Article

Letting Your Hair Down with Party Pills

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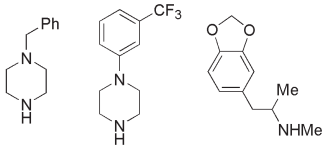
Over the past few years several piperazine analogue drugs have caught the attention of the public, authorities and media in NZ, with many questions being raised about both their safety and legal status. Two of the more common drugs in this class are 1-benzylpiperazine (BZP, **1**) and *m*-trifluoromethylphenylpiperazine (TFMPP, **2**). With comparisons being made to amphetamine and ecstasy (methylenedioxymethamphetamine – MDMA, **3**) they have been classed as stimulants, reported and advertised as giving feelings of euphoria, alertness, and a desire to socialise.[1] Side effects include a *hangover* (similar to that of alcohol), dry mouth and urine retention. The legal status of these drugs varies throughout the world and until recently little scientific research had been conducted on their use as recreational drugs. Until April 2008 the purchase and use of *Party Pills* containing BZP and TFMPP was legal in NZ, providing a unique opportunity for their study.



1 **2** **3**

The detection of *Party Pill* and other recreational drugs is important in workplace and roadside drug testing, forensic casework, and court disputes, *e.g.* child custody cases. From a forensic toxicology perspective it is essential to have a method that is simple, robust, accurate and reproducible. Traditionally gas chromatography mass spectrometry (GC-MS) has been the method of choice for the types of analyses mentioned above and it has an extensive history of use in the forensic field. GC-MS, however, requires time-consuming sample preparation or is otherwise limited to volatile compounds.[2] Liquid chromatography tandem mass spectrometry (LC-MS/MS) allows the detection and quantification of many different drugs in biological matrices and often requires less sample preparation. Indeed, according to the review by Maurer,[3] LC-MS/MS has the potential to become the *golden standard* for forensic and clinical analysis.

The ESR Forensic Toxicology group at Kenepuru have developed several LC-MS/MS methods for the analysis of prescription drugs and drugs of abuse. Included in this suite of analyses are two methods for the detection of prescription sedatives, amphetamine-type stimulants, and opiates in hair. The first of these methods simultaneously detects and quantifies drugs such as the amphetamines: **3** and methamphetamine (MA, *N*-methyl-1-phenyl-2-propanamine) and the opiates morphine, heroin (6-acetylmorphine, 6-MAM), and codeine. The second method detects and quantifies sedatives such as the benzodiazepine type drugs: 7-aminoclonazepam, temazepam, oxazepam, alprazolam, diazepam, and the non-benzodiazepine sedative zopiclone.

The adaptation of the amphetamines/opiates method to include the detection and quanititation of BZP (**1**) in hair using LC-MS/MS was undertaken by Natasha Lucas at ESR as part of her Waikato MSc studies. The assay was validated according to current best practice for quantitative methods following guidelines promulgated by the Society of Hair Testing, an international group of hair analysis experts.

Hair testing is becoming common, especially in workplace drug testing, drug facilitated sexual assaults, and child custody disputes. In some European countries hair tests are used to determine abstinence from drug use before driving licences are reissued following bans for driving under the influence of drugs. Although analysis of hair cannot determine the exact time of exposure, or the level of impairment, it does offer long-term exposure information not offered by other biological specimens, such as blood and urine.

There are many different forms of samples that can be collected for use in drug analysis. These include blood (and its components, *e.g.* plasma, serum), urine, oral fluid (saliva), sebum, hair, nails, and skin. Specimens of hair and nails can be useful for determining drug-use patterns. Examples of uses of such specimens include workplace drug testing (hair, sweat, oral fluid), criminal investigations (hair, sweat, oral fluid), child custody disputes and divorce cases (hair), and roadside testing for drug-impaired drivers (oral fluid).[4] The use of hair, nails and oral fluid for drug testing is becoming more popular because it is non-invasive. Fig.1 shows the various biological specimens and the time period in which they are useful for the detection of drugs. Blood and oral fluid are useful for immediate detection, and can give an indication of levels of impairment in a user. Urine can allow detection (depending on the drug) for days. Hair and nails are proving valuable as they can give a history of a person's drug use, although they cannot give information on the level of impairment, or the precise time of ingestion.

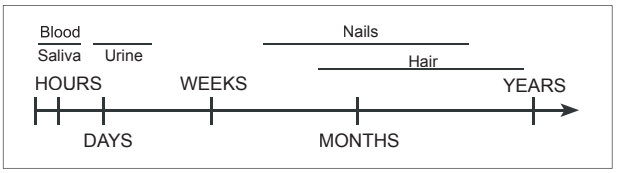


Fig. 1. Timeline of detection of drugs in biological specimens

Urine is the most widely used biological sample for toxicological analysis. Urine demonstrates use of a drug but gives little or no idea of when the drug was taken or the magnitude of any pharmacological effect, and therefore cannot be used to determine the level of impairment. Urine analysis can also take a long time, as there is often a need to identify metabolites owing to extensive transformation of the drug during metabolism. It is possible to detect drugs/metabolites in urine from hours until days after use.

Blood has many advantages as a biological matrix, as it can provide information about distribution, metabolism, and pharmacokinetics, and it can be used to measure drug levels almost immediately after administration.[5] Plasma is often the most common choice for blood analysis, although in autopsy cases plasma often cannot be obtained and so whole blood is used. With major advances in sample preparation, chromatography, and detectors, the use of whole blood as a matrix for quantification and identification has become widespread.[5]

Testing of hair for toxins began with heavy metals in the 1950's and was extended in the late 1970's with methods for the analysis of drugs being published. It is proving valuable in toxicological analysis, as it can give an idea of a person's drug history (months to years; see Fig. 1) and is also non-invasive. It is particularly useful in cases such as suspected drug-facilitated sexual assaults where a urine or blood sample was not taken soon enough.[6]

The methods of incorporation of drugs in hair are still rather unclear. The simplest explanation is by passive transfer from the blood into the hair follicle during formation. However, the multi-compartmental model[7] seems to be the most widely accepted.[7,8] This comprises:

- *Passive Transfer:* This occurs via passive diffusion from the blood stream into growing hair cells at the base of the follicle. The drug is retained in the interior of the hair (medulla) during keratogeneisis (formation of keratin, hardening of hair). Hair grows approximately one cm every 28 days[8] so that drugs deposited into the hair will be found approximately one cm from the scalp one month after the drug was taken.
- *Transfer from Sweat and Sebum:* Drugs are transferred into the hair after formation through sweat and sebum. Drugs have been found in sweat in higher concentrations than are found in the blood, so this offers an explanation to the higher concentrations sometimes found in the hair.[7]
- *Transfer from External Environment:* Drugs are passed into the hair from the environment. This could be through air, water or hair treatments (dying, perming, *etc*.). Drugs such as amphetamine, cannabis, heroin and cocaine are often smoked and hence transferred into the hair.
- *Intradermal Transfer:* Very lipid-soluble drugs such as tetrahydrocannibinol (THC, cannabis) are deposited into skin layers and transferred to hair.
- *Transfer from Melanin:* Drugs could possibly bind to melanin-related sites in the skin, which could result in drug uptake in the hair.

For analysis using hair, decontamination of the surface of the hair is a vital step. It is important to clear the hair of drugs deposited via air or hair treatments as mentioned in the multi-compartmental model above. The reviews by Boumba *et al*.[8] and Pragst, and Musshoff and their groups[9] give comprehensive overviews of the methods of drug incorporation into hair mentioned above, as well as the structure of hair and methods of drug detection.

Determining a person's drug history is important in drug-related deaths, as it can help establish whether or not they were a chronic or naive user, and this can often shed some light on why they might have died. Also, detection in hair can help determine whether someone may have been using particular drugs at the time of an earlier (criminal) incident. This can help support or refute other information. Other applications of hair analysis involve child custody disputes and drink spiking incidents.[6]

In the last year ESR has analyzed 80 samples for amphetamines, 25 for opiates and 17 for BZP. The majority of these analyses were undertaken to allow parents to prove abstinence from drug use and gain access to their children. A summary of results is presented in Table 1. The range of BZP concentrations detected in the hair was similar to that found for the amphetamines, from around 0.4 to 37 ng/mg of hair analyzed. There has been no work done as yet to directly correlate the amount of BZP found in the hair to the amount of drug taken. In all cases when a hair sample is taken from a living person, a drug use questionnaire is completed. From this information we anticipated a positive test for BZP in only 2 out of the 7 positive tests, whilst no one volunteered the use of illegal drugs.

Table 1. Drug analyses performed by ESR over 12 months

Drug	No of Tests	Positives	% Positive
Party Pills - BZP	17	7	41 %
Methamphetamine	80	32	40 %
MDMA	80	6	7.5 %
Codeine	25	7	28 %
Morphinea	25	3	12 %
Heroin (6-MAM)*	25	3	12 %

[a]The heroin marker 6-MAM was present in all cases where morphine was detected.

Whilst the number of hair cases/samples analyzed for BZP is small by comparison with amphetamines, the percentage of positive tests is similar to that of methamphetamine. It would be anticipated that prior to its reclassification, BZP would have been used amongst the general population more widely than methamphetamine. Following reclassification, we anticipate more requests to analyze hair samples for BZP.

References

1. Maurer, H. H.; Kraemer, T.; Springer, D.; Staack, R. F. *Theraputic Drug Monitoring* **2004**, *26*, 127-131.
2. Marquet, P.; Lachatre, G. *J. Chromat. B* **1999**, *733*, 93-118.
3. Maurer, H. H. *Clinical Biochem.* **2005**, *38*, 310-318.
4. Cone, E.J. *Forensic Sci. Int.* **2001**, *121*, 7-15.
5. Moeller, M.R.; Steinmeyer, S.; Kraemer, T. *J. Chromatog. B.* **1998**, *713*, 91-109.
6. Irving, R.C.; Dickson, *Forensic Sci. Int.* **2007**, *166*, 58-67.
7. Henderson, G.L. *Forensic Sci. Int.* **1993**, *63*, 19-29.
8. Boumba, V.A.; Ziavrou, K.S.; Vougiouklakis, T. *Int. J. Toxicology* **2006**, *25*, 143-163.
9. Pragst, F.; Balikova, M.A. *Clin. Chim. Acta* **2006**, *370*, 17-49; Musshoff, F.; Madea, B. *Forensic Sci. Int.* **2007**, *165*, 204-215.