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Application Note

Analysis of Drug-infused Papers by RADIAN™ ASAP

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This is an Application Brief and does not contain a detailed Experimental section.

For Forensic Use Only

Abstract

It has been reported that UK prisons have had a significant issue with drug misuse for decades, which causes increased levels of violence, has a negative effect on rehabilitation and puts increased strain on medical resources.[1] Although traditional illegal drug use persists in prisons, the use of novel psychoactive substances (NPS) has dramatically increased over the past few years, in particular the use of synthetic cannabinoids.[2]

Recently there have been reports of paper and other materials infused with drugs being smuggled into UK prisons.[1] Reducing drug supply to prisoners is a crucial part of minimizing drug use and therefore the negative effects that drug use in prison causes.[2] An effective method of testing materials received by inmates, may greatly assist in this process.

This study assesses the potential of using the RADIAN ASAP Mass detector, a compact device based on Atmospheric Solids Analysis Probe-Mass Spectrometry (ASAP-MS), as a simple, yet rapid, screening tool for drug infused paper. Paper samples (infused and drug free) were extracted, and ASAP-MS analysis was performed acquiring data at four differing cone voltages to generate data for both precursor and product ions.

Data was processed using LiveID™ 2.0 Software which matches acquired data to a spectral library.

Benefits

- Simple and easy-to-use
- Direct analysis (no chromatography)
- Minimal sample preparation
- Enhanced specificity by incorporating fragmentation data
- Rapid analysis. Real-time library matching with LiveID 2.0
- Compact benchtop instrument

Introduction

Drug misuse within UK prisons is prevalent and a major concern; it has been reported to contribute to increased levels of aggression and violence amongst inmates and putting an increased strain on valuable medical resources, as inmates suffer adverse effects or injury.[1,3] Therefore, drug misuse has a negative effect on the stability, security, and the overall effectiveness of the penal system.

It has been reported that prisons in England and Wales have had a problem with illegal drugs for decades; a 2010 survey of prisoners suggested that 30% had used cannabis in prison, more than a fifth had used heroin and a tenth had used cocaine.[2] While traditional illegal drug substances continue to be widespread in prisons, the emergence of potent NPS have significantly exacerbated the issue. It has been reported that the use of NPS is widespread, with estimates from 60% to 90% of the prison population in England and Wales.[2] In particular, synthetic cannabinoids, which mimic the psychoactive effects of cannabis, are especially popular among prisoners with these compounds being highlighted as a concern in 64% of men's prisons in England.[3] NPS substances are not easily detected by traditional screening methods as they are chemically distinct, can vary and will change over time; this presents analytical challenges for detection systems and laboratories assigned with this testing.[1]

In recent years it has been reported that paper and other materials infused with drugs have been smuggled into UK prisons, including letters to inmates impregnated with drugs including NPS such as etizolam and synthetic

cannabinoid receptor agonists.[1,3] There have been reports of observations by prison staff of prisoners licking, chewing, and smoking their letters.[3] Reducing inmates access to drugs is a key consideration in the overall strategy to minimize drug use in prisons by tackling supply and demand. An effective screening method of testing materials received prior to release to inmates, may both reduce drug use and act as a deterrent.

RADIAN ASAP, a small footprint system from Waters that combines the simplicity of ASAP with the specificity of MS, has previously demonstrated promise as a rapid screening technique for the analysis of seized drug samples.[4] The aim of this study was to assess the potential of using the RADIAN ASAP mass detector as a simple, yet rapid, screening tool for drug infused paper.

Experimental

Materials and Sample Preparation

Certified reference material for 17 drug substances were obtained from either Merck Life Science (Dorset, UK) or Cayman Chemical (Michigan, USA). CRM was typically used at the supplied concentration of 1 mg/mL in methanol (or acetonitrile) or diluted to 0.2 mg/mL in methanol.

The paper samples that were used in this study were: 80 gsm white paper, newspaper, greetings card, envelope, and "glossy" magazine, and included samples that were free from ink and included ink. Two alternative methods were used to infuse the papers with common drug substances for one minute:

- Pipetting method – A 50 µL aliquot of the CRM (0.2 mg/mL and 1 mg/mL) was pipetted onto 1x1 cm pre-cut paper squares, which were then placed on a glass tile for 30 minutes to dry
- Soaking method – Paper samples cut into 4x4 cm sections were placed in a 100 mL beaker with 4 mL of diluted CRM (1 mg/mL in methanol), which were then placed on a glass tile for 30 minutes to dry. Once dry, a 6 mm hole-punch was used to take samples from the larger square

Drug-free papers were prepared by the same two methods, using methanol-only in place of reference material.

Infused and drug-free paper samples prepared using both preparation methods were extracted using the following method:

- Pre-cut square samples or hole-punched samples were placed into individual screw cap glass vials with 500

µL of methanol and sonicated

- Following sonication, the solvent was transferred to a clean screw cap vial

RADIAN ASAP Analysis

Sampling procedure - 'dipping' method

For each sample a new glass capillary was selected and cleaned using the automated RADIAN ASAP bakeout procedure that is provided within the software. A 'dipping' method was used for each sample ***i.e.***, the cleaned capillary was held just below the surface of the liquid sample to a depth approximately 1 cm for 5 seconds, after which the capillary was placed into the holder and inserted into the RADIAN ASAP source. The parameters used in the analytical method used to acquire data are shown below in Table 1. For this study, each sample was analysed in triplicate (the same glass capillary was used for three cycles of 'dip and detect').

Analytical Method

Parameter	Setting
Ionization mode:	ASAP+
Corona pin:	3 µA
Desolvation gas and temperature:	Nitrogen at 600 °C
Cone voltage:	15, 25, 35, 50 V
Acquisition mode:	Full scan MS over the range *m/z* 50–600 – continuum mode
Scan speed:	5 Hz

Table 1. Analytical parameters used to acquire data using the RADIAN ASAP.

Data processing with LiveID 2.0

Data was processed using LiveID 2.0 library matching software, which enables real-time library matching or post-acquisition processing of data files. LiveID software compares the acquired spectral data against a prepared reference library using a reverse fit model. LiveID calculates an average match score considering all four cone voltages (maximum is 1000), for this study a match score of 850 or greater was used as a threshold for indication of a positive identification.

Results and Discussion

ASAP-MS provides a direct analysis technique yielding mass spectrometry data without chromatographic separation; this is performed by the process of ASAP ionization. The process involves the volatilization of the sample loaded onto the glass capillary using heated nitrogen gas and subsequent ionization via a corona discharge.

For all substances evaluated in this study, ionization resulted in protonation $[M+H]^+$ of the analyte. Mass detection was performed using full scan over the range *m/z* 50–600. Four cone voltages (15, 25, 35, 50 V) were applied, to generate fragmentation by in-source collision-induced dissociation (CID). The combination of precursor and the generated fragment ions provide a spectral fingerprint for each analyte, thus increasing specificity and accuracy of drug identification. Figure 1 shows data for the CRM for Ketamine and illustrates the mass spectrometry information that can be generated using this technique.

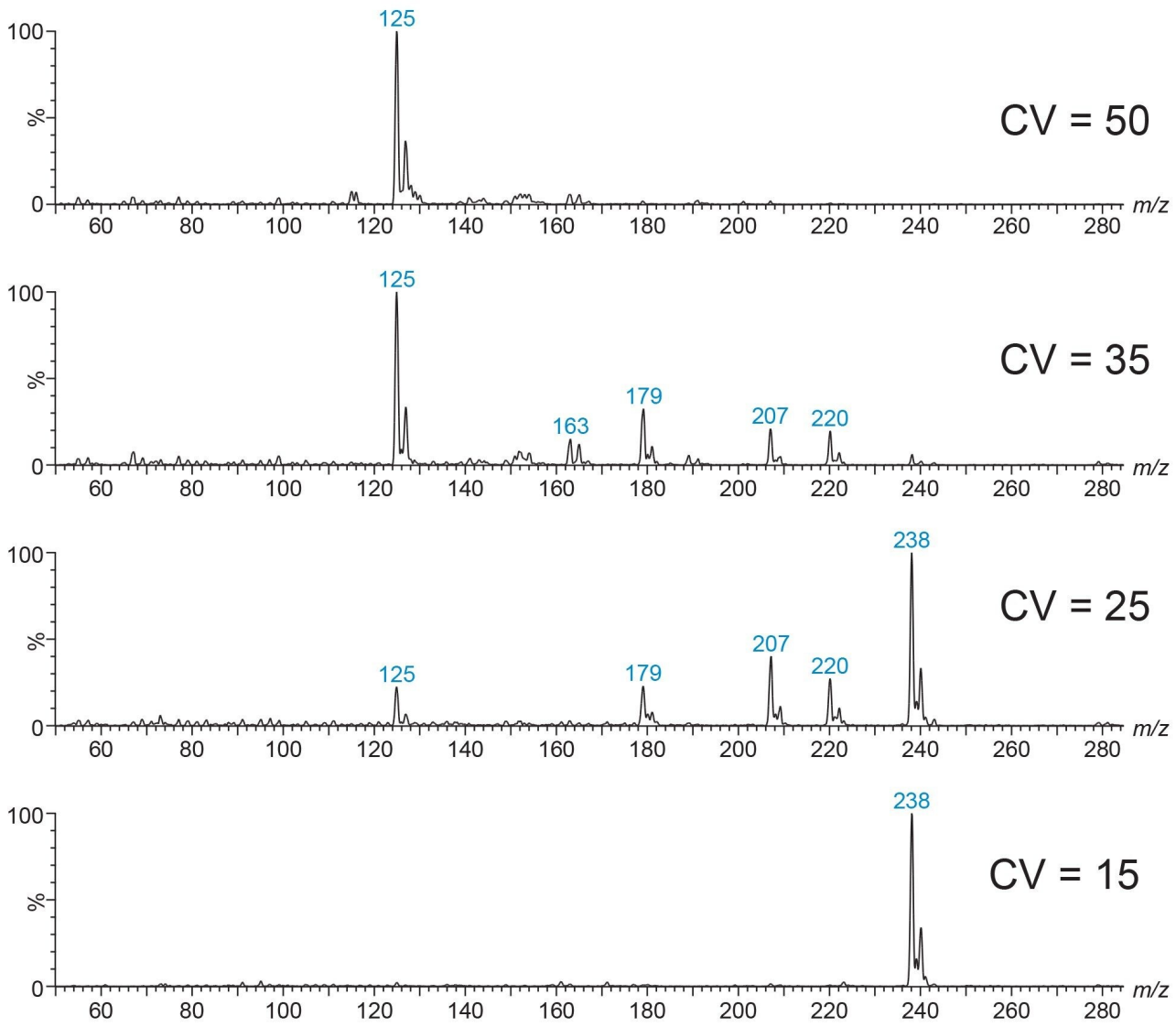

Figure 1. RADIAN ASAP analysis of Ketamine CRM. Data is collected at four cone voltages to generate a spectral fingerprint. The lowest cone voltage (15 V) typically contains the ionized precursor molecule, in this example ASAP ionization resulted in the generation of the $[M+H]^+$ species at m/z 238.

Paper samples (white paper, 80 gsm) were prepared in triplicate, by spiking individually with 17 common drug substances at 1 mg/mL, using the pipetting method. Samples were extracted for five minutes and then analyzed and the mean match score of the three 'dip and detect' analyses were calculated. All drugs were correctly identified with mean match scores ranging from 853 to 996 (Figure 2). The drug-free blank papers, which were

treated with methanol only, did not result in any library match scores greater than 850 and were therefore deemed negative.

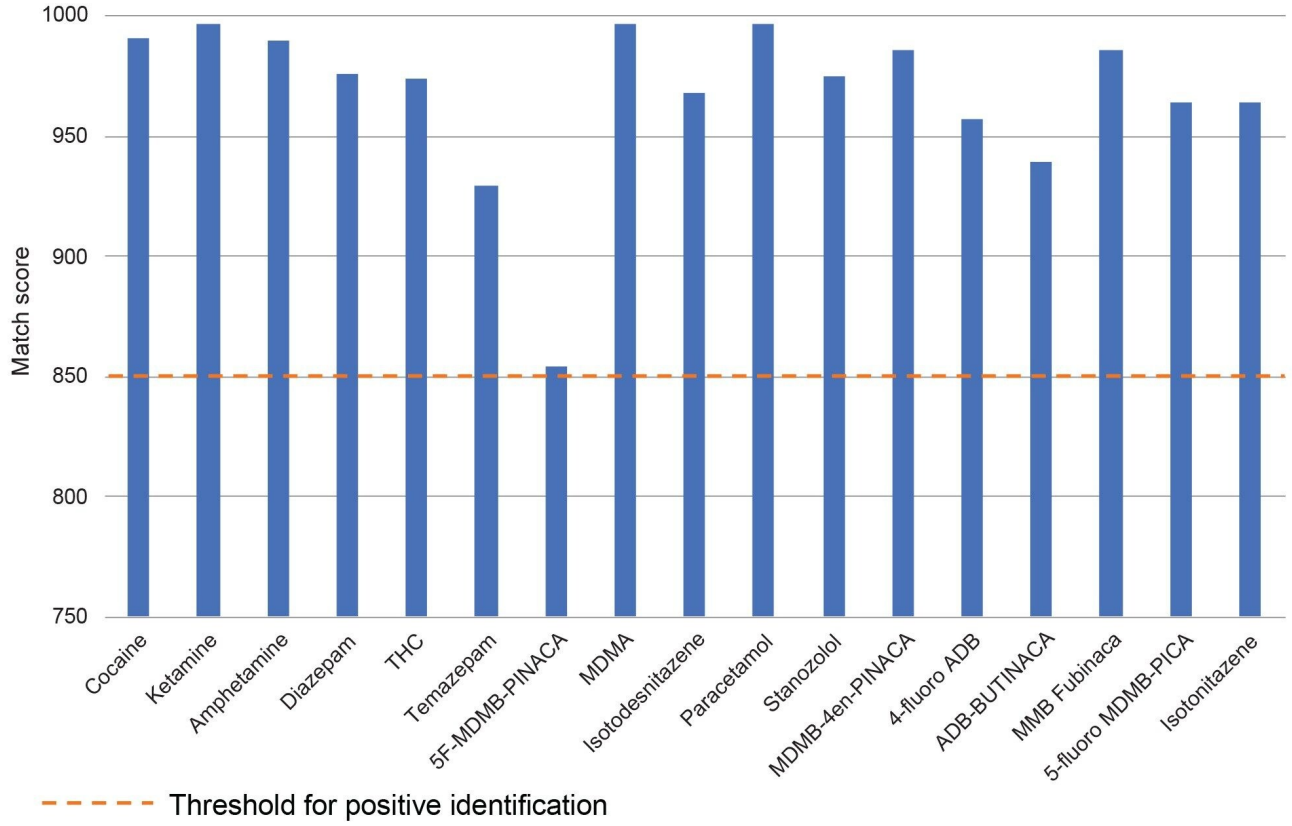

Figure 2. Average match scores for spiked paper samples (n=3) infused with 1 mg/mL methanolic solutions of various reference materials, using a 5-minute extraction time.

In addition to the common drugs of abuse, several NPS were also included in this study; these substances are of particular interest, as the use of NPS in prison communities has been reported to be endemic.[3] Figure 3 illustrates an example of typical LiveID data obtained for a paper sample infused with the synthetic cannabinoid 5-Fluoro MDMB-PICA; for this sample a high confidence match score of 969 was obtained.

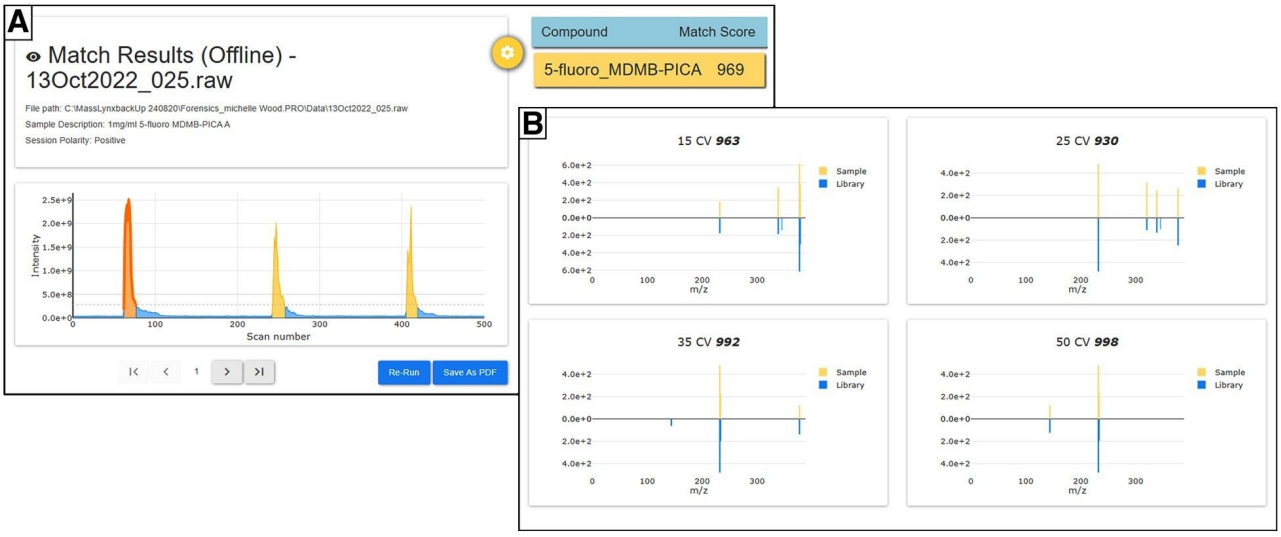

Figure 3. LiveID analysis of a sample prepared, infused with 50 uL of 5-Fluoro MDMB-PICA using the pipetting method. Panel A shows three "dip and detect" paper replicates for the infused sample and the match score 969 (maximum 1000) obtained for the first replicate. Panel B displays the detail for this spectral match; all four cone voltages are used in the identification process with a weighted mean (lowest cone voltage with the highest weighting) used.

In this study, samples at a lower concentration were also assessed; 6 samples were infused with individual drugs at 0.2 ng/mL. These samples resulted in slightly lower match scores than those infused with 1 mg/mL, however the match scores obtained still exceeded the threshold used for positive identification.

For the extraction method, different sonication times were evaluated; 80 gsm white paper samples that had been initially spiked with drugs using both methods were sonicated for 5, 10, and 15 minutes. The increased sonication times showed no significant difference in the library match scores which were obtained, therefore, to reduce the overall analysis time a 5-minute sonication time was subsequently applied.

For improved ease and consistency of sampling, the use of a hole-punch was also assessed. The match scores for the hole-punched samples were lower than those obtained using the 1x1 cm square sample, but still exceeded the minimum 850 threshold. It is likely that the lower responses are reflective of the smaller surface area sampled, however, both sampling techniques were found to be suitable for this analysis.

As initial testing was based on in-house spiked paper samples which were free of ink, the study was extended to

include evaluation of differing paper types, thickness, and the potential effect on spectral data in the presence of inks. Paper samples that contained inks, included ballpoint pen (plain paper and greeting card), printed text (newspaper and 'glossy' magazine) and printed image (greeting card). The 5-minute extraction method was confirmed to be suitable for all paper types tested, with no significant difference in match factors when compared to results from 80 gsm white paper. Furthermore, the presence of inks was found to not have a significant effect on the match scores obtained. This is advantageous as materials received by inmates are likely to contain some form of ink, for example letters and children's drawings.[3]

For most of the samples tested, a match against a single substance was returned. However, some exceptions were noted, for example, isotonitazene which was identified with a mean score of 964 also resulted in a match score >850 for its isomer protonitazene. In these instances, the compound that the papers were infused with was always detected with a match score greater than the positive threshold. This is not of great concern, as the procedure detailed is intended for use as a preliminary fast screening technique, and differentiation may be achieved if required, using a confirmatory method such as LC-MS/MS. In our laboratory for example, isotonitazene and protonitazene can be differentiated by retention time using LC-MS/MS.[5]

Conclusion

RADIAN ASAP is an easy-to-use, rapid, and accurate direct mass spectrometry screening technique, which provides mass spectral data directly, without the requirement for chromatographic separation. The technique has shown promise as a simple screen for common illegal drugs and NPS (including synthetic cannabinoids and synthetic opioids) infused into paper samples.

The extraction method is both quick and simple and has been demonstrated to be efficient for differing paper-types, thicknesses, and treatments. Sampling paper by use of a hole-punch further simplifies the sample preparation process. RADIAN ASAP and LiveID 2.0 library matching takes less than two minutes for each sample. The presence of inks and other treatments did not appear to interfere with the detection of drug substances.

This technique shows promise to be an effective screening tool to both reduce access to drugs in prisons and act as a deterrent. To further evaluate the feasibility of this application to screen drug-infused papers, future work is planned to analyze authentic seized papers.

References

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