR spectra of mixtures.

Chromatographic Techniques

Although IR, NMR and some MS techniques may be used to identify pure or purified samples of barbiturates, the reviewers consider that chromatography will be a necessary part of any analytical scheme to identify these compounds, either prior to IR spectrometry (in order, for example, to distinguish cyclobarbitone and heptabarbitone\textsuperscript{294}) or prior to IR or MS in order to obtain pure samples. It may also be necessary to use a chromatographic screening technique in order to reduce the final identification problem. Confirmation of final identification by chromatography using an authentic standard may also be desirable.
TLC, GLC (often with prior derivative formation) and, more recently, HPLC have been widely used for the analysis of barbiturates; these topics have been reviewed by Gough and Baker.44

Mass Spectrometry

EI MS has been found to be of limited value for barbiturate identification as few of these compounds give characteristic molecular ions.284,297,298 Fales et al.299 used both EI and CI MS in a study of eight important barbiturates and showed that they could be distinguished. GC - MS of the alkyl derivatives of barbiturates has been shown to provide considerably more data for the identification of barbiturates300,306,301; however, some barbiturates give the same derivative on methylation and prior screening by an alternative technique may be necessary.44 Jones and Whitehouse300 observe that CI MS was not specific for isomeric barbiturates and studied the anion mass spectra under CI conditions of 30 barbiturates and obtained simple spectra that distinguished all the compounds except butalbarbitone and allylbutyrlbarbitone.

Concluding Remarks

Forensic drug identification has progressed in the last three decades from the application of simple testing procedures to the almost routine use of the most powerful instruments available in modern chemistry. This is simply a reflection of the ever increasing pressure on the analyst unequivocally and rapidly to identify the drugs in any sample presented. The reviewers consider this trend likely to continue and it behoves analysts not only to keep up-to-date with the scientific literature, but also to keep abreast of developments in analytical instrumentation.

Most common drugs can be simply and rapidly identified by existing techniques, but with the misuse of drugs increasing world-wide, continual method development is necessary if forensic laboratories, already in many instances overstretched, are to be able to keep up with a workload of increasing amount and complexity. However, certainty of identification must not be compromised in a search for analytical speed.

It is outside the scope of this review to discuss presentation of evidence, but it is clearly necessary for the expert to be totally familiar with the legal status of the substances identified. The haphazard growth of legislative control of drugs, and the pitfalls encountered in the UK, up to implementation of the comprehensive Misuse of Drugs Act 1971, have been extensively reviewed.302 For the forensic analyst it is salutary and necessary to remember that the outcome of nearly every positive identification of a controlled drug is a sworn witness statement, which may require oral presentation of evidence in Court. It is then that the expertise possessed by the analyst is truly tested. Useful discussions on the role of the expert witness, from both the UK303-306 and the USA,307,308 have been presented.

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