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An Early Warning System for Illicit Drug Use at Large Public Events: Trace Residue Analysis of Discarded Drug Packaging Samples

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| Journal: | <i>Journal of the American Society for Mass Spectrometry</i> |
| Manuscript ID | js-2021-002328.R2 |
| Manuscript Type: | Research Article |
| Date Submitted by the Author: | 16-Aug-2021 |
| Complete List of Authors: | West, Henry; The University of Melbourne, School of Chemistry Fitzgerald, John; The University of Melbourne, School of Social and Political Sciences Hopkins, Katherine; The University of Melbourne, School of Chemistry Li, Eric; Agilent Technologies Australia Pty Ltd Clark, Nicolas; North Richmond Community Health Tzanetis, Stephanie; Harm Reduction Victoria Greene, Shaun; Austin Health, Victorian Poisons Information Centre Reid, Gavin; The University of Melbourne, School of Chemistry |
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An Early Warning System for Illicit Drug Use at Large Public Events: Trace Residue Analysis of Discarded Drug Packaging Samples

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53 **Author Contributions:** The manuscript was written through contributions of all authors. All
54 authors have given approval to the final version of the manuscript.
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Abstract

Inspired by Locard's exchange principle, which states "every contact leaves a trace", a trace residue sampling strategy has been developed for the analysis of discarded Drug Packaging Samples (DPS), as part of an early warning system for illicit drug use at large public events including music/dance festivals. Using Direct Analysis in Real Time (DART) -mass spectrometry (MS) and -tandem mass spectrometry (MS/MS), rapid and high-throughput identification and characterization of a wide range of illicit drugs and adulterant substances was achieved, including in complex poly-drug mixtures and at low relative ion abundances. 1362 DPS were analyzed either off-site using laboratory-based instrumentation, or on-site and in close-to-real-time using a transportable mass spectrometer housed within a mobile analytical laboratory, with each analysis requiring less than one minute per sample. 92.2% of DPS yielded positive results for at least one of 15 different drugs and/or adulterants, including cocaine, MDMA, and ketamine, as well as numerous Novel Psychoactive Substances (NPS). 52.6% of positive DPS were found to contain polydrug mixtures, and a total of 42 different drug and polydrug combinations were observed throughout the study. For analyses performed on-site, reports to key stakeholders including event organizers, first aid and medical personnel, and peer-based harm reduction workers could be provided in as little as 5 minutes after sample collection. Following risk assessment of the potential harms associated with their use, drug advisories or alerts were then disseminated to event staff and patrons, and subsequently to the general public, when substances with particularly toxic properties were identified.

Introduction

The use of psychoactive drugs is common in society, including at large public events such as music and dance festivals. Due to their unknown origins, compositions and lack of regulation, the use of these substances presents risks of harm, including toxicity, overdose and death [1,2]. These risks may be exacerbated when a drug sample has an unknown quantity, or is adulterated via the addition of other pharmaceutically active compounds to dilute, bulk up or otherwise improve the appearance and/or apparent quality of the product [3,4,5]. Additional risk exacerbation occurs when a drug is mis-sold, e.g., counterfeited pharmaceuticals [6,7], or when conventional illicit drugs e.g., cocaine, MDMA and ketamine, are adulterated with or substituted for structurally related analogues or NPS such as synthetic cathinones, synthetic cannabinoids, or novel synthetic opioids such as fentanyl and its analogues [8]. Due to their ever increasing number and structural diversity (up to December 2020, 1,047 NPS have been reported [9]), the pharmacological properties of many NPS, and their subsequent potential for adverse side-effects, are poorly understood [10-16]. Therefore, to provide effective public health and harm reduction interventions at large public events there is a need for rapid qualitative and quantitative identification and characterization of the drug substances and combinations that are in use, including NPS that have not previously been reported or characterized [17,18]. Information regarding the presence and/or concentrations/amounts of these substances, and assessment of their potential adverse effects can then be relayed to relevant stakeholders e.g., first aid and medical personnel, peer-based harm reduction workers, and/or event patrons.

Throughout Europe since the 1990's [18,19], more recently in the UK [20,21] and New Zealand [22], and at a single location on two occasions to date in Australia [23,24], drug checking (also known as pill testing) services have been established on-site at music festivals or off-site at fixed locations within the community, for people who use drugs to be informed about the identity, and in some instances, purity, of drugs in their possession, prior to consumption. As an alternative to simple colorimetric reagent tests, which generally lack sensitivity and specificity to reliably detect or confidently identify the enormous number of substances currently available, a range of more sophisticated analytical techniques have been employed for drug checking in these services, including "Fourier Transform – InfraRed" (FTIR) spectroscopy [25-29]. The potential benefits of FTIR include minimal sample preparation,

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3 minimal sample amount (low mg quantities), speed of analysis (<1 minute per sample), relative
4 low establishment cost, and simple operational complexity (i.e., low technical expertise
5 requirement). The presence of the predominant drug and/or other inert filler compounds that
6 are present can be presumptively but confidently determined by searching the resultant data
7 against pre-defined reference libraries of authentic compounds [26]. However, FTIR is not
8 useful for the detection of potentially highly harmful drugs present at low levels relative to
9 inert filler compounds or other major drugs that may be present (i.e., in poly drug mixtures),
10 or when a drug is not included in the reference library (e.g., new psychoactive substances
11 (NPS)). Furthermore, FTIR is largely a qualitative analysis technique, such that critical
12 quantitative information regarding drug purity or dose is typically not obtained [26, 30]. Raman
13 spectroscopy, also used previously for the analysis of illicit substances in field-based drug
14 analysis applications, can suffer from similar limitations [25,30,31]. Therefore, additional
15 approaches and resources are typically required to provide further and more precise
16 information regarding drug compositions and purity e.g., by using a team of highly-trained
17 chemists to extract and to perform additional chemical analysis of the drugs, to reduce the
18 potential for false negative (and false positive) results.
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33 In contrast, mass spectrometry (MS) is widely acknowledged as the gold standard analytical
34 measurement technology for forensic drug analysis, including for drug monitoring [30,32].
35 MS, when coupled with established chromatographic separation techniques, namely Gas
36 Chromatography (GC)-MS [33] and Liquid Chromatography (LC)- Electrospray Ionization
37 (ESI)-MS or -Tandem Mass Spectrometry (MS/MS) [34-36] are capable of providing
38 definitive qualitative and quantitative drug identifications with high sensitivity and specificity,
39 including for low level components within poly-drug mixtures, by matching the observed
40 retention times, mass-to-charge ratio's and/or characteristic fragmentation patterns for each
41 drug against information contained within reference libraries generated from authentic
42 standards [35,36]. MS techniques are also capable of *de-novo* identification and
43 characterization of novel drug substances that appear on the market.
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53 Commercially available portable or transportable GC-MS instrumentation have also been
54 employed for on-site drug testing applications, e.g., at clandestine laboratories by police
55 forensic services, or off-site in police stations [37] or drug checking services. However, these
56 'hyphenated' MS methods can suffer from relatively low throughput capability due to the need
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3 for time consuming sample extraction/processing steps and long chromatographic analysis
4 times (often 10's of minutes per sample), can have relatively high establishing or ongoing
5 operating costs, and require significant expert technical knowledge for operation and
6 maintenance. Therefore, they are not considered fit for purpose for use in drug checking
7 applications requiring high throughput, or in close to real time harm reduction initiatives, in
8 varied settings such as music festivals or supervised drug consumption facilities.
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15 To address these limitations, various alternative MS based strategies employing ambient
16 ionization techniques for direct sample introduction have been investigated [32], including
17 Desorption ElectroSpray Ionization (DESI) [38,39], DART [40-44], Low Temperature Plasma
18 (LTP) ionization [45-47], Paper-Spray (PS) ionization [48-51], and Atmospheric Solids
19 Analysis Probe (ASAP) [52]. Importantly, these approaches enable rapid MS and MS/MS data
20 acquisition with little or no requirement for sample preparation or separation prior to sample
21 introduction [32], and with trace-level sensitivity. Furthermore, they can be interfaced with
22 portable mass spectrometry instrumentation [38,39,51-56] for both qualitative and quantitative
23 analysis of pharmaceutical and illicit drug substances including synthetic cathinones [38] and
24 fentanyl and fentanyl-analogues [51]. For example, thermal desorption (TD)-DART-MS has
25 previously been shown to have nanogram level limits of detection for the identification of
26 fentanyl, fentanyl analogues, and a variety of other opioids, and also enable detection of
27 fentanyl down to 0.1% by mass in the presence of heroin [43]. Furthermore, TD-DART-MS
28 has previously been shown to enable identification of the contents of bulk drug packaging
29 samples seized by law enforcement, via analysis of swabs taken from the exterior of the
30 packaging material (when single to tens of micrograms of drugs are present). In this report, the
31 drugs identified from the exterior of the drug packaging were found to be 92% accurate with
32 the contents [54]. To date, however, DART-MS has not been applied for harm reduction
33 applications at large public events, including on-site and in close to real time.
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50 Here, as part of developing an early warning monitoring system for illicit drug use at large
51 public events, including on-site and in close to real time, we describe the development of a
52 high-throughput and rapid qualitative identification and characterization strategy using DART-
53 MS and -MS/MS, involving trace-residue sampling of illicit drug substances found within
54 discarded Drug Packaging Samples (DPS). Applications involving both laboratory-based ultra-
55 high-resolution and accurate mass spectrometry (UHRAMS) instrumentation, as well as in a
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field deployed mobile analytical laboratory using a transportable triple quadrupole mass spectrometer instrument are demonstrated. Importantly, the results from this study are shown to enable rapid risk assessments of the potential harms associated with the identified substances, with subsequent dissemination of drug alerts or advisories to event staff and patrons, and to the general public, when substances with particularly toxic properties were identified.

Materials and Methods

Sample collection. 1362 samples tentatively assigned as discarded DPS were collected during routine waste collection at multiple large public events including music / dance festivals and other entertainment events, as well as other public locations where illicit drug use was likely to occur, between September 2019 and March 2020, and in November, 2020 throughout metropolitan Melbourne and in regional Victoria, Australia. The number of patrons at each event ranged in size from 2,000 – 80,000 people. The types of DPS obtained are listed in **Table 1**, with reusable, re-sealable plastic ziplock bags being the most prevalent (90%), consisting of a variety of sizes and with a variety of distinguishing manufacturers or distributors features such as line thickness and color, and branding or logos.

Table 1. Summary of drug packaging sample (DSP) types.

| Sample type | No visible residue (86%) | Visible residue ¹ (11%) | Measurable ² (3%) | Total |
|--------------------------|-----------------------------|---------------------------------------|---------------------------------|-------|
| Ziplock bag | 1061 | 134 | 32 | 1227 |
| Clingwrap | 37 | 5 | 0 | 42 |
| Foil | 18 | 4 | 0 | 22 |
| Pill casing/ gel capsule | 18 | 7 | 8 | 33 |
| Other ³ | 35 | 3 | 0 | 38 |
| Total | 1169 | 153 | 40 | 1362 |

¹defined as containing trace amounts of powder or crystals.

²defined as containing greater than 5 mg of material.

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3 ³Samples categorised as “other” included air and water balloons, small glass and plastic food
4 or confectionary containers (e.g., ‘kinder surprise’ plastic eggs), feminine hygiene product
5 applicators, adhesive tape, etc.
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10 **Sample Preparation.** Samples were prepared for analysis by lightly dry swabbing the surface
11 area of the DPS (e.g., the interior of the ziplock bags) using commercially available cotton tip
12 applicators (Swisspers, Kingsgrove, NSW, Australia). For samples containing visible residue
13 and measurable amounts of material, the cotton tip applicators were gently flicked after
14 swabbing to displace any loose material. The swabs were then cut to a length such that the
15 widest circumference of the head aligned vertically with the tip of the DART probe when
16 placed into a custom sample holder. See the Supplemental Information for additional detail.
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24 **Direct Analysis in Real Time (DART) sample introduction.** Samples were introduced for mass
25 spectrometry analysis using a DART source (IonSense, MA, USA) interfaced to the mass
26 spectrometers using a Vapur® interface with Nitrogen as the source gas. Variables that could
27 affect the practical application and performance of the DART source for trace residue drug
28 analysis were evaluated and optimized during initial method development (see the
29 Supplemental Information for additional detail). To ensure even desorption and the acquisition
30 of a mass spectrum that was representative of the entire surface that had been swabbed,
31 tweezers were used to manually rotate the stem of the cotton tip applicator containing the
32 samples during data acquisition.
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41 **Mass Spectrometry Analysis** Two mass spectrometers were used in this study. For off-site
42 laboratory-based analysis, a Thermo Scientific Q Exactive Plus Orbitrap mass spectrometer
43 (Bremen, Germany) was used, while for on-site close to real time analysis, an Agilent Ultivo
44 triple quadrupole mass spectrometer housed within a custom modified mobile analytical
45 laboratory was employed (see the Supplemental Information for additional detail).
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51 **Identification of illicit drug compounds and adulterants.** Given that the majority of the DPS
52 contained no visible residue, all results in this study are reported as qualitative identifications
53 only. For off-site laboratory-based analysis, the identities of all drugs and adulterants within
54 DPS were initially assigned based on manual comparison of their accurate mass values
55 determined from their $[M+H]^+$ ions in positive ionization mode, against a list of >700 drugs,
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3 adulterants and common contaminants that were of possible interest to this study, then
4 definitively confirmed by manual comparison of their HCD-MS/MS spectra against reference
5 spectra contained within the mzCloud Advanced Mass Spectral Database [57], or against
6 MS/MS spectra previously reported in the literature. Blank cotton swabs were run every 5
7 samples as controls. Positive identifications were assigned only if the MS signal intensity for
8 the precursor ion of interest was 10x the background level in the blank spectra. For on-site
9 close to real time analysis, definitive identification of MS peaks observed at nominal m/z values
10 corresponding to potential expected drug substances, or abundant MS peaks not present in the
11 blanks, was achieved by acquisition of their MS/MS spectra followed by *de novo* analysis then
12 comparison against reference spectra contained within the mzCloud Advanced Mass Spectral
13 Database, or against MS/MS spectra previously reported in the literature. For samples that
14 contained visible residue, conventional ESI-MS was used to confirm that the drug compositions
15 observed by DART were consistent. However, although DART-MS has previously been shown
16 to enable detection of low concentration drugs in the presence of higher concentration species,
17 we cannot exclude the possibility that competitive ionization via DART could inhibit the
18 detection of all drugs of interest that may be present at very low concentrations.
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32 Results and Discussion

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36 ***Off-site laboratory-based DART-MS and -MS/MS for rapid trace residue illicit drug***
37 ***monitoring.*** Representative spectra acquired by trace residue swabbing and DART-MS
38 analysis of four different DPS ziplock bags, each containing no visible residue, are shown in
39 **Figure 1** (the inset to Figure 1A shows a photograph of the clear plastic ziplock bag from which
40 the swab was taken, measuring 30 x 24 mm, that had been torn open). For the MS spectrum in
41 Figure 1A, tentative assignment of the ions at m/z 194.1174, 238.0991 and 304.1543 were made
42 for 3,4-Methylenedioxyamphetamine (MDMA) (calculated m/z 194.1181), ketamine
43 (calculated m/z 238.0999) and cocaine (calculated m/z 304.1549), respectively, based on close
44 agreement with their molecular formulas, then definitively confirmed by manually matching
45 the product ion m/z and relative ion abundances observed within their HCD-MS/MS product
46 ion spectra against the reference spectra of authentic compounds contained in the mzCloud
47 database that had been acquired under approximately the same collision energies
48 (**Supplemental Figures S1A-C**, respectively). These three drugs were the most commonly
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3 observed compounds during our study (see below for further discussion and summary of the
4 overall dataset).
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8 MDMA (commonly known as ecstasy or molly) is the drug most commonly associated with
9 recreational use at music festivals and similar events, where it is reported to give rise to user
10 effects including an enhanced sense of well-being and sensory perception [58,59]. Ketamine is
11 a dissociative anesthetic that is commonly consumed for its mild psychedelic effects [60], while
12 cocaine is a stimulant whose use can result in feelings of euphoria [61]. The DPS giving rise
13 to the DART-MS spectra in Figure 1B was found to contain cocaine along with the common
14 adulterants, levamisole and lidocaine (Figure 1B), while those giving rise to the spectra in
15 Figures 1C and 1D were found to contain MDMA adulterated with the substituted cathinones
16 [15] eutylone and ethylone, respectively. Confirmatory HCD-MS/MS spectra for each of these
17 adulterants are shown in Supplemental Figure S1D-G(also see below for further discussion
18 regarding these compounds). Importantly, the spectra in Figure 1, obtained by averaging 100
19 scans, were each acquired in only 7 seconds while the individual HCD-MS/MS spectra were
20 each acquired in only 10 seconds each. Thus, the identity of all the substances in each DPS
21 using this trace residue sampling and analysis approach were definitively confirmed in less
22 than one minute of total analysis time each, indicative of the high-throughput capabilities of
23 DART-MS and MS/MS for illicit drug monitoring. Including sample preparation time (i.e.,
24 swabbing of the DPS), it required only two people to analyze up to 80 samples per hour.
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39 For these off-site laboratory-based analyses, in which samples were batch collected and then
40 transported to the laboratory for analysis, reporting of results regarding the identity of the
41 drug(s) and/or their packaging appearance were then provided to key Event Management Team
42 (EMT) stakeholders including event organizers, first aid and medical personnel, peer-based
43 harm reduction workers, and security contractors, amongst others, in as little as 60 min after
44 sample collection, depending upon the distance between the event and the laboratory. Risk
45 assessments of the potential harms associated with the identified substances were then
46 performed by this group, and, as deemed appropriate or necessary, alerts were then provided
47 to event personnel, event patrons, and subsequently to the general public (see the section on
48 Substituted Cathinones below), to reduce their risk of drug-related harm when substances with
49 particularly toxic properties were identified.
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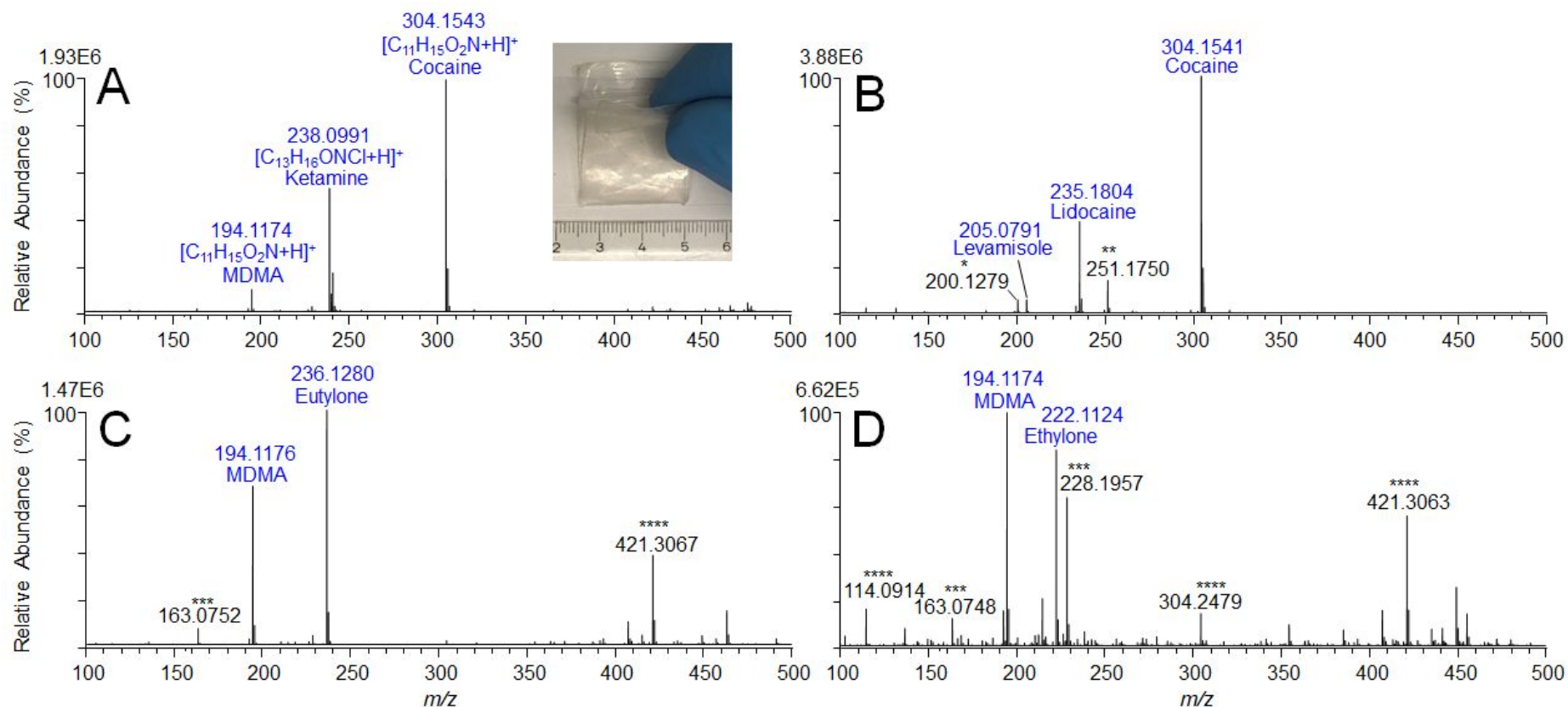


Figure 1. DART-MS spectra from several discarded DPS ziplock bags containing no visible residue, found to contain (A) cocaine, ketamine and MDMA (the inset to panel A shows a photograph of the bag), (B) cocaine adulterated with levamisole and lidocaine, (C) MDMA adulterated with ethylone, and (D) MDMA adulterated with ethylone. * in-source cocaine fragment. ** in-source oxidation product of lidocaine. *** in-source fragment of MDMA **** background ions.

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5 An overall summary of the results obtained from the off-site laboratory-based analysis of DPS
6 are shown in **Figure 2**. A total of 1,315 samples were analyzed, with 92.2% (N=1,212)
7 providing positive results for at least one pharmacologically active compound. A total of 15
8 different drugs and/or adulterants were identified, including cocaine, MDMA, ketamine,
9 levamisole, lidocaine, methamphetamine, amphetamine, several NPS including 2-
10 fluorodeschloroketamine (2-FDCK), tiletamine, the synthetic cathinones ethylone, eutylone
11 and N-ethylpentylone, the endogenous hallucinogen N,N-dimethyltryptamine (DMT), 3,4-
12 methylenedioxyamphetamine (MDA), and methylphenidate.
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21 Of the DPS samples that tested positive, cocaine (58.9%) was the most commonly observed
22 drug, followed by MDMA (58.2%) and ketamine (32.8%). However, polydrug mixtures were
23 more common than single drugs, with 52.6% of positive samples containing more than one
24 substance, in 42 different drug combinations. Of the total positive samples, 39.0% tested for
25 two substances, 12.0% tested for three active compounds and 1.6% tested for four active
26 compounds. MDMA/cocaine (n=227), ketamine/cocaine (n=98) and
27 MDMA/ketamine/cocaine (n=89) were the three most common combinations. Although
28 cocaine was observed as the most common drug, it was present only 24.2% (n=173) of the time
29 as a single drug i.e., without other co-present drugs or adulterants, and was instead found to be
30 co-present with MDMA 48.6% of the time, and co-present with ketamine 31.2% of the time.
31 Note that these percentages include all cocaine + MDMA combinations and cocaine + ketamine
32 combinations, including those where cocaine + MDMA + ketamine were all present.
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43 Additionally, cocaine was found to be co-present with the common adulterants levamisole
44 (without lidocaine) 9.9% of the time, co-present with lidocaine (without levamisole) 4.3% of
45 the time, and co-present with both levamisole and lidocaine 1.3% of the time. Similarly,
46 MDMA was observed to be present only 40.7% of the time as a single drug, and 49.2% of the
47 time to be co-present with cocaine (n=347), and 22.8% of the time with ketamine. MDMA was
48 also found to be co-present with levamisole (no lidocaine) and with lidocaine (no levamisole)
49 1.3% and 1.8% of the time, respectively, and co-present with both lidocaine and levamisole
50 0.1% of the time. In addition, MDMA was found to be co-present 1.3% of the time with one
51 of the synthetic cathinone drugs, eutylone, ethylone or N-ethylpentylone. Finally, ketamine
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3 was observed only 22.4% of the time as a single drug, while being present 56.0% of time with
4 cocaine, and 40.5% of the time with MDMA.
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8 Notably, a significantly greater fraction of samples that contained polydrug mixtures were
9 identified in this study, compared to reports from other recent pill testing/drug checking
10 operations in New Zealand and Australia, that could be expected to share similar drug
11 distribution and/or recreational drug user demographics and usage patterns to those within the
12 events and locations from which the current samples were obtained [22-24]. This difference is
13 likely explained by the different analytical measurement technologies that were employed. For
14 example, in contrast to the study performed here using highly sensitive mass spectrometry-
15 based methods, the results from these other reports were obtained using FT-IR spectroscopy,
16 suggesting a potential bias and/or limitations of that technique against the detection of
17 substances present in polydrug mixtures, particularly those at low levels relative to inert filler
18 compounds or other major drugs that may be present. This highlights the critical requirement
19 for analytical measurement technologies that are capable of accurately identifying and
20 characterizing all the components of complex drug mixtures, including those at low relative
21 ion abundances, if the results are to be used for health promotion and harm reduction purposes.
22 Due to the nature of the sample type (i.e., DPS) and the anonymized sample collection strategy
23 employed here, it was not possible to evaluate whether or not the identified substances were
24 the same as those expected by the consumer.
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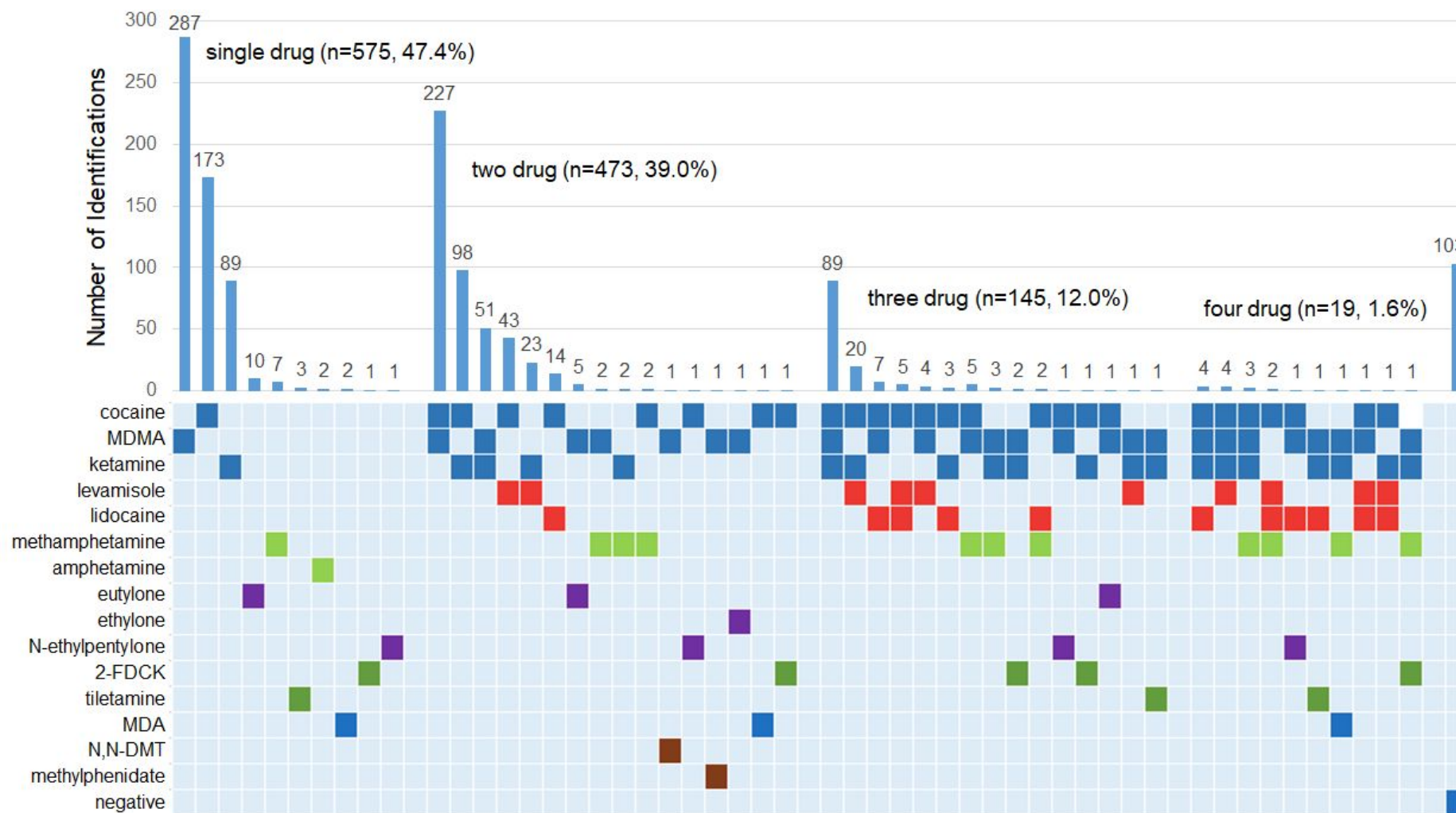


Figure 2. Illicit drugs, and drug combinations observed by trace residue laboratory-based DART-MS analysis of DPS samples. The colors highlight the various different classes of drugs that are present.

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5 ***Adulterants and bulking agents.*** Lidocaine and levamisole are well-documented adulterants
6 of cocaine [4] and were the two most commonly observed in our study. Lidocaine, a local
7 anesthetic, is commonly added to cocaine to enhance the characteristic numbing effect that is
8 often associated with 'high-quality' cocaine, while levamisole is used as a bulking and
9 enhancing agent due to its white, fluffy powdery appearance, similar to cocaine. Previously,
10 levamisole was used as a worming agent in humans; however, it was withdrawn from market
11 after being linked to serious cardiovascular issues including agranulocytosis, the depletion of
12 white blood cells, as well as vasculitis leading to inflammation and destruction of blood vessels
13 [62]. Both complications have been reported amongst cocaine users who have consumed
14 cocaine laced with levamisole [5]. Whilst levamisole was primarily observed here to be co-
15 present with cocaine, 23 ketamine-containing samples also tested positive for the presence of
16 levamisole, revealing the complexity of the illicit market when drugs are readily substituted
17 and the consumers have little awareness of the materials they are consuming. Other adulterants
18 observed in this study included nicotinamide, commonly known as vitamin B3, and
19 dimethylsulfone (DMS). Nicotinamide is readily available from most pharmacists and
20 supermarkets, with potential negative effects reportedly occurring only after doses exceeding
21 3 grams/day [63]. DMS, a common bulking agent in methamphetamine samples [64], was
22 observed in 5 samples in this component of the study, all of which also contained
23 methamphetamine and corresponding to 17% of all the methamphetamine samples identified.
24 However, a previous study investigating the impact of DMS on rats did not identify any adverse
25 effects from exposure [65].
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43 ***Substituted Cathinones.*** Substituted cathinones, colloquially known as "bath salts", are
44 synthetic analogues of cathinone, a naturally occurring stimulant found in the plant khat, and
45 are a class of NPS of concern for recreational drug use [66]. A wide range of substituted
46 cathinones have been reported [11,66]. Like most NPS, the pharmacokinetic and
47 pharmacodynamic properties, as well as the short-term and long-term effects of many
48 substituted cathinones are poorly understood. Common symptoms associated with their use
49 include agitation, tachycardia, paranoia and seizure/tremors, hyperthermia, and multiorgan
50 system failure [66,67]. In a 2015 study of drug use among nightclub/festival-attending young
51 adults in New York City, analysis of donated hair samples revealed that of the samples
52 containing MDMA, 47.9% contained butylone, and 10.4% contained methylone. Furthermore,
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3 the hair samples from 41.2% of respondents who reported no lifetime use of NPS or unknown
4 pills or powders, tested positive for the synthetic cathinones butylone, methylone, alpha-
5 pyrrolidinovalerophenone (α -PVP), 5- or 6-(2-aminopropyl)benzofuran (5/6-APB), or 4-
6 fluoroamphetamine (4-FA) [68]. This suggests that many MDMA-users may be
7 unintentionally using synthetic cathinones or other NPS, and that the risks associated with the
8 use of MDMA may be further exacerbated through adulteration or substitution with synthetic
9 cathinone drugs.
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17 During this study, three substituted cathinones were identified: ethylone, eutylone and N-
18 ethylpentylone. Eutylone was the most commonly observed and was found to be present in 16
19 samples (10 times as a single drug, 5 times in combination with MDMA (Figure 1C), and once
20 in combination with MDMA and cocaine). Ethylone was observed in only one sample, co-
21 present with MDMA (Figure 1D), while N-ethylpentylone was observed in a total of 4 samples.
22 As a demonstration of the general utility of the cotton tip ‘swabbing’ method described here
23 for the detection and identification of synthetic cathinone drugs from a variety of DPS
24 materials, including those containing polydrug mixtures and at varying relative ion abundances,
25 the DART-MS spectra from the four N-ethylpentylone-containing samples are shown in
26 **Figure 3**. Figure 3A shows the spectrum obtained through swabbing of a ziplock bag, that was
27 found to contain N-ethylpentylone at moderate relative ion abundance and co-present with
28 cocaine, MDMA and lidocaine, while Figure 3B shows the result from swabbing a piece of
29 aluminum foil found to contain N-ethylpentylone at low relative ion abundance and co-present
30 with MDMA and cocaine. In contrast, Figure 3C shows the spectrum obtained by analysis of
31 an intact green, speckled pill imprinted with ‘UPS’ (approximately 8 mm x 10 mm x 3 mm in
32 size), that was collected as a discarded ‘ground find’ (also called an ‘unattached seizure’ i.e., a
33 sample not associated with any identifiable individual or police investigation) at a large multi-
34 day music festival. To ensure that the sample subjected to analysis would be representative of
35 the entire pill, the pill was initially broken into several small pieces from which representative
36 small scrapings were taken prior to pulverizing into a fine powder, followed by trace residue
37 swabbing. It would be of interest in a subsequent study to systematically examine the
38 heterogeneity associated with the distribution of different drug substance or amounts, in this
39 type of sample. DART-MS and MS/MS (Supplemental Figure S1H) of this sample resulted in
40 the identification of only N-ethylpentylone. The risk associated with N-ethylpentylone use is
41 exemplified by a previous case study from a patient who died from an accidental N-
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3 ethylpentylone overdose, that reported several complications including hyperthermia,
4 disseminated intravascular coagulation (DIC) (blood clots forming throughout cardiovascular
5 system), kidney and liver failure, and with the cause of death being cardiac arrest [2]. In this
6 instance, as the pill had a clearly definable appearance, and following risk assessment of the
7 potential harm associated with this drug at the multi-day event, an initial “patron alert” was
8 sent via messaging app within 12 hours of its initial identification to warn patrons of its
9 presence, and subsequently a “public drug alert” was made by the Department of Health &
10 Human Services State Government of Victoria in March 2020 [69], the first of its kind within
11 the State of Victoria. We note that the specific format and delivery of such alerts is a subject
12 that requires ongoing consideration and evaluation. Finally, the spectra in Figure 3D resulted
13 from analysis of a sample found to contain N-ethylpentylone and cocaine, obtained by
14 swabbing a toilet roll holder in a public toilet cubicle at a popular social location (i.e., not a
15 music/dance festival) on which traces of a white powder was observed. This demonstrated
16 ability to obtain positive identifications from a diversity of DPS materials, as well as directly
17 from surfaces in which drugs had been in contact, indicates that the developed sampling
18 technique is quite versatile, with almost any surface being able to be swabbed. The co-presence
19 of cocaine with three of the four N-ethylpentylone containing samples (and especially in the
20 context of the visible powder on the toilet roll holder from which the sample in Figure 3C was
21 acquired) suggested that the means of administration was likely to be insufflation, which could
22 result in the N-ethylpentylone entering the body at a higher rate and level than from oral
23 ingestion. As a result of this increased potential harm, combined with information regarding a
24 number of N-ethylpentylone associated hospitalizations that occurred around the same period
25 of time as the sample collection, a “public drug advisory” was subsequently made by the
26 Department of Health & Human Services State Government of Victoria in December 2020
27 [70].
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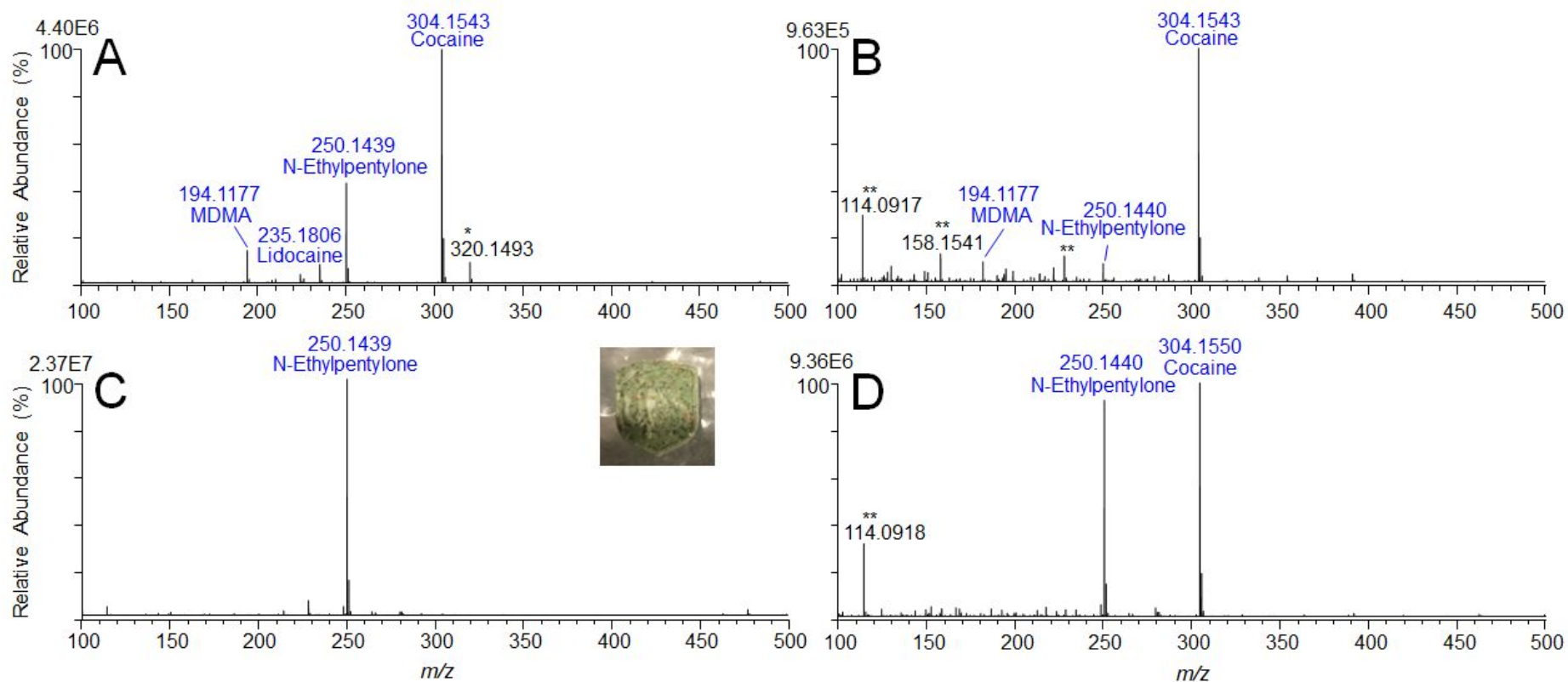


Figure 3. DART-MS of DPS samples containing N-ethylpentylone obtained from dry swabs of, (A) a ziplock bag, (B) aluminium foil, (C) a green speckled 'UPS' pill (the inset shows a photograph of the pill), and (D) residue, found on a toilet roll holder in a public toilet cubicle. * in-source oxidation product of cocaine. ** background ions.

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5 ***Ketamine analogues.*** Two analogues of the dissociative anesthetic ketamine were identified
6 throughout this study, namely 2-fluorodeschloroketamine (2-FDCK), and tiletamine. 2-FDCK,
7 an analogue where the chlorine has been substituted with a fluorine [71], was present in six
8 DPS, once by itself, once with cocaine, once with cocaine and ketamine, twice with MDMA
9 and ketamine, and once in a quaternary mixture with methamphetamine, MDMA and ketamine
10 (**Supplemental Figure S2A**). The co-presence of ketamine in four of the six DPS suggests that
11 the 2-FDCK was introduced as an adulterant or substitution for ketamine. As with many NPS,
12 little is understood regarding the effects, metabolism and potential toxicity of 2-FDCK.
13 However, a study of halogen substitution on metabolism by cytochrome P450 2B6 (the major
14 metabolic enzyme for hepatic metabolism of ketamine, operating through N-demethylation)
15 found that the Michaelis Constant (K_m) was more than double that for 2-FDCK compared to
16 ketamine (17 +/- 1 for ketamine and 40 +/- 3 for 2-FDCK) [72], suggesting that 2-FDCK has a
17 lower binding affinity for the enzyme, which may result in a lower metabolism rate. Potential
18 for harm exists therefore, if users re-administer after a period of time, thinking it to be ketamine.
19 Tiletamine, another structural analogue of ketamine in which the chlorophenyl ring is
20 substituted for a thiophene ring, was observed in a total of five samples, three times by itself,
21 once co-present with MDMA and ketamine, and once with MDMA, ketamine and lidocaine
22 (**Supplemental Figure S2B**). Although tiletamine is often combined with zolazepam (trade
23 name Telazol) for use as a veterinary anesthesia [73], and human fatalities associated with use
24 of the tiletamine/zolazepam combination have been documented [74], zolazepam was not
25 observed in any of the samples analyzed here. The HCD-MS/MS spectra used to definitively
26 confirm the identifications of 2-FDCK and Tiletamine are shown in Supplemental Figures S1I
27 and S1J, respectively.

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46 ***Other illicit substances.*** Methamphetamine was observed in 20 DPS in several different
47 combinations, including in quaternary drug mixtures containing i) 2-FDCK, ketamine and
48 MDMA, as shown in Supplemental Figure S2A, and ii) 3,4-Methylenedioxyamphetamine
49 (MDA), MDMA and Ketamine (**Supplemental Figure S3A**). Confirmatory HCD-MS/MS
50 spectra for methamphetamine and MDA are shown in Supplemental Figures S1K and S1L,
51 respectively. MDA, observed in a total of 4 samples, is the primary metabolite of MDMA, but
52 is also a potential synthetic by-product of MDMA, often observed at very low levels relative
53 to MDMA. In this study however, MDA was observed in three samples without MDMA being
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3 present, and was co-present with MDMA at similar intensity in the remaining sample,
4 suggesting its deliberate adulteration or substitution for MDMA. Methylphenidate, commonly
5 known under its trade name 'Ritalin', is a stimulant drug and a first line medication for
6 management of ADHD, that was observed in one DPS co-present with MDMA (Supplemental
7 Figures S3B and S1M). With similar reported effects to amphetamines, the recreational use of
8 this substance is well documented [75]. Dimethyltryptamine (DMT), a psychoactive compound
9 related to other psychedelic tryptamines including psilocin and bufotenine, was observed in
10 one DPS co-present at low level with MDMA (Supplemental Figures S3C and S1N). Finally,
11 two samples were found to contain amphetamine (an example is shown in Supplemental
12 Figures S3D and S1O). The potent synthetic hallucinogen 25C-NBOMe, a member of the
13 phenethylamine N-benzyl methoxy class of compounds, in combination with 4-
14 fluoroamphetamine (4-FA) was recently found to be responsible for several fatal overdoses in
15 Melbourne, Victoria, Australia throughout July 2016 to January 2017 [76], while another
16 NBOMe, 25I-NBOMe, has been implicated in at least one other death in Australia [77].
17 Fortunately, none of these potentially fatal NBOMe drugs, or 4-fluoroamphetamine, were
18 observed in our study. A subsequent study on a different cohort of samples collected from a
19 different illicit drug user demographic group (to be reported elsewhere), did result in the
20 detection of 4-FA, indicating this drug can be readily observed using our technique. Finally,
21 although several health alerts were recently released in New South Wales, Australia with
22 warnings of possible acetyl fentanyl and fentanyl-laced cocaine and ketamine samples [78,79],
23 no fentanyl containing samples were observed in our study. However, based on prior reports
24 regarding the detection sensitivity of fentanyl drugs using DART-MS [43], we are confident
25 that our method would have detected fentanyl drugs if they were present.
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45 ***Proof of concept implementation of on-site 'close to real time' DART-MS and -MS/MS***
46 ***analysis for trace residue illicit drug monitoring.*** To reduce the time delay between DPS
47 collection and analysis, such that results could be obtained and reported in close to real time, it
48 is desirable to perform the analyses on-site, i.e., proximal to where the sample collection
49 occurs. To achieve this, portable or transportable mass spectrometry instrumentation is
50 required. Therefore, in a proof-of-concept demonstration of the practical utility of our trace
51 residue DPS sampling and analysis approach for on-site drug monitoring in close to real time,
52 a compact triple quadrupole mass spectrometer equipped with a DART ionization source was
53 installed in a customized mobile analytical laboratory and then transported for use at a one-day
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3 music festival event (approx. 15,000 patrons) (**Supplemental Figure S4**). During this proof-
4 of-concept study 47 DPS were analyzed, with 44 (93.6%) testing positive for at least one
5 pharmaceutically active compound (**Figure 4**). Eight different drugs in 10 combinations were
6 identified (79.5% as single drugs and 20.5% as two-drug mixtures). Albeit a proof-of-concept
7 implementation on a limited scale, this is the first report describing the field deployment of a
8 transportable DART-MS system interfaced with a compact triple quadrupole mass
9 spectrometer for on-site close to real time drug monitoring at music festivals or large public
10 events, anywhere in the world.
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19 Example MS and MS/MS spectra resulting from analysis of DPS collected and analyzed on-
20 site at this event are shown in **Figure 5**. The first (Figures 5A and 5B) was from a sample
21 containing no visible residue, that was found to contain cocaine, while a second (Figure 5C and
22 5D), resulting in the identification of methiopropamine, was acquired from a swab taken from
23 a discarded ground find consisting of a ziplock bag containing four capsules that themselves
24 contained off-white colored powder. Methiopropamine is a structural analogue of
25 methamphetamine where the phenyl ring has been substituted with a thiophene ring. In this
26 case, as a low-resolution triple quadrupole instrument was used for the analysis, only a nominal
27 mass was obtained, so direct accurate mass based searching against the mzCloud database
28 could not be performed. Instead, the similarity in the experimentally observed CID-MS/MS
29 fragmentation behavior for methiopropamine with other amphetamines, especially
30 methamphetamine, enabled a proposed structure to be initially assigned by *de novo*
31 interpretation of the MS/MS spectrum, that subsequently allowed its accurate mass to be
32 calculated. Then, the spectrum was matched against that the reference spectrum contained
33 within the mzCloud database for definitive identification, despite us not having observed this
34 drug throughout our previous off-site laboratory-based study. In this instance, less than 5
35 minutes elapsed between running and identifying this unknown sample, and subsequent
36 reporting to on-site medical and first-aid personnel. Similar to other NPS observed in this study,
37 the pharmacology and toxicological effects of methiopropamine, first detected in 2011 in
38 Finland, are largely unknown, but has loosely been characterized as being amphetamine-like,
39 but with hallucinatory effects. Although acute toxicity related to use of methiopropamine was
40 first reported in the UK in 2014 [80], and a fatality associated with the use of methiopropamine
41 was reported in Australia in 2015 [81], this is the first report of the identification of this drug
42 in the context of its recreational use at Australian music festivals.
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5 Subsequent analysis of each of the samples run on-site was performed using our off-site
6 instrumentation. Importantly, each of the substances identified, and their approximate relative
7 ion abundances, were qualitatively similar on both systems, indicating that the instrumentation
8 used for the on-site monitoring was fit for purpose, despite its inferior performance
9 specifications. Collectively, these results clearly highlight the potential for on-site transportable
10 mass spectrometry-based instrumentation to identify new and potentially harmful compounds
11 in close to real time, as part of an early warning system for monitoring and responding to illicit
12 drug use at large public events.
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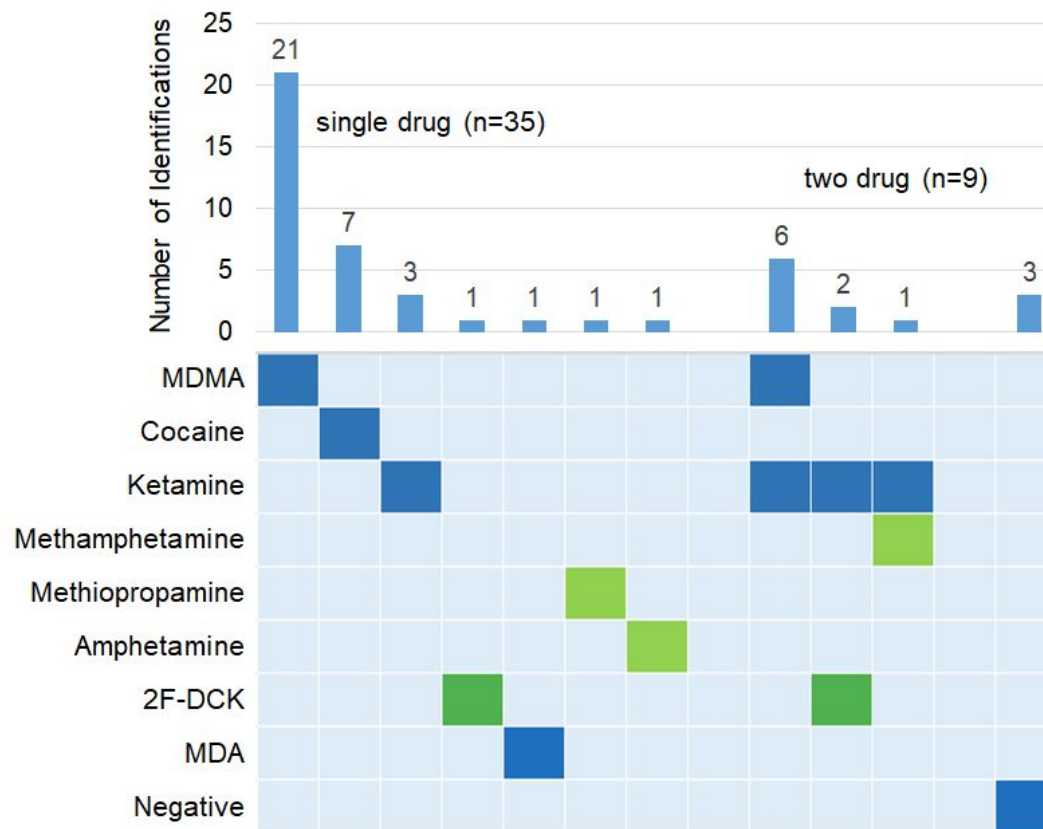


Figure 4. Illicit drugs and drug combinations observed by on-site close to real time mobile laboratory-based DART-MS monitoring of DPS samples. The colors highlight the various different classes of drugs that are present.

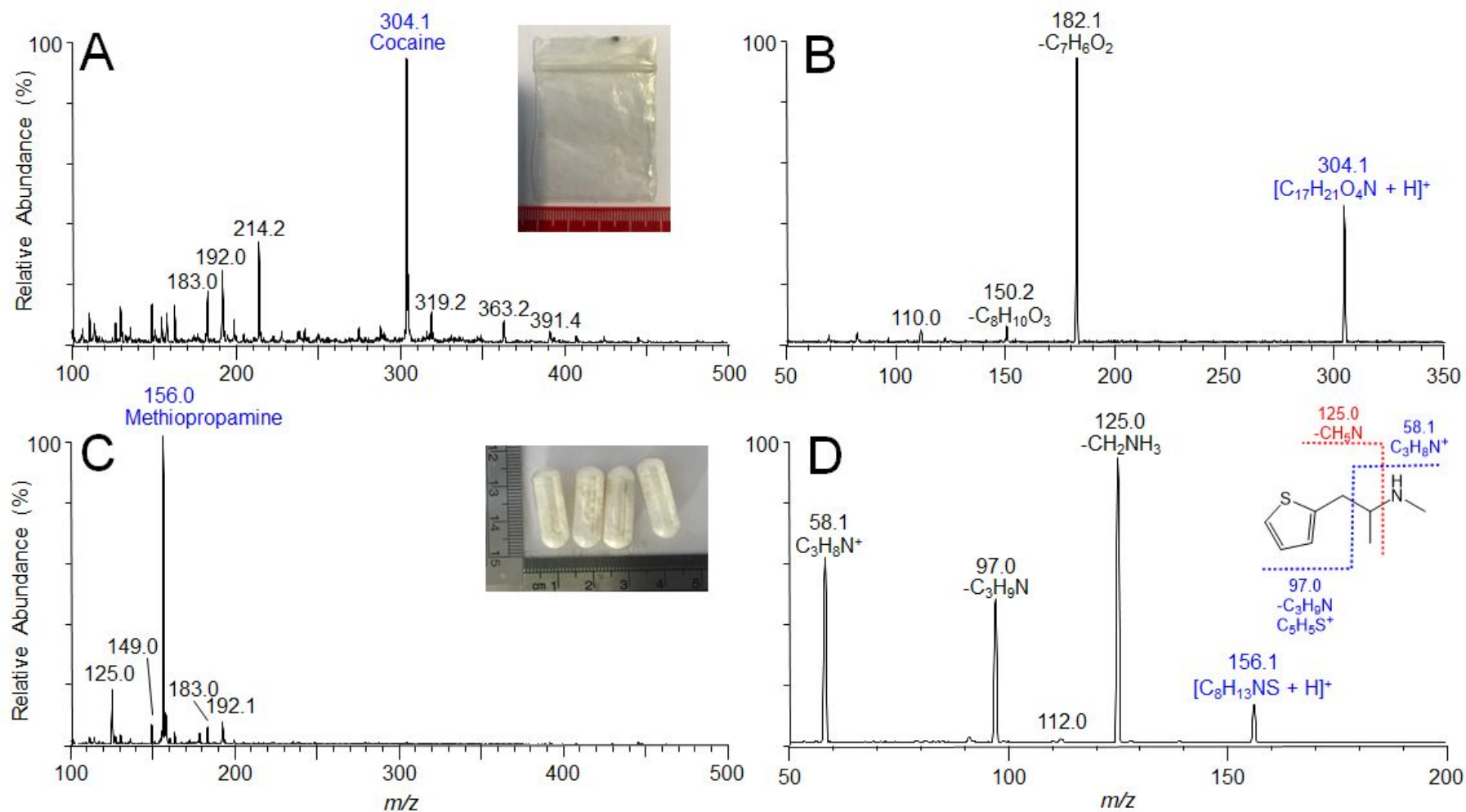


Figure 5. On-site close to real time DART-MS and -MS/MS analysis of illicit drug samples. (A) MS spectrum of a ziplock bag with no visible residue, found to contain cocaine. (B) CID-MS/MS spectrum of cocaine (m/z 304.1) from panel A. (C) MS spectrum of a capsule containing an off-white powder, found to contain methiopropamine. (D) CID-MS/MS spectra of methiopropamine (m/z 156.0) from panel C.

Conclusions

The use of illicit drug substances carries risks that can be exacerbated by adulteration or misidentification, or when present in complex poly-drug mixtures. Of particular relevance to the rationale for developing the sample analysis strategy reported herein, including the capability for being deployed on-site and in close to real time, are the dual and interacting challenges of being able to identify illicit substances, particularly NPS, when drug users are combining substances and trying out new substances within the milieu of a rapidly changing illicit drug marketplace. Using DART-MS and -MS/MS for sensitive, rapid and high-throughput ‘population’ level monitoring of a wide range of illicit drug substances found within discarded DPS at large public events, we determined that the majority of samples collected during our study contained polydrug mixtures, including NPS, thereby demonstrating that this method has the capacity to address these dual challenges. Importantly, the results obtained are shown to be a critical input for the provision of harm reduction information to first aid and medical personnel and event patrons, and subsequently to the general public, when substances with particularly toxic properties, or in particular harmful combinations, are identified.

Supporting Information

The Supporting Information, containing additional details of the experimental methods used in this study, and additional DART-MS and MS/MS spectra of selected compounds discussed in the text, is available free of charge on the ACS Publications website.

Acknowledgements

We thank Graham Hilder for his expert assistance with customization of the mobile analytical laboratory, and Jim Tsiotinas and Tom Hennessy (Agilent Technologies Australia) for their collaboration and loan of the Ultivo triple quadrupole mass spectrometer used in this study. Funding for this study was provided by the University of Melbourne Proof of Concept Fund and from the Victoria State Government Department of Health.

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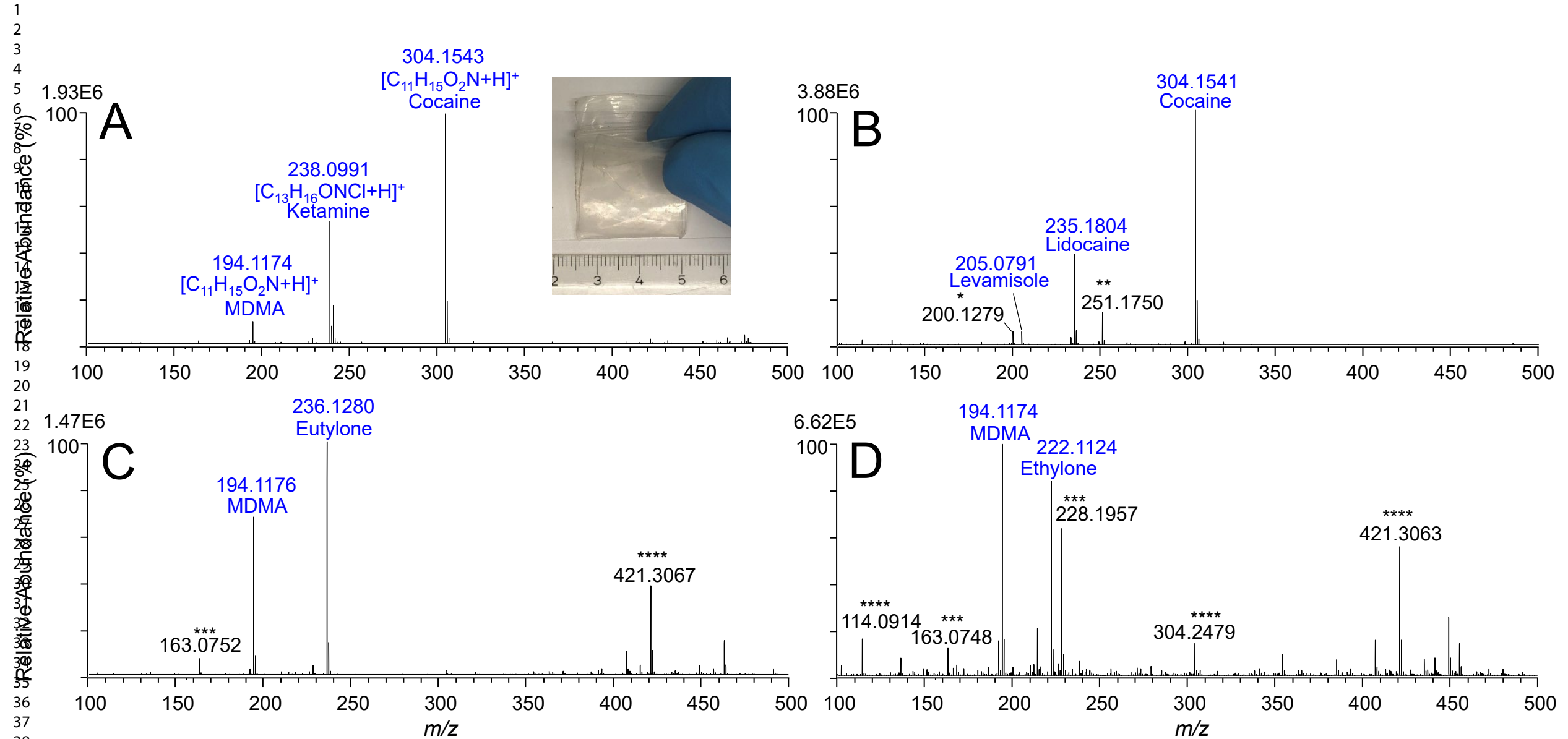
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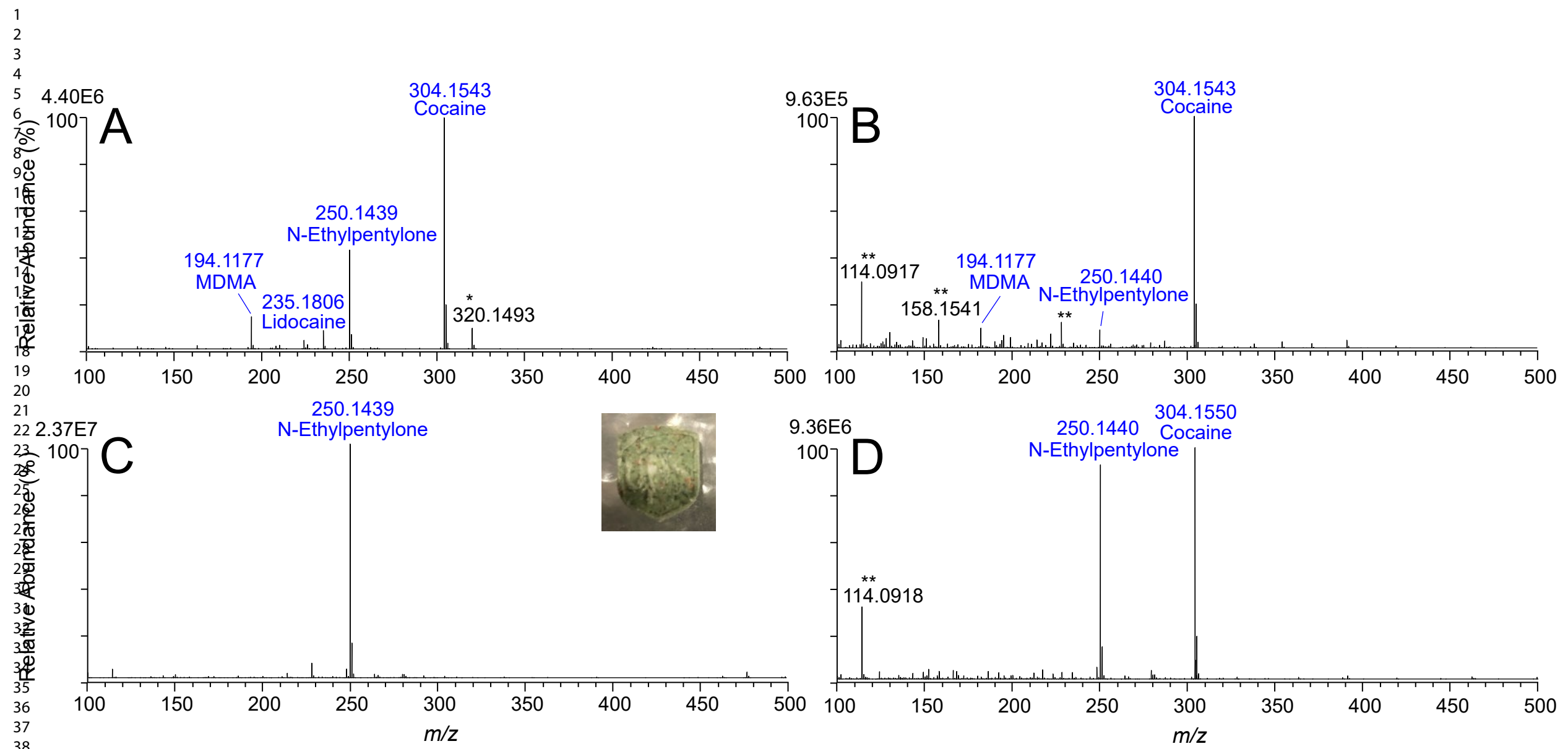
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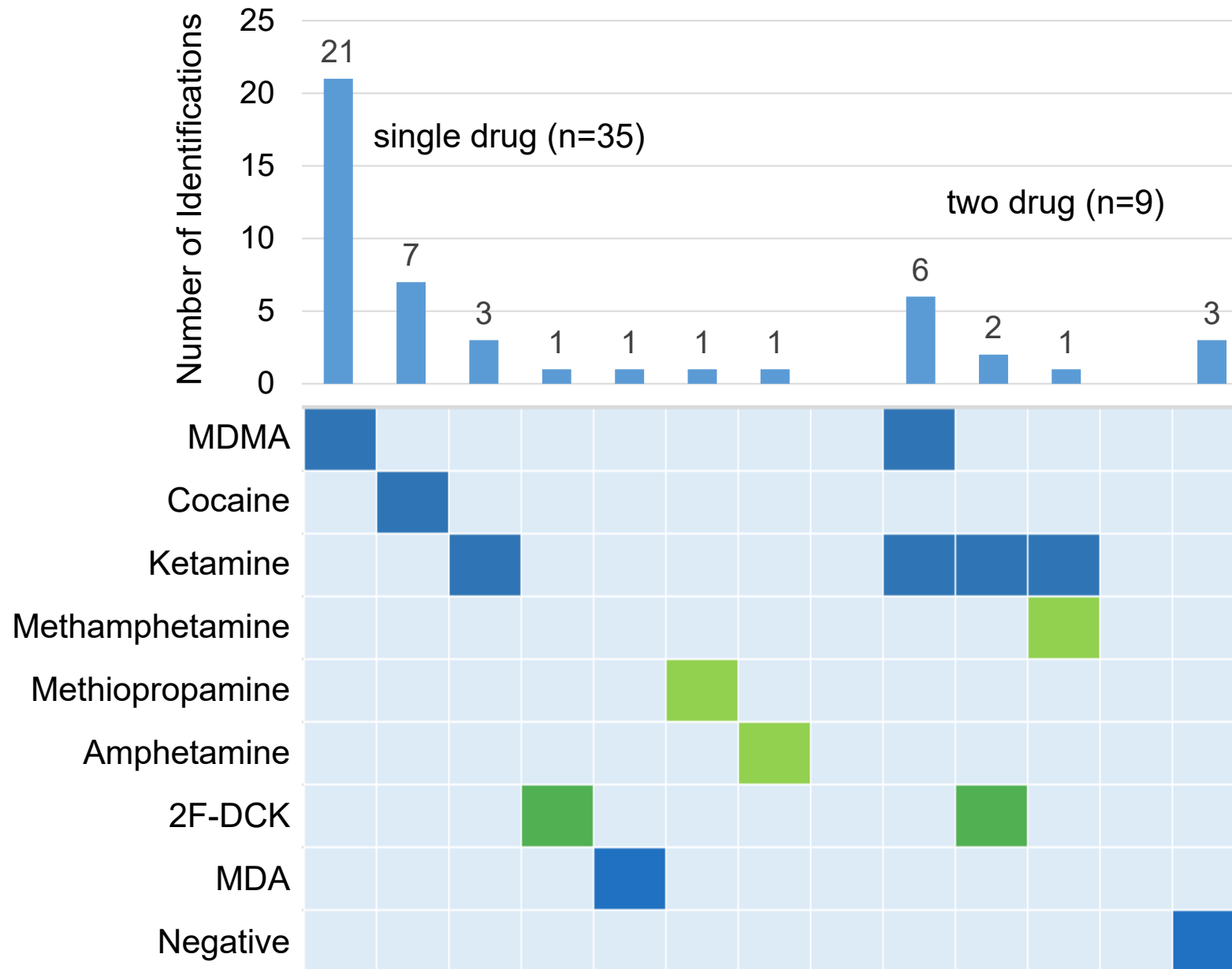
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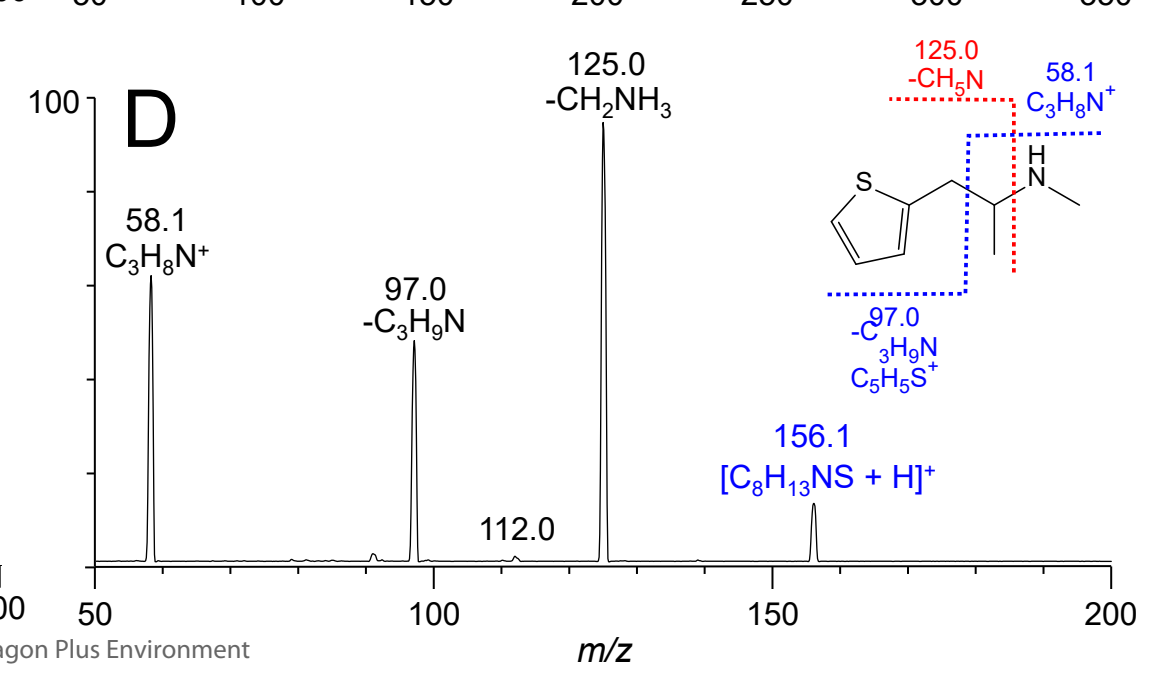
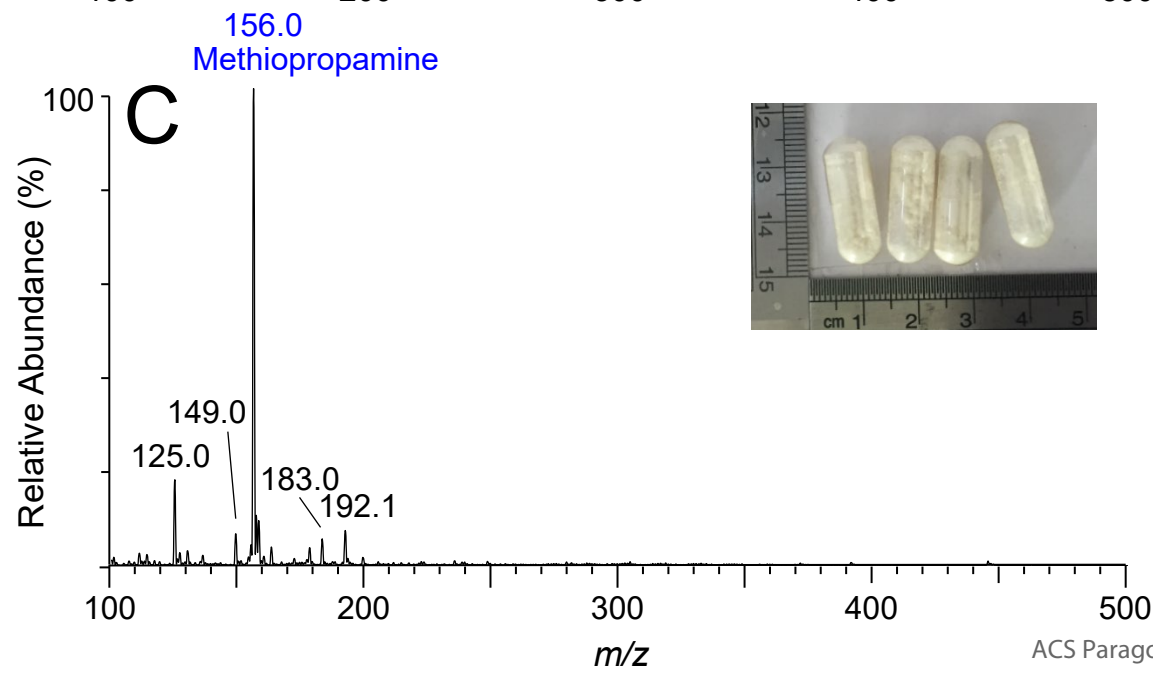
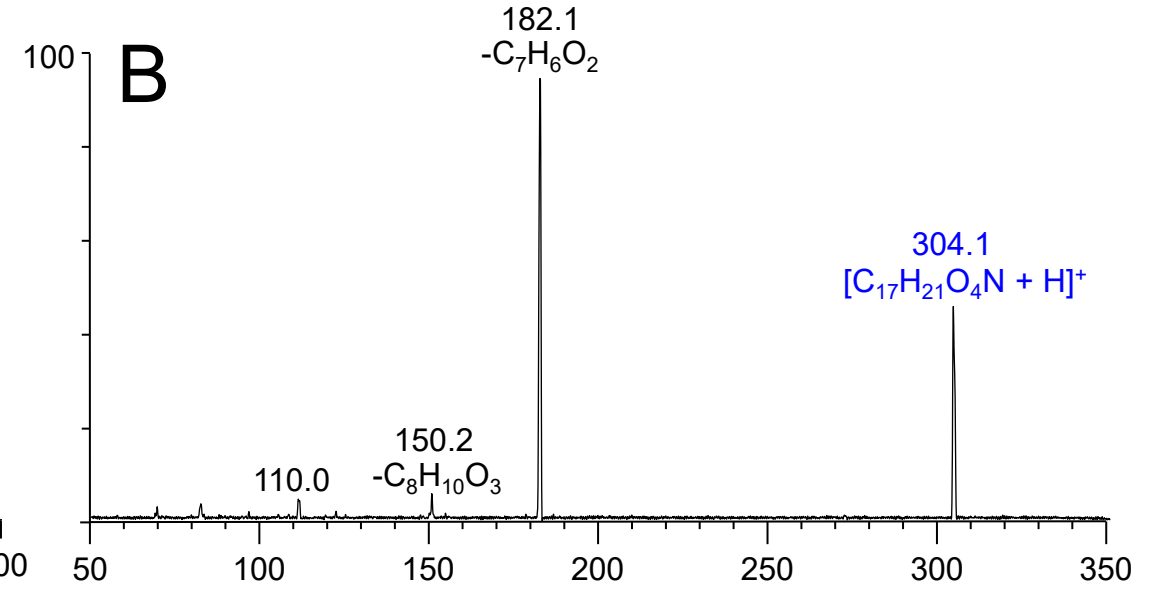
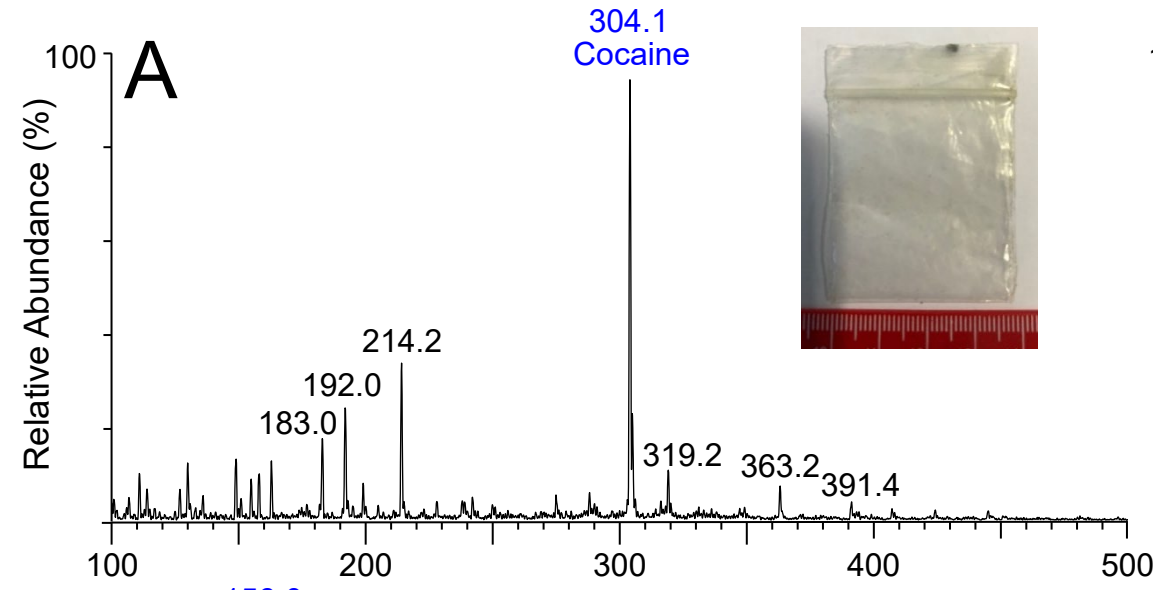
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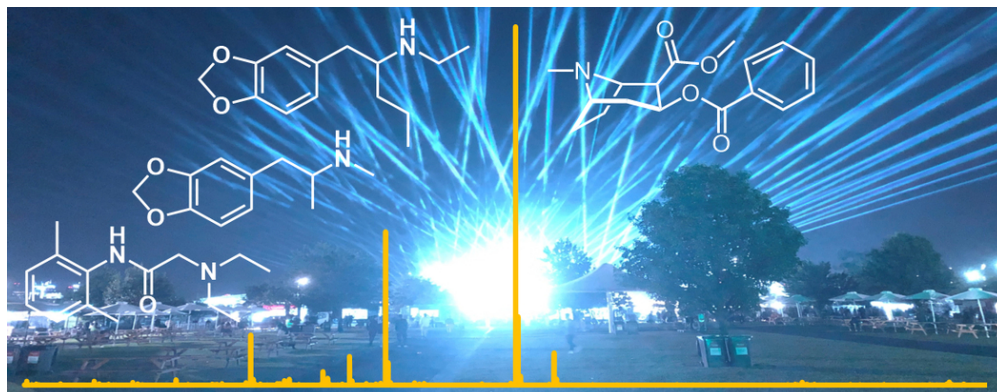
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An Early Warning System for Illicit Drug Use at Large Public Events: Trace Residue Analysis of Discarded Drug Packaging Samples

Henry West, John Fitzgerald, Katherine Hopkins, Eric Li, Nicolas Clark, Stephanie Tzanetis, Shaun L. Greene, Gavin E. Reid.

DART-MS spectrum from a mixture of illicit drug substances found at a music/dance festival

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