AN ALTERNATIVE WORKFLOW FOR DRUG ANALYSIS

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INTRODUCTION

- **The increase in number, diversity and potential toxicity of drugs is a major concern; it also presents significant challenges for forensic laboratories who are involved in the analysis of seized substances and need to turnaround results quickly**
- **Guidelines stipulate that two independent tests are required for seized drug analysis[1]; traditionally a screen *e.g.,* colorimetric or TLC, followed by a confirmatory technique *e.g.,* GC-MS. However, these workflows can lead to bottlenecks and backlogs, therefore analytical methods that can provide rapid, reliable results are of interest**
- **The aim of this study was to assess the potential of an alternative workflow comprising a rapid screen based on Atmospheric Solids Analysis Probe-Mass Spectrometry (ASAP-MS), followed by UPLC-Time of Flight-Mass Spectrometry (UPLC-TOF-MS), for confirmatory analysis (Figure 1)**

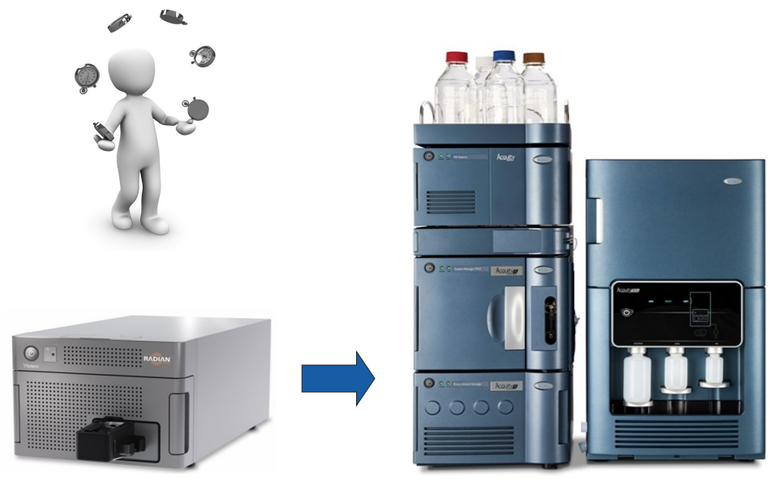

Figure 1. Workflow using Waters™ Radian™-ASAP™-MS and Waters™ ACQUITY™ I-Class UPLC™ and TOF-MS

SAMPLES AND PREPARATION

- Certified reference material (CRM) were analysed using ASAP-MS
- Seized materials (tablets, pills, powders), from music festivals and pharmaceuticals, were analysed using both techniques. Seized materials were prepared by dissolving in methanol:water (50:50 v/v). These samples were further diluted 1:20 with 100% methanol, prior to analysis using ASAP-MS
- The 1:20 methanolic solutions were further diluted (1:1000) with mobile phase A and subsequently analysed by the UPLC-TOF-MS confirmatory method (Table 1)

EXPERIMENTAL

Screening with ASAP-MS

The system comprises a RADIAN™-ASAP (Figure 2) with LiveID™ for data processing

- Direct MS analysis (separation without chromatography), is performed by the process of ASAP ionisation (Figure 3). The process involves the volatilisation of the sample with the use of a heated desolvation gas and a corona discharge for ionisation, typically resulting in the generation of protonated species
- The application of four cone voltages (15, 25, 35, 50 V), in positive ionisation mode, generates fragmentation by in-source collision-induced dissociation (CID)[2]. The combination of the precursor and the generated fragment ions provide a spectral fingerprint for each analyte, thus increasing specificity (Figure 4)
- LiveID compares the acquired spectral data against a prepared reference library; this matching can be performed in near real-time with a result provided in seconds. For a positive identification, a minimum match score of 850 (from a maximum 1000) was applied using a reverse fit model

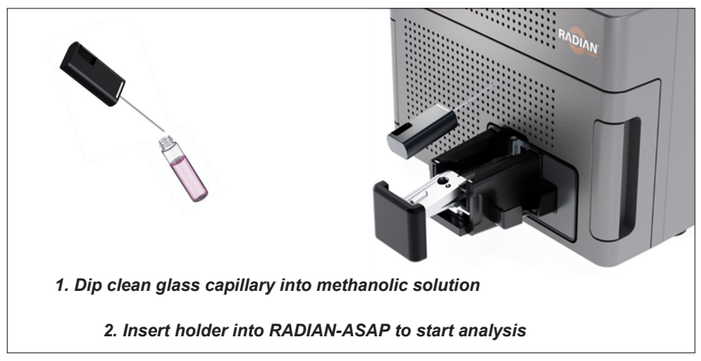

Figure 2. Data acquisition using the RADIAN-ASAP (Waters)

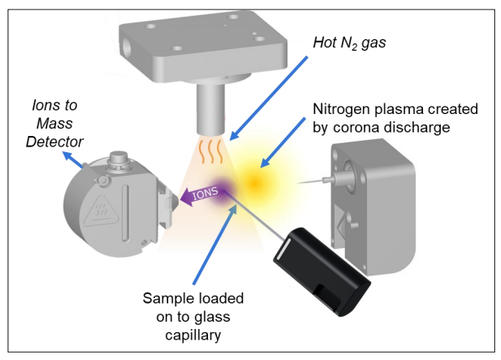

Figure 3. ASAP-MS ionisation process

Confirmation with UPLC-TOF-MS

The system comprises an ACQUITY™ UPLC™ I-Class™ and an ACQUITY™ RDa™ with waters_connect™ (UNIFI™) informatics

- Chromatographic separation was achieved in 9.5 minutes and accurate mass data was acquired in full scan with fragmentation in positive ionisation mode[3] (Table 1)
- This acquisition mode involves the simultaneous collection of data under two energy conditions; the low energy (function 1) provides accurate mass of the precursor ion while the elevated energy (function 2) leads to the generation of specific accurate mass fragment ions for additional confirmatory purposes
- Resultant data were compared with a library comprising >100 drug substances. Identification was based on retention time (±0.35 minutes of reference retention time), detection of a precursor mass, and the presence of at least one diagnostic fragment ion

Column (Temp.)	ACQUITY UPLC HSS T3, 2.1×100mm (45°C)
Mobile Phase A	5mM ammonium formate pH 3.0
Mobile Phase B	Acetonitrile with 0.1% formic acid
Analysis Time	9.5 minute gradient elution
Ionisation Mode	ESI Positive
Acquisition Range	*m/z* 50 - 2000
Acquisition Mode	Full scan accurate mass (with fragmentation)
Fragmentation Cone Voltage	70 - 130 eV

Table 1. Summary of LC/MS conditions used with ACQUITY RDa

RESULTS AND DISCUSSION

ASAP-MS and UPLC-TOF-MS were used for their ability to rapidly screen and confirm, respectively, potential drug substances in seized materials

Overall, there was excellent qualitative agreement with concordance

- Performance of ASAP-MS was initially evaluated with the analysis of CRM. All match scores were >870, with sensitivity and specificity both >95% for identified analytes
- Analysis of the seized materials, resulted in 93% of samples obtaining a match score >850 for one or more components. A total of 74 positive identifications of 7 substances were made from the 80 seized samples tested, and are shown in Table 2
- Confirmation analysis of the seized materials with UPLC-TOF-MS, resulted in 95% of samples matching one or more components to the library. A total of 79 positive identifications of 8 substances were made from the 80 seized samples tested, and are shown in Table 2
- Figure 5 summarises the substances and their identification frequency, in the seized materials tested, for the two analytical techniques
- Both techniques were negative for the same samples tested

There were a small number of discordant results:

- One discordant result was owing to differences in the content of the respective reference libraries, sildenafil, see Table 2
- Four samples tested positive by UPLC-TOF-MS, but were initially classed as negative by ASAP-MS. This discordance was owing to the difference in sensitivity of detection, as their match scores were below the minimum threshold applied during processing for the rapid screen
- Two of the pharmaceutical preparations did not yield any match with either analytical technique but gave a visible peak by UPLC-TOF-MS. The data was submitted for structural elucidation and returned a proposed component. This was subsequently confirmed through analysis of relevant CRM

MDMA
Ketamine
Cocaine
2C-B
Flualprazolam
Paracetamol
Caffeine
Sildenafil*

Table 2. List of compounds identified using both techniques

Sildenafil identified by the UPLC-TOF-MS only*

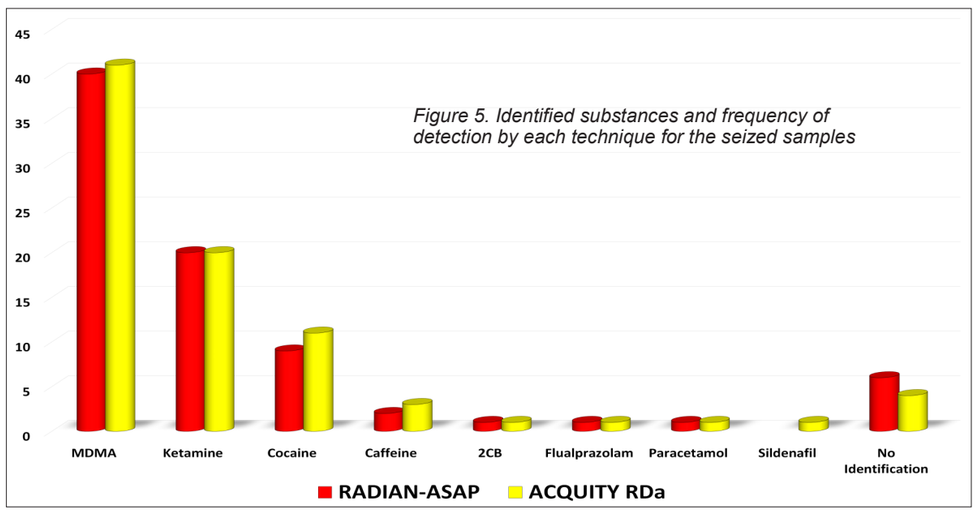

Figure 5. Identified substances and frequency of detection by each technique for the seized samples

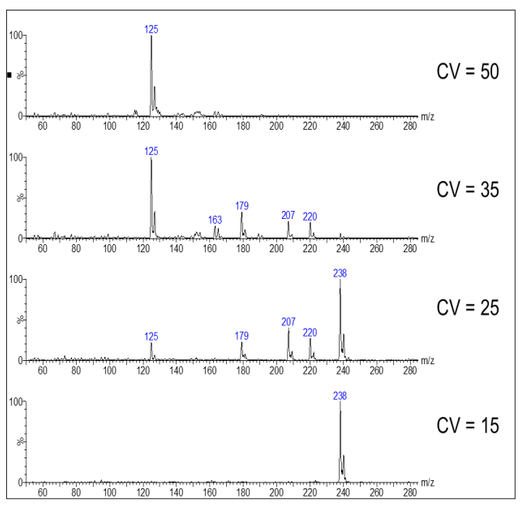

Figure 4. Spectral fingerprint generated by ASAP-MS for Ketamine CRM

CONCLUSION

- **ASAP-MS is an easy-to-use, rapid and accurate direct MS screening technique. Identification of single or multiple components in seized materials was provided within seconds and shows potential for a rapid triage of samples to improve laboratory workflow**
- **UPLC-TOF-MS was used for confirmation in this study and has been proven to be a powerful complementary technique, offering multi-analyte detection and identification, with the added ability to identify unknown substances through structural elucidation**
- **ASAP-MS and UPLC-TOF-MS are two adaptable techniques that can be used for seized drug analysis, either in combination or as standalone systems**

References

1. Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), www.swgdrug.org/
2. M. Wood; RADIAN ASAP with LiveID-Fast, Specific, and Easy Drug Screening. Waters Application Note Library Number, 720007125EN
3. M. Wakefield and E. Todd; Seized Drug Screening using the ACQUITY RDa Detector. Waters Application Note Library Number, 720007097EN

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