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Nota de aplicación

# Seized Drug Screening with RADIAN™ ASAP

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*For forensic toxicology use only.*

Este es un resumen de la aplicación y no contiene una sección experimental detallada.

## Abstract

Illicit drug use and trafficking causes harm, instability, and violence; analysis of seized samples significantly supports programs which aim to control the use, trafficking, and distribution of illegal drug substances.[1] The increase in the number of seized samples requiring analysis has placed pressure on forensic drug laboratories to provide reliable results quickly.

Traditional workflows used by these laboratories consist of a presumptive screen followed by confirmatory analysis. The screening analyses typically used can cause sample bottlenecks and backlogs, as they can be time consuming, lack selectivity or result in a high number of false positives. Therefore, screening techniques which are robust, fast, and efficient are of considerable interest to forensic drug laboratories.

The RADIAN ASAP has already demonstrated great promise as a rapid, accurate triage of seized drug samples.[2] This study further evaluates the suitability of using RADIAN ASAP for the screening analysis of seized drug samples.

## Benefits

- Simple and easy-to-use with minimal sample preparation

- Direct analysis (no chromatography)
- Enhanced specificity by incorporating fragmentation data
- Rapid analysis with real-time library matching using LiveID™ 2.0
- Compact benchtop instrument

## Introduction

The analysis of seized drug samples plays a vital role in the effectiveness of national and international programs which aim to control the use, trafficking, and distribution of illegal drug substances. Drugs can be seized from many places, including night-time venues, music events and prisons, by police forces and border control. The increase in number of samples seized and submitted for analysis along with diversity and potential toxicity of drugs, places a huge burden on drug control and forensic chemistry laboratories to produce results quickly.

Industry guidelines, such as those set by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) provide recommendations for the minimum analytical requirements required for laboratories to provide a reliable and scientifically supported identification of a drug in a seized sample.[3] At a minimum, an analytical workflow should consist of at least two independent techniques. Techniques are categorized based on the level of selectivity, with Category A being the highest level of selectivity and Category C being the lowest (Figure 1). Traditional workflows usually comprise of presumptive screening analysis such as TLC, FTIR, or colorimetric tests; followed by confirmatory analysis from a more selective method such as gas chromatography in combination with mass spectrometry (GC-MS). However, TLC analysis can be time-consuming, FTIR results lack selectivity with mixtures and colorimetric tests are not available for all drugs or result in a high rate of false positives. This can result in an increase in samples requiring analysis by GC-MS, which can lead to sample bottlenecks and backlogs. Consequently, screening techniques which are easy-to-use, robust, fast, and efficient, and can be quickly updated are of interest to forensic drug laboratories.

| | |
|---|---|
| **Category A**<br><br>**(Selectivity through structural information)** | Infrared spectroscopy |
| | Mass spectrometry |
| | Nuclear magnetic resonance spectroscopy<br>Raman spectroscopy |
| | X-Ray diffractometry |
| **Category B**<br><br>**(Selectivity through chemical and physical characteristics)** | Capillary electrophoresis |
| | Gas chromatography |
| | Ion mobility spectrometry |
| | Liquid chromatography |
| | Microcrystalline tests |
| | Supercritical fluid chromatography |
| | Thin layer chromatography |
| | Ultraviolet/visible spectroscopy |
| | Macroscopic examination (cannabis only) |
| | Microscopic examination (cannabis only) |
| **Category C**<br><br>**(Selectivity through general or class information)** | Color tests<br>Fluorescence spectroscopy |
| | Immunoassay<br>Melting point |
| | Pharmaceutical identifiers |

*Figure 1. Examples of analytical techniques applied for seized drug analysis which include instrumental and non-instrumental tests. Category A techniques have the highest selectivity; if a Category A technique is not used, a minimum of three tests must be applied, two of which must be from Category B.*

RADIAN ASAP, a small footprint system from Waters that combines the simplicity of Atmospheric pressure Solids Analysis Probe (ASAP) with the specificity of MS, has previously demonstrated promise as a rapid screening technique for the analysis of seized drug samples. The aim of this study was to further assess the use of the RADIAN ASAP mass detector as a simple, yet rapid, screening tool for seized materials. Two hundred and twenty-nine unknown samples that had been confiscated at music events/night-time venues by police, were analyzed. The established Forensic Toxicology High-Resolution Mass Spectrometry (HRMS) Screening Solution was used for subsequent confirmatory analysis; this method incorporates a 15-minute chromatographic separation and analysis with the Xevo™ G3 QTof Mass Spectrometer.[4]

# Experimental

## Material and Sample Preparation

A series of unknown/suspect materials (n=229) confiscated at music events/night-time venues were supplied by the UK police. The samples were split into 23 groups based on seizure and appearance of sample, as shown in Table 1. Within the sample set there was two groups of powders and one group of capsules, all other samples were a variety of pills.

| Group | Material type | Number of samples | Appearance |
|---|---|---|---|
| 642 | Pill | 13 | Spherical blue pill |
| 124 | Pill | 6 | Yellow cartoon character |
| 170 | Pill | 7 | Brown/red square |
| 516 | Pill | 2 | Pale pink rectangle |
| 517 | Pill | 2 | Orange car logo |
| 537 | Pill | 3 | Fluorescent pink drink logo |
| 665 | Pill | 7 | White long rectangle |
| 663 | Pill | 24 | White long rectangle |
| 734 | Pill | 7 | Pink and white phone logo |
| 146 | Pill | 9 | Pink film character |
| 717 | Pill | 8 | Grey, one side skull, one side double PP |
| 147 | Pill | 15 | Beige, one side skull, one side double PP |
| 257 | Pill | 8 | Beige film logo |
| 728 | Pill | 3 | Red and white soda logo |
| 686 | Powder | 1 | Beige powder |
| 252 | Pill | 4 | Purple and pink phone logo |
| 615 | Pill | 6 | Orange, film logo |
| 641 | Capsule | 9 | Red and Yellow capsule |
| 657 | Pill | 11 | White, one side skull, one side double PP |
| 662 | Powder | 9 | White powder in individual bags |
| 616 | Pill | 16 | Pink 100 logo |
| 148 | Pill | 17 | Pink 100 logo |
| 716 | Pill | 42 | Yellow Octopus |

*Table 1. Sample groups analysed, including material type, number of samples and appearance.*

Seized pills/material were simply added to a glass vial with 5 mL methanol and sonicated for 10 minutes. Prior to analysis, each sample was further diluted 1:20 with methanol. If additional dilutions were required these were also made using methanol.

For capsular material, contents were emptied and added to a glass vial with 5 mL methanol and sonicated for 10 minutes. Prior to analysis, each sample was further diluted 1:20 with methanol. If additional dilutions were required these were also made using methanol.

For seized powders/crystalline material, the material was added to a glass vial with 5 mL methanol and sonicated for 10 minutes. Prior to analysis, each sample was diluted 1:20 with methanol. If additional dilutions were required these were also made using methanol.

## 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 in the analytical method that were used to acquire data are shown below in Table 2. For this study, each sample was analyzed in triplicate (the same glass capillary used for three cycles of 'dip and detect').

| 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 2. 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 seized drug reference library using a reverse fit model. LiveID calculates an average match score (maximum score is 1000) considering all four cone voltages, for this study a match score of 850 or greater was used as a threshold for indication of a positive identification.

### Forensic Toxicology HRMS Screening Solution Analysis

Samples were further diluted 1:1000 with 5 mM Ammonium formate at PH3, prior to analysis with an established HRMS screening method, which utilizes a 15-minute chromatographic separation and Xevo G3 QTof Mass Spectrometry. Identification was based on reference retention time (RT), accurate mass for the precursor, and fragment ion information, generated through data independent analysis (DIA) using $MS^E$.

## Results and Discussion

ASAP-MS provides a direct analysis technique yielding mass spectrometry data without chromatographic separation, which 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 2 shows data for the CRM for MDMA and illustrates the mass spectrometry information that can be generated using this technique.

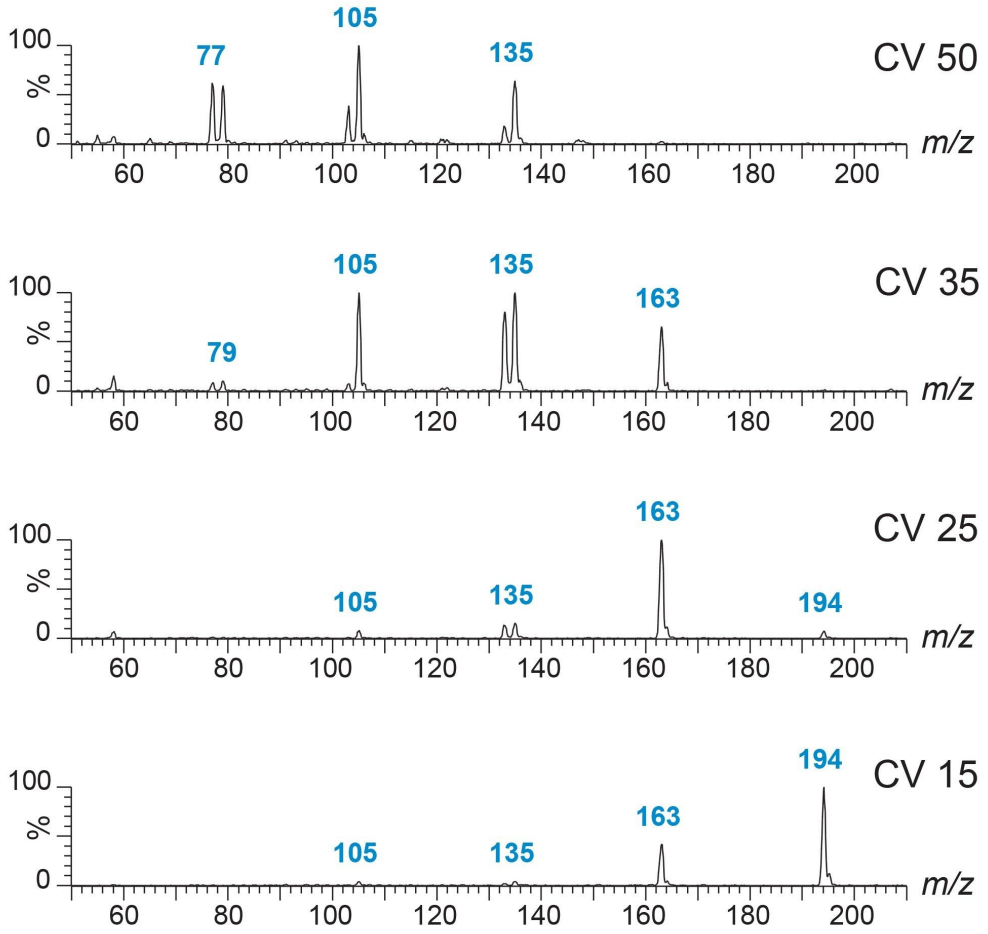


*Figure 2. RADIAN ASAP analysis of MDMA CRM. Data is collected at four cone voltages to generate a spectral fingerprint. The lowest cone voltage (15 V) typically contains the ionized precursor molecule, in this example ASAP ionization resulted in the generation of the $[M+H]^+$ species at m/z 194.*

The results for all 229 seized samples analysed, resulted in a positive match (>850) for one or more compounds in the seized drug reference library indicating 100% sensitivity for true positives. In total there were 252 positive identifications within the sample set of groups, which involved only seven substances: MDMA (73.9%), caffeine (4.3%), etizolam (4.3%), flualprazolam (4.3%), amphetamine (4.3%), paracetamol (4.3%), and cocaine (4.3%). MDMA was detected in over half of the samples and in most cases was the only compound detected; this is likely due to the locations and events that these samples were seized from.

The results obtained from screening with the RADIAN ASAP showed good qualitative agreement with the Forensic Toxicology HRMS Screening Solution. It is likely that any discrepancies between the results was due to the differences in analytical sensitivity and in library content. Two groups of samples resulted in additional compounds being positively identified by RADIAN ASAP analysis, however the main compound identified by HRMS was also positively detected. For group 170, the main components identified by HRMS analysis were not identified by RADIAN ASAP analysis. Figure 3 shows an example of the LiveID result obtained for one of the samples from this group; caffeine was positively identified. HRMS analysis resulted in the identification of 4 additional compounds including: methoxetamine, TFMPP, MDMA and 4-methylethcathinone. MDMA and TFMPP were identified on the RADIAN ASAP with a match score lower than the 850 cut-off which suggests these compounds were at low levels within the sample. Differences in the two-screening methods library content also had an impact for this group, as 4-methylethcathinone and methoxetamine are not currently included in the RADIAN ASAP seized drug reference library. We have previously described the quick process of updating the RADIAN library, which can enable laboratories to keep their reference libraries up to date with new emerging compounds.[5]


*Figure 3. LiveID analysis of a seized sample. Panel A shows three "dip and detect" replicates for the sample and the match score 985 (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 the weighted mean (lowest cone voltage with the highest weighting) used.*

The 229 samples were confiscated in 23 different groups of varying number of samples (Table 1); HRMS analysis

showed there was little variation in the intensity of response between samples within the same group or between the different groups of the same compound identification. RADIAN ASAP analysis showed good agreement with this as the positive match scores did not vary significantly between inter-sample triplicate analysis, inter-group samples or compound intra-group samples (Figure 4). Therefore, demonstrating that the RADIAN ASAP is a reliable and robust screening technique which provides reproducible data.

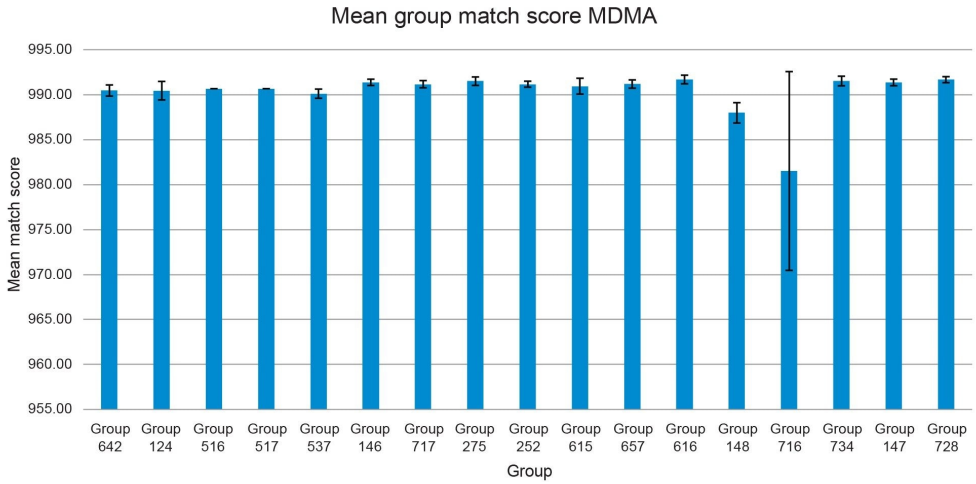


*Figure 4. Average match scores for groups of seized drug samples in which MDMA was positively identified, with standard deviation.*

## 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 to be suitable as a simple screen for common illegal drugs in seized suspect materials, including pills, powders, and capsular material.

The sample preparation method is both quick and simple. RADIAN ASAP analysis and LiveID 2.0 library matching is also very fast, taking less than two minutes for each seized drug sample. Reproducibility of the RADIAN ASAP data is good which demonstrates its ability to reliably screen seized drug samples and potentially

reduce forensic drug chemistry laboratory backlogs.

## References


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