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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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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 colorometric 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,

minimal sample amount (low mg quantities), speed of analysis (<1 minute per sample), relative low establishment cost, and simple operational complexity (i.e., low technical expertise requirement). The presence of the predominant drug and/or other inert filler compounds that are present can be presumptively but confidently determined by searching the resultant data against pre-defined reference libraries of authentic compounds [26]. However, FTIR is not useful for the detection of potentially highly harmful drugs present at low levels relative to inert filler compounds or other major drugs that may be present (i.e., in poly drug mixtures), or when a drug is not included in the reference library (e.g., new psychoactive substances (NPS)). Furthermore, FTIR is largely a qualitative analysis technique, such that critical quantitative information regarding drug purity or dose is typically not obtained [26, 30]. Raman spectroscopy, also used previously for the analysis of illicit substances in field-based drug analysis applications, can suffer from similar limitations [25,30,31]. Therefore, additional approaches and resources are typically required to provide further and more precise information regarding drug compositions and purity e.g., by using a team of highly-trained chemists to extract and to perform additional chemical analysis of the drugs, to reduce the potential for false negative (and false positive) results.

In contrast, mass spectrometry (MS) is widely acknowledged as the gold standard analytical measurement technology for forensic drug analysis, including for drug monitoring [30,32]. MS, when coupled with established chromatographic separation techniques, namely Gas Chromatography (GC)-MS [33] and Liquid Chromatography (LC)- Electrospray Ionization (ESI)-MS or -Tandem Mass Spectrometry (MS/MS) [34-36] are capable of providing definitive qualitative and quantitative drug identifications with high sensitivity and specificity, including for low level components within poly-drug mixtures, by matching the observed retention times, mass-to-charge ratio's and/or characteristic fragmentation patterns for each drug against information contained within reference libraries generated from authentic standards [35,36]. MS techniques are also capable of *de-novo* identification and characterization of novel drug substances that appear on the market.

Commercially available portable or transportable GC-MS instrumentation have also been employed for on-site drug testing applications, e.g., at clandestine laboratories by police forensic services, or off-site in police stations [37] or drug checking services. However, these 'hyphenated' MS methods can suffer from relatively low throughput capability due to the need

for time consuming sample extraction/processing steps and long chromatographic analysis times (often 10's of minutes per sample), can have relatively high establishing or ongoing operating costs, and require significant expert technical knowledge for operation and maintenance. Therefore, they are not considered fit for purpose for use in drug checking applications requiring high throughout, or in close to real time harm reduction initiatives, in varied settings such as music festivals or supervised drug consumption facilities.

To address these limitations, various alternative MS based strategies employing ambient ionization techniques for direct sample introduction have been investigated [32], including Desorption ElectroSpray Ionization (DESI) [38,39], DART [40-44], Low Temperature Plasma (LTP) ionization [45-47], Paper-Spray (PS) ionization [48-51], and Atmospheric Solids Analysis Probe (ASAP) [52]. Importantly, these approaches enable rapid MS and MS/MS data acquisition with little or no requirement for sample preparation or separation prior to sample introduction [32], and with trace-level sensitivity. Furthermore, they can be interfaced with portable mass spectrometry instrumentation [38,39,51-56] for both qualitative and quantitative analysis of pharmaceutical and illicit drug substances including synthetic cathinones [38] and fentanyl and fentanyl-analogues [51]. For example, thermal desorption (TD)-DART-MS has previously been shown to have nanogram level limits of detection for the identification of fentanyl, fentanyl analogues, and a variety of other opioids, and also enable detection of fentanyl down to 0.1% by mass in the presence of heroin [43]. Furthermore, TD-DART-MS has previously been shown to enable identification of the contents of bulk drug packaging samples seized by law enforcement, via analysis of swabs taken from the exterior of the packaging material (when single to tens of micrograms of drugs are present). In this report, the drugs identified from the exterior of the drug packaging were found to be 92% accurate with the contents [54]. To date, however, DART-MS has not been applied for harm reduction applications at large public events, including on-site and in close to real time.

Here, as part of developing an early warning monitoring system for illicit drug use at large public events, including on-site and in close to real time, we describe the development of a high-throughput and rapid qualitative identification and characterization strategy using DART-MS and -MS/MS, involving trace-residue sampling of illicit drug substances found within discarded Drug Packaging Samples (DPS). Applications involving both laboratory-based ultra-high-resolution and accurate mass spectrometry (UHRAMS) instrumentation, as well as in a

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

[1]defined as containing trace amounts of powder or crystals.

[2]defined as containing greater than 5 mg of material.

[3]Samples categorised as "other" included air and water balloons, small glass and plastic food or confectionary containers (e.g., 'kinder surprise' plastic eggs), feminine hygiene product applicators, adhesive tape, etc.

Sample Preparation. Samples were prepared for analysis by lightly dry swabbing the surface area of the DPS (e.g., the interior of the ziplock bags) using commercially available cotton tip applicators (Swisspers, Kingsgrove, NSW, Australia). For samples containing visible residue and measurable amounts of material, the cotton tip applicators were gently flicked after swabbing to displace any loose material. The swabs were then cut to a length such that the widest circumference of the head aligned vertically with the tip of the DART probe when placed into a custom sample holder. See the Supplemental Information for additional detail.

Direct Analysis in Real Time (DART) sample introduction. Samples were introduced for mass spectrometry analysis using a DART source (IonSense, MA, USA) interfaced to the mass spectrometers using a Vapur® interface with Nitrogen as the source gas. Variables that could affect the practical application and performance of the DART source for trace residue drug analysis were evaluated and optimized during initial method development (see the Supplemental Information for additional detail). To ensure even desorption and the acquisition of a mass spectrum that was representative of the entire surface that had been swabbed, tweezers were used to manually rotate the stem of the cotton tip applicator containing the samples during data acquisition.

Mass Spectrometry Analysis Two mass spectrometers were used in this study. For off-site laboratory-based analysis, a Thermo Scientific Q Exactive Plus Orbitrap mass spectrometer (Bremen, Germany) was used, while for on-site close to real time analysis, an Agilent Ultivo triple quadrupole mass spectrometer housed within a custom modified mobile analytical laboratory was employed (see the Supplemental Information for additional detail).

Identification of illicit drug compounds and adulterants. Given that the majority of the DPS contained no visible residue, all results in this study are reported as qualitative identifications only. For off-site laboratory-based analysis, the identities of all drugs and adulterants within DPS were initially assigned based on manual comparison of their accurate mass values determined from their $[M+H]^+$ ions in positive ionization mode, against a list of >700 drugs,

adulterants and common contaminants that were of possible interest to this study, then definitively confirmed by manual comparison of their HCD-MS/MS spectra against reference spectra contained within the mzCloud Advanced Mass Spectral Database [57], or against MS/MS spectra previously reported in the literature. Blank cotton swabs were run every 5 samples as controls. Positive identifications were assigned only if the MS signal intensity for the precursor ion of interest was 10x the background level in the blank spectra. For on-site close to real time analysis, definitive identification of MS peaks observed at nominal *m/z* values corresponding to potential expected drug substances, or abundant MS peaks not present in the blanks, was achieved by acquisition of their MS/MS spectra followed by *de novo* analysis then comparison against reference spectra contained within the mzCloud Advanced Mass Spectral Database, or against MS/MS spectra previously reported in the literature. For samples that contained visible residue, conventional ESI-MS was used to confirm that the drug compositions observed by DART were consistent. However, although DART-MS has previously been shown to enable detection of low concentration drugs in the presence of higher concentration species, we cannot exclude the possibility that competitive ionization via DART could inhibit the detection of all drugs of interest that may be present at very low concentrations.

Results and Discussion

Off-site laboratory-based DART-MS and -MS/MS for rapid trace residue illicit drug monitoring. Representative spectra acquired by trace residue swabbing and DART-MS analysis of four different DPS ziplock bags, each containing no visible residue, are shown in **Figure 1** (the inset to Figure 1A shows a photograph of the clear plastic ziplock bag from which the swab was taken, measuring 30 x 24 mm, that had been torn open). For the MS spectrum in Figure 1A, tentative assignment of the ions at *m/z* 194.1174, 238.0991 and 304.1543 were made for 3,4-Methylenedioxymethamphetamine (MDMA) (calculated *m/z* 194.1181), ketamine (calculated *m/z* 238.0999) and cocaine (calculated *m/z* 304.1549), respectively, based on close agreement with their molecular formulas, then definitively confirmed by manually matching the product ion *m/z* and relative ion abundances observed within their HCD-MS/MS product ion spectra against the reference spectra of authentic compounds contained in the mzCloud database that had been acquired under approximately the same collision energies (**Supplemental Figures S1**A-C, respectively). These three drugs were the most commonly

observed compounds during our study (see below for further discussion and summary of the overall dataset).

MDMA (commonly known as ecstasy or molly) is the drug most commonly associated with recreational use at music festivals and similar events, where it is reported to give rise to user effects including an enhanced sense of well-being and sensory perception [58,59]. Ketamine is a dissociative anesthetic that is commonly consumed for its mild psychedelic effects [60], while cocaine is a stimulant whose use can result in feelings of euphoria [61]. The DPS giving rise to the DART-MS spectra in Figure 1B was found to contain cocaine along with the common adulterants, levamisole and lidocaine (Figure 1B), while those giving rise to the spectra in Figures 1C and 1D were found to contain MDMA adulterated with the substituted cathinones [15] eutylone and ethylone, respectively. Confirmatory HCD-MS/MS spectra for each of these adulterants are shown in Supplemental Figure S1D-G(also see below for further discussion regarding these compounds). Importantly, the spectra in Figure 1, obtained by averaging 100 scans, were each acquired in only 7 seconds while the individual HCD-MS/MS spectra were each acquired in only 10 seconds each. Thus, the identity of all the substances in each DPS using this trace residue sampling and analysis approach were definitively confirmed in less than one minute of total analysis time each, indicative of the high-throughput capabilities of DART-MS and MS/MS for illicit drug monitoring. Including sample preparation time (i.e., swabbing of the DPS), it required only two people to analyze up to 80 samples per hour.

For these off-site laboratory-based analyses, in which samples were batch collected and then transported to the laboratory for analysis, reporting of results regarding the identity of the drug(s) and/or their packaging appearance were then provided to key Event Management Team (EMT) stakeholders including event organizers, first aid and medical personnel, peer-based harm reduction workers, and security contractors, amongst others, in as little as 60 min after sample collection, depending upon the distance between the event and the laboratory. Risk assessments of the potential harms associated with the identified substances were then performed by this group, and, as deemed appropriate or necessary, alerts were then provided to event personnel, event patrons, and subsequently to the general public (see the section on Substituted Cathinones below), to reduce their risk of drug-related harm when substances with particularly toxic properties were identified.

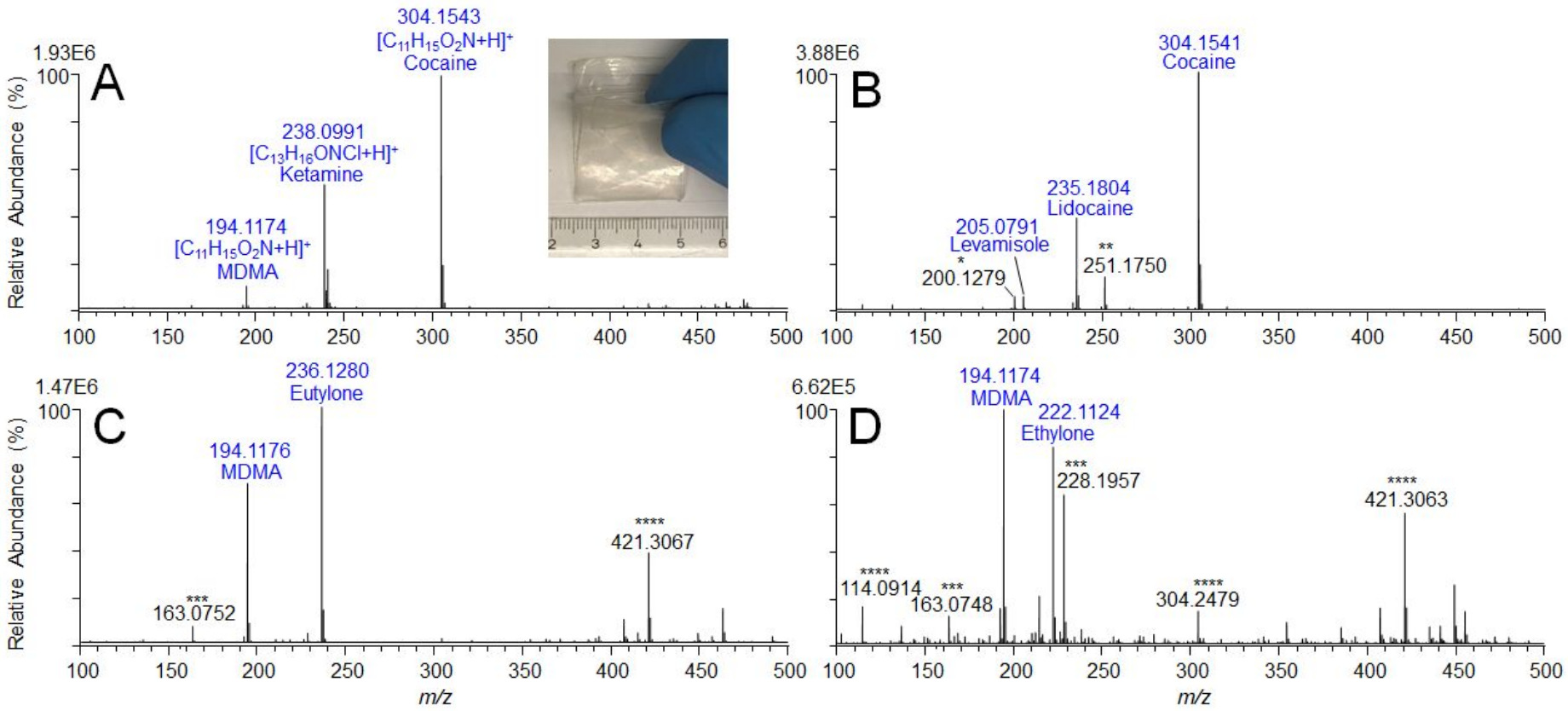

Figure 1. DART-MS spectra from several discarded DPS ziplock bags containing no visible residue, found to contain (A) cocaine, ketamine and MDMA (the inset to panel A shows a photograph of the bag), (B) cocaine adulterated with levamisole and lidocaine, (C) MDMA adulterated with eutylone, and (D) MDMA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

An overall summary of the results obtained from the off-site laboratory-based analysis of DPS are shown in **Figure 2**. A total of 1,315 samples were analyzed, with 92.2% (N=1,212) providing positive results for at least one pharmacologically active compound. A total of 15 different drugs and/or adulterants were identified, including cocaine, MDMA, ketamine, levamisole, lidocaine, methamphetamine, amphetamine, several NPS including 2-fluorodeschloroketamine (2-FDCK), tiletamine, the synthetic cathinones ethylone, eutylone and N-ethylpentylone, the endogenous hallucinogen N,N-dimethyltryptamine (DMT), 3,4-methylendioxyamphetamine (MDA), and methylphenidate.

Of the DPS samples that tested positive, cocaine (58.9%) was the most commonly observed drug, followed by MDMA (58.2%) and ketamine (32.8%). However, polydrug mixtures were more common than single drugs, with 52.6% of positive samples containing more than one substance, in 42 different drug combinations. Of the total positive samples, 39.0% tested for two substances, 12.0% tested for three active compounds and 1.6% tested for four active compounds. MDMA/cocaine (n=227), ketamine/cocaine (n=98) and MDMA/ketamine/cocaine (n=89) were the three most common combinations. Although cocaine was observed as the most common drug, it was present only 24.2% (n=173) of the time as a single drug i.e., without other co-present drugs or adulterants, and was instead found to be co-present with MDMA 48.6% of the time, and co-present with ketamine 31.2% of the time. Note that these percentages include all cocaine + MDMA combinations and cocaine + ketamine combinations, including those where cocaine + MDMA + ketamine were all present.

Additionally, cocaine was found to be co-present with the common adulterants levamisole (without lidocaine) 9.9% of the time, co-present with lidocaine (without levamisole) 4.3% of the time, and co-present with both levamisole and lidocaine 1.3% of the time. Similarly, MDMA was observed to be present only 40.7% of the time as a single drug, and 49.2% of the time to be co-present with cocaine (n=347), and 22.8% of the time with ketamine. MDMA was also found to be co-present with levamisole (no lidocaine) and with lidocaine (no levamisole) 1.3% and 1.8% of the time, respectively, and co-present with both lidocaine and levamisole 0.1% of the time. In addition, MDMA was found to be co-present 1.3% of the time with one of the synthetic cathinone drugs, eutylone, ethylone or N-ethylpentylone. Finally, ketamine

was observed only 22.4% of the time as a single drug, while being present 56.0% of time with cocaine, and 40.5% of the time with MDMA.

Notably, a significantly greater fraction of samples that contained polydrug mixtures were identified in this study, compared to reports from other recent pill testing/drug checking operations in New Zealand and Australia, that could be expected to share similar drug distribution and/or recreational drug user demographics and usage patterns to those within the events and locations from which the current samples were obtained [22-24]. This difference is likely explained by the different analytical measurement technologies that were employed. For example, in contrast to the study performed here using highly sensitive mass spectrometry-based methods, the results from these other reports were obtained using FT-IR spectroscopy, suggesting a potential bias and/or limitations of that technique against the detection of substances present in polydrug mixtures, particularly those at low levels relative to inert filler compounds or other major drugs that may be present. This highlights the critical requirement for analytical measurement technologies that are capable of accurately identifying and characterizing all the components of complex drug mixtures, including those at low relative ion abundances, if the results are to be used for health promotion and harm reduction purposes. Due to the nature of the sample type (i.e., DPS) and the anonymized sample collection strategy employed here, it was not possible to evaluate whether or not the identified substances were the same as those expected by the consumer.

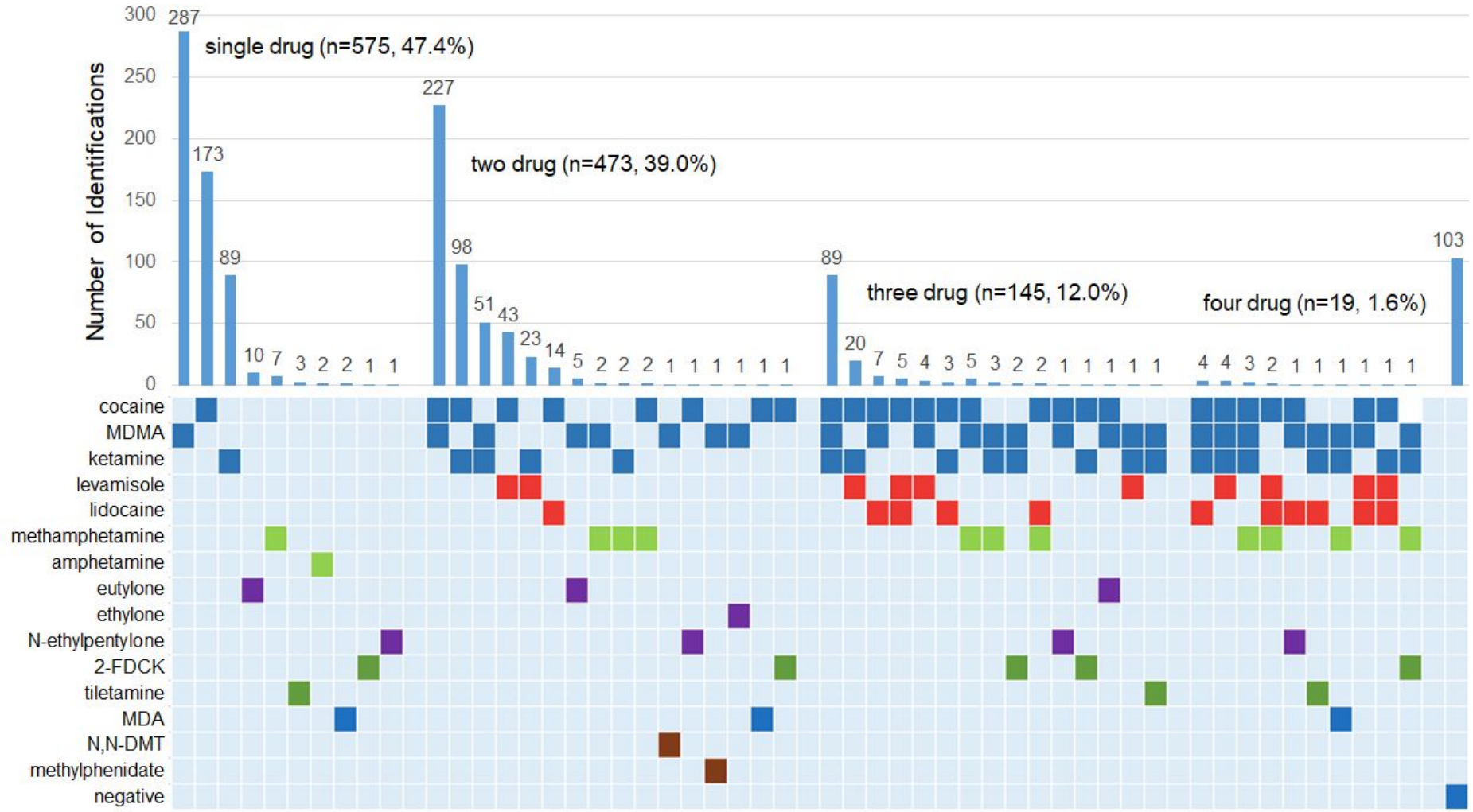

Figure 2. Illicit drugs, and drug combinations observed by trace residue laboratory-based DART-MS analysis of DPS samples. The colors highlight the various different classes of drugs that are present.

Adulterants and bulking agents. Lidocaine and levamisole are well-documented adulterants of cocaine [4] and were the two most commonly observed in our study. Lidocaine, a local anesthetic, is commonly added to cocaine to enhance the characteristic numbing effect that is often associated with 'high-quality' cocaine, while levamisole is used as a bulking and enhancing agent due to its white, fluffy powdery appearance, similar to cocaine. Previously, levamisole was used as a worming agent in humans; however, it was withdrawn from market after being linked to serious cardiovascular issues including agranulocytosis, the depletion of white blood cells, as well as vasculitis leading to inflammation and destruction of blood vessels [62]. Both complications have been reported amongst cocaine users who have consumed cocaine laced with levamisole [5]. Whilst levamisole was primarily observed here to be co-present with cocaine, 23 ketamine-containing samples also tested positive for the presence of levamisole, revealing the complexity of the illicit market when drugs are readily substituted and the consumers have little awareness of the materials they are consuming. Other adulterants observed in this study included nicotinamide, commonly known as vitamin B3, and dimethylsulfone (DMS). Nicotinamide is readily available from most pharmacists and supermarkets, with potential negative effects reportedly occurring only after doses exceeding 3 grams/day [63]. DMS, a common bulking agent in methamphetamine samples [64], was observed in 5 samples in this component of the study, all of which also contained methamphetamine and corresponding to 17% of all the methamphetamine samples identified. However, a previous study investigating the impact of DMS on rats did not identify any adverse effects from exposure [65].

Substituted Cathinones. Substituted cathinones, colloquially known as "bath salts", are synthetic analogues of cathinone, a naturally occurring stimulant found in the plant khat, and are a class of NPS of concern for recreational drug use [66]. A wide range of substituted cathinones have been reported [11,66]. Like most NPS, the pharmacokinetic and pharmacodynamic properties, as well as the short-term and long-term effects of many substituted cathinones are poorly understood. Common symptoms associated with their use include agitation, tachycardia, paranoia and seizure/tremors, hyperthermia, and multiorgan system failure [66,67]. In a 2015 study of drug use among nightclub/festival-attending young adults in New York City, analysis of donated hair samples revealed that of the samples containing MDMA, 47.9% contained butylone, and 10.4% contained methylone. Furthermore,

the hair samples from 41.2% of respondents who reported no lifetime use of NPS or unknown pills or powders, tested positive for the synthetic cathinones butylone, methylone, alpha-pyrrolidinovalerophenone (α-PVP), 5- or 6-(2-aminopropyl)benzofuran (5/6-APB), or 4-fluoroamphetamine (4-FA) [68]. This suggests that many MDMA-users may be unintentionally using synthetic cathinones or other NPS, and that the risks associated with the use of MDMA may be further exacerbated through adulteration or substitution with synthetic cathinone drugs.

During this study, three substituted cathinones were identified: ethylone, eutylone and N-ethylpentylone. Eutylone was the most commonly observed and was found to be present in 16 samples (10 times as a single drug, 5 times in combination with MDMA (Figure 1C), and once in combination with MDMA and cocaine). Ethylone was observed in only one sample, co-present with MDMA (Figure 1D), while N-ethylpentylone was observed in a total of 4 samples. As a demonstration of the general utility of the cotton tip 'swabbing' method described here for the detection and identification of synthetic cathinone drugs from a variety of DPS materials, including those containing polydrug mixtures and at varying relative ion abundances, the DART-MS spectra from the four N-ethylpentylone-containing samples are shown in **Figure 3**. Figure 3A shows the spectrum obtained through swabbing of a ziplock bag, that was found to contain N-ethylpentylone at moderate relative ion abundance and co-present with cocaine, MDMA and lidocaine, while Figure 3B shows the result from swabbing a piece of aluminum foil found to contain N-ethylpentylone at low relative ion abundance and co-present with MDMA and cocaine. In contrast, Figure 3C shows the spectrum obtained by analysis of an intact green, speckled pill imprinted with 'UPS' (approximately 8 mm x 10 mm x 3 mm in size), that was collected as a discarded 'ground find' (also called an 'unattached seizure' i.e., a sample not associated with any identifiable individual or police investigation) at a large multi-day music festival. To ensure that the sample subjected to analysis would be representative of the entire pill, the pill was initially broken into several small pieces from which representative small scrapings were taken prior to pulverizing into a fine powder, followed by trace residue swabbing. It would be of interest in a subsequent study to systematically examine the heterogeneity associated with the distribution of different drug substance or amounts, in this type of sample. DART-MS and MS/MS (Supplemental Figure S1H) of this sample resulted in the identification of only N-ethylpentylone. The risk associated with N-ethylpentylone use is exemplified by a previous case study from a patient who died from an accidental N-

ethylpentylone overdose, that reported several complications including hyperthermia, disseminated intravascular coagulation (DIC) (blood clots forming throughout cardiovascular system), kidney and liver failure, and with the cause of death being cardiac arrest [2]. In this instance, as the pill had a clearly definable appearance, and following risk assessment of the potential harm associated with this drug at the multi-day event, an initial "patron alert" was sent via messaging app within 12 hours of its initial identification to warn patrons of its presence, and subsequently a "public drug alert" was made by the Department of Health & Human Services State Government of Victoria in March 2020 [69], the first of its kind within the State of Victoria. We note that the specific format and delivery of such alerts is a subject that requires ongoing consideration and evaluation. Finally, the spectra in Figure 3D resulted from analysis of a sample found to contain N-ethylpentylone and cocaine, obtained by swabbing a toilet roll holder in a public toilet cubicle at a popular social location (i.e., not a music/dance festival) on which traces of a white powder was observed. This demonstrated ability to obtain positive identifications from a diversity of DPS materials, as well as directly from surfaces in which drugs had been in contact, indicates that the developed sampling technique is quite versatile, with almost any surface being able to be swabbed. The co-presence of cocaine with three of the four N-ethylpentylone containing samples (and especially in the context of the visible powder on the toilet roll holder from which the sample in Figure 3C was acquired) suggested that the means of administration was likely to be insufflation, which could result in the N-ethylpentylone entering the body at a higher rate and level than from oral ingestion. As a result of this increased potential harm, combined with information regarding a number of N-ethylpentylone associated hospitalizations that occurred around the same period of time as the sample collection, a "public drug advisory" was subsequently made by the Department of Health & Human Services State Government of Victoria in December 2020 [70].

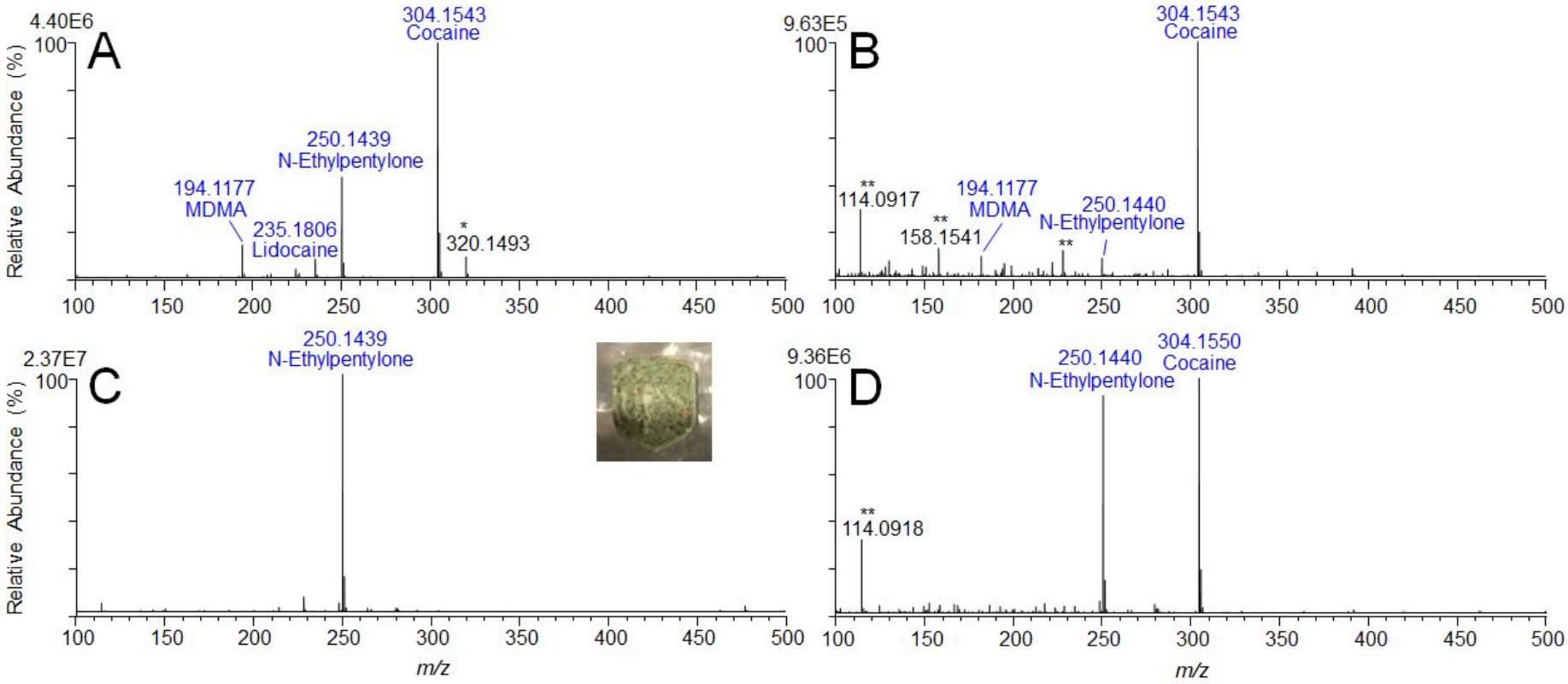

Figure 3. DART-MS of DPS samples containing N-ethylpentylone obtained from dry swabs of, (A) a ziplock bag, (B) aluminium foil, (C) a green speckled 'UPS' pill (the inset shows a photograph of the pill), and (D) residue, found on a toilet roll holder in a public toilet cubicle. * in-source oxidation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

Ketamine analogues. Two analogues of the dissociative anesthetic ketamine were identified throughout this study, namely 2-fluorodeschloroketamine (2-FDCK), and tiletamine. 2-FDCK, an analogue where the chlorine has been substituted with a fluorine [71], was present in six DPS, once by itself, once with cocaine, once with cocaine and ketamine, twice with MDMA and ketamine, and once in a quaternary mixture with methamphetamine, MDMA and ketamine (**Supplemental Figure S2**A). The co-presence of ketamine in four of the six DPS suggests that the 2-FDCK was introduced as an adulterant or substitution for ketamine. As with many NPS, little is understood regarding the effects, metabolism and potential toxicity of 2-FDCK. However, a study of halogen substitution on metabolism by cytochrome P450 2B6 (the major metabolic enzyme for hepatic metabolism of ketamine, operating through N-demethylation) found that the Michaelis Constant (K_m) was more than double that for 2-FDCK compared to ketamine (17 +/- 1 for ketamine and 40 +/- 3 for 2-FDCK) [72], suggesting that 2-FDCK has a lower binding affinity for the enzyme, which may result in a lower metabolism rate. Potential for harm exists therefore, if users re-administer after a period of time, thinking it to be ketamine. Tiletamine, another structural analogue of ketamine in which the chlorophenyl ring is substituted for a thiophene ring, was observed in a total of five samples, three times by itself, once co-present with MDMA and ketamine, and once with MDMA, ketamine and lidocaine (Supplemental Figure S2B). Although tiletamine is often combined with zolazepam (trade name Telazol) for use as a veterinary anesthesia [73], and human fatalities associated with use of the tiletamine/zolazepam combination have been documented [74], zolazepam was not observed in any of the samples analyzed here. The HCD-MS/MS spectra used to definitively confirm the identifications of 2-FDCK and Tiletamine are shown in Supplemental Figures S1I and S1J, respectively.

Other illicit substances. Methamphetamine was observed in 20 DPS in several different combinations, including in quaternary drug mixtures containing i) 2-FDCK, ketamine and MDMA, as shown in Supplemental Figure S2A, and ii) 3,4-Methylenedioxyamphetamine (MDA), MDMA and Ketamine (**Supplemental Figure S3**A). Confirmatory HCD-MS/MS spectra for methamphetamine and MDA are shown in Supplemental Figures S1K and S1L, respectively. MDA, observed in a total of 4 samples, is the primary metabolite of MDMA, but is also a potential synthetic by-product of MDMA, often observed at very low levels relative to MDMA. In this study however, MDA was observed in three samples without MDMA being

present, and was co-present with MDMA at similar intensity in the remaining sample, suggesting its deliberate adulteration or substitution for MDMA. Methylphenidate, commonly known under its trade name 'Ritalin', is a stimulant drug and a first line medication for management of ADHD, that was observed in one DPS co-present with MDMA (Supplemental Figures S3B and S1M). With similar reported effects to amphetamines, the recreational use of this substance is well documented [75]. Dimethyltryptamine (DMT), a psychoactive compound related to other psychedelic tryptamines including psilocin and bufotenine, was observed in one DPS co-present at low level with MDMA (Supplemental Figures S3C and S1N). Finally, two samples were found to contain amphetamine (an example is shown in Supplemental Figures S3D and S1O). The potent synthetic hallucinogen 25C-NBOMe, a member of the phenethylamine N-benzyl methoxy class of compounds, in combination with 4-fluoroamphetamine (4-FA) was recently found to be responsible for several fatal overdoses in Melbourne, Victoria, Australia throughout July 2016 to January 2017 [76], while another NBOMe, 25I-NBOMe, has been implicated in at least one other death in Australia [77]. Fortunately, none of these potentially fatal NBOMe drugs, or 4-fluoroamphetamine, were observed in our study. A subsequent study on a different cohort of samples collected from a different illicit drug user demographic group (to be reported elsewhere), did result in the detection of 4-FA, indicating this drug can be readily observed using our technique. Finally, although several health alerts were recently released in New South Wales, Australia with warnings of possible acetyl fentanyl and fentanyl-laced cocaine and ketamine samples [78,79], no fentanyl containing samples were observed in our study. However, based on prior reports regarding the detection sensitivity of fentanyl drugs using DART-MS [43], we are confident that our method would have detected fentanyl drugs if they were present.

Proof of concept implementation of on-site 'close to real time' DART-MS and -MS/MS analysis for trace residue illicit drug monitoring. To reduce the time delay between DPS collection and analysis, such that results could be obtained and reported in close to real time, it is desirable to perform the analyses on-site, i.e., proximal to where the sample collection occurs. To achieve this, portable or transportable mass spectrometry instrumentation is required. Therefore, in a proof-of-concept demonstration of the practical utility of our trace residue DPS sampling and analysis approach for on-site drug monitoring in close to real time, a compact triple quadrupole mass spectrometer equipped with a DART ionization source was installed in a customized mobile analytical laboratory and then transported for use at a one-day

music festival event (approx. 15,000 patrons) (**Supplemental Figure S4**). During this proof-of-concept study 47 DPS were analyzed, with 44 (93.6%) testing positive for at least one pharmaceutically active compound (**Figure 4**). Eight different drugs in 10 combinations were identified (79.5% as single drugs and 20.5% as two-drug mixtures). Albeit a proof-of-concept implementation on a limited scale, this is the first report describing the field deployment of a transportable DART-MS system interfaced with a compact triple quadrupole mass spectrometer for on-site close to real time drug monitoring at music festivals or large public events, anywhere in the world.

Example MS and MS/MS spectra resulting from analysis of DPS collected and analyzed on-site at this event are shown in **Figure 5**. The first (Figures 5A and 5B) was from a sample containing no visible residue, that was found to contain cocaine, while a second (Figure 5C and 5D), resulting in the identification of methiopropamine, was acquired from a swab taken from a discarded ground find consisting of a ziplock bag containing four capsules that themselves contained off-white colored powder. Methiopropamine is a structural analogue of methamphetamine where the phenyl ring has been substituted with a thiophene ring. In this case, as a low-resolution triple quadruple instrument was used for the analysis, only a nominal mass was obtained, so direct accurate mass based searching against the mzCloud database could not be performed. Instead, the similarity in the experimentally observed CID-MS/MS fragmentation behavior for methiopropamine with other amphetamines, especially methamphetamine, enabled a proposed structure to be initially assigned by *de novo* interpretation of the MS/MS spectrum, that subsequently allowed its accurate mass to be calculated. Then, the spectrum was matched against that the reference spectrum contained within the mzCloud database for definitive identification, despite us not having observed this drug throughout our previous off-site laboratory-based study. In this instance, less than 5 minutes elapsed between running and identifying this unknown sample, and subsequent reporting to on-site medical and first-aid personnel. Similar to other NPS observed in this study, the pharmacology and toxicological effects of methiopropamine, first detected in 2011 in Finland, are largely unknown, but has loosely been characterized as being amphetamine-like, but with hallucinatory effects. Although acute toxicity related to use of methiopropamine was first reported in the UK in 2014 [80], and a fatality associated with the use of methiopropamine was reported in Australia in 2015 [81], this is the first report of the identification of this drug in the context of its recreational use at Australian music festivals.

Subsequent analysis of each of the samples run on-site was performed using our off-site instrumentation. Importantly, each of the substances identified, and their approximate relative ion abundances, were qualitatively similar on both systems, indicating that the instrumentation used for the on-site monitoring was fit for purpose, despite its inferior performance specifications. Collectively, these results clearly highlight the potential for on-site transportable mass spectrometry-based instrumentation to identity new and potentially harmful compounds in close to real time, as part of an early warning system for monitoring and responding to illicit drug use at large public events.

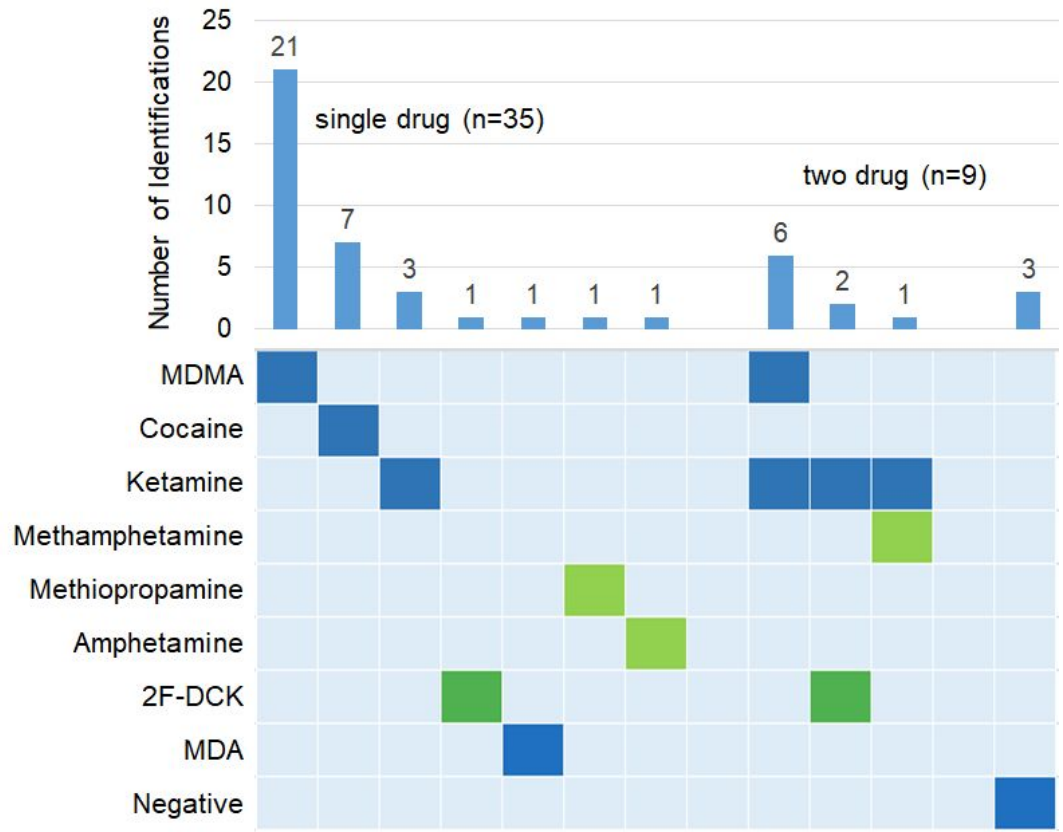

Figure 4. Illicit drugs and drug combinations observed by on-site close to real time mobile laboratory-based DART-MS monitoring of DPS samples. The colors highlight the various different classes of drugs that are present.

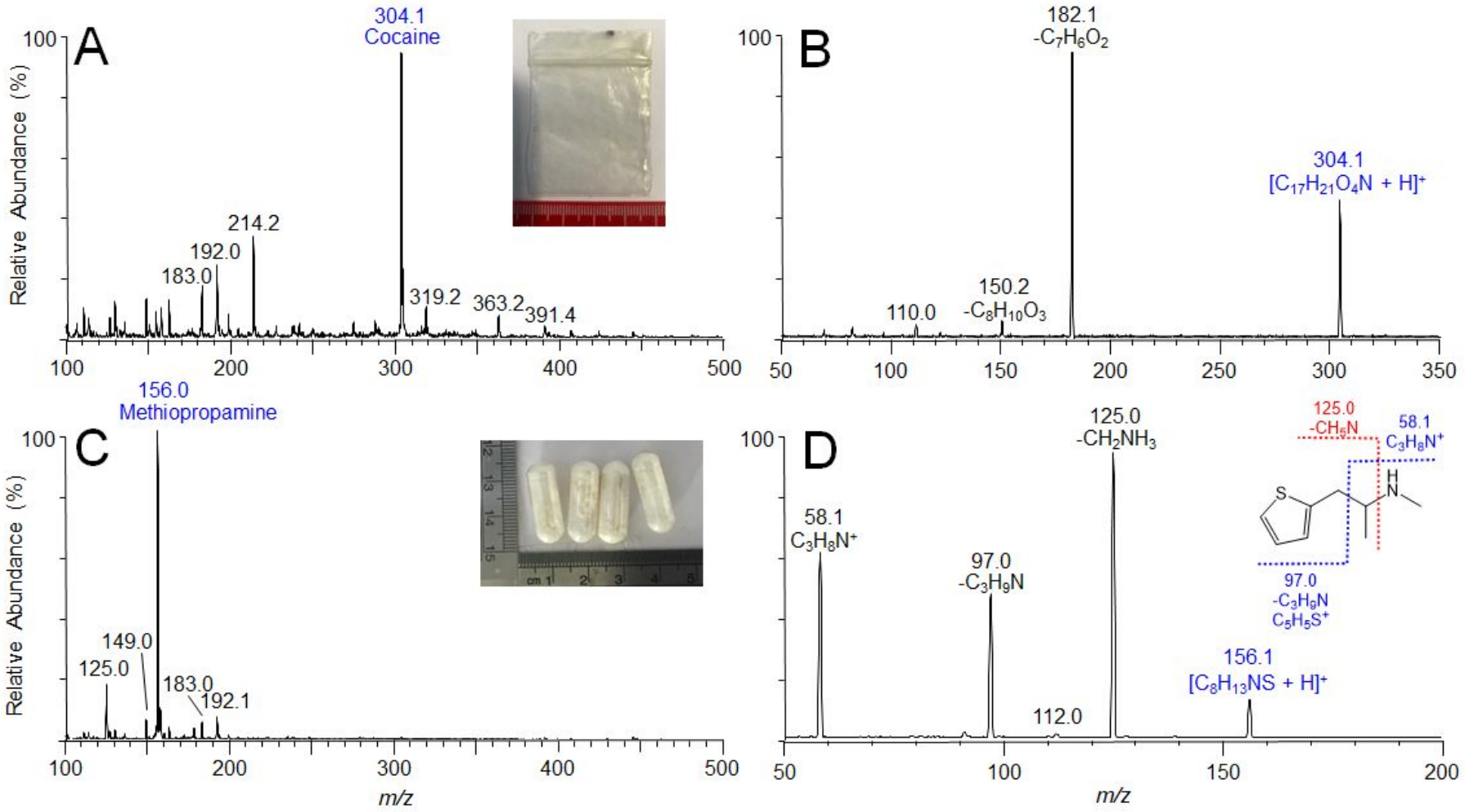

Figure 5. On-site close to real time DART-MS and -MS/MS analysis of illicit drug samples. (A) MS spectrum of a ziplock bag with no visible residue, found to contain cocaine. (B) CID-MS/MS spectrum of cocaine (*m/z* 304.1) from panel A. (C) MS spectrum of a capsule containing an off-white power, found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

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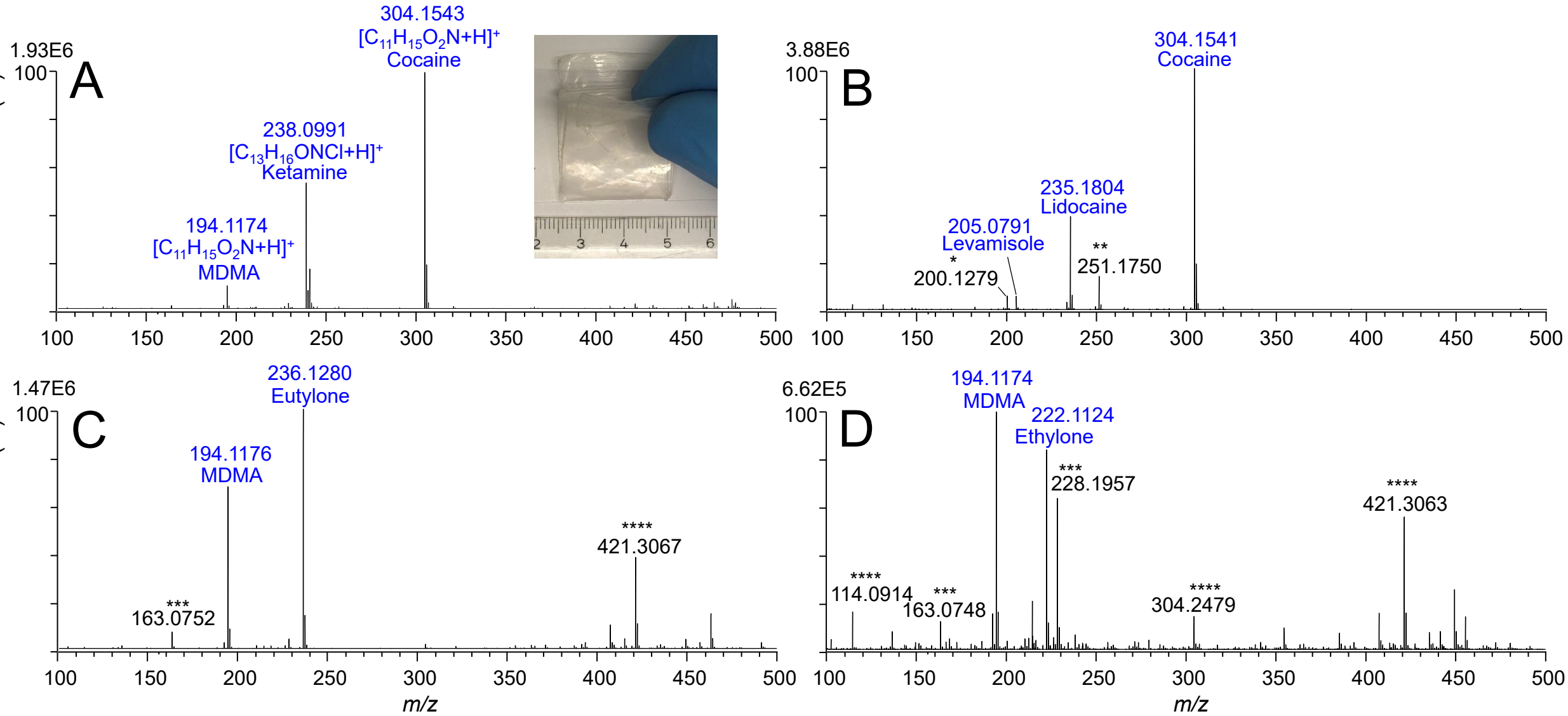
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100
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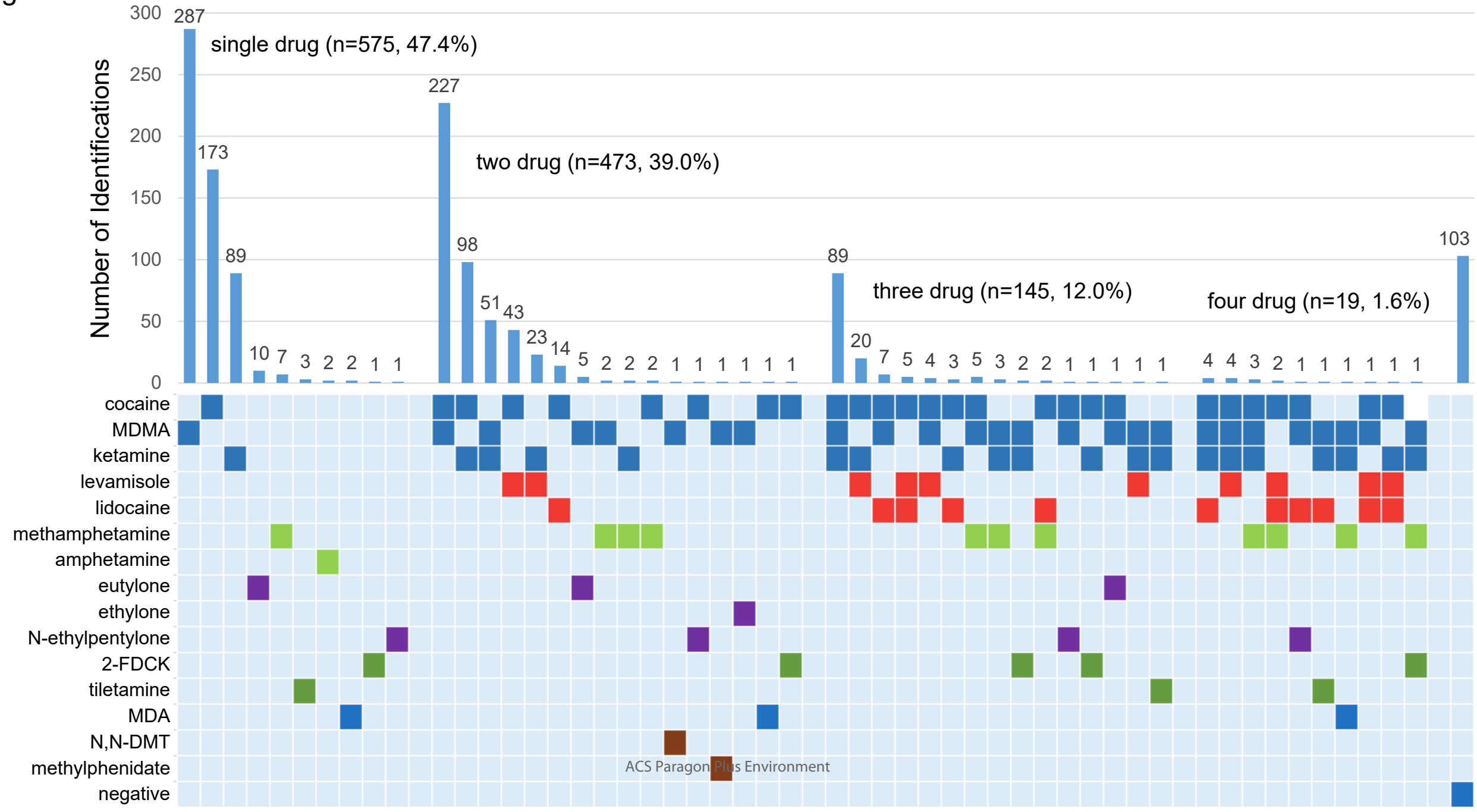
Cocaine
238.0991
[C13H16ONCl+H]+
Ketamine
194.1174
[C11H15O2N+H]+
MDMA
B
3.88E6
304.1541
Cocaine
235.1804
Lidocaine
205.0791
Levamisole
*
200.1279
**
251.1750
C
1.47E6
236.1280
Eutylone
194.1176
MDMA
***
163.0752
****
421.3067
D
6.62E5
194.1174
MDMA
222.1124
Ethylone
***
228.1957
****
421.3063
****
114.0914
***
163.0748
****
304.2479
Relative Abundance (%)
m/z
100
150
200
250
300
350
400
450
500

Figure 2


Number of Identifications
300
250
200
150
100
50
0
287
173
89
10
7
3
2
2
1
1
single drug (n=575, 47.4%)
227
98
51
43
23
14
5
2
2
2
1
1
1
1
1
1
two drug (n=473, 39.0%)
89
20
7
5
4
3
5
3
2
2
1
1
1
1
1
three drug (n=145, 12.0%)
4
4
3
2
1
1
1
1
1
1
103
four drug (n=19, 1.6%)
cocaine
MDMA
ketamine
levamisole
lidocaine
methamphetamine
amphetamine
eutylone
ethylone
N-ethylpentylone
2-FDCK
tiletamine
MDA
N,N-DMT
methylphenidate
negative
ACS Paragon Plus Environment

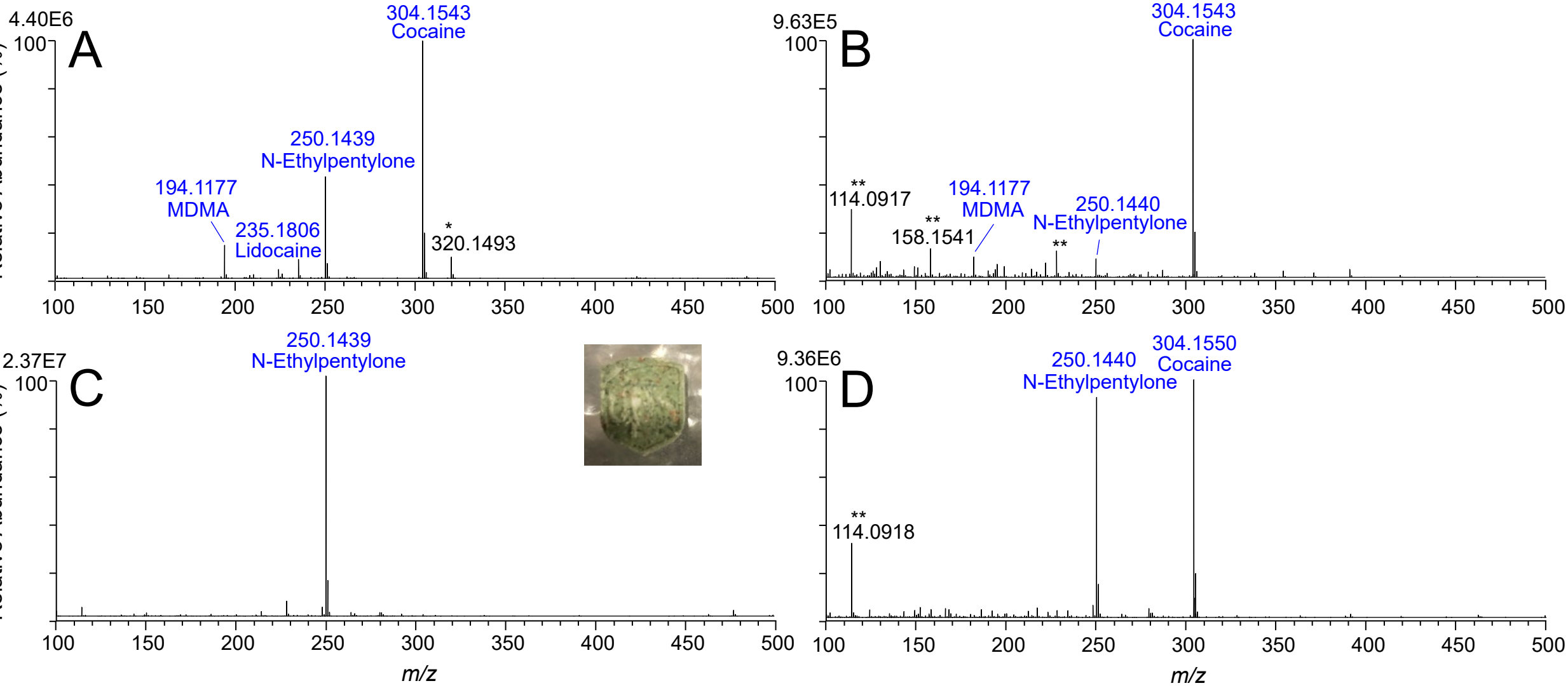
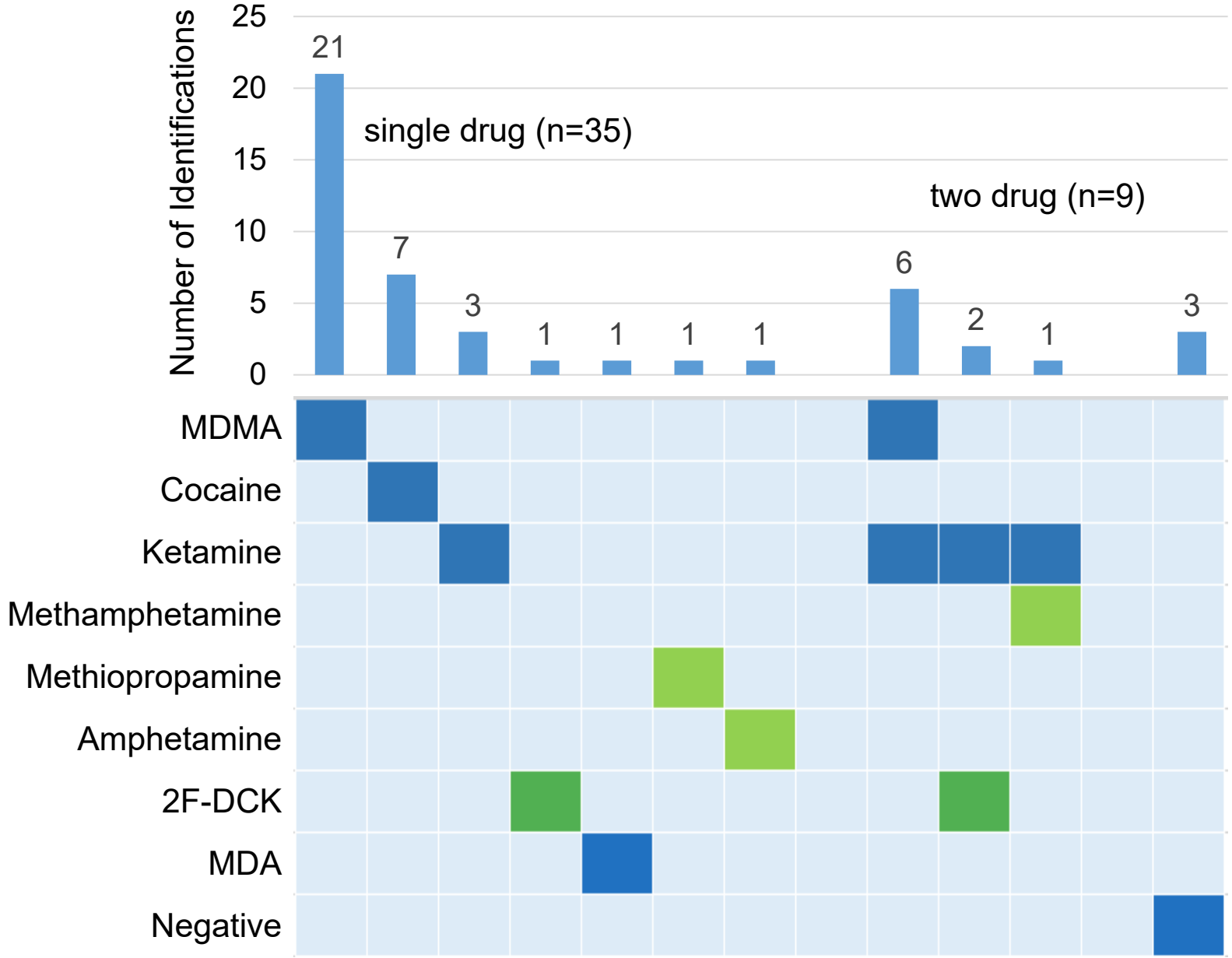
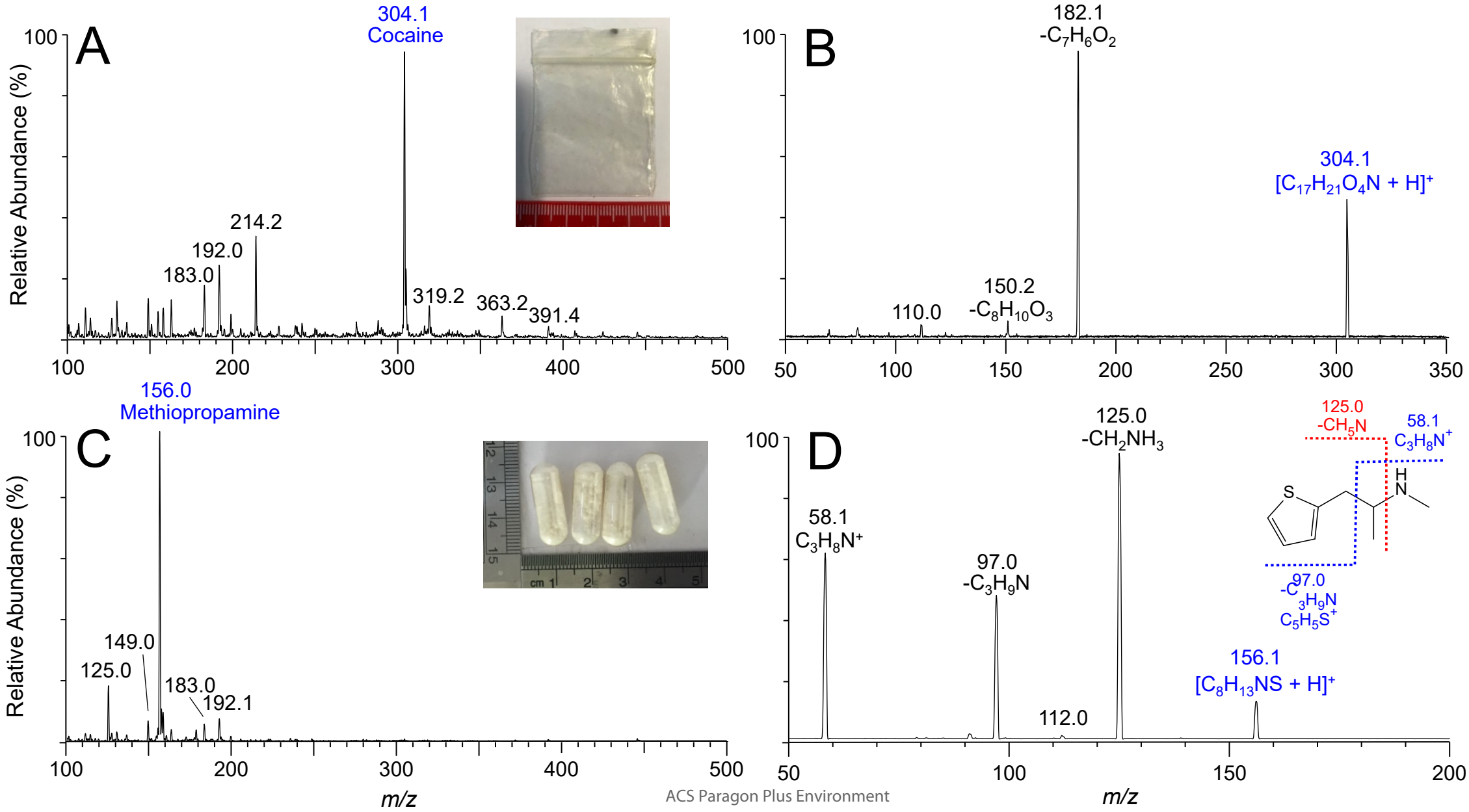
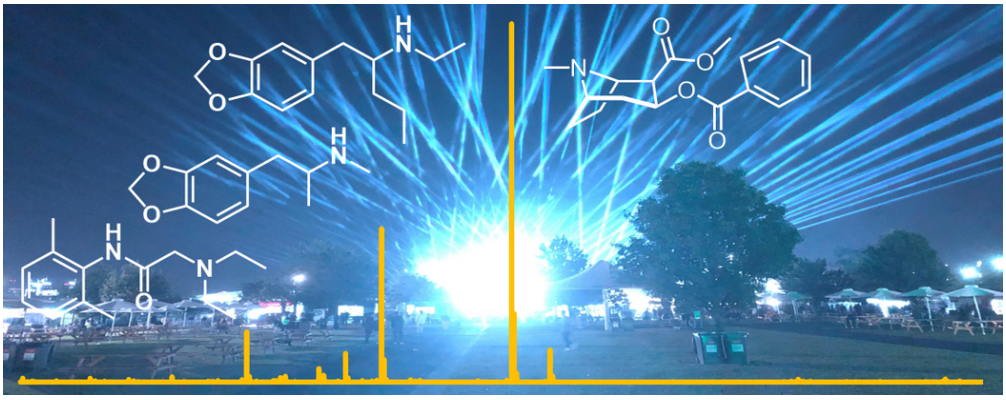

A
4.40E6
100
304.1543
Cocaine
250.1439
N-Ethylpentylone
194.1177
MDMA
235.1806
Lidocaine
*
320.1493
B
9.63E5
100
304.1543
Cocaine
**
114.0917
194.1177
MDMA
**
158.1541
250.1440
N-Ethylpentylone
**
C
2.37E7
100
250.1439
N-Ethylpentylone
D
9.36E6
100
250.1440
N-Ethylpentylone
304.1550
Cocaine
**
114.0918
Relative Abundance (%)
m/z
100
150
200
250
300
350
400
450
500

Figure 4


Number of Identifications
25
20
15
10
5
0
21
7
3
1
1
1
1
6
2
1
3
single drug (n=35)
two drug (n=9)
MDMA
Cocaine
Ketamine
Methamphetamine
Methiopropamine
Amphetamine
2F-DCK
MDA
Negative


A
304.1
Cocaine
214.2
192.0
183.0
319.2
363.2
391.4
Relative Abundance (%)
100
200
300
400
500
B
182.1
-C7H6O2
304.1
[C17H21O4N + H]+
150.2
-C8H10O3
110.0
50
100
150
200
250
300
350
C
156.0
Methiopropamine
149.0
125.0
183.0
192.1
Relative Abundance (%)
m/z
D
125.0
-CH2NH3
58.1
C3H8N+
97.0
-C3H9N
112.0
156.1
[C8H13NS + H]+
125.0
-CH5N
58.1
C3H8N+
97.0
-C3H9N
C5H5S+
S
H
N
m/z



For Table of Contents Use Only

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

88x34mm (300 x 300 DPI)