Mini review

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Reference materials for new psychoactive substances

Roland P. Archer,[a] Ric Treble[b] and Keith Williams[c]*

Historically, the appearance of new psychoactive materials (and hence the requirement for new reference standards) has been relatively slow. This position has now changed, with 101 new psychoactive substances reported to EMCDDA-Europol since 2006. The newly reported materials, and associated metabolites, require properly certified reference materials to permit reliable identification and quantification.

The traditional approach and timescales of reference material production and certification are being increasingly challenged by the appearance of these new substances. Reference material suppliers have to adopt new strategies to meet the needs of laboratories. This situation is particularly challenging for toxicology standards as the metabolism of many of these substances is initially unknown.

Reference material production often involves synthesis from first principles. While it is possible to synthesis these materials, there can be significant difficulties, from synthetic complexities through to the need to use controlled materials. These issues are examined through a discussion of the synthesis of cathinones. Use of alternative sources, including pharmaceutical impurity materials or internet sourced products, as starting materials for conversion into appropriately certified reference materials are also discussed.

The sudden appearance and sometimes brief lifetime in the market place of many of these novel legal highs or research chemicals present commercial difficulties for reference material producers. The need for collaboration at all levels is highlighted as essential to rapid identification of requirements for new reference materials. National or international commissioning or support may also be required to permit reference material producers to recover their development costs.

Keywords: legal highs; reference standards; cathinones synthesis; MDAI; new psychoactives

Introduction

Traditionally, the appearance of new psychoactive materials has been a relatively rare occurrence. Designer fentanyls appeared in the 1980s, primarily in the USA, and a range of Ecstasy-type materials appeared in the 1990s. The situation in 2011 is rather different. There has been a rapid growth in new psychoactive materials appearing in the 'drug scene', primarily marketed via the Internet. These 'new' substances, legal highs, or research chemicals can be divided into two broad categories: designer drugs that are substances that have been deliberately synthesized to avoid control under various countries' drugs legislation (e.g. mephedrone and the related cathinones), and other substances that have previously been reported in the scientific literature. The latter group includes materials specifically synthesized for research studies and potential pharmaceutical products that were never fully released to the market place.

Table 1 lists the number of new psychoactive substances reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) between 2006 and 2010.

This rapid growth in the rate of appearance of new substances shows no signs of abating, with some new materials appearing to be deliberately 'designed' to circumvent legal controls in the UK. One of the authors of this paper coined the phrase 'a chemicals arms race'[6] to describe the cycle of identification of new chemicals, followed by legislation to control them, followed by the appearance of new designer variants intended to avoid the legal control. This ever-changing field can easily be observed by searching on the Internet for products being offered for sale by head shops, and 'research chemical' suppliers. Many of the new substances appearing on the market appear to be derived from the scientific literature of neurochemical research, in particular the work of J. W. Huffman and D. E. Nichols's research groups.

Two groups of analytical scientists are particularly interested by the appearance of new psychoactive substances; forensic drug scientists and toxicologists, both forensic and clinical. New materials provide analytical challenges for them and reference standards are sought from a variety of sources, including specialist reference material providers, chemical suppliers, and pharmaceutical manufacturers and, on occasion, analysts may resort to 'in house' characterization of seized materials.

Meanwhile, quality standards and accreditation bodies are placing ever greater emphasis on the necessity of using properly certified and characterized reference materials to underpin routine laboratory operations. This increasing need for reference materials is challenging for reference material suppliers and the rapid growth of 'legal highs' entering the market in recent years is compounding the issue.

* *Correspondence to: Keith Williams, LGC Standards, Queens Road, Teddington, Middlesex, TW11 0LY UK. E-mail: keith.williams@lgcstandards.com*

a *States Analyst's Laboratory, Longue Rue, St. Martin's, GUERNSEY, GY4 6LD*

b *LGC Forensics, Queens Road, Teddington, Middlesex, TW11 0LY*

c *LGC Standards, Queens Road Teddington, Middlesex TW11 0LY*

Table 1. New psychoactive substances reported in Annex 2 of the EMCDDA–Europol Annual Reports on the Implementation of Council Decision 2005/387/JHA between 2006 and 2010

	New Substances Reported
2006[1]	7
2007[2]	16
2008[3]	13
2009[4]	24
2010[5]	41

There is also increasing emphasis from laboratories' customers on timely provision of reliable analytical results. As a result, there is pressure on laboratories and their reference material suppliers to ensure that their analyses and products are both fit for purpose and readily available.

The requirement for reference materials

In order to allow analysts to present reliable evidence to courts, and to allow toxicologists (both clinical and forensic) to correctly attribute specific symptoms, causes of impairment, or death to a specific substance, it is important to have confidence in the identification of the material in question.

Considering the arsenal of purification and analytical techniques available to chemists, there appears to be no limit to what can be identified. Often the only limiting factor is the quantity of material available for analysis.

Purification techniques such as preparative liquid chromatography (Prep LC) and recent advances in counter current chromatography (CCC) allow for separation of molecules with only very minor structural differences. Once a relatively pure material has been isolated, techniques such as high resolution mass spectrometry (HR-MS), infrared (IR) and nuclear magnetic resonance (NMR) can be employed to solve even the most complex structural puzzles. In rare occasions, more specialized techniques such as single crystal X-Ray Diffraction (XRD) may be employed to determine absolute configuration of enantiopure materials.

Use of primary analytical techniques, such as NMR and HR-MS, to establish chemical identity of novel materials is usually not feasible for routine laboratories. It is often possible to obtain analytical reference data for new substances, such as IR and mass spectra, from other laboratories. However, for definitive identification, chromatographic retention times are usually also required. To achieve this, it is necessary to have access to a reliably characterized reference material. This approach is strongly advocated by international quality standards such as ISO 17025, and by guidance from bodies such as the European Network of Forensic Science Institutes (ENFSI). For these reasons, access to reference standards is extremely desirable for laboratories carrying out forensic testing.

Accreditation and other expert bodies do recognize that fully characterized reference materials may not always be available, particularly for new psychoactive substances. If a laboratory has access to suitable analytical techniques, it can deploy these to create what has been termed an 'in-house reference material'. ENSFI has produced guidelines on how such material can be produced using material from law enforcement seizures with sufficient surety to allow it to be defined and used.[7] This self-certification is not ideal, and ENSFI recommends that such materials are used only for identification purposes.

The production of in-house reference materials requires significant effort and analytical capability and routine laboratories often do not have the luxury of time, quantity of substance, or advanced analytical resources required. The capital investment, running cost, operation, and data interpretation requirement of advanced analytical instruments is prohibitively expensive for most routine laboratories. Therefore, these organizations have an absolute requirement for access to reference materials to assist in identification.

For quantitative analysis, it is essential to have an accurately characterized reference material to ensure that detector response can be related to the quantity of the target substance. This is particularly important in forensic toxicology where accurate quantification of target substances is required to allow appropriate interpretation to be developed from the results.

For all these reasons, analytical laboratories are reliant on reference standard suppliers to stay abreast of developments in the field of legal highs, designer drugs, and what are increasingly being marketed as 'research chemicals'.

Production and types of reference materials

It is not necessary for all materials used within a laboratory to be primary certified reference materials. Such highly characterized materials require significant investment in production and can take years to produce. Their availability is therefore limited and, even where available they are usually too expensive to be used in a laboratory on a routine basis. For the identification and quantification of drugs, the use of well-characterized and defined secondary reference materials is normally fit for purpose.

Although university groups do produce novel chemicals for pharmacology research purposes, these are not available in sufficient quantity to permit conversion into reference materials.

The traditional route for the commercial production of a drug reference material is a lengthy process, typically taking between six and twelve months to complete. Typically, the target compound is either synthesized by the reference material supplier or is acquired from a reputable source in the form of an uncertified chemical. After purchase or production and purification, the bulk material is homogenized and subjected to detailed examination using various analytical techniques to verify its identity and characterize the relevant parameters of the material. Where necessary, further purification steps are used to ensure that this starting bulk material is of sufficient quality to allow its certification as a primary reference material, as defined by ISO Guide 34[8] or similar. ENFSI guidelines,[7] for example, suggest that purity in excess of 98% is desirable. It is critical that an appropriate range of analytical techniques is applied to guarantee that the bulk material is sufficiently well characterized to allow it to become a primary drug reference material. For example, gas chromatographic analysis alone will not identify the presence of insoluble or non-volatile impurities.

Once the bulk material has been characterized, it is dispensed into the units that the end user will require. These end units are typically small amounts of powdered material in a vial or standard concentration solutions, ready for further dilution, or for use in spiking. Further testing for homogeneity between finished units is then conducted, together with stability testing and preparation of a certificate providing the relevant details of the material prepared.

This traditional approach results in an extended timeline for reference material production, which is unhelpful in a rapidly changing field of new psychoactive substances.

This approach describes the traditional approach to the production of a reference material as either a solid material or a

pre-made solution ready for quantitative laboratory use. Under ILAC-G7 : 6 2009,[9] allowance is made for the use of qualitative reference materials, particularly of drug metabolites, that can be produced through the administration of parent compounds to a target species or hepatocyte culture and subsequent detailed characterization of collected matrix samples or hepatocyte digest to confirm the identity of the target metabolites. This approach can only be used for the qualitative identification of metabolites. For a successful quantitative analysis, any substance produced through this biological route would have to be subjected to the rigorous examination detailed earlier for the production of pure reference substances.

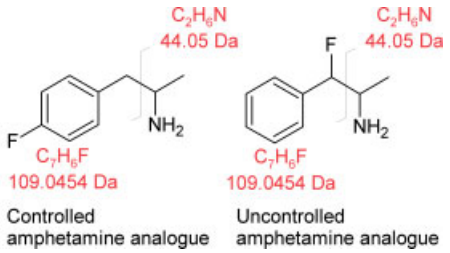


Figure 1. Structures of 4-fluoroamphetamine (controlled) and β-fluoroamphetamine (uncontrolled) showing the expected fragmentation in the EI mass spectrum.

Materials required

The number of new substances reported to the EMCDDA in 2010[5] suggests that forensic drug scientists could be looking for in excess of 40 new reference materials each year. However, this makes the assumption that a laboratory will invest in acquiring materials in order to develop the capability to definitively identify all new psychoactive substances that might be encountered in the future. Unless exceptionally well resourced, forensic drugs laboratories cannot afford this aspiration, except when there is a clear and adequately funded customer requirement for the identification of non-controlled new psychoactive substances.

There are already many obscure compounds covered by drug control legislation around the world. For example, within the legislation used to control drugs within the United Kingdom, Schedule II of the Misuse of Drugs Act 1971 contains the names of hundreds of individual substances which are controlled, together with a number of 'generic controls'. In order to prevent minor modifications to the chemical structure of certain named chemicals to produce a legal version (a designer drug), generic controls define a chemical 'backbone' and a range of modifications of this core structure which will all fall under legal control. Ecstasy (MDMA), for example, is not specifically named in UK legislation, but is covered by a generic control on ring substituted phenethylamine derivatives which was prepared long before Ecstasy became popular. Other drug groups under generic control in the UK include the barbiturates, cannabinoid receptor agonists, cathinones, fentanyls, pethidines, piperazines, steroids, and tryptamines.[10] The number of compounds potentially covered by these generic controls is very large, so that a precautionary approach of obtaining reference materials for all materials that might be encountered is not feasible.

Consequently, a 'chicken and egg' situation develops. Reference material suppliers don't produce a material if there is no perceived demand but laboratories don't seek a reference material until they need it, so that the potential demand isn't appreciated. When the need does arrive, many laboratories cannot formally identify a material due to a lack of an appropriately characterized reference material.

In addition to controlled materials, analysts can require reference standards to enable them to discriminate between closely related materials. One such example is fluoroamphetamine. The main positional isomers of fluoroamphetamine could be expected to be 2, 3 or 4-fluoroamphetamine. All three are controlled in the UK under the generic phenethylamine controls laid out in the Misuse of Drugs Act 1971. Although the fragmentation of these by electron impact (EI) would theoretically produce identical mass spectra, in reality there are slight differences that allow for discrimination.[11]

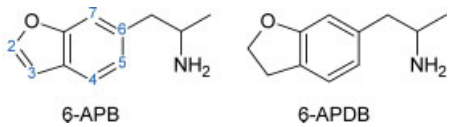


Figure 2. Chemical structures of 6-APB and 6-APDB.

However, β-fluoroamphetamine (Figure 1) is not covered in the Misuse of Drugs Act. From the expected fragmentations of fluoroamphetamine it can be established that β-fluoroamphetamine would theoretically provide a very similar EI mass spectrum with both drugs fragmenting to produce a stable fluoro-tropylium ion (m/e/109). It should also be noted that any β-substituted amphetamine which introduces a new chiral centre into the molecule could exist as either one of two diastereomeric forms, or a mixture of both. While one would expect these to give almost identical MS data, their retention times could differ and hence both diastereoisomers would be required for confirmation.

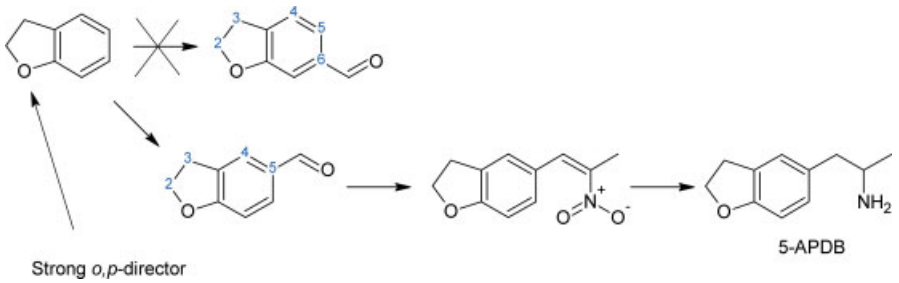
It is clearly desirable to be able to distinguish between uncontrolled and controlled isomers of fluoroamphetamine.

Another example is a product named BenzoFury, which is available via the Internet. This material is closely related to Ecstasy, but with one oxygen atom replaced by a carbon. It is alleged to have similar effects to Ecstasy, but falls outside the UK's legal controls, so that it is a designer legal high. Initially there was some disagreement between suppliers as to whether this product was 6-(2-aminopropyl)benzofuran (6-APB) or 6-(2-aminopropyl)-2,3-dihydrobenzofuran (6-APDB) (Figure 2). Most now agree that the correct designation is 6-APB.

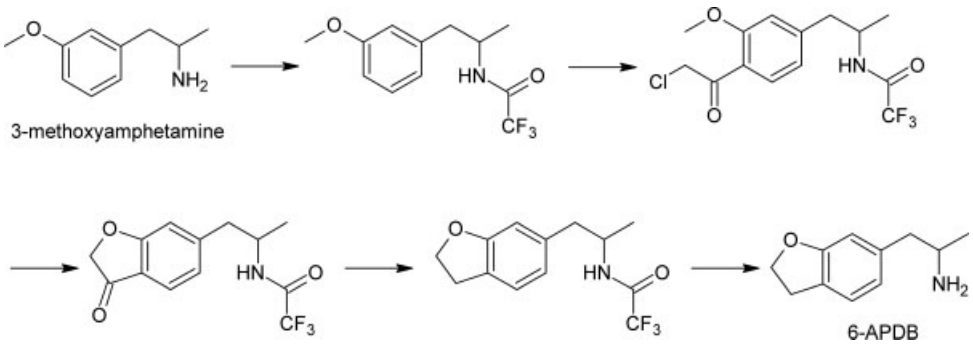
However, from an ease-of-synthesis point of view, neither of these are particularly likely. Nichols first synthesized and evaluated the 5- and 6-isomers of APDB.[12] His research showed that there was little difference in terms of activity between the two positional isomers. However, when one looks at the synthesis of 5-APDB (Synthetic Scheme 1) there are just three synthetic steps from dihydrobenzofuran.

The synthesis of 6-APDB cannot follow the same method. The *ortho, para* directing effects of the oxygen atom prevents substitution in the *meta* position and the intermediate aldehyde needed for 6-APDB is not formed. The procedure used by Nichols uses 3-methoxyamphetamine as a precursor and has five synthetic steps (Synthetic Scheme 2).

In addition, the synthesis of 6-APDB requires the use of the controlled substance 3-methoxyamphetamine as a precursor, providing an additional unnecessary complication. One could conclude that, as the isomers have similar relative activities and the 5-isomers are simpler to make, commercial Benzofury is more



Scheme 1. Synthesis of 5-APDB from dihydrobenzofuran.



Scheme 2. Synthesis of 6-APDB from 3-methoxyamphetamine.

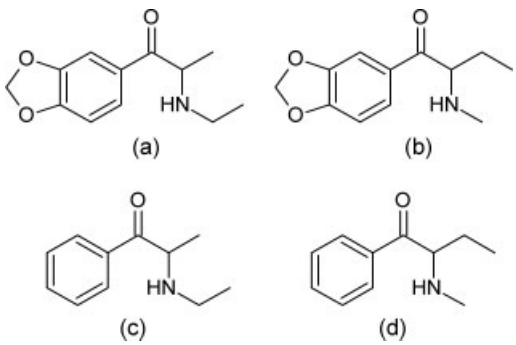


Figure 3. Ethylone (a), butylone (b), ethcathinone (c), and buphedrone (d).

likely to be 5-APB. At the time of writing, the illicit material that found has indeed been identified as 5-APB.

If some or all of these substances become controlled, there will be a requirement to be able to discriminate between 5-APB, 6-APB, 5-APDB, and 6-APDB. Consequently, to address the marketing of a single legal high, four related but different reference materials are likely to be required.

Such drug isomers highlight one of the primary limitations of mass spectrometry as an identification technique. Although the molecular structures of these substances are not identical, their molecular formula is. HR-MS is unable to distinguish between these isomers. It is possible to identify the isomer via its fragmentation using other ionization techniques where fragmentation is observed, but this can only be achieved by comparison of the mass spectrometry data of known materials, hence reference standards are needed. In some cases, such as ethylone (a) and butylone (b), and ethcathinone (c) and buphedrone (d) (Figure 3), the retention time produced by routine gas chromatography (GC) analysis is of limited value due to the closeness of the values. Using authentic standards is it possible to optimize GC separations and identify these drugs by minor differences in the EI-MS data.

In cases such as fluoromethcathinone ([16] and Figure 4), there are no differences in the EI-MS data and the retention times of the 3- and 4- isomers are so similar that using reference standards is the only way to ensure that the correct isomer is identified by GC-MS.

Reference materials for toxicologists

Different analytical disciplines require differing reference materials. Forensic drugs scientists are primarily interested in the identification of parent compounds but forensic and clinical toxicologists face the additional complicating factor of the body's metabolism of the psychoactive materials. The metabolism of each new psychoactive material is likely to be unknown. If a similar compound has already been studied, it may be possible to predict the pattern of metabolites from existing knowledge. Kamata *et al.*[13] illustrated that the metabolism of the cathinone, methylone followed a similar pattern to the corresponding phenethylamine, MDMA (Ecstasy).

While the assumption of similar metabolism may hold for structurally similar compounds, when an entirely new group of materials becomes available, it is unlikely that its metabolism can be predicted with any degree of certainty. Sobolevsky *et al.*[14] reported that the synthetic cannabinoid receptor agonist JWH-018 is extensively metabolized in the body, so that virtually none of the parent compound is eliminated via the urine, and tentatively identified the presence of 13 different metabolites. Their publication, claiming to be the first to identify these metabolites, is dated as becoming available online on 28 April 2010. This is some 16 months after the presence of JWH-018 in smoking mixtures was first reported by THC-Pharm and the University of Freiburg, thereby revealing that synthetic cannabinoid receptor agonists were already in widespread use. As stated earlier, a reference

material typically takes a minimum of six months to produce. If publication of metabolic studies in the peer-reviewed literature is awaited before beginning work on preparing standards, the lead time from the identification of a new psychoactive substance through to availability of reference materials for the metabolites is therefore likely to be around two years. Metabolites of one of the JWH compounds (JWH-018) first became commercially available as reference materials in mid- to late 2010, slightly ahead of this prediction. Their appearance is assisting forensic toxicology and workplace drug testing laboratories to develop analytical protocols to detect the use of such materials. However, there has been a long delay between the appearance of the new drug and laboratories having the ability to screen for use in impairment testing.

For new pharmaceuticals, a delay before standards for metabolites become available is also undesirable. However, metabolic studies will have been conducted by the pharmaceutical developer before the pharmaceutical is released and toxicologists can be reasonably confident that, for a commercial pharmaceutical which can be expected to be in clinical use for many years, reference materials for major metabolites will become available. The situation with legal highs is rather different, as a material may go in and out of fashion before the metabolic pattern is identified, and the necessary reference materials synthesized and certified.

Traditionally, GC-MS in the Electron Impact (EI) full-scan mode has been the primary mass spectrometry tool for the screening of toxicology samples. Although compromising sensitivity, this approach allowed toxicologists to identify the presence of an unknown material in a blood, urine, or other specimens. Once the presence of the unknown material had been identified, the resultant mass spectrum, in conjunction with other ionisation and spectroscopic techniques such as chemical ionization (CI) could allow a skilled operator to make a tentative identification of the unknown material. Alternatively, LC with ultraviolet diode array detection can also provide some structural information.

The shift from full-scan mass spectrometry towards the more sensitive targeted screening of samples through selected ion monitoring (SIM) or multiple reaction monitoring (MRM) runs the risk that the new legal highs will not be detected as, quite simply, the samples are not being examined for the relevant target materials. The absence of reference materials prevents the appropriate validation of methodology and validation of legally (and clinically) defensible identification and quantitative methodology. For example, the traditional approach to workplace drug testing is to use an immunoassay screen followed by GC-MS confirmation in the SIM mode to maximize the trueness of the final analytical result. The parameters required for the correct identification of a target analyte have been defined by many guidelines such as *European Laboratory Guidelines for Legally Defensible Workplace Drug Testing*.[15] These guidelines precisely define acceptable criteria for the legally defensible identification of a target substance, such as amphetamine, Ecstasy or mephedrone. However, if no suitable reference material had existed for mephedrone to identify a retention time and suitable ions for SIM or SRM, the selectivity of these two mass spectrometry techniques is such that it is unlikely that the instrument would produce any response to the presence of mephedrone in the sample.

Commercial considerations

Before producing a reference material, commercial suppliers need to be convinced that production costs can be recovered through the sale of sufficient units. However, the lifetime of a legal high is unpredictable and may only be measured in months before the material falls out of favour and disappears. This potentially short window of laboratory interest, combined with the relatively limited number of potential customers can reduce the commercial attractiveness of the production of a material if the traditional approach to reference material production is employed.

Other factors may inhibit production. Looking at the materials detailed earlier, the synthesis of β-fluoroamphetamine requires the use of fluorinating reagents that are extremely toxic. The hazards involved in synthesis will drive up the cost of any standard. The complexities in the synthesis of 6-APB and related materials have already been mentioned.

A further difficulty arises from the range of materials required by end users. Forensic drugs scientists require access to reference materials for parent substances. Toxicologists would ideally like access to at least one major Phase 1 metabolite and, with the increasing utilization of LC-MS/MS, access to any conjugates of the parent and major metabolite. To facilitate quantification and to address the well known issues of ion suppression/enhancement in LC-MS/MS analysis, access to deuterium labelled forms of the parent drug, metabolites, and conjugates is also desirable. Therefore the appearance of a new legal high potentially generates a requirement for the production and certification of a number of new reference materials. While unlabelled parent compounds may be relatively easy to source and produce, the design and production of suitable deuterium-labelled metabolites and conjugates is a more costly and time-consuming process.

Combining all these factors, there are significant issues facing producers of reference materials to address the market needs generated by new psychoactive substances. The traditional, relatively slow, reactive approach is no longer appropriate and ultimately results in a mismatch between laboratories' needs and reference material suppliers' processes.

Solutions to the market mismatch

Before producing any reference material, a need for the material must be identified. The traditional approach of waiting for end-user laboratories to ask for a new material only exacerbates the delays in the provision of reference materials. Suppliers need to work far more closely with appropriate agencies, such as 'front line' forensic laboratories, as well as systematically examining other sources of information on the internet, including legal high vendors' websites and various Internet drug discussion forums.

Collaboration and co-ordination between suppliers is also desirable, to prevent several suppliers working on the same high profile compound, while failing to address other related targets.

Production of standards

Once a target material has been identified, there are several possible routes to producing a reference material.

Option 1: Synthesis of materials

The synthesis of standards for some new psychoactive substances can be relatively trivial. This can be attributed to the fact that most of the new psychoactive substances are synthesized in 'underground' laboratories which prefer simple and inexpensive synthetic routes using easily obtainable starting materials.

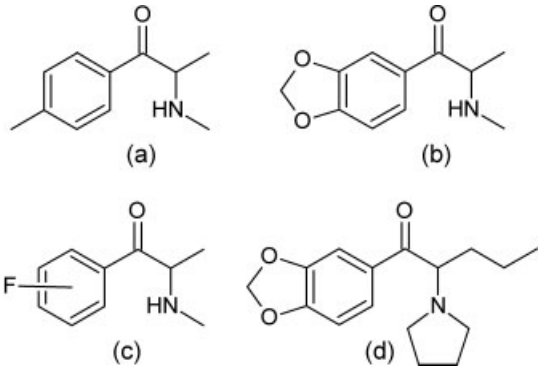
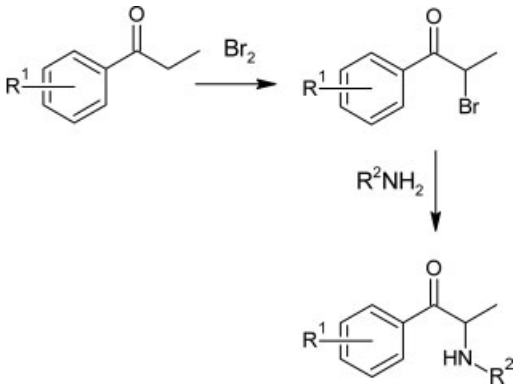
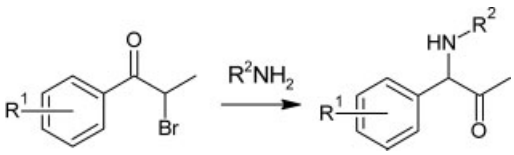


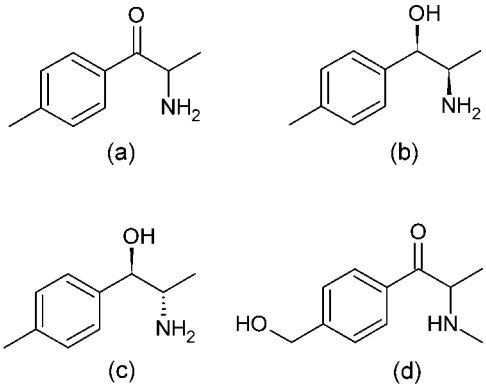
Figure 4. Cathinone structures (mephedrone (a), methylone (b), fluoromethcathinone (c), and methylenedioxypyrovalerone (MDPV) (d).



Scheme 3. General preparation of cathinones.



Scheme 4. Formation of an isocathinone derivative.



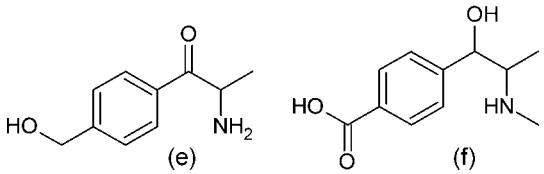


Figure 5. Metabolites of mephedrone.[20].

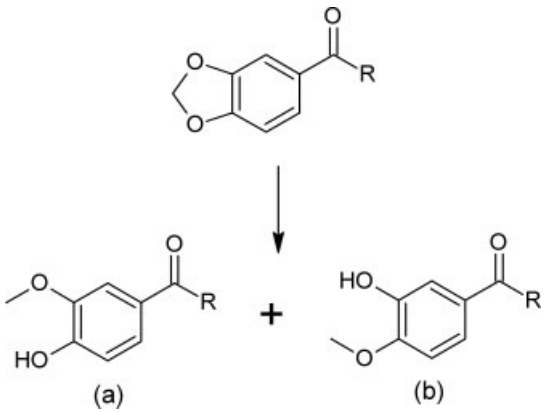


Figure 6. Metabolism of the methylenedioxy moiety of cathinones to metabolites (a) and (b).

Therefore a complex or multistep synthesis is only likely to be attempted by such laboratories for substances with particularly high efficacy and, therefore, potential value.

The synthesis of the currently popular cathinones does present some problems. The appearance and widespread adoption of the cathinones as drugs was primarily a result of their availability from internet vendors. Initially, compounds such as mephedrone and methylone (Figure 4) were often sold by name. Vendors then began to introduce new cathinones such as fluoromethcathinone[16] and methylenedioxypyrovalerone (MDPV).[17]

Generic control of the cathinones was introduced in the UK in April 2010, with a subsequent adjustment to the controls to address naphyrone (naphthylpyrovalerone) which was not covered by the initial controls and had begun to appear as a 'designer cathinone'. Although these materials are now controlled in many jurisdictions, they are still encountered as undeclared ingredients of supposedly legal-high products or as materials being knowingly sold as controlled drugs.

N-substituted cathinone synthesis is relatively trivial from propiophenones. One can prepare most cathinones in two synthetic steps (Synthetic Scheme 3).[16]

However, with certain cathinones, care has to be taken to avoid an isomerisation reaction occurring during the synthesis (Synthetic Scheme 4).[18]

Perhaps one reason why cathinone itself has rarely been encountered as a drug of abuse is the difficulty in synthesizing primary beta-keto amines. Dimerization occurs in basic media and aromatisation is also observed.[19] We also observed an isomerization similar to that for cathinone in the N-substituted cathinones.

Mephedrone was the most popular of the cathinones and, even after legal controls were introduced, it appears to still be in use, albeit at a reduced level. Reference standards for mephedrone are commercially available. However, there is a pressing need for reference materials for the metabolites of mephedrone to assist toxicologists.

Six metabolites (a-f) have been identified in the urine of rats and are illustrated in Figure 5.[20]

The synthesis of these compounds introduces several issues. Thus 4-methylcathinone (a) suffers the issues associated with cathinone synthesis.[18,19] However we found that these issues

Figure 7. Orginal packaging and presentation of MDAI.

can be avoided if the freebase is not formed at any point in the reaction.

The major synthetic issues arise from the requirements for the 4-hydroxymethyl (d,e) or 4-carboxylic acid metabolites (f), as these are unfavoured synthetically. A study and synthesis of the metabolites of pyrovalerone[21] showed that the carboxylic acid metabolite would require five synthetic steps and the hydroxymethyl metabolite would require seven synthetic steps from commercially available materials.

Equally difficult is the synthesis of the metabolites of the methylenedioxycathinones, such as methylone, ethylone and butylone.[22] In humans, there are two metabolites of the methylenedioxy moiety:

One of the metabolites, (metabolite (a), Figure 6) has a straightforward synthesis; however, the other presents a synthetic challenge. There are therefore significant technical obstacles to the provision of reference materials for metabolites of these novel compounds.

Option 2: Grey-market sourcing

One way to short circuit the production process is to obtain materials directly from Internet retailers and to then convert them into reference materials. However, obtaining materials from unregulated sources has its problems.

The MDAI story

MDAI (methylenedioxyaminoindane) is one of the materials being offered by Internet suppliers of 'research chemicals', sometimes under the name of 'Sparkle'. It is structurally related to methylenedioxyamphetamine (MDA). Material was purchased from an Internet supplier and was received within two weeks of placing the order with a stated purity of 97%+. It arrived in a ziplock plastic bag, shown in Figure 7.

Initial examination by GC-MS gave a single peak, suggesting high purity. However, when the material was also examined by NMR and total carbon nitrogen and oxygen analysis, problems were identified. The initial NMR spectrum was poor (Figure 8) and the CNO analysis returned values of C 35.17%, H 5.86%, N 2.87% against expected values of C 67.78, H 6.26 and N 7.90.

The material was purified through recrystalization of the hydrochloride salt and a white crystalline powder was obtained (Figure 9). This represented only 25% of the original material.

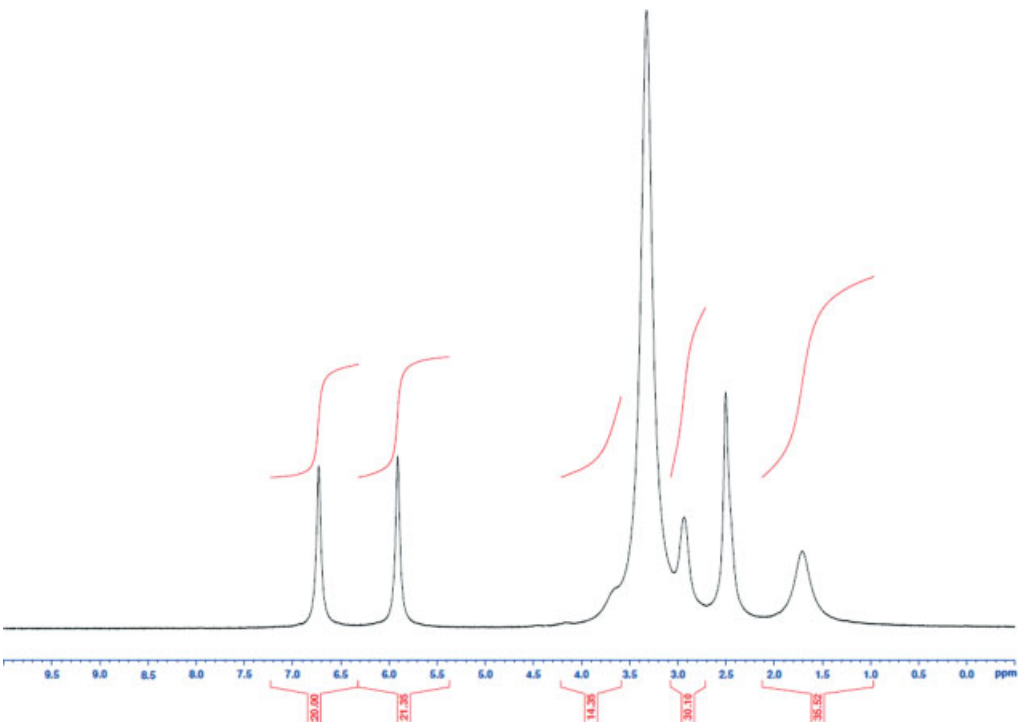


Figure 8. NMR spectrum of purchased MDAI.

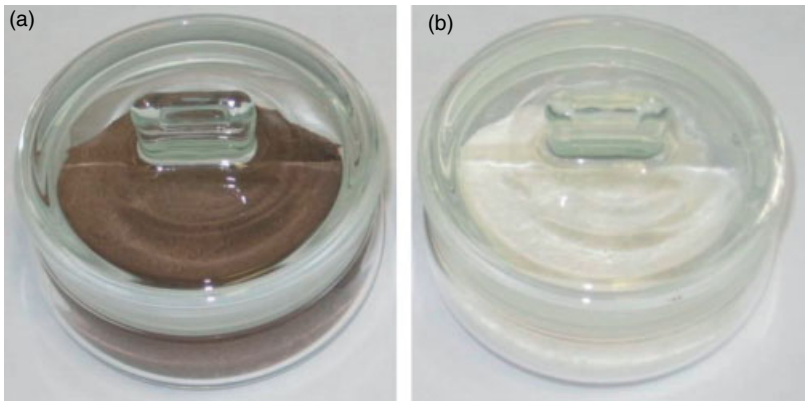


Figure 9. Comparison of original purchased MDAI (a) and final purified material (b).

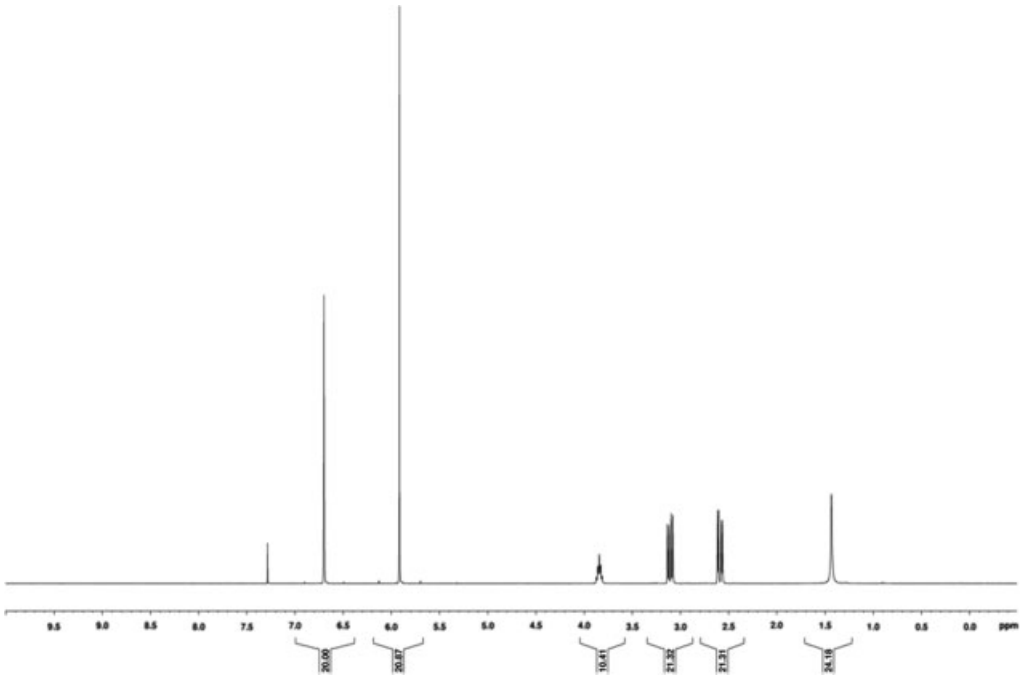


Figure 10. NMR spectrum of final MDAI reference material.

This final material gave a satisfactory NMR Spectrum (Figure 10), mass spectrum (Figure 11), a CNO analysis that was in close agreement to the theoretical value (C 67.65%, H 6.24%, N 7.76%) and a certified purity of 99.7%.

This material was then subjected to further analysis before conversion into an appropriately certified reference material.

Option 3: Pharmaceutical Impurities

One untapped source of reference materials is products listed in catalogues of organizations producing pharmaceutical impurities, intended for use in monitoring the quality of pharmaceutical products. While most of these materials are of little interest to forensic toxicologists, some materials being sold as pharmaceutical impurities are coincidentally also either drugs in their own rights or metabolites of drugs. Although not a new psychoactive substance, one example is nordothiepin, the desmethyl metabolite of dothiepin (Dosulepin INN). This material has traditionally been difficult for forensic toxicologists to obtain, but it is readily available as a pharmaceutical impurity reference material. Such materials have the benefit of already carrying a significant degree of characterisation and certification.

A more responsive approach

In order to respond to laboratories' urgent needs for reference materials for novel psychoactive compounds, there needs to be a change in the approach to production of reference materials. Producers need to be more proactive in identifying the materials that are likely to be required, and to search more diligently for existing sources of raw materials. Where custom synthesis will definitely be required, such as metabolites and deuterated forms, a mechanism for 'pump-priming' at national or international level will be desirable to encourage and support commercial reference material suppliers to take on the commercial risk of producing difficult materials with an uncertain level of demand. Without these changes, laboratories will continue to struggle to find sources of reference materials for novel compounds.

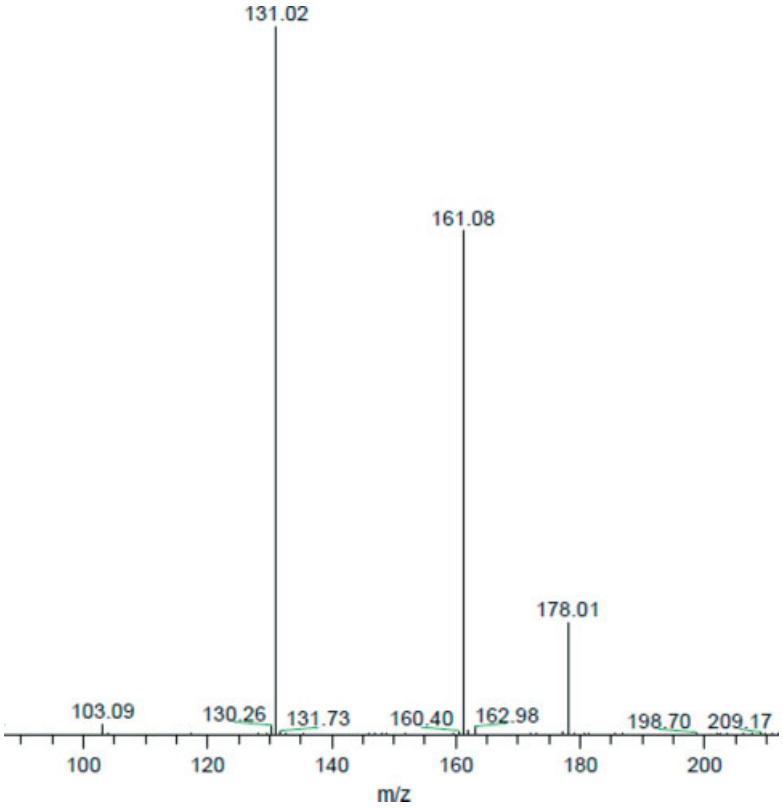


Figure 11. Mass spectrum of purified MDAI.

Conclusions

The challenge posed to laboratories, reference material producers, and legislative bodies by these new and emerging psychoactive substances shows no signs of abating. No single approach to reference material production is likely to fully satisfy the requirements of all forensic drugs and toxicology laboratories. Reference material suppliers will have to work in conjunction with end users to prioritize the manufacture and supply of high quality, appropriately certified drug and metabolite reference materials. This is likely to require national or international authorities to commission the production of certain key, commercially unviable materials.

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