NDLERF

Hair drug testing – Hair drug analysis to identify cases of drug facilitated sexual assault

(Boast, Dayman, Young, Felgate)

Final report

Dr Gregory Dayman, Dr Lyndall Young, Peter Stockham,
Danielle Butzbach, Chris Kostakis,
Elizabeth Gebler-Hughes, Scott Janes

Funded by the National Drug Law Enforcement Research Fund
An Initiative of the National Drug Strategy

Hair drug testing – Hair drug analysis to identify cases of drug facilitated sexual assault

(Boast, Dayman, Young, Felgate)

Final report

Dr Gregory Dayman

Dr Lyndall Young

Peter Stockham

Danielle Butzbach

Chris Kostakis

Elizabeth Gebler-Hughes

Scott Janes

The project was a prospective cohort study comparing hair drug analysis with blood/urine drug analysis in people who allege sexual assault where there is a belief that drug facilitation of that assault may be present.

Funded by the National Drug Law Enforcement Research Fund, an initiative of the National Drug Strategy

Produced by the National Drug Law Enforcement Research Fund (NDLERF)
GPO Box 2944, Canberra, Australian Capital Territory 2601

ISBN: 978-1-922009-71-5

ISSN: 1449-7476

The research on which this report is based was funded by the National Drug Law Enforcement Research Fund, an initiative of the National Drug Strategy.

Contents

Tables

Background information

This was a joint project between Yarrow Place Rape and Sexual Assault Service, Forensic Science South Australia and Dr Tanya Boast, College of Emergency Medicine, advanced trainee. Dr Boast initiated the project, achieved Ethics Approval from the Women and Children's Health Network (WCHN) and was responsible for gaining the funding from National Drug Law Enforcement Research Fund (NDLERF). She was not able to continue the project due to work commitments.

Yarrow Place Rape and Sexual Assault Service is the adult sexual assault service in Adelaide, Australia. The service provides counselling, medical and forensic services to people aged 16 years and over who report that they have been sexually assaulted. Yarrow Place is a community based service of Women and Children's Health Network.

Forensic Science South Australia (FSSA) provides a comprehensive, coordinated range of services associated with the justice system. Within FSSA there are five main groups: Pathology, Chemistry and Materials, Biology, Toxicology Group and Administration.

The project was guided by a Reference Group comprising of:

Barry Newell
Senior Project Officer
Alcohol and Drug Policy Unit
Licensing Enforcement Division
Western Australian Police

Dr Mark Stephenson
Acting Chief Chemist
Queensland Health's Forensic Toxicology

Maria Borzycki
NDLERF research officer
Australian Institute of Criminology

Dr Paul Kirkbride
Chief Scientist
Australian Federal Police
Forensic and Data Centres

Bernie Farrington
South Australian Police

Executive summary

Between 2011 and 2013, a prospective cohort study was undertaken aiming to compare hair drug analysis with blood and urine drug analysis in people who alleged drug-facilitated sexual assault (DFSA). This was a joint project between Yarrow Place Rape and Sexual Assault Service, Forensic Science South Australia (FSSA) and Dr Tanya Boast (Advanced Trainee, College of Emergency Medicine).

Between August 2011 and September 2013, doctors working at Yarrow Place recruited clients who made allegations of DFSA. After the attending doctor provided information about the project and obtained written consent, the participant provided blood and urine samples for analysis and completed a questionnaire regarding the incident and any related alcohol/drug consumption. The participants were followed up after a period 4 weeks to 6 months, at which time they provided information regarding any drug consumption since the assault. During this appointment the Yarrow Place doctor collected hair samples from each client.

All samples were processed by FSSA. Drugs reported as present were confirmed to be present in a second extraction and analysis. Each blood sample was subjected to a number of tests, including ethanol concentration measurements, screening for drugs of abuse (amphetamines, THC, opiates and cocaine), basic drug screening (antidepressants, anti-psychotics, benzodiazepines, sedatives, stimulants, analgesics and anti-hypertensives among others) and analysis for GHB. All urine samples were subjected to testing for the presence of drugs and their conjugated forms. Hair samples were incubated with methanol at 45°C. Drugs present in the samples were extracted into the methanol, which was then removed and analysed.

The recruitment period yielded 32 participants, 19 of whom were enrolled within 72 hours. A further 7 clients were enrolled within a week, while the rest were recruited between 16–20 days post-assault. The vast majority experienced amnesia of the event, unknown drug consumption status or an unknown drug source. However, 4 participants reported known or suspected drugs (such as benzodiazepines). Drink and food spiking were the suspected sources of drug consumption in 10 cases and 1 case respectively. The most common reason for concern was a period of memory loss, but clients also reported vaginal/anal soreness, waking in a strange place, concerns about removal of clothing and informed by another person that sexual intercourse had taken place, among other reasons.

The majority of clients documented medication use, most commonly antidepressants, while 6 reported illicit drug use (e.g. THC, amphetamine, MDMA). Thirty-one clients documented alcohol consumption, with 23 of these reporting harmful levels of consumption. Blood samples were taken from 22 clients, with 10 of these testing positive for drugs and/or alcohol. In contrast, 20 of 25 urine samples tested positive and in 14 cases, there were drugs unaccounted for by the clients. Hair samples were taken from 10 of 32 participants between 30 to 60 days post-assault. Drugs unaccounted for by clients were found in 50% of these.

Statistical interpretation of the data was not completed due to limited sample size. The enrolment rate was approximately 30% of that anticipated prior to study commencement and only approximately 30% of participants went on to have subsequent hair analysis undertaken. Despite measures to improve recruitment including staff notifications, a follow-up reminder system and obtaining ethics approval to allow the enrolment of 16 and 17 year olds, participant enrolments remained low leading to cessation of the study in September 2013.

The circumstance under which the participants found themselves at recruitment may have led to some omissions of known ingested substances. When compared with recent Australian series, there are some features of the results worth noting. Firstly the high rates of reported harmful alcohol consumption levels and rates of prescription medication use and the high rate of detection of alcohol and/or drugs (80%). The small population and selection bias, along with the improvement of detection methods since the comparison studies, may account for some of the high rates reported in this study.

Overall, there was some encouraging data in regards to detection of unreported substances in hair samples and it was concluded that analysis of hair samples may provide useful information in cases of DFSA when drugs are missed in blood and urine samples.

Methods

Recruitment

The recruitment period for the project was between August 2011 and September 2013. A total of 561 clients were seen by the medical service in this timeframe. Of these, 422 had some form of forensic medical examination. The data for those clients requesting a forensic examination is more complete, so this group will be used for the next analysis. Of the group who had a forensic medical examination, 71 (16.8%) people presented with concerns that they had been sexually assaulted without their knowledge and 90 (21.3 %) people believed they had been drugged. A total of 226 (53.6%) people reported having (knowingly) consumed alcohol and/or drugs prior to the alleged assaults.

People attending for medical services after a sexual assault were assessed in relation to the allegation made. If the allegation was of a suspected DFSA, information was provided by the attending doctor about the research project and consent was gained by those interested in participating. Photographs were shown to potential participants of the amount of hair required to be taken including a photograph of a head of hair after the taking of a hair sample.

It must be noted however, that the research was being conducted on people after a recent sexual assault. There are a multitude of decisions that people need to make post-assault, at a time when the majority are anxious, distressed, tired and possibly recovering from the impacts of alcohol and/or drug use. It was not always appropriate in these circumstances to enrol people in a study.

The doctors recruiting people into the study were the permanent Yarrow Place doctors and casual doctors working on the after-hours panel. The doctors collecting the hair samples were the small group of permanent daytime doctors.

When the person agreed to be in the study, the following process was undertaken:

1. The doctor provided information and obtained written consent for participation. If this was not considered appropriate, a pamphlet was provided to the person for consideration at a later date.
2. The participant, with the assistance of the doctor, completed Questionnaire 1 (see Appendix A), regarding their experience, symptomatology, and alcohol and drug consumption the week prior to, on the day of and in the period since the assault but before the medical examination.
3. If the timing of the enrolment was within prescribed time limits, the doctor collected additional blood (<72 hours) and urine (<96 hours) samples for the purpose of the study labelled with the participant code. The samples were locked in the Yarrow Place refrigerator used to store forensic samples.
4. The questionnaires were stored in a separate folder at Yarrow Place.
5. A recall was added to Yarrow Place's patient management software (Medical Director 3) so that a reminder about the follow-up visit could be sent.
6. The participant was seen between 4 weeks and 6 months after the assault. At this visit, the participant with the assistance of the doctor completed Questionnaire 2 (see Appendix B) about drug use, both illicit and prescribed since the assault and factors that may affect the hair analysis (bleaching, haircut, perm etc).
7. Hair samples were taken and labelled with the participant code and locked in the refrigerator.
8. Participants who did not make an appointment were contacted in an attempt to encourage them to complete the process.

Sample analysis

The samples were either taken to FSSA by the police when taking the forensic kits to be processed, by FSSA staff collecting the samples or by Yarrow Place staff delivering the samples to FSSA.

All instrument sequence lists (which detailed vial positions and contents of vials on instrument autosamplers) were checked by another analyst.

Drugs reported as present were confirmed to be present in a second extraction and analyses of the sample for confirmation.

Further details regarding methodology for quantification of substances are available at Appendix C.

Quantification of ethanol in blood and urine

Ethanol concentrations were determined in blood and urine using a protein precipitation and gas chromatography-flame ionisation detector method. All samples were analysed in duplicate with each replicate performed by a different analyst on a different day and analysed on a different instrument. Calibration curves were produced using known concentrations of ethanol in aqueous solution and used n-propanol as the internal standard. The calibration range was 0.010–0.300% w/v. Levels below 0.01% w/v were reported as negative. Protein precipitation was achieved using a sodium tungstate solution. Other low molecular weight molecules detectable using this analysis included acetone and iso-propanol. The instruments used were Perkin-Elmer Clarus 500 gas chromatographs and flame ionisation detectors with 180cm x 2mm internal diameter glass column packed with a) 0.2% Carbowax 1500 on GRAPHPAK-GC 80/100, or b) 5% Carbowax 20M on CARBOPAK B 60/80.

Screening for drugs of abuse by ELISA

ELISA (enzyme linked Immuno Sorbent Assay) is a solid phase immunoassay designed to rapidly detect and quantify a specific compound in a sample by means of a high-affinity capture antibody. This analysis was performed on a TRITURUS automated system and screened blood and urine for 4 classes of drugs of abuse: amphetamines, THC (cannabis), opiates and cocaine. Blood samples were diluted with a phosphate buffered saline solution and analysed against drug-free blood spiked with analytes at known concentrations. Urine samples were not diluted. The low-positive spike contained 0.020mg/L methamphetamine, 0.010mg/L morphine, 0.050mg/L benzoylecgonine (cocaine metabolite) and 0.005mg/L THC-COOH (metabolite from THC, active compound in cannabis). The high-positive spike contained 0.080mg/L methamphetamine, 0.040mg/L morphine, 0.200mg/L benzoylecgonine and 0.020mg/L THC-COOH. Any samples with responses indicating drug levels at or above the low-positive control were then subjected to confirmatory tests by LC-MS methods. One limitation to this method was that it responds to methamphetamine and MDMA but not amphetamine. However, detection of amphetamine was accomplished using the Basic Drugs screening method.

Screening for basic drugs by LC-MS

This method was primarily designed to extract blood for a range of chemically basic and neutral drugs from blood, but it is applicable to other biological liquid samples. The drugs detected included anti-depressants, anti-psychotics, benzodiazepines, sedatives, stimulants, analgesics, antihypertensives, among others (a full list can be seen at Appendix C). The drugs were extracted using liquid–liquid extraction with ammonia and butyl chloride. Analysis was performed on an Agilent 1200 liquid chromatograph coupled to a 6520 quadrupole-time of flight (QTOF) mass spectrometer. The LC column used was a Waters Acquity BEH C18, 1.7um, 3.0 mm x 50 mm with a Phenomenex 3mm C18 guard column. The mobile phase consisted of 0.1% formic acid and acetonitrile run in a reverse phase gradient. The mass spectrometer acquired data in auto-MSMS mode (ie data dependant acquisition using MS mass peak intensity and a preferred target list to select MSMS

precursors). Confirmation of identity was by comparison of accurate mass measurements and MSMS spectral matching with authentic standards. For blood samples, an approximate concentration was obtained by comparison against an extracted blank blood sample that was spiked with known concentrations of common drugs (see Table 1), or against a neat standard for those not listed in Table 1.

Table 1: Concentrations of drugs in spiked blank blood

Analyte	Approx Concentration (ug/mL)	Analyte	Approx Concentration (ug/mL)
Olanzapine	0.2	Nitrazepam	0.1
Oxycodone	0.08	Citalopram	0.1
Quetiapine	0.4	Sertraline	0.1
Risperidone	0.07	Lignocaine	1.0
Haloperidol	0.04	Alprazolam	0.1
Clonazepam	0.1	ODM-venlafaxine	0.2
Duloxetine	0.3	Oxazepam	0.4
Fentanyl	0.02	Propoxyphene	0.3
Amlodipine	0.02	Temazepam	0.4
Fluoxetine	0.2	Nordiazepam	0.5
Tramadol	0.5	Diazepam	0.4
Paliperidone	0.05	Midazolam	0.4
Venlafaxine	0.2	Nortriptyline	0.2
7-aminonitrazepam	0.1	Dothiepin	0.1
7-aminoclonazepam	0.1	Metoclopramide	0.1
Methadone	0.2	Ketamine	1.0
Amitriptyline	0.2	Doxylamine	0.2
Mirtazapine	0.1		

For any cases where a drug was detected and confirmed, the analysis was repeated to reconfirm the drugs presence (ie exclude contamination). This method was used for both blood and hydrolysed urine samples.

Enzyme hydrolysis of urine samples

Many drugs are excreted from the body in urine in the form of glucuronide conjugates, with very little parent drug present. This method was designed to revert the glucuronide conjugate form of a drug back to the parent drug; that is, if temazepam-glucuronide was in urine, to convert it back to temazepam, which is more easily detected using current methods.

Urine samples were incubated with β-glucuronidase enzyme for 5 hours at 55°C. The β-glucuronidase enzyme breaks the chemical bond between a drug and the glucuronide moiety. A quality-control drug, isotopically labelled D3-codeine-glucuronide, which should be detected as D3-codeine, was also included to monitor enzymatic activity. The urine samples were then extracted and analysed using the basic drug screening described above. Numerous benzodiazepines showed increased detection rates using this method compared with not using enzyme hydrolysis.

Analysis for gamma-hydroxybutyrate (GHB, fantasy) in urine

Gamma-hydroxybutyrate (GHB) is a naturally occurring metabolite of gamma-aminobutyric acid (GABA) found in the central nervous system and peripheral tissues. GHB, a central nervous system depressant, is reportedly abused recreationally for its euphoric and relaxation effects, and potentially for the purposes of DFSA due to its sedative and amnesic effects at higher doses. Blood samples in this project were not analysed for GHB

since it has a very short circulatory half-life (0.3–1 hour). It has a slightly longer detection time in urine, with an approximate 12 hour detection window before levels return to endogenous concentrations. Consideration of this GHB detection window should be considered before excluding this drug as a possible DFSA agent.

The biological sample was acidified with concentrated sulphuric acid (0.25 mL). Under acidic conditions, GHB readily cyclises to form GBL and was isolated from the sample by solvent extraction using butyl chloride (2mL). The extract was transferred to a 2mL GC vial and analysed by GC-MS (Perkin Elmer Clarus 500 instrument). D6-GHB was added to the sample and used as an internal standard to ensure accurate recoveries.

A spiked calibration curve, a blank and a quality-control sample were included with each batch run. Calibration curves were constructed and used to calculate the GHB concentration in the sample. None of the urines in this project were positive for GHB.

Analysis of drugs in hair by LC/QQQ

Human head hair grows at approximately 1cm per month; however, there are variations in this between people At any one time, around 85% of hair is actively growing, with other hair strands at other stages where growth has stopped (sometimes for several months) before the hair strand falls out and another one begins. Drugs may therefore be present as broad dilute bands in the hair, rather than as a narrow concentrated band. Hair growth is supported by nutrients from the blood, which also contains drugs circulating in the body. The principle of testing for drugs in hair is that as the hair shaft is forming, drugs present in the blood are also being incorporated in the hair shaft. The drugs are then able to be extracted from the hair, detected and correlated to a time window related to the location of the drug in the hair length.

Hair samples collected from the scalp were measured and a segment suitable for analysis selected. For example, if the previous 1 month (4 weeks) was to be tested, a 1cm segment of hair closest to the scalp was measured and cut for analysis. Hair segments were cut into 1–3mm pieces and approximately 20mg of hair was incubated in methanol for 18 hours at 45°C to extract the drugs from the hair. The methanol was then removed from the hair and analysed using an Applied Biosystems 4000Q-Trap with and Agilent 1200 LC system. Two different methods were used. For the analysis of THC, the LC column used was an Agilent XDB-C18 1.8um 4.6mm x 50mm fitted with a Phenomenex 3mm x 4mm Gemini guard column. A reverse phase gradient elution program with 0.1% formic acid and methanol as mobile phases was used. The mass spectrometer was used in multiple reaction monitoring mode (MRM) with 3 MRMs obtained for THC. For the analysis of the other targeted drugs, a Phenomenex Luna PFP 3um 50mm x 4.6mm column with a PFP 5um 4mm x 2.0mm guard was used, using the same mobile phases in a reverse phase gradient. The mass spectrometer was used in MRM mode, with 2 MRMs obtained for each drug. Deuterated internal standards were also used, along with calibration curves of all analytes up to approximately 600pg/mg. The lower limit of quantitation with this method was 20pg/mg for all analytes.

The analytes targeted in this method were amphetamine, MDMA, methamphetamine, pethidine, morphine, codeine, heroin, MAM (heroin metabolite), clobazam, temazepam, triazolam, oxazepam, nordiazepam, 7-aminonitrazepam, nitrazepam, midazolam, lorazepam, 7-aminoflunitrazepam, flunitrazepam, 7-aminoclonazepam, clonazepam, bromazepam, alprazolam, diazepam, zopiclone, zolpidem, ketamine, methadone, fentanyl, benzoylecgonine, cocaine and oxycodone. The lower limit of quantitation for this method was 20pg/mg; however, some drugs were able to be confirmed to be present at levels lower than this.

Attempts to develop a method for the analysis of GHB in hair were not successful due to lack of sensitivity using the available instrumentation and lack of a suitable literature method at the time. Literature reports describe difficulties in discrimination of single GHB ingestion events from endogenous GHB hair concentrations as problematic. The recent purchase of more sensitive GC-MS instrumentation may assist future developments in this area.

Results

The study recruited a total of 32 participants during the two year period between August 2011 and September 2013.

Results from Questionnaire 1

Time from sexual assault to enrolment was recorded. A total of 28.1% (9/32) of participants were recruited within 24 hours, a further 31.3% (10/32) recruited within 24 to 72 hours, 21.9% (7/32) within 3 to 7 days and the remaining 18.8% (6/32) of participants were recruited in a range from 16 to 20 days post assault.

Amnesia for the sexual assault was documented in 71.9% (23/32) of cases.

Suspected drug used in the sexual assault was unknown in most cases (78.1%, 25/32), with benzodiazepines (9.4%, 3/32), GHB, THC, amphetamines and alcohol suspected in the remaining cases (3.1%, 1/32).

Source of the drug used was also unknown in most cases (65.6%, 21/32), followed by suspected drink (31.3%, 10/32) and food spiking (3.1%, 1/32).

Medication use was documented by the majority of participants (62.5%, 20/32), with antidepressants (37.5%, 12/32), benzodiazepines (12.5%, 4/32), antipsychotics and prescription opioids (6.3%, 2/32), and anticonvulsant medication (3.1%, 1/32) recorded. No medication use was recorded for 15.6% (5/32) of participants.

Illicit drug use was reported by 18.8% (6/32) of participants with THC (9.4%, 3/32), amphetamine (6.3%, 2/32) and MDMA use (3.1%, 1/32) recorded.

Alcohol use was reported by 96.9% (31/32) or participants, with harmful level consumption (>60gm or unknown amount) most frequently reported by participants (71.9%, 23/32) and ranges between 60 to 170gm documented. Above recommended level consumption (>20gm) was the next most common use recorded (18.8%, 6/32). Safe level alcohol use was documented in 6.3% (2/32) of participants.

Symptoms experienced by participants were recorded with loss of memory the most common symptom (26/32, 81.2%), followed by confusion and drowsiness (19/32, 59.4%), black out and nausea (18/32, 56.3%), weakness and passing in and out of consciousness (13/32, 40.6%), dizziness (11/32, 34.4%), lack of muscle control (7/32, 21.9%), out of body experiences (5/32, 15.6%), and impaired judgement, reduced inhibitions and vomiting (4/32, 13%). Other symptoms recorded as experienced by participants were tremor, sweating and aggression (2/32, 6.3%) and insomnia, anxiety, blurred vision and double vision (1/32, 3.1%).

Reasons for concerns about sexual assault were also documented with a period of memory loss (23/32, 71.9%), vaginal and/or anal soreness (15/32, 46.9%), waking in a strange place (15/32, 46.9%), clothing removed and/or dishevelled (11/32, 34.4%), concerns regarding people in the environment such as a party or club (8/32, 25%), vaginal discharge (7/32, 21.9%), informed by another person that sexual intercourse had taken place (6/32, 18.8%), a known sexual assault (4/32, 13%), memory blank only (3/32, 9.4%) and wet clothing (1/32, 3.1%) recorded. A Table of the participant symptoms and concerns is provided at Appendix E.

Results from blood, urine and hair samples

Blood testing was undertaken for 68.8% of cases (22/32) with drugs and/or alcohol detected in 45.5% of samples (10/22). Positive blood alcohol levels were detected in two participant samples (22 and 49) both of which were collected within 24 hours post assault. Drugs unaccounted for by the participant questionnaires were identified in 22.7% (5/22) of the blood samples (3, 8, 20, 26, 27). These samples were collected within a

range of 10–43 hours post assault, with THC (43 hours), methamphetamine (10 hours), lorazepam (40 hours), amlodipine (37 hours) and nordiazepam (40 hours) identified.

Urine testing was undertaken for 78.1% of participants (25/32) with alcohol and/or drugs detected in 80% of samples (20/25). Alcohol was detected in four of the urine samples, all of which were collected within a range of 12–22 hours after ingestion. A total of 56% (14/25) of urine samples contained drugs, which were not accounted for by the participants, from 8 drug groups with a total of 27 drugs detected. The most common unreported drugs detected were in urine were benzodiazepines (40.7%, 11/27), amphetamines (14.8%, 4/27), THC and opioids (11.1%, 3/27), antihistamines and antidepressants (7.4%, 2/27,) and anti-nausea and anti-hypertensive medication (3.7%, 1/27). Urine samples which were positive for unaccounted for drugs were collected in a range of 10 to 87 hours post assault.

Hair testing was undertaken for 31.3% of participants (10/32) with sampling taking place in a range of 30 to 60 days post-sexual assault. Drugs that were unaccounted for from the participant questionnaires were found in 50% (5/10) of the hair samples, with a total of 8 unaccounted drugs identified. Benzodiazepine drugs were the most often (50%, 4/8), followed by opioids (37.5%, 3/8) and methamphetamine (12.5%, 1/8). No other drugs, including THC were identified from the hair analyses. Oxycodone and codeine were each identified twice in participant hair samples for which the corresponding participant urine or drugs samples were negative for these drugs (participants 3 and 24 for oxycodone and 24 and 27 for codeine). Methamphetamine was detected in a hair sample for which there were no urine or blood testing available.

A summary of the toxicology results for blood, urine and hair are presented in Table 2, with detailed information regarding hair samples presented in Table 3.

Table 2: Positive results from drug analysis of blood, urine and hair

Participant no.	Reported ingestion	Time from assault to urine/blood collection (hours)	Positive results from specimen samples		
			Blood	Urine	Hair
1	Paracetamol Vitamins Alcohol (80-100g) *Paracetamol/Codeine* *Temazepam*	12	Nil	Nil	Codeine
2	Alcohol (20g)	72	Nil	**Mirtazapine#**	N/A
3	Ibuprofen Codeine *Diazepam* *Sertraline* *Doxylamine*	43	Codeine **THC-COOH**	Codeine Morphine **THC-COOH**	Codeine Morphine Diazepam Nordiazepam **Oxycodone***
4	Alcohol (60 g) Paracetamol Ibuprofen	14	Nil	0.075% Alcohol	N/A
5	Alcohol (50g)	20	Nil	**Pholcodine**	N/A
6	Alcohol (90g) Diazepam Paracetamol/codeine THC	37	Diazepam	Codeine Diazepam Nordiazepam Oxazepam Temazepam	N/A
7	Alcohol (80 g) Fluvoxamine Paracetamol	80	Nil	Fluvoxamine **Pholcodine** **Chlorpheniramine**	N/A

Table 2: Positive results from drug analysis of blood, urine and hair cont.					
Participant no.	Reported ingestion	Time from assault to urine/blood collection (hours)	Positive results from specimen samples		
			Blood	Urine	Hair
8	Alcohol (115 g) Fenofexadine Thyroxine Sertraline Vitamin D	10	**Methamphetamine** Sertraline#	**Methamphetamine** **Amphetamine** Sertraline#	N/A
10	Alcohol (150 g) Oral contraceptive	18	Nil	**Amphetamine**	N/A
11	Alcohol (185g) Diazepam Azathioprine Azithromycin *Metronidazole* *Paracetamol/Codeine*	720	N/A	N/A	Diazepam Nordiazepam*
12	Alcohol (90 g) Oxycontin Amphetamines	456	N/A	N/A	N/A
13	Alcohol (60g) Desvenlafaxine Oral contraceptive	87	N/A	ODM-Venlafaxine **Nordiazepam** **Temazepam** **Oxazepam**	**Diazepam** **Nordiazepam***
15	Alcohol MDMA	42	Nil	Nil	N/A
16	Alcohol (50g) Fluoxetine Oral contraceptive	384	N/A	N/A	N/A
17	Alcohol (280g) Amphetamine	456	N/A	N/A	N/A
18	Alcohol (40-60g)	12	Nil	0.036% Alcohol **THC-COOH**	N/A
19	Alcohol (120g) Fluoxetine Salbutamol	64	Fluoxetine#	Fluoxetine# **Amphetamine** **Codeine** **Lorazepam**	N/A
20	Alcohol (110g) Thyroxine Oral contraceptive Temazepam Paracetamol	40	**Lorazepam**	**Lorazepam**	**Codeine***
21	Alcohol (70 g)	97	Nil	Nil	N/A
22	Alcohol (170 g) Paracetamol	22	0.046% Alcohol	0.112% Alcohol	N/A
23	Alcohol (40 g) Escitalopram Valproate Quetiapine	20	N/A	**Midazolam#** **Metoclopramide** **THC-COOH** Citalopram# Quetiapine **Doxylamine**	N/A

Table 2: Positive results from drug analysis of blood, urine and hair cont.					
Participant no.	Reported ingestion	Time from assault to urine/blood collection (hours)	Positive results from specimen samples		
			Blood	Urine	Hair
24	Alcohol (80 g) Quetiapine Fluvoxamine Morphine Paracetamol Promethazine *Ibuprofen*	21	Quetiapine	Quetiapine # Fluvoxamine **Oxazepam** **Temazepam**	**Codeine** Morphine **Nordiazepam** **Oxycodone***
25	Alcohol (30 g) Fluvoxamine	47	Nil	N/A	N/A
26	Candesartan/ Hydrochlorothiazide Atenolol Temazepam Escitalopram	24	**Amlodipine** Citalopram# Temazepam	**Amlodipine** **Amitriptyline** Atenolol Citalopram# **Oxazepam** Temazepam Nortriptyline	N/A
27	Alcohol (30g) Citalopram	40	Citalopram# **Nordiazepam**	Citalopram# **Oxazepam** **Nordiazepam** **Temazepam**	**Codeine** **Oxazepam*** **Nordiazepam***
41	Alcohol (80g) Infliximab	120	N/A	N/A	N/A
43	Alcohol (80g) Oral contraceptive	34	Nil	Nil	Nil
44	Alcohol (100g) Fluoxetine Azathioprine Oral contraceptive	50	N/A	Fluoxetine#	N/A
45	Alcohol (60g) Desvenlafaxine	672	N/A	N/A	**Methamphetamine**
46	Alcohol (95g)	107	Nil	Nil	Nil
48	Alcohol THC	132	Nil	Nil THC-COOH	N/A
49	Alcohol (90g) THC	15	0.025% Alcohol	0.074% Alcohol	N/A

Bold font indicates unaccounted for substances

Italics indicate substances ingested subsequent to collection of blood and urine samples

Nordiazepam is a diazepam metabolite

Oxazepam may be present as a result of oxazepam ingestion or as temazepam or diazepam metabolite

Temazepam may be present as a result of temazepam ingestion or as a diazepam metabolite

Morphine may be present as a result of morphine or heroin use or as a codeine metabolite

* Indicates substance present <LOQ

# Indicates metabolites of this substance also detected

Table 3: Detailed results from drug analysis of hair samples			
Participant no.	Time from assault to sample taken days (approx weeks)	Timeframe	Drugs detected
1	40 (5–6 weeks)	0–12 weeks	Codeine
3	60 (6–7 weeks)	0–2 weeks	Codeine, morphine, diazepam, nordiazepam, oxycodone*
		2–4 weeks	Codeine, morphine, diazepam, nordiazepam*, oxycodone*
		4–6 weeks	Codeine, morphine, diazepam, nordiazepam*, oxycodone*
		6–8 weeks	Codeine, diazepam, nordiazepam, morphine*
		8–12 weeks	Codeine, diazepam, nordiazepam, morphine*
11	30 (4–5 weeks)	0–2 weeks	Diazepam, nordiazepam*
		2–4 weeks	Diazepam, nordiazepam*
		4–6 weeks	Diazepam, nordiazepam*
		6–8 weeks	Diazepam, nordiazepam*
		8–12 weeks	Diazepam, nordiazepam*
13	30 (4–5 weeks)	0–2 weeks	Nordiazepam*
		2–4 weeks	Diazepam, nordiazepam*
		4–6 weeks	Nil
		6–8 weeks	Nil
		8–12 weeks	Nordiazepam*
20	36 (5–6 weeks)	0–2 weeks	Nil
		2–4 weeks	Nil
		4–6 weeks	Nil
		6–8 weeks	Nil
		8–12 weeks	Codeine*
24	46 (6–7 weeks)	0–2 weeks	Codeine, morphine, nordiazepam, oxycodone
		2–4 weeks	Nordiazepam, oxycodone
		4–6 weeks	Codeine, nordiazepam, oxycodone
		6–8 weeks	Codeine, nordiazepam, oxycodone
		8–12 weeks	Codeine, nordiazepam, oxycodone
27	30 (4–5 weeks)	0–4 weeks	Codeine
		0–12 weeks	Codeine, Oxazepam, nordiazepam*
43	32 (4–5 weeks)	0–4 weeks	Nil
		4–8 weeks	Nil
45	30 (4–5 weeks)	0–4 weeks	Methamphetamine*
		4–8 weeks	Methamphetamine*
46	30 (4–5 weeks)	0–8 weeks	Nil

* Indicates substance present <LOQ

Discussion

This study had a small sample due to recruitment problems and for this reason, statistical interpretation of the data has not been undertaken. Enrolments into the study were about one-third the number anticipated prior to commencement, with 32 subjects enrolled in total. Of these, only about one-third of subjects consented to subsequent hair analysis (10/32). A number of strategies were implemented throughout the study in an attempt to improve enrolment: the age group was expanded to include 16 and 17 year old subjects, emergency departments in Adelaide were alerted to the study and provided with information to facilitate enrolment, the study was advertised within the Yarrow Rape and Sexual Assault Service and doctors and counsellors working within the service were actively involved in recruitment of participants and doctors were tasked with enrolment and follow up of potential participants who had not been enrolled at their initial presentation to Yarrow Place. A questionnaire was devised for those enrolled participants who declined subsequent hair analysis. These participants provided reasons for not continuing in the study including: they did not want their hair cut, thought the amount of hair required was too much, did not want reminders of the sexual assault, had work commitments or decided that hair analysis was no longer necessary. All participants stated that they would have undergone hair analysis if this was possible at the time of enrolment into the study (ie at the time of first presentation for the sexual assault for most participants). One participant indicated she may have pursued hair analysis if collection of the sample had been possible through her hairdresser. Recruitment into the study ceased in September 2013 when the study was terminated due to ongoing poor recruitment.

Some features of the raw data obtained from the study are worth noting. In considering these results, it is important to remember that determination of previously ingested substances was reliant upon the recall of the participants and at a time when they may be potentially distressed, tired or uncomfortable. Therefore, it is possible that substances detected in biological samples and marked as unaccounted for could in fact be the result of participants omitting some items when reporting previously ingested substances.

The rate of alcohol use by study participants was high, with harmful or above recommended level alcohol consumption reported in 90.6% of study participants compared with recent alcohol use noted in 77% of participants in another Australian series (1). Similarly, reported rates of prescription medication use was high (62.5% vs 49%), although the reported rate of illicit drug use was slightly lower (18.8% vs 26%). The detection rate for drugs in participant urine samples was also high compared with another large series (2). Alcohol and/or drugs detected in 80% of samples (vs 63%), with unaccounted for drugs detected in 56% of the urine samples provided. Also notable was the high rate of urines positive for unaccounted benzodiazepines (40.7% vs 4.8%), amphetamines (14.8% vs 1.9% and 2.8% cocaine) and opioids (11.1% vs 0.69%), along with the relatively lower rates of THC detected (11.1% vs30.3%) (2). Regarding detection of substances in hair samples, it is interesting to note that no THC was detected in any samples, despite reported regular ingestion by some participants and blood and urine samples testing positive to its metabolites. This could be attributed to the relatively low levels of THC and its metabolites incorporated into hair (13, 14). Additionally, hair samples were unable to be tested for GHB due to lack of sensitivity in the available instrumentation as described previously and therefore these results do not exclude the presence of GHB in the specimens.

The high rates of substances detected in this study may be accounted for by the low overall study numbers and a possible selection bias due towards participants who have experienced a more significant or prolonged episode of memory loss or intoxication. Additionally, the rapidly improving nature of technologies for drug detection in biological materials could account for increased rates of detection compared with studies undertaken several years previously. However, it is not possible to exclude the possibility that the rate of use of some drugs to facilitate sexual assault was actually increased among the population of patients presenting to Yarrow Place.

In contrast to other studies that indicate little benefit in sampling blood or urine more than 48 hours after the reported assault, this study detected drugs in 28% (4/14) of urine samples taken outside of this timeframe, including benzodiazepines, antidepressants, THC-COOH, amphetamine and opioids (3, 4). This finding

supports the opinion that the use of increasingly sensitive instruments and testing for drug metabolites can considerably extend the forensic utility of urine sampling (5). The 22.7% of blood samples that tested positive for drugs unaccounted for by participant histories were all collected within 48 hours.

Despite the small sample size, analysis of the 10 hair samples collected in this study detected unaccounted for drugs in 50% of cases (5/10). The type and percentage of drugs detected in hair were similar to those found in other series, with benzodiazepine drugs predominating, along with other sedatives such as opioids (6). The detection of amphetamine in the hair sample of participant 45 is reflected in the drugs found by Villain (2007) who also did not exclusively find sedative drugs.

Specific case discussions

Participant 2 reported consuming 20gm of alcohol followed by a 6 hour period of memory loss. Mirtazapine was detected in urine taken 72 hours after the possible DFSA. This drug has sedative effects, especially at the commencement of therapy and can potentiate the sedative effects of alcohol (7). Mirtazapine is an uncommon drug in the setting of DFSA, but has been detected in previous series (4, 8).

Participant 3 was informed she had been drugged with THC in food which was detected in blood and urine 43 hours later. She experienced symptoms likely related to THC, but also described loss of memory, nausea, hallucinations and coming in and out of consciousness. THC-COOH was not detected in the hair, reflecting the difficulty this study had in detection of this drug. Oxycodone was detected using this matrix (weeks 0–6, with hair sampling at week 8). This may be an occurrence where hair was able to provide evidence of drug ingestion outside of the detection interval for blood and urine.

Participants 5 and 7 both had pholcodine detected in urine (at 20 and 80 hours respectively). These participants described drowsiness, loss of memory, blackout weakness and nausea. Both had consumed alcohol (50 and 80gm), which can potentiate the sedative effects of this drug (7). Cough agents have been detected in samples of 18 cases in other large series of DFSA (4).

Participants 8 and 10 both reported alcohol use, with amphetamine detected in urine samples, with methamphetamine also detected in the blood and urine for subject 8 who suspected this drug was used and reported symptoms consistent with psycho-stimulants. Participant 10 reported a symptom complex much more related to harmful alcohol consumption which may be accounted for by the higher level use at the time of the assault. Urinary amphetamines have been detected in other series at rates similar to those found in this study (13% vs 14%) (8).

Participant 45 had hair sampled 28 days after consuming 60gm of alcohol and experienced symptoms including sedation and a blackout. The sample detected methamphetamine in segments 0–4 weeks and 4–8 weeks. It is not clear from the literature if a one-off episode of amphetamine use can be reliably detected in hair (9). If the drug detected in the hair was from one off use at the time of the reported assault, the detection of amphetamine in both segments could be explained by the variable growth rate of individual hair follicles in the sample, hair cutting technique with some hair strands cut closer to the scalp and the possible migration of drug up or down the individual hair strand in the time prior to sampling.

Participant 23 was prescribed psychoactive medication and consumed 40gm of alcohol poured by another person. She experienced a nine-hour period of memory loss and reported confusion, nausea and aggression upon regaining her memory in hospital. Urine taken 20 hours later was positive for midazolam, metoclopramide, THC-COOH and doxylamine, with blood not sampled. It is not possible to exclude the possibility that the first two agents were given in hospital to the participant prior to her enrolment in the study. The use of midazolam in the context of DFSA is reported where it can be given orally (10). THC and doxylamine have both been reported in the context of DFSA as well (8).

Participants 13 and 27 both reported symptoms of memory loss, drowsiness and confusion, with urine samples testing positive for benzodiazepine drugs, some of which may be metabolites. These two participants had benzodiazepine drugs detected in their hair in segments related to the timing of the reported assault, with

nordiazepam detected also for participant 13 in the 0–2 week segment. For these participants, hair sampling did not seem to provide any additional information above urine testing alone. Participant 20 consumed 110gm of alcohol and had a blackout lasting 8 hours. Urine and blood tested positive for lorazepam with these samples collected 40 hours after the reported assault. Hair sampling done at 36 days was negative for lorazepam, a drug for which the literature provides conflicting reports about the success of being able to detect one off use in hair (11, 12). Participant 20 also had codeine detected in the hair in the 8–12 week segment, but this period of time was not enquired about in either participant questionnaire, so its significance is unknown.

Participant 24 reported passing in and out consciousness and memory loss associated with harmful-level alcohol consumption and suspected benzodiazepine drink spiking. Benzodiazepine drugs were found in urine collected 21 hours after the assault. Nordiazepam, codeine and oxycodone were detected in the hair sample collected 46 days after the assault; however, these drugs were found throughout segments from 0–12 weeks making attribution of ingestion to a one-off episode impossible.

Problems encountered with the study

Recruitment of participants for this study commenced in August 2011 and continued until the cancellation of the study in September 2013, with a rate of enrolment of participants being approximately three participants every two months. The rate of subsequent hair analysis was approximately 30% among the enrolled participants. This pattern of enrolment was only a fifth of the participant numbers originally hoped for.

The reduced enrolment rate may have been due to a number of factors including fewer clients presenting to Yarrow Place with a history of a possible DFSA (90 during the recruitment phase), refusal by a number of potentially suitable participants to enrol in the study (35.6%, 32/90) and missed opportunities to enrol eligible potential participants at the initial medical examination.

At the medical service level, a number of measures were instituted to try to improve recruitment: Medical Officers tasked with enrolment were reminded of the importance of doing so at regular clinical meetings, staff responsible for reviewing the medical notes from recent sexual assault examinations were tasked with recruitment of potential participants if this was not undertaken at the initial medical examination, a reminder system was implemented to ensure that recruited participants who failed to attend follow up study appointments received contact from the investigators to check if the participant wished to continue with the study, counselling staff who saw clients who had not seen a doctor were educated about the study and invited to forward contact details of potential participants for possible enrolment. Ethics approval was sought and gained to allow enrolment of 16 and 17 year old participants into the study. Despite these measures, enrolment into the study and participation in subsequent hair sampling remained below expectations.

Benefits arising from this study

Despite the low recruitment rate, there were several constructive outcomes of this project. The data suggests that existing hair-testing methodologies at FSSA can provide potentially useful information regarding unreported substances to DFSA investigators. In terms of analytical strategies, it suggests that in order to detect some drugs, instrument sensitivity needs to be improved and/or new methodologies developed, particularly if single-dose events are to be detected (eg THC and lorazepam). Partly as a result of this work, Flinders University and FSSA are collaborating on a PhD project in which the student will be examining ways to improve sampling, extraction and detection methodologies. The finding that the detection of drugs in urine for periods greater than 48 hours may result in changes to sampling times. In terms of method development and improvements, access to DFSA urines as part of this project was instrumental for the validation of improved screening techniques at FSSA. This project reenforced relationships between staff at Yarrow Place and FSSA, and has lead to a greater understanding of each other's roles in serving the community and the Justice System of South Australia. Future research collaborations are currently in discussion.

Conclusion

The analysis of hair in some cases of DFSA can provide useful additional information and may detect drugs missed by traditional matrices such as blood and urine. However, interpretation of the significance of unexpected findings in all cases must be carefully considered in relation to reported ingestion by participants and the potential for omissions in recall. This study brought about significant improvements in processes for drug detection for the FSSA laboratory, which will have ongoing benefits to the criminal justice system in South Australia. It also highlighted the methodological difficulties of detecting some drugs in the hair. This will be a focus of ongoing research at FSSA. The disappointing recruitment for this project may be in part accounted for by the issues that make reporting and follow-up rates for sexual assault victims low in general. It may be that retention of recruited participants could be improved in future studies with changes in methodology.

References

Hurley M, Parker H & Wells D (2006). The epidemiology of drug facilitated sexual assault. *Journal of Clinical Forensic Medicine*, 13: 181–185.

Slaughter L (2000). Involvement of drugs in sexual assault. *Journal of Reproductive Medicine*, 45(5): 425–430.

Kintz P (2007) Bioanalytical procedures for detection of chemical agents in hair in the case of drug-facilitated crimes. *Annals Bioanalytical Chemistry*, 388: 1467–1474.

Scott-Ham M & Burton F (2005). Toxicological findings in cases of alleged drug-facilitated sexual assault in the United Kingdom over a 3-year period. J*ournal of Clinical Forensic Medicine*, 12: 175-186.

Scott KS (2009). The use of hair as a toxicological tool in DFC casework. *Science and Justice*, 49: 250–253.

Villain M (2007). Applications of hair in drug facilitated crime evidence, in: P Kintz (Ed.), *Analytical and Practical Aspects of Drug Testing in Hair*, CRC Press, 2007.

MIMS Australia, viewed online 29/10/13.

Du Mont J, Macdonald S, Rotbard N, Bainbridge D, Asllani E, Smith N & Cohen MM (2010). Drug-facilitated sexual assault in Ontario, Canada: Toxicological and DNA findings. *Journal of Forensic and Legal Medicine*, 17: 333–338.

Kintz P (2012). Value of the concept of minimal detectable dosage in human hair. *Forensic Science International,* 218: 28–30.

Kelso P (2000). 'Male nurse used sedative to kill and rape', *The Guardian*, 18 May. Viewed online 29/10/13 www.theguardian.com/uk/2000/may/18/paulkelso

Kintz P, Villain M, Cirmele V, Pepin G & Ludes B (2004). Windows of detection of lorazepam in urine, oral fluid and hair, with a special focus on drug-facilitated crimes. *Forensic Science International* 145: 131–135.

.Gaulier JM, MErcerolle F, SAuvage F, Lamballais F & Lachatre G (2010). Soumission chimique: Des difficulties a chaque etape, illustrees par un cas de viol en reunion sous lorazepam, *18eme congress de la Societe Francaise de Toxicologie Analytique*: 48.

Han E, Yang W, Lee S, Kim E: in S Choi H, Lee S, Chung H & Song JM (2011). Establishment of the measurement uncertainty of 11-nor-D9-tetrahydrocannabinol-9-carboxylic acid in hair. *Forensic Science International*, 206: e85–92.

Minoli M, Angeli I, Ravelli A, Gigli F & Lodi F (2012). Detection and quantification of 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid in hair by GC/MS/MS in Negative Chemical Ionization mode (NCI) with a simple and rapid liquid/liquid extraction. *Forensic Science International*, 218: 49–52.

Appendix A – Questionnaire 1

Hair Drug Testing – Questionnaire 1

Date	Age	Gender

Date and Time of Alleged Sexual Assault

_____

Place of Alleged Sexual Assault (if known)

_____

Can you attend a follow-up appointment at Yarrow Place 4 weeks after your alleged assault? (please circle)
Yes
No

Suspected drug (leave blank if not known)

_____

How it was taken (leave blank if not known) eg in drink, in food

_____

Tick symptoms experienced (maybe more than one)

Confusion	In and out of consciousness
Dizziness	Out of body experience
Loss of memory	Weakness
Impaired judgment	Nausea
Reduced inhibitions	Vomiting
Drowsiness	Low heart rate
Lack of muscle control	Hallucinations
Black out	Aggression

Reasons why concerns re sexual assault:

Clothing removed/dishevelled	Memory blank only
Vaginal/anal soreness	Awoke in strange place
Vaginal discharge	Memory blank
Informed re sexual intercourse	Concerns re people in party, club, etc environment

List your regular medications

_____

Are you undergoing radiotherapy to your head or chemotherapy?
Yes
No

Before Alleged Sexual Assault (Up to One Week)

Did you have your regular medications?

Yes
No

Did you have any other prescription medications? eg temazepam, digesic

Yes
No

If yes, which ones?

_____

Did you have any over the counter medications? eg panadeine, antihistamines

Yes
No

If yes, which ones?

_____

Did you have any illicit or party drugs? (include those smoked, injected, etc)

Yes
No

If yes, which ones?

_____

On Day of Alleged Sexual Assault

Did you have your regular medications?

Yes
No

Did you have any other prescription medications? eg temazepam, digesic

Yes
No

If yes, which ones?

_____

Did you have any over the counter medications? eg panadeine, antihistamines

Yes
No

If yes, which ones?

_____

Did you have any illicit or party drugs? (include those smoked, injected, etc)

Yes
No

If yes, which ones?

_____

Did you have any alcohol?

Yes
No

If yes what did you drink (estimate standard drinks)

_____

Between what times?

_____

After Alleged Sexual Assault

Did you have your regular medications?

Yes
No

Did you have any other prescription medications? eg temazepam, digesic

Yes
No

If yes, which ones?

_____

Did you have any over the counter medications? eg panadeine, antihistamines

Yes
No

If yes, which ones?

_____

Did you have any illicit or party drugs? (include those smoked, injected, etc)

Yes
No

If yes, which ones?

_____

Did you have any alcohol?

Yes
No

If yes what did you drink (estimate standard drinks)

_____

Between what times?

_____

Thank you for your time

Appendix B – Questionnaire 2

Hair Drug Testing Questionnaire 2

Date

_____

List your regular medications

_____

If these are different to previous, could you please indicate what date they were changed

_____

Since your last visit to Yarrow Place

Did you have your regular medications?

Yes
No

Did you have any other prescription medications? eg temazepam, digesic

Yes
No

If yes, which ones?

_____

Did you have any over the counter medications? eg panadeine, antihistamines

Yes
No

If yes, which ones?

_____

Did you have any illicit or party drugs? (include those smoked, injected, etc)

Yes
No

If yes, which ones?

_____

Concerns re another episode of drink spiking

Yes
No

If yes, when did this occur?

_____

Since your last visit to Yarrow Place

Did you have your hair cut?

Yes
No

Have you had your hair bleached?

Yes
No

Have you had any other treatments to your hair? eg perm, colouring, etc

Yes
No

Once again, thank you for your time

Appendix C – Basis drug screen

[#] Analytes as indicated may only be detected at greater than therapeutic concentrations.
[*] For some drugs in this group there is limited or no blood concentration data. These drugs may not be detected at concentrations resulting from typical use/abuse.

Basic Drug Screen

Antidepressants

Agomelatine [#]
Amitriptyline
Citalopram
Clomipramine/Desmethylclomipramine
Desipramine
Dothiepin
Doxepin/Nordoxepin
Duloxetine
Fluoxetine/Norfluoxetine
Fluvoxamine
Imipramine
Mianserin
Mirtazapine
Moclobemide
Nefazodone
Nortriptyline
Paroxetine
Protriptyline
Reboxetine
Sertraline
Trimipramine
Venlafaxine/O-desmethylvenlafaxine

Antihistamines

Azatadine [#]
Brompheniramine
Chlorpheniramine
Cyclizine
Cyproheptadine
Diphenhydramine
Doxylamine
Loratadine
Methdilazine [#]
Pheniramine
Promethazine
Trimeprazine
Triprolidine

Cardiac/ Antihypertensives

Amlodipine [#]
Clonidine [#]
Diltiazem
Felodipine [#]
Flecainide
Lignocaine
Metoprolol
Perhexiline
Propranolol
Quinidine
Verapamil

Amphetamines/Stimulants/ Hallucinogens

Amphetamine
Atomoxetine
Atropine
Chlorphentermine
Cocaine
Ephedrine
Fenfluramine
LSD (lysergide) [#]
MDA (methylenedioxyamphetamine)
MDEA (methylenedioxyethamphetamine)
MDMA (methylenedioxymethamphetamine)
Mephentermine
Mescaline
Methamphetamine
Methylphenidate
PCP (phencyclidine)
Phendimetrazine
Phenmetrazine
Phentermine
PMA (paramethoxyamphetamine)
Pseudoephedrine
Sibutramine [#]

Anaesthetic Agents

Dexmedetomidine [#]
Benzocaine [#]
Bupivacaine
Prilocaine
Procaine
Ropivacaine

Antipsychotics

Amisulpride
Aripiprazole
Chlorpromazine
Clozapine
Droperidol
Flupentixol
Fluphenazine [#]
Glutethimide
Haloperidol
Olanzapine
Paliperidone/hydroxybenzoylpaliperidone
Pericyazine
Pimozide
Prochlorperazine
Promazine
Quetiapine
Risperidone/hydroxybenzoylrisperidone
Sertindole
Thioridazine
Thiothixene [#]
Trifluoperazine
Ziprasidone
Zuclopenthixol (cis)

Benzodiazepines/Sedatives

Alprazolam
Bromazepam
Buspirone [#]
Clobazam
Clonazepam/7-aminoclonazepam
Desalkyl-flurazepam
Diazepam
Flunitrazepam/7-aminoflunitrazepam
Flurazepam [#]
Ketamine
Lorazepam
Methaqualone
Midazolam
Nitrazepam/7-Aminonitrazepam
Nordiazepam
Oxazepam
Phenazepam
Temazepam
Triazolam
Zaleplon
Zolpidem
Zopiclone [#]

Miscellaneous

Acetylmethadol (LAAM)
Amidopyrine
Anastrazole
Bupropion/Hydroxy-/Threo-/Erythro-
Carbamazepine
Cisapride
Clopidogrel
Donepezil
Eletriptan
Etoricoxib
Hydroxychloroquine
Hyoscine [#]
Lamotrigine
Malathion
Memantine
Metoclopramide
DEET (N,N-Diethyl-3-methylbenzamide)
Nevirapine
Oxymetazoline [#]
Pholcodine
Pizotifen
Quinine
Rizatriptan
Sildenafil
Strychnine
Sumatriptan
Tadalafil
Thebaine
Trimethoprim
Tropisetrone
Vardenafil

Narcotic Analgesics

Alfentanil [#]
Buprenorphine [#]/Norbuprenorphine [#]
Codeine
Dextromethorphan
Dextromoramide
Dextropropoxyphene
Dihydrocodeine
Fentanyl [#]
Hydrocodone
Methadone
Oxycodone
Pentazocine
Pethidine
Tramadol/O-desmethyltramadol

Anti-Hyperkinetic agents

Benzhexol [#]
Benztropine
Biperiden
Orphenadrine
Procyclidine
Tetrabenazine [#]

Other Synthetic/Illicit/Drugs of Abuse *

2-Fluoromethamphetamine
3,4-Dimethoxyamphetamine
4-Fluoromethamphetamine
4-Methylthioamphetamine
5-Methoxy-α-methyltryptamine
BZP (benzylpiperazine)
CPCPP (1-(3-chlorophenyl)-4-(3-chloropropyl)piperazine)
DOI (2,5-dimethoxyiodoamphetamine)
Diethylcathinone
Diethyltryptamine
JWH-018
JWH-073
JWH-200
MDPV (methylenedioxypyrovalerone)
Mephedrone
Methoxyphenamine
Methylone
N-Ethylamphetamine
N-Ethylcathinone
N,N-Dimethylamphetamine
N,N-Dimethyltryptamine
TFMPP (N-(α,α,α-trifluoromethylphenyl)piperazine)

Note: Method validation at FSSA has verified that this methodology is suitable for detection of DFSA agents in hydrolysed urine at concentration at or below those recommended by the Society of Forensic Toxicologists.

Appendix D – Detailed methodology for quantification of detected substances

For all quantitative methods (hairs, opiates, amphetamines, THC), quality control (QC) samples were prepared by another analyst and were analysed at the beginning and end of a run sequence to check for a) correct preparation of standards and b) drift of instrument response throughout the run sequence. QC results should be within 20% of the nominal value to report an accurate result for validated methods, or to use 2 significant figures in the concentration for methods where validation was in progress.

For all quantitative results, samples were analysed in duplicate and each duplicate result had to be within 12% of the mean of the duplicates to be accepted. The average result was reported.

Quantification of Amphetamines in biological samples by liquid chromatography-mass spectrometry (LC-MS)

Amphetamines are potent psychomotor stimulants and may be sniffed, swallowed or injected. They induce exhilarating feelings of power, strength, energy, self-assertion, focus and enhanced motivation. The need to eat or sleep is diminished. This method covered the detection, confirmation and quantification of amphetamine, methamphetamine, ephedrine, pseudoephedrine, phentermine, mephentermine, MDMA (methylenedioxymethamphetamine, or ecstasy), MDA (methylenedioxyamphetamine), PMA (paramethoxyamphetamine) and phenylephrine. The quantification ranges for these drugs were 0.01–0.50mg/L. If the concentration was above this range, the sample was diluted and reanalysed. Results below 0.01mg/L were reported as negative. Urine samples were reported as ‘detected’ or ‘not detected’.

Sample preparation was performed using solid-phase extraction with mixed-mode UCT XTRACT cartridges (200mg/3mL) with subsequent analysis performed on an Agilent 1200 liquid chromatograph coupled to an Applied Biosystems 4000Q-Trap mass spectrometer. The LC column used was a Phenomenex Luna PFP 3um, 50mm x 4.6mm with PFP 5um, 4mm x 2.0mm guard column. A reverse phase gradient elution with 0.1% formic acid and methanol as mobile phases was used. The mass spectrometer was used in multiple reaction monitoring mode (MRM) with 3 MRMs obtained for each drug. Deuterated internal standards were also used.

Quantification of Codeine and Morphine in biological samples by LC-QTOF

Morphine is the principle alkaloid of opium. It is the main narcotic analgesic used for relief of moderate to severe pain. Diamorphine (heroin), hydromorphine and oxymorphone are closely related morphine derivatives and all are liable to produce dependence. Morphine can be found in the blood from administration of morphine itself, heroin which is rapidly metabolised to monoacetylmorphine (MAM) which is then metabolised to morphine, or as a metabolite of codeine. Codeine is much less liable to produce dependence. Its potency is considered 1/10 to 1/6 as potent as morphine. This assay incorporated the extraction, confirmation and quantification of morphine and codeine in biological samples.

The analytes were extracted using mixed-mode solid phase extraction with mixed-mode UCT XTRACT cartridges (200mg/3mL) with subsequent analysis performed on an Agilent 6520 QTOF LC-MS. The LC

column used was an Agilent Extend C18 4.6mm x 50mm column with a C18 4mm x 3mm guard column. A reverse phase gradient elution with pH 9 20mM ammonium formate buffer and methanol as mobile phases was used. The QTOF was used in both MS scan and targeted MS/MS modes using accurate mass measurements. The concentration range for the method was 0.01–0.6mg/L. If the concentration was above this range, the sample was diluted and reanalysed. Results below 0.01mg/L were reported as negative. Urine samples were reported as 'detected' or 'not detected'.

Quantification of THC and THC-COOH in biological samples by LC/QQQ

Δ9-tetrahydrocannabinol (Δ9-THC, or THC) is the most active constituent of marijuana (Cannabis sativa) and is contained in various parts of the plant in amounts that vary from only traces to as high as 12% by weight. Blood Δ9-THC concentrations reach a maximum a short time after cannabis use and then decrease rapidly. Low concentrations of Δ9-THC may be detected for up to a day following Cannabis use depending on dose and frequency of use. 11-nor-9-carboxy-Δ9-THC (Δ9-THC-COOH, or THC-COOH) is the major metabolite of Δ9-THC in blood and urine. It may be detected in blood for up to several days and urine for up to several weeks after cannabis use. The lower limit of quantitation (LOQ) for this method was set at 2ug/L (0.002mg/L) for THC and 5ug/L (0.005mg/L) for THC-COOH and was shown to be linear up to 150ug/L for THC and 300ug/L for THC-COOH. THC is not excreted to any appreciable extent in urine.

The blood and urine samples underwent sample preparation using liquid–liquid extraction using a pH 4 potassium dihydrogen phosphate buffer and 20% ethyl acetate in hexane. Analysis was performed on an Applied Biosystems 4000Q-Trap with and Agilent 1200 LC system. The LC column used was an Agilent XDB-C18 1.8um 4.6mm x 50mm fitted with a Phenomenex 3mm x 4mm Gemini guard column. A reverse phase gradient elution with 0.1% formic acid and methanol was used. The mass spectrometer was used in MRM mode, with 3 MRMs obtained for each drug. Deuterated internal standards were also used.

Appendix E – Table of symptoms and concerns reported by participants

Participant no.	Symptoms	Concerns
1	S1, S3, S6, S8, S10, S12	C2, C3, C4, C8
2	S3, S6, S8, S12, S17	C1, C2, C3, C4, C8
3	S1, S2, S3, S4, S5, S6, S7, S10, S11, S12, S15	C6
4	S2, S8, S10, S11, S12, S13	C4, C7
5	S3, S6, S8, S11, S12	C4, C7
6	S3, S8	C1, C2, C4
7	S1, S2, S3, S6, S8, S10, S11, S12	C1, C2, C3, C4
8	S2, S5, S12, S17	C2
10	S1, S2, S3, S6, S7, S8, , S11, S12, S13, S14	C2, C4, C8, C9
11	S3, S6	C1, C2, C4, C8
12	S1, S3, S6, S8, S9, S11	C4, C6
13	S1, S2, S3, S4, S6, S7, S8, S11, S12, S13	C3, C4, C5, C6, C7
15	S7, S10, S11	C10
16	S2, S3, S6, S10, S11, S12, S13	C1, C2, C10
17	S1. S3, S5, S6, S9, S12, S15, S17	C10
18	S3	C2, C8, C9
19	S1, S3, S6, S8, S9	C4, C6, C7
20	S9	C1, C3, C4, C6
21	S3, S8, S9, S12, S13	C4, C6, C7
22	S1, S3, S8, S12	C3, C4
23	S1, S3, S11, S12, S16	C1, C2, C8
24	S1, S3, S6, S7, S10, S11, S12, S16	C1, C2, C4
25	S1, S2, S7, S8, S10, S11, S12	C2, C3, C4
26	S1, S2, S3, S4, S6, S8, S9, S12	C4, C7
27	S1, S3, S5, S6, S8, S12	C4
41	S1, S2, S3, S6, S10	C10
43	S1, S2, S3, S6, S7, S8, S10, S12	C1, C3, C4
44	S1, S3, S10, S12, S13	C3, C4
45	S1, S2, S3, S4, S5, S6, S7, S8, S10, S12	C1, C3, C4, C6, C7
46	S1, S2, S3, S6, S8, S10, S12, S13	C2, C4, C7, C8
48	S3	C9
49	S3	C4, C9

Code	Symptom
S1	Confusion
S2	Dizziness
S3	Loss of memory
S4	Impaired judgement
S5	Reduced inhibitions
S6	Drowsiness
S7	Lack of muscle control
S8	Black out
S9	Out of body experience
S10	In and out of consciousness
S11	Weakness
S12	Nausea
S13	Vomiting
S14	Low heart rate
S15	Hallucinations
S16	Aggression
S17	Other
C1	Clothing removed/dishevelled
C2	Vaginal/anal soreness
C3	Awoke in strange place
C4	Memory blank
C5	Memory blank only
C6	Informed re sexual intercourse
C7	Concerns re people in party/club/environment
C8	Vaginal discharge
C9	Other
C10	Memory of sexual assault