ANALYSIS OF POOLED ANONYMOUS URINE FROM A NIGHTCLUB USING UPLC® -TOF-MS^E

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INTRODUCTION

- **Recreational drug use is common in the UK, particularly amongst clubbers and others within the night-time economy.**
- **The British Crime Survey of 2010/2011 estimated that 8.8% of the adult population had used illicit drugs in the last year[1].**
- **An on-line survey, conducted over the same period by the dance magazine *MixMag*[2], showed significantly higher use in the population who frequent the night-time economy reporting that 50-75% of clubbers had used MDMA (Ecstasy), cocaine or mephedrone over the previous year.**
- **In a more recent survey of attendees at a London nightclub, 41% of those surveyed claimed to have used mephedrone over the last month.**
- **In a study designed to assess the feasibility of using pooled urine to confirm what drugs are currently being used, a series of samples were collected using an adapted portable urinal at a London nightclub[3].**
- **Samples were analysed using a variety of analytical techniques; this paper presents the results of screening using a method based on UPLC in combination with TOF-MS^E.**

METHODS

Sampling site and urine collection

Setting: A large south London nightclub catering predominantly for men who have sex with men (MSM; 'gay') in July 2011.
Urinal: A portable standalone four-person urinal; use was voluntary and anonymous—other standard toilets were also available within the club.
Samples: Pooled urine samples were collected from the urinal (using a manual vacuum pump) during two club promotions:

Event 1: Friday/Saturday (11 pm - 4 am)
Collection times: 2 am (sample #1),
3 am (sample #2) and 4 am (sample #3)

Event 2: Saturday/Sunday (11 pm - 10 am)
Collection time: 10 am (sample #4).

Sample preparation: Dilution 5-fold with mobile phase.

Instrumentation

Waters® ACQUITY UPLC® System in combination with the XEVO™ G2 QTOF Mass Spectrometer in MS^E mode (**Figure 1**).

Column:	ACQUITY UPLC HSS C18 column
Mobile Phases:	(A) Ammonium formate, pH 3 (B) Acetonitrile containing 0.1% formic acid
Run Time:	15 min gradient
Injection volume:	10 µL
Ionisation:	ES+
Acquisition mode:	MS^E mode. Collision energy ramped from 10 to 40 eV
Acquisition range:	*m/z* 50 — 1000
Resolution:	20,000

Data processing

POSI±IVE™ software was used in targeted analysis mode.
Data were matched to a database for 950 drugs/metabolites (Waters).

RESULTS AND DISCUSSION

Data was collected using a Waters XEVO G2 QTOF in MS^E mode; this involves the rapid alternation between two functions and provides the exact mass of the parent ion in addition to fragment ions for additional confirmatory purposes. Acquired data were compared to a comprehensive database, prepared under the same conditions, containing 950 drugs and metabolites. All substances have an associated RT, >65% of entries have additional fragment ions. Substance identification is thus based on retention time and an exact mass 'fingerprint' for each analyte, the latter comprising exact mass of the precursor ion and up to four fragment ions.

Pooled urine analysis

A total of 72 parent drugs and their metabolites were detected in the four samples. Detected drugs could be broadly divided into the following categories:

- Classical recreational drugs
- Novel psychoactive compounds
- Potential adulterants
- Prescription/over-the-counter medications

Each of the four samples contained several of the substances listed in **Table 1**. Detection of the metabolites confirmed presence of drugs that were actually being used and metabolized by individuals rather than measuring unused drug materials that had simply been discarded into the urinal.

A number of potential adulterants were also detected in the samples these were: diliazem, levamisole, caffeine, lidocaine and quinine.

Group	*Drug*	*Metabolite(s) detected*
Classical	Amphetamine	+
	Cocaine	+
	Ketamine	+
	Methamphetamine	
	Morphine	
	MDMA	+
	GHB/GBL[#]	
Novel	Mephedrone (4-MMC)	+
	NRG-2 (4-MEC)	
	2-AI	
	TFMPP	+

Table 1. *Classical and novel psychoactive substances found in the four pooled urine samples.*
[#]Analysed by GC/MS.

Prescription/over-the-counter medications included anti-depressants, benzodiazepines and other sedatives, anti-histamines, anti-malarials, anti-virals, nasal decongestants, analgesics and proton pump inhibitors.

Figure 2 shows the screening results for one of the urine samples following MS^E analysis and database matching. Sample #4 showed many of the same drugs as detected in the samples from the previous event but also screened positive for TFMPP and 4-methylethcathinone (4-MEC; NRG-2). **Figure 3** below shows the data for this latter substance.

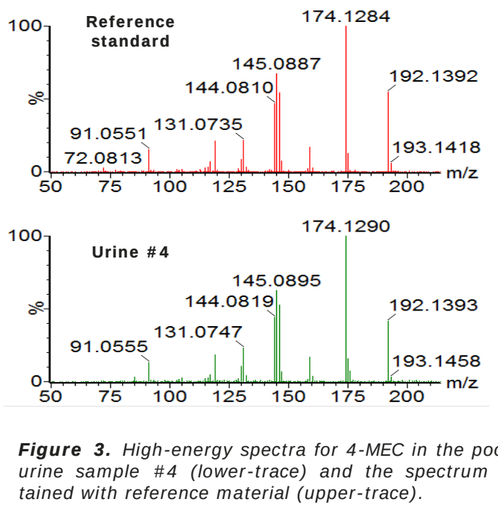


Figure 3. *High-energy spectra for 4-MEC in the pooled urine sample #4 (lower-trace) and the spectrum obtained with reference material (upper-trace).*

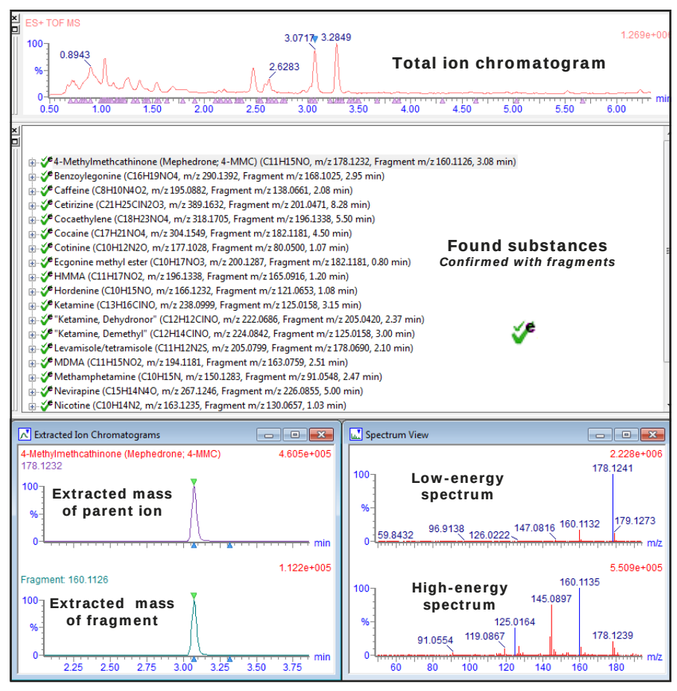


Figure 2. *Nightclub urine sample #2 showing presence of numerous illicit substances including: mephedrone (4-methylmethcathinone); amphetamines (including MDMA, MDA, methamphetamine); cocaine and metabolites; ketamine and metabolites in addition to paracetamol, levamisole and the anti-retroviral drugs, atazanavir and nevirapine.*

CONCLUSIONS

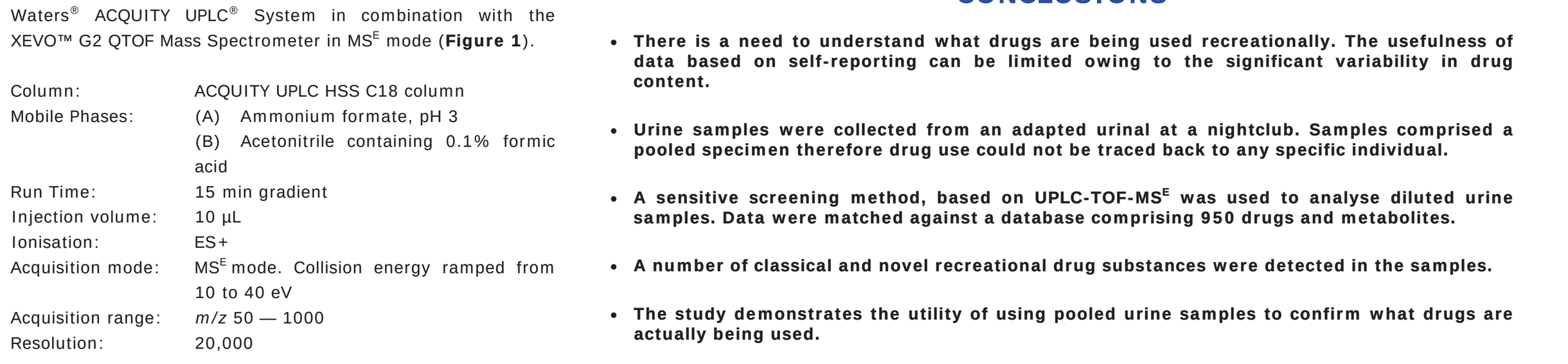
- **There is a need to understand what drugs are being used recreationally. The usefulness of data based on self-reporting can be limited owing to the significant variability in drug content.**
- **Urine samples were collected from an adapted urinal at a nightclub. Samples comprised a pooled specimen therefore drug use could not be traced back to any specific individual.**
- **A sensitive screening method, based on UPLC-TOF-MS^E was used to analyse diluted urine samples. Data were matched against a database comprising 950 drugs and metabolites.**
- **A number of classical and novel recreational drug substances were detected in the samples.**
- **The study demonstrates the utility of using pooled urine samples to confirm what drugs are actually being used.**
- **The technique has the potential to be developed to utilise free-standing urinals to establish trends in drug use across different geographical areas, over time and different days of the week.**

References

1.Drug misuse declared: findings from the 2010/2011 British Crime Survey. HOSB (2011). Smith K, Flatley J. http://www.homeoffice.gov.uk/publications/science-research-statistics/research-statistics/home-office-science/consult-drug-misuse-12?view=Standard&pubID=1033970 (last accessed 25th May 2012).

2.Drugs survey. Winstock A. MixMag 238: 50-59 (2011).

3.Taking the pissoir-a novel way of knowing what drugs are being used in the nightclubs. JRH Archer, Dargan PL, Hudson S, Davies S, Puchnarewicz M, Kicman AT, Ramsey J, Measham F, Wood M, Johnston A, Wood DM. submitted to British Medical Journal (In preparation, May 2012).



Compound	Formula	RT	Fragment ions	
4-Fluoroamfetamine (4-FA)	C9H12FN	2.6	f:109.0454	f:137.0767

Figure 1. MS^E analysis concept. *Data shows analysis of a 4-Fluoroamphetamine (4-FA) reference standard for appending to database. With MS^E, full exact mass is collected simultaneously under low and high-energy conditions (**panel A**). Fragmentation of the parent molecule occurs within the T-Wave collision cell of the instrument (**panel B**). Low (lower-trace) and high energy (upper-trace) spectra are always available for every component (**panel C**). The structure of observed fragment ions are verified prior to their addition into the database along with retention time (RT) and elemental formula for automatic determination of the exact mass of the parent molecule (**panel D**).*