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REVIEW ARTICLE

Toxicology in clinical laboratories: challenging times

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ABSTRACT

In recent years, there have been a number of significant developments in toxicology within clinical laboratories, both with the available instrumentation and in the range of compounds abused by the drug using communities. There have also been developments in the regulation of forensic science in the UK which may in time impact clinical toxicology. This review is designed to provide an update of these changes within toxicology to the more general pathology laboratory audience. For detailed information in specific areas, the reader is referred to the references in the text. In the preparation of this review, the references held by the author as part of his practice of an analytical toxicologist in the NHS were supplemented by a full literature search in Medline and Embase and a review of pertinent UK and European Governments and regulatory agency websites for recent documentation.

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Introduction

The scope of toxicology services within the NHS varies widely from trust to trust, but should follow the guidelines [1] published by the ACB/NPIS in 2014. This paper lists analytes in two groups. The first should be available in a District General Hospital laboratory. They give recommendations for the acceptable turnaround time for a number of clinically warranted analytes, most of which are easily achievable. There are some possible exceptions, in particular paraquat, which is now seldom seen and therefore the need of having an assay on standby is debateable. Coverage of the assays in the second group is variable and will rely on a regional or supra-regional laboratory to plug the gaps on what is available locally. In the same way, drug screening varies widely from trust to trust. Many addiction services prefer to use near patient testing, often without any laboratory oversight, as the mental health trust may have no arrangement with the local acute trust and its POCT coordinator.

Drug misuse: the scale of the problem

Drug misuse is a serious problem across Europe. Data in the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) 2016 report [2] estimate over a million illicit drug seizures across the continent.

The EMCDDA report states the most widely abused drug to be cannabis with 83.2 million or 24.8% of adults (aged 15–64) using cannabis in their lifetime and 22.1 million or 6.6% of adults (aged 15–64) using cannabis in the last year. Cocaine was the second most abused drug with a lifetime use of 17.1 million or 5.1% of adults. This was followed by ecstasy and the amphetamines with lifetime use in adults of 13.0 and 12.0 million, respectively. Opioids are less common with 1.3 million problem adult users but responsible for about 40% of the drug treatment requests. This latter group (which includes heroin) is found in 82% of fatal overdoses.

They also report a lifetime use of 8.0% of the novel psychoactive substances (NPS) in younger adults aged 15–24. This latter group of drugs is a cause for concern as there has been an explosion in the number of drugs seized across Europe in the last few years. The total number of new drugs seized rose from 29 in 2009 to a peak of 101 in 2014 with (hopefully) a plateaux being reached as the number in 2015 was 98. In total this gives a total of 467 new drugs available within Europe in 6 years. Of these new drugs the most numerous are the synthetic cannabinoids (SC) with 157 compounds and the cathinones with 93 [2]. These drugs will be considered in more detail later in the review.

The pattern of drug use across Europe is not consistent with different areas favouring different drugs: for example, cocaine and ecstasy use is relatively high in the UK, whereas other countries have considerably higher rates of opioid use [2]. Similar variations are likely to occur in drug use across the UK. Informal conversations between NHS toxicology services suggest there are regional variations in drug use which may simply reflect availability from local dealers. Data from the 2014/15

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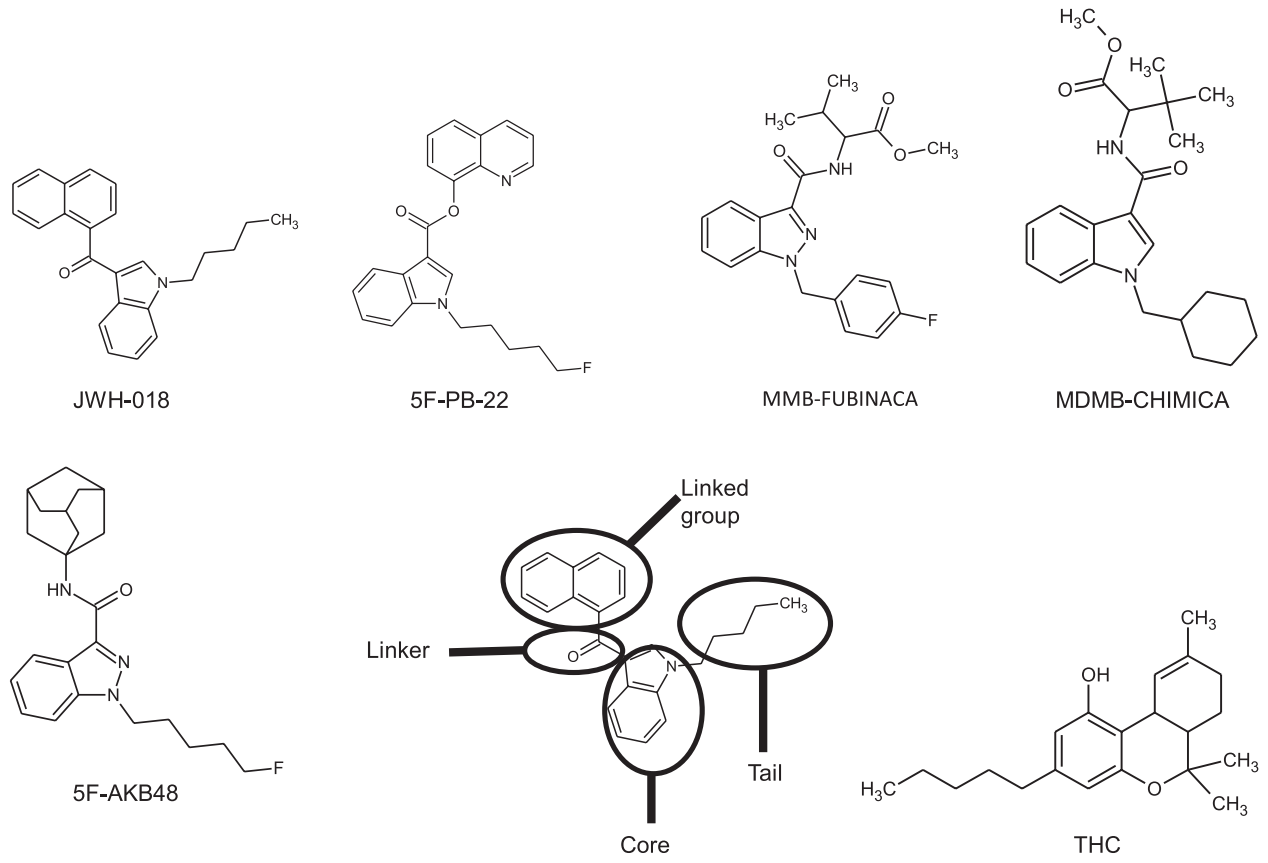

Figure 1. Generic structure of the synthetic cannabinoids [14], with a few examples of reported compounds with THC for comparison.

Crime Survey for England and Wales indicate that drug use is more common in the urban areas and is associated with increased visits to clubs, bars and nightclubs. The survey reports decreasing levels of drug abuse overall from the late 1990s, but does not give any detail on regional variation [3].

In addition to illicit drugs, there is a growing problem with the diversion of prescribed drugs for illicit purposes. Recent data from the UK Advisory Council on the Misuse of Drugs [4] report opioids and benzodiazepines to be the most diverted drugs with increasing amounts of gabapentin and pregabalin. This report suggests some regional variation with dihydrocodeine possibly more popular in Scotland and benzodiazepine use particularly high in Northern Ireland [4]. An acknowledgement of this problem is reflected in the addition of a question about misuse of prescription only medication in the most recent Crime Survey for England and Wales [3]. Interestingly, only a quarter of people illicitly using these drugs took another drug during the year compared to 83% of those using NPS [3].

In summary, the main drugs of abuse have remained constant over the last few years. However, there have been important developments with the dramatic increase in the range of NPS available, largely over the Internet, and with diversion of prescribed drugs for illicit use.

Problems in detection

The EMCDDA data [2] suggest there is little change in the most common drugs (cannabis, cocaine, amphetamine, ecstasy and the opioids) and these can be reliably detected in a range of matrices (urine, oral fluid and hair) for monitoring purposes. There are however issues with each of these matrices which will be considered in a later section.

Detection of the NPS or diversion of prescription drugs is problematical. Anecdotal evidence suggests one of the reasons these drugs are taken is to avoid detection by the current technology. Therefore, clear and regular communication should take place between the toxicology laboratory and the service users, who are often in different NHS organisations in different geographical locations. Clinically, there are potentially serious implications of NPS use as the adverse effects will be unknown and can be potentially serious [5, for review]. This has led to ongoing (the IONA study) research into the acute medical toxicity associated with these compounds: these include a number of serious adverse effects including metabolic and respiratory acidosis and reduced consciousness [6].

The following section considers a few of the classes of NPS and prescription drugs in more detail and discusses some of the issues in the detection of these drugs in clinical samples.

Cannabis and synthetic cannabinoids

Cannabis is a widely used drug, with reports of use going back into pre-history. It contains a number of active compounds; the main ingredient for its desired effects

is Δ9-tetrahydrocannabinol (THC) which is a weak agonist at the human cannabinoid CB1 and CB2 receptors. Cannabis contains a number of other substances including cannabidiol which modulates the effects of THC on the receptors [7, for review]. This modulatory effect may be decreasing over time as the potency of cannabis increases, for example the THC content in herbal cannabis has risen from 2% in 1995, to 7% in 2009 and to 13% in 2015/16 [8] with decreases in cannabidiol. There are data to suggest that experienced cannabis users are able to estimate the THC (but not cannabidiol) content of the cannabis used, but that this discrimination is not observed in recreational cannabis users [9], so increasing the chance of adverse effects in naive users. THC may remain detectable in blood for several days after last use [10], with data from France [11] and the UK [10] suggesting an increased risk of responsibility for a fatal road traffic accident, though this was considerably lower than the risk associated with alcohol use.

Finally, a recent study has highlighted the difficulty in obtaining a positive urine screen for cannabis from passive smoking (which is a common excuse for explaining a positive drug screen). A few positive screens were obtained in volunteers seated in a sealed room with a group of people smoking large amounts of cannabis, but these were close to the time of exposure and only occurred when the exposure was obvious to the volunteer [12].

The synthetic cannabinoids (SC) bind to the CB1 and/or the CB2 receptors with a much higher affinity than THC and consequently exert much more potent effects. They tend to be sold as 'herbal' products and consist of plant material with the drugs sprayed on to them [13,14]. As a group they are commonly referred to as 'Spice'. The chemical structures show some similarities to each other and to THC with a non-polar carbon tail coupled to core, which is in turn coupled to a further ring via a small linking group [14] Figure 1. It can be seen that these are complex molecules: original compounds such as JWH-018 were developed for research use in legitimate laboratories, whereas the more recent derivatives have been developed and produced in clandestine laboratories. It is thought that the majority of these laboratories are located in China and generally produce drugs of high purity [14].

A recent report from an 'outbreak' of SC use in New York was described in the popular press as a 'zombie' outbreak due to the behaviour of the 33 affected persons. The agent responsible (MMB-FUBINACA or methyl 2-(1-(4-fluorobenzyl)-1*H*indazole-3-carboxamido)-3-methylbutanoate) was detected in samples from 8 of the patients who were treated in hospital and in a product AK-47 24 Karat Gold which was marketed as an herbal incense product [15]. There have been a number of lurid reports of their use in the UK popular press, with concerns of heavy use in prisons [16–18] and exposed in a recent (February 2017) Panorama programme on the BBC [19].

A number of other adverse effects have been noted with use of these compounds including sudden or slow death due to 5F-PB-22 use [20], whilst Castenato et al. [13] in a review report nausea and vomiting, shortness of breath or depressed breathing, hypertension, tachycardia, chest pain, muscle twitches, acute renal failure, anxiety, agitation, psychosis, suicidal ideation and cognitive impairment. It should be borne in mind that the patients suffering from adverse reactions may be a minority of the users: similar problems are noted with therapeutic drugs, for example tramadol a widely used opioid has an incidence of between 1 in 1000 and 1 in 10,000 for a number of side effects including respiratory depression, convulsions, hallucinations and blurred vision [21].

Interestingly, a study examining reasons for taking the SCs in a group of persons undergoing treatment for substance use reported curiosity (91%), feeling relaxed or getting high (89%), relaxation (71%) and getting high without being detected in a drug screen (71%). As a group these persons had higher rates of other substance use and higher scores in depression and psychiatric distress measures [22], but it is not clear whether the SC use caused the symptoms, or the symptoms promoted SC use.

Detection of these drugs is challenging as they are often extensively metabolised with little if any of the parent compound detected in urine. Hydroxylation was the most common metabolic route for 7 SCs of the JWH type (aminoalkylindoles) with some of the alkyl chains being carboxylated [23]. 5F-PB-22 mentioned above produced 22 metabolites in a human tissue cell culture, some of which were identical to the 20 metabolites of the close analogue PB-22 [24].

Benzodiazepines

A number of benzodiazepine derivatives have begun to make an appearance [25]. They are considered to be synthesised following the details in patent records for the individual drugs or are therapeutic drugs in other countries [26]. Phenazepam, a therapeutic drug in Russia, has been implicated in a number of adverse outcomes in the UK [27], where it may be passed off as diazepam. It is likely to be detected by benzodiazepine immunoassay screens but like the SCs above it is mainly present in the urine as a metabolite. Chrichton et al. [28] gave details of 29 fatalities in the UK involving the drug with the concentration in urine ranging from 0.007 to 0.049 mg/L and that of 3-hydroxyphenazepam ranging from 0.017 to 0.264 mg/L.

Opiates

The traditional opiate of abuse (heroin) continues to be popular, but there are a number of synthetic opioid drugs that are being abused. These include tramadol

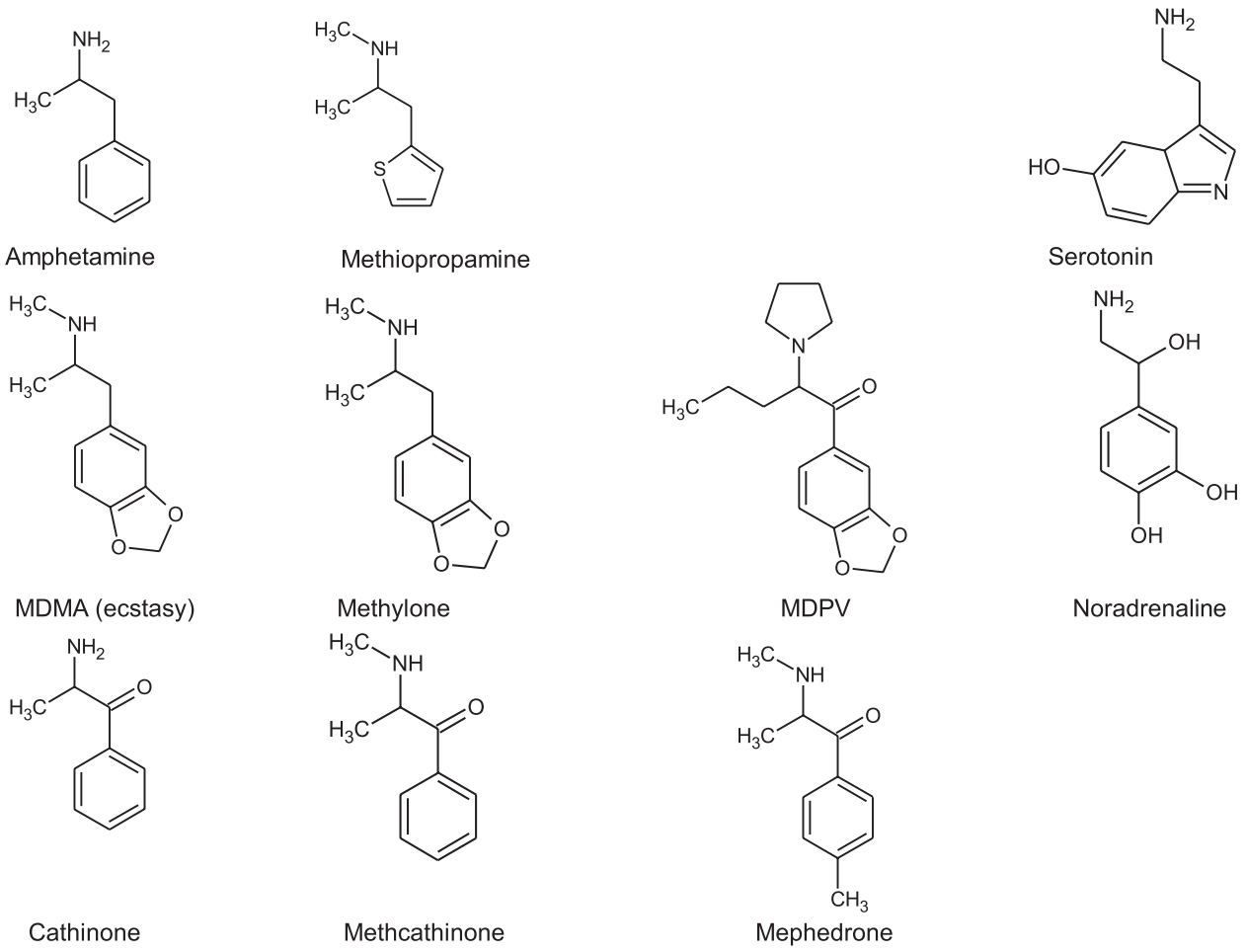

Figure 2. Structures of amphetamine, ecstasy and cathinone and a number of derivatives, with the structures of serotonin and noradrenaline for comparison.

and its active metabolite [29,30], fentanyl and its various illicit derivatives [31,32], as well as the opiate substitution medication methadone and buprenorphine.

Cathinones

This group of compounds commonly referred to as 'Bath Salts' or 'Plant Food' is based on cathinone which is a naturally occurring drug found in Khat. This plant is indigenous to East Africa and the Arabian Peninsula, and chewing of the leaves for euphoric effects has taken place for centuries in countries such as Ethiopia, Somalia, Yemen and Kenya [33]. The synthetic derivatives became widely used in the mid-2000s, which was probably linked to the low quality of the ecstasy and cocaine available at the time [34]. A large number of compounds have appeared including mephedrone, methcathinone, MDPV (methylenedioxypyrovalerone), methiopropamine and methylone (Figure 2): these show similarities to amphetamine, ecstasy and cathinone and to the neurotransmitters serotonin and noradrenaline. As a group, these compounds have been linked to the development of a number of adverse effects including delusions, hallucinations and potentially dangerous behaviour [33].

Gabapentin and pregabalin

Both these drugs are being increasingly abused in the UK [4]. Worldwide misuse rates of gabapentin of 1.1% in the general population and 22% in drug misuse clinics and withdrawal symptoms are reported on stopping the drug [35]. Pregabalin was noted to have a frequent side effect of euphoria during development and there are reports of pregabalin tablets being crushed and swallowed or injected to maximise the effect [36 for review].

Alcohol

Whilst there is considerable attention made by the Government and Media to illicit drug use, the drug causing the biggest financial drain on the health service is alcohol. In 2013/14, it was estimated in England there were just over 1 million hospital admissions due to alcohol-related disease, which was just under twice that in 2003/4 [37].

Laboratory detection of alcohol misuse has traditionally relied on biomarkers such as the mean corpuscular volume and gamma glutamyl transferase which have relatively poor specificity and sensitivity [38, for example]. Measurement of blood or urine alcohol can detect recent

Table 1. Window of detection and cut-off values: for example, drugs in urine and oral fluid.

Drug	Urine window of detection	Workplace confirmatory cut off (µg/L) [65]	Oral fluid window of detection	Workplace confirmatory cut off (µg/L) [66]	Comments
Amphetamine	2–4 days	200	1–2 days	30	Half-life decreased if urine acidic
Methamphetamine	3–5 days	200	1 day	30	
Ecstasy	1–3 days	200	1 day	30	
Diazepam	2–40 days or 10 days	100	0–7 days	10	
Flunitrazepam	5 days	100	<6 h	10	
Cocaine	2–4 days (7 days as metabolite)	100	0.5–1 day	8	
Cannabis	15–30 days, 3 days for single use	15	1–2 days	2	
Buprenorphine	7 days	2		++	Measure metabolites to avoid issues with urine spiking
Methadone	1–4 days	75+ as EDDP		20	Measure metabolite (EDDP) to avoid issues with urine spiking
Morphine	2–4 days	300	0.5–1 day	2	
6-acetyl morphine (6-MAM)	1.5 days	10	<0.5 days	2	
Acetylcodeine	0.5 days			2	
Fentanyl	3 days	++		++	
Pregabalin	3–5 days	++		++	

Notes: The windows of detection are approximate as they depend on the dose, inter-individual differences in metabolism and excretion and the length of time the drug has been used. These are based on the recommendations from a number of sources [65,66,93–95], but as will be noted from a study of these references, there are considerable differences between each report. As analytical methods become more sensitive and cut-offs are lowered, these will increase. ++under discussion.

use, but is limited by the rapid clearance of alcohol from the body (on average around 190 mg/L per hour). A number of new biomarkers are available, some of which have been used in a forensic or research setting for many years. Of these, two are entering clinical use: Carbohydrate deficient transferrin (CDT) is able to detect marked alcohol use over a period of time. An intake of 6–10 units per day for a week can be detected and CDT will remain increased for 9–15 days after stopping intake [39,40], but it will not detect irregular binge drinking. Ethyl glucuronide and sulfate, which can be measured in serum and urine, are minor metabolites of ethanol but have a longer window of detection of around three days. However, they suffer from poor specificity at low levels with false positives due exposure to alcohol from a number of sources including use of perfume and alcohol-based hand washes [41,42]. In addition, ethyl glucuronide may be produced by bacteria in UTI [43]. Therefore, a cut-off is generally used to distinguish alcohol intake from innocent sources [41]. A further marker which appears to have promise is phosphatidylethanol (PEth) that may show a relationship between alcohol use and the amount of PeTH use [44,45], but use may be compromised by its relative instability in blood [46].

Matrix choices

A number of matrices are available for drugs of abuse testing. Each of these offers advantages and disadvantages in the clinical setting. In addition to urine, oral fluid and hair (which are discussed below), blood [10,47], breath [48], nails [49] and sweat [50] may be used as alternative matrices.

Urine

Urine is a readily available fluid and can easily be collected in large volumes. Drugs and/or their metabolites are often present in relatively high concentrations and may be detectable for several days after last exposure. Urine is a relatively clean fluid and can be used for screening with little or no sample pre-treatment. Observing the collection is not practical in the majority of cases so sample swapping or adulteration is a problem. A number of substances such as bleach, salt, lemon juice and commercial adulterants such as Klear, Whizzies, Urine Luck and Stealth can be added to the urine to mask the presence of drugs, particularly when using immunoassays [51]. There are a number of tests [52] to detect these adulterants (including the simple smelling of the sample), the most useful of all is measurement of urine creatinine, where continually low creatinine values (<1.8 mmol/L) may be due to dilution or excessive drinking [53].

Oral fluid

This offers the advantage that collection is easily observed without invading patient privacy and can be undertaken without the need of a bathroom. However, adulteration is possible as there are saliva cleaning mouthwashes available and the sample may be contaminated by recent food and drink [51].Drugs may be adsorbed into the device

(e.g. chewing gum) used to stimulate fluid flow and the stimulation itself can alter the relative levels of drugs in the fluid compared to resting flow. There are also data to suggest that drugs may be adsorbed by the collection device [54]. In general, the detection window is shorter than urine [55] and the pattern of the drug and metabolites may be different from urine [56]. Whilst NPS tend to be detected in urine (or post-mortem fluids) in the first instance, there are published examples: for example, a number of synthetic cathinones (including methedrone and mephedrone) can be reliably detected as the parent drug [57]. Finally, it is possible that oral fluid testing may become more common outside toxicology as it can be used to measure a range of hormones and metabolites as well as for therapeutic drug monitoring [58].

Hair

Hair testing offers a number of advantages over urine and oral fluid testing in that exposure to a drug can be examined over time. The technique is based on the premise that hair grows on average 1 cm per month and that drugs and their metabolites circulating in the blood are incorporated into the keratin structure of the hair as it is built up in the follicle. There are a number of issues with testing as the incorporation may be affected by natural hair colour as well as bleaching and dyeing [59, for review]. Analysis is complex; the hair must be carefully washed to remove any drug on the outside of the hair without extracting any from the hair strand. The extraction procedure to remove the drug from the hair material must not destroy any of the drugs and there are difficulties in preparing standards/QC samples that accurately reflect the binding of drugs incorporated into hair during growth. The Society of Hair Testing (SoHT) provides recommendations for testing and cut-offs [60].

Legislation

The supply and possession of drugs is controlled in the UK by the Misuse of Drugs Act (1971) [61]. The drugs legislation was amended and strengthened by a considerable number of Acts of Parliament, culminating in the Psychoactive Substances Act (2016) [62] which aimed to restrict the production, sale and supply of NPS. Whilst this has had an effect on the number of UK-based websites offering drugs for sale (though the author has noted one site is now based in India), anecdotally users were stockpiling NPS before the act came into place and may be returning to the more traditional drugs or NPS sourced by local dealers. Prior to the latter Act of Parliament, changes in the drugs being offered for sale (particularly via the Internet) where apparent when a drug or group of drugs became scheduled [63]. This arguably decreased the chances of detecting the NPS as laboratories would have to start looking for a new set of drugs which were not available via legitimate sources.

A further legal requirement is to have chain of custody procedures in place: this enables collection of samples in a controlled fashion, with a clear record of everything that has happened from when it left the client/patient to its arrival in the laboratory and the testing processes once there [64]. Within a clinical laboratory, it may be difficult to ensure all staff stick to these requirements as such samples are likely to be relatively infrequent.

Instrumentation advances

Traditionally, drug screening was performed by chemical testing and thin layer chromatography. With the advent gas chromatography, particularly when coupled to mass spectrometry and of automated immunoassay-based methods, the previous methods began to fall out of favour, though they may still be of use in specific circumstances.

The second change began with the advent of liquid chromatography – tandem mass spectrometry (LC-MS/MS) which enabled screening for specific drugs with a high degree of specificity, with much reduced sample pre-treatment processes.

The third change is in progress with the advent of accurate mass, high resolution mass spectrometry to the clinical laboratory.

General issues

All the assays for toxicology will employ cut-offs. This is the concentration of the drug below which the result will be reported as negative, even though there may be traces of the drug present. Historically, this was required as the methods lacked the necessary specificity to accurately identify and quantify low levels of the drugs. Cut-offs for employment screening are well defined for the traditional drugs of abuse by the European Workplace Drug Testing Society in urine, oral fluid and hair [65–67], but non-European countries may use US-based cut-offs [68], which are different for a few of the drugs. Considerable variation exists in the cut-offs used for clinical work, where there are no clear guidelines: for example, laboratories in the NE of England were using three different cut-offs for urine amphetamine screening prior to 2016 (see Table 1).

In particular, the point of testing devices (instant POTs) may not clearly state the cut-offs used in their method and the clinical users of the system may be unaware of their existence. The clients of substance misuse services however will be aware of them as evidenced by the need to easily detect dilute urine samples.

Care must be taken to ensure the method is suitable for its purpose; a drug screen with a cut-off suitable for monitoring drug use in a recovery services clinic will not be suitable for detection of low levels of drugs in a neonate born to a mother with suspected drug use. For general clinical purposes, it is usual to employ a general

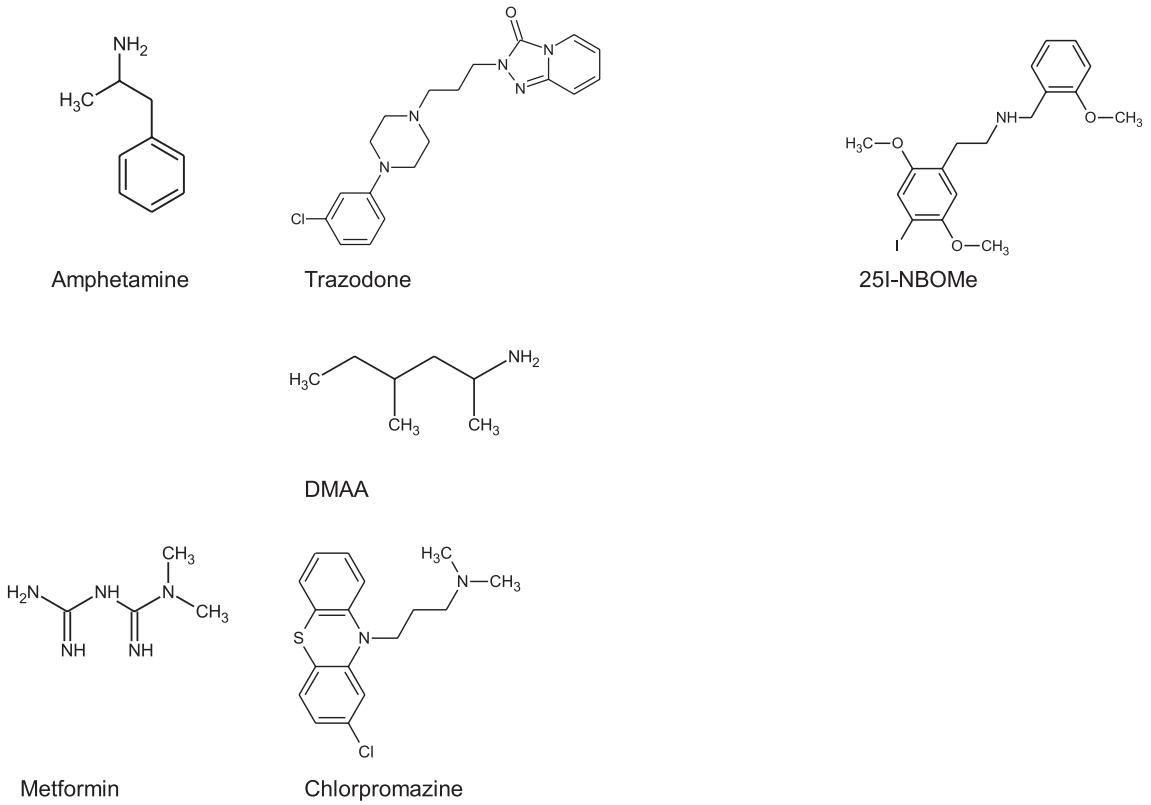

Figure 3. Structures of several compounds reported to give false positive amphetamine immunoassay screens [70] and a drug 25I-NBOMe (2-(4-Iodo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine) used for its psychedelic effects which was not detected by immunoassay [72].

screening method (often immunoassay based) followed by confirmation of any positive screen with a specific chromatography-based method.

Immunoassay screens

A number of laboratory-based immunoassay screens are in wide use in the UK and internationally and the automated assay systems offer the advantage of rapid throughput with relatively little operator involvement. In addition, there are a large number of instant POTs which can test for more than one class of drug at a time. These devices have the advantage of being portable and can be used in the clinic to confront a patient on their drug use during consultation.

The immunoassays as a group use a broad spectrum antibody directed towards a group of compounds: for example, an immunoassay screen for opiates may be able to detect morphine and codeine and dihydrocodeine, but not able to detect low levels of oxycodone. False positives can be common: for example, a study involving over 8000 urine samples reported an overall false positive rate (compared to MS-based methods) of 14.6%, with unacceptably high rates for ecstasy and phencyclidine (of 100%) [69]. A review of false positive data showed differences in false positive rate between the differing immunoassay systems [70] and highlighted particular issues with amphetamine methods – but this is not the case in all studies [71]. Amphetamine is a relatively simple molecule which has structural similarities with a large number of compounds and there are reports of false positives with (for example) metformin, trazodone, dimethylethylamine (DMAA – a widely available energy supplement), whilst some of the false positives are obvious when comparing the structures, others are less so (Figure 3). On the other hand, the detection rate for the amphetamine/ecstasy-type NPS is not universal and depends on the method used [72].

Other immunoassay screens also suffer from false positive and negative issues with some buprenorphine methods suffering from a high false positive rate [73] and if targeted to the parent compound will not identify spiked samples [74]. It may also lead to false negatives as not all patients prescribed buprenorphine have detectable levels of the drug in their urine [75] but will have detectable levels of norbuprenorphine and the glucuronide metabolites.

False positive benzodiazepine screens have been observed due to nefopam and its metabolites [76] and sertraline [77].

In summary, the immunoassay screens offer the advantage of quick and easy methodology, which in the laboratory can be incorporated into an automated system. False positive and negative results can cause confusion and lead to incorrect treatment decisions if the limitations of the technique are not known.

GC-MS

GC-MS has a number of advantages: firstly, the results of the analysis on one manufacturer's equipment can be applied to other manufacturer's equipment which has led to the development of large libraries of compounds [78,79]. Thus, an unknown peak can be tentatively identified by reference to one of these databases; obviously, this would need confirmation by the analysis of the appropriate standard preparation.

There are a number of disadvantages to GC-MS. The common form of GC-MS uses electron ionisation (EI) which fragments all ions on arrival at the mass spectrometer and thus may not include the parent ion. Thus, coelution of compounds may produce a confusing picture with a number of fragment ions, but no parent ion to guide the interpretation. Chemical ionisation is a form of EI where the ionisation is transferred from a reagent gas (for example, methane and/or ammonia) and provides a softer ionisation with more likelihood of observing the parent ion. This latter form of ionisation is more useful with GC-MS/MS where further identification is possible from the fragmentation patterns [80].

A problem with all GC analyses is the compound of interest has to be sufficiently volatile and thermally stable to pass through the GC at temperatures up to 250 C. This can limit the range of drugs and metabolites that can be identified [81]. To increase the volatility, compounds may be derivatised, by attaching large organic groups by interaction between specific chemical groups. For example, gamma hydroxybutyrate (GHB), the 'date rape' drug, can be analysed by GC-MS after reaction with *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) which converts the small hydrophilic molecule with few specific MS fragments into a large volatile molecule with specific MS fragments [82]. In a similar fashion, acetic anhydride can be used to derivatise amphetamine-related compounds [83].

A further development in toxicology has been the introduction of an enzymatic method for the analysis of ethylene glycol. This method has been shown to give comparable results with GC-MS and enables rapid screening for the compound in clinical samples [84]. This decreases the time required to perform the analysis to around half an hour [1], and allows screening for the compound 24/7 without the need to use specialist equipment.

A major issue with GC use in the clinical laboratory is the increasing lack of exposure of the technique to all staff and its perceived complexity. Therefore, it is increasingly becoming the preserve of small numbers of staff in specialist centres, which is likely in time give rise to a lack of suitably trained staff. Whilst the advances in liquid chromatography coupled to mass spectrometry (discussed below) may reduce the need for the technique, there will still be a role for GC-MS in the analysis of small molecules that either do not ionise well or those that are too small to produce unique fragments.

Advantages and disadvantages of LC-MS/MS

There are numerous references to the theory of LC-MS/MS in the literature and current texts (for example 80). A number of issues with technique however need to be highlighted if it is to be used successfully in toxicology. LC-MS/MS is not designed for detection of large numbers of drugs but is ideally suited to measure the low levels of one or two drugs after appropriate sample pre-treatment and optimised chromatography. The technique will suffer from isobaric interferences (i.e. co-elution of compounds of close molecular mass) for example [85], and with the advent of a new NPS an isobaric interference may suddenly appear in an established and fully validated assay. Some substances (particularly conjugated metabolites) will undergo in-source fragmentation; therefore, careful development of the chromatography is required to resolve all metabolites from the parent drug [86]. Ion suppression must not be ignored; it is likely that this will differ for different drugs of the same class. A further issue that may become apparent is loss of deuterium from internal standards [87]; this can be overcome by use of internal standards containing carbon 13, but these are not commonly available and generally cost more to produce than the deuterated equivalents. The LC-MS/MS must be tuned with the appropriate standard chemical; this may be difficult as metabolites may not be available or be prohibitively expensive. It may be possible to use MS settings from the literature, but these do not readily transfer between different makes of MS [88].

LC-HRMS advantages and disadvantages

High resolution mass spectrometry is based on two techniques: time of flight (TOF) and Orbitrap-based systems. These are able to detect all compounds that will ionise in the sample, but this will include compounds of no interest to the toxicologist (for example, hormone metabolites and/or amino acids). The ability to measure the mass of the compound to four (or more) decimal places dramatically increases the specificity of the identification of compounds. It is possible to develop libraries of compounds which may be possible to share between users of other LC-HRMS systems; however, a system of assessing the accuracy of compound identification would have to be developed by the users [89]. Furthermore, sophisticated software allows for prediction of fragmentation of novel compounds (without need to purchase a standard to tune the system) and the results can be retrospectively searched for a new drug of abuse.

It is clear that LC-HRMS increases the detection rate for drugs of abuse, particularly in the identification of NPS. However, care must be taken in method development

and result interpretation; a study comparing LC-MS/MS and LC-HRMS showed 19 false positives identified by LC-HRMS when screening for 29 compounds in 152 urine samples from a population with chronic pain. They noted that the false positive rate was decreased by the use of deuterated internal standards and retention time matching [90]. Some of the caveats that applied to LC-MS/MS apply to this technique as ion suppression, in-source fragmentation and deuterium loss will still occur and need to be controlled by careful interpretation of the results.

Forensic science regulator and ISO

Laboratories in the UK will be aware of the transition of the accreditation from CPA to ISO:15189 following the incorporation of CPA into UKAS in 2009. There are also issues with toxicology services, where the Forensic Science Regulator (FSR) is 'expecting' that forensic science providers gain accreditation with UKAS to the standard ISO:17025 [91]. This is a general standard, so a guidance document, ILAC-G19, is being used to ensure the provisions of ISO:15189 and 17025 make the accreditation suitable when providing toxicology analysis in a forensic setting [92]. Whilst the majority of forensic testing would not be undertaken by a clinical laboratory, drug screens undertaken for clinical purposes may be used by Social Services in Child Protection proceedings, or even used in prosecutions by the Police. Therefore, it may be prudent for clinical laboratory toxicology services to include ILAC G-19 to cover instances where they are involved in legal work (particularly if they undertake second sample analysis for Road Traffic Act alcohol).

Toxicology is currently in 'interesting times' with rapid explosion in the number and type of abused drugs, enormous advances being made in the instrumentation and a tighter regulatory framework in which to operate.

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