An Analytical Platform for Near Real-Time Drug Landscape Monitoring using Paraphernalia Residues

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Abstract: Deaths attributed to drug overdoses are constantly on the rise, but drug trends are frequently changing and often differ across geographical regions. Current analytical techniques are limited in their abilities to rapidly identify drugs that would inform both public health and law enforcement officials about the evolving drug landscape. The work presented here outlines an analytical platform that utilizes ambient ionization mass spectrometry and additional techniques (*e.g.,* tandem mass spectrometry) to qualitatively analyze trace residues from drug paraphernalia to quickly detect both drugs and cutting agents. To demonstrate proof-of-concept, samples collected from syringe service programs throughout the state of Maryland were analyzed by direct analysis in real time – mass spectrometry (DART-MS) to provide rapid, near complete chemical profiles (drugs, cutting agents, and other compounds of interest). To obtain a more complete chemical profile, it was found that a small subset of samples (7.5 %) benefited from additional analysis by either direct analysis in real time – tandem mass spectrometry (DART-MS/MS) or liquid chromatography – tandem mass spectrometry (LC-MS/MS). This additional analysis enabled confirmation of the presence or absence of questioned compounds, assisted in identification of new compounds, and provided isomer differentiation without hindering the rapid reporting of results. This analytical platform utilizing DART-MS and, where necessary, tandem mass spectrometry techniques, was found to detect a wide range of drugs and cutting agents in a manner that can better inform public health and public safety personnel about the drug landscape in "near real-time".

1. Introduction

Over the past several decades there has been a dramatic increase in overdose related deaths in the United States.[1, 2] Due to fluctuating drug supplies, the emergence of novel compounds, and the addition of cutting agents, monitoring the drug landscape that leads to such overdoses has become more challenging. A common method for trying to understand the landscape is to pool information from numerous sources such as drug reports from forensic laboratories,[3] surveys from emergency medical services, toxicology reports from medical examiners, and reports from poison control centers databases.[4] While comprehensive, this approach is retrospective and puts public health and public safety officials in a reactive (instead of proactive) position. Another method that is employed to understand the drug landscape is collecting information from drug users through verbal statements[5] and social media accounts.[6] While this approach provides more timely information, it is based on the assumption that users know what they are taking. Substance use information can also be gathered by analyzing collected materials such as biological samples from patients in emergency rooms,[7] seized materials from offenders[8] and waste materials (*e.g.,* discarded drug packaging[9] and wastewater[10, 11]).

To analyze these samples, agencies have successfully used a variety of analytical techniques, such as gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS).[12-14] Even though these techniques have limitations such as long analysis times and extensive sample preparation,[15] they are frequently utilized by analytical laboratories to analyze relevant samples.[16] With the influx of novel psychoactive substances (NPSs) and the prevalence of fentanyl, these limitations lead to backlogs and delay forensic results by weeks to months.[17, 18]

To address the delay between sample collection and data reporting, some public health and law enforcement agencies have begun implementing novel technologies for rapid in-field testing.[15, 19] Spectroscopic techniques, such as Fourier-transform infrared (FTIR) and Raman spectroscopy, are often deployed[16] because they are portable, relatively inexpensive, easy to use, and have large libraries.[15] These techniques can lead to inconclusive results when compounds are present at low percentages of the overall mixture.[20] Additionally, cutting agents and compounds that have high fluorescence can inhibit detection.[15] Another common approach to analyzing street samples is the use of color tests,[16] which are fast and simple to conduct, but require bulk material and prior

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knowledge of the substance, such as possible drug class. Color tests are susceptible to subjective interpretation and suffer from poor reproducibility. They also use toxic chemicals, which pose safety concerns, and are limited in the type of compounds they can detect.[15, 21, 22] Lateral flow immunoassays (LFIs), which are paper-based antibody tests, are also used by law enforcement and public health agencies to rapidly analyze street samples.[23] With this approach, aqueous solutions of samples are directly absorbed onto the paper-based tests enabling detection of one or more specific analytes in minutes.[24] LFIs are inexpensive, easy to use, and are highly sensitive and selective.[23, 25] However, the high degree of compound selectivity can also be a limitation for non-targeted applications like drug screening.

For instances where the overall drug landscape (including cutting agents) is of interest, current laboratory and field-based techniques are often inadequate. They require bulk or biological materials, have extensive sample preparation procedures and lengthy analysis times, target specific compounds, and/or do not provide rapid results. Therefore, there is a need to identify technologies that could address these limitations by enabling rapid, non-targeted analysis of a wide range of samples while also being safe, sensitive, and specific.

Ambient ionization techniques require limited to no sample preparation and in combination with mass spectrometry, can provide results within minutes, and can detect a wide range of compounds in one analysis.[26, 27] Specifically, direct analysis in real time – mass spectrometry (DART-MS) has been used to address several questions in forensic science within the fields of seized drug analysis and toxicology[28] due to its ability to detect materials at low levels (nanogram detection limits).[29, 30] This level of sensitivity allows for the probing of trace residues instead of bulk material, thereby reducing the risk of exposure to toxic substances. Trace residues can be collected using a wipe-based analysis approach,[31] which enables collection of samples from a wide range of surfaces. Previous research has shown there are residues on drug paraphernalia that can be used to determine the presence of a drug(s).[32] The combination of trace residues collection and DART-MS analysis has also been successfully demonstrated using discarded paraphernalia at festivals.[9]

Although ambient ionization mass spectrometry techniques can overcome many of the challenges presented by other techniques, they are not without their limitations. Lack of chromatography, which gives these techniques their speed, can also be a constraint when structurally similar compounds are present in a mixture. Oftentimes identification of an exact isomer (*e.g.*, *ortho*-,

meta-, *para-*) is not possible. While this information may not be necessary for public health and public safety applications, where identifying a compound or its isomer may be sufficient, isomeric information can be critical when determining the legality of compounds. Lack of chromatography can also lead to competitive ionization – a phenomenon where a compound with high proton affinity consumes all available charge, hindering the detection of other compounds with lower affinities. This phenomenon is not often seen when analyzing illicit drugs but has been reported in instances where high levels of fentanyl can mask the presence of low levels of heroin.[33] Due to these factors, DART-MS is often referred to as a screening tool used for triaging of samples. When it is necessary to identify a new compound or differentiate between isomers, additional analysis can be done following initial screening by DART-MS. These additional analyses could include rapid targeted methods such as direct analysis in real time – tandem mass spectrometry (DART-MS/MS) or lengthier traditional methods such as liquid chromatography – tandem mass spectrometry (LC-MS/MS). In this work we describe the development of an analytical platform for near-complete chemical profiling of collected trace residues to provide near real-time results to public safety and public health agencies with proof-of-concept supported by real world samples collected through the creation of a pilot study with Syringe Service Programs in Maryland.

2. Methods

2.1. Sample collection and preparation

Trace residues were collected by Syringe Service Programs (SSPs) across the state of Maryland. Used drug paraphernalia from SSP participants were sampled by SSP personnel using either a dry meta-aramid wipe (Smiths Detection Inc., Edgewood, MD, USA) or a cotton swab (Puritan Medical Products Company LLC, Guilford, ME, USA), shown in Figure 1A and Figure 1B, respectively. The paraphernalia was sampled by wiping or swabbing its exterior (*e.g.*, plastic bag, capsule, etc.) using firm force in a unidirectional manner. The wipes or swabs were then placed in individual coin envelopes labelled with a unique identifier, date of collection, site location, and type of item sampled (*e.g.*, empty bag, capsules, or pipe). Samples were mailed from the SSPs to the laboratory for analysis. All samples were anonymized, and the research was deemed to not be human subjects research. It should be noted that syringes were not sampled in this proof-of-concept study to minimize potential handling hazards for SSP personnel.

Once the samples were received at the laboratory they were cataloged and prepared for analysis. Wipes were trimmed to remove the area that did not come in contact with paraphernalia (outlined in Figure 1A.) and placed in 2 mL glass vials. Cotton swabs were also placed in 2 mL glass vials and the wooden stem was trimmed to fit within the vial (outlined in Figure 1B.). Samples were extracted by adding a 1 mL aliquot of acetonitrile to the vials which were capped and vortexed for 10 s. The wipe or cotton swab tip was removed to prevent reabsorption onto the substrate.
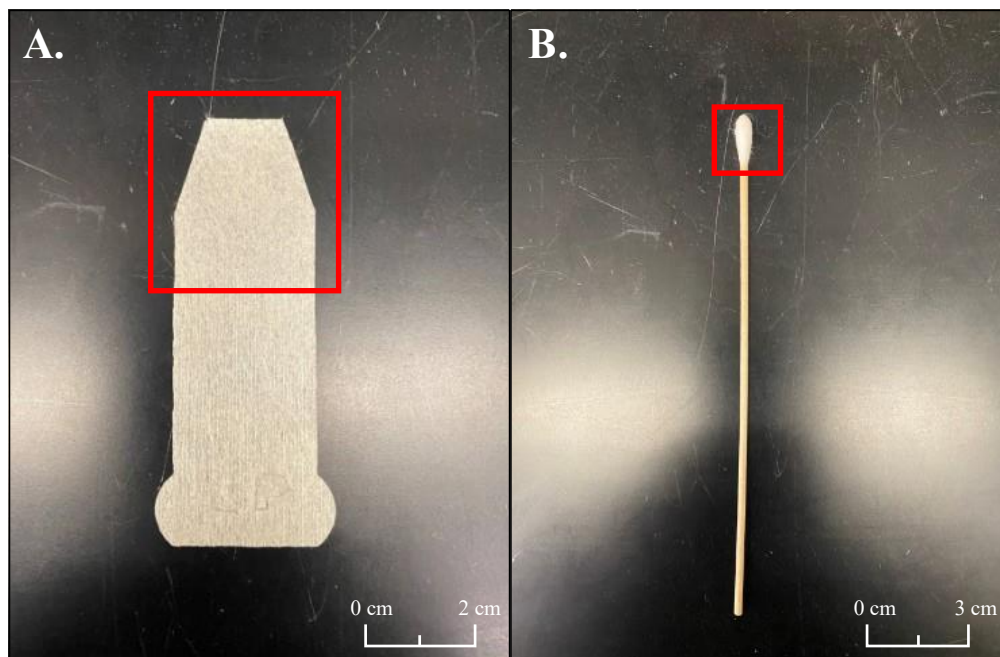
Figure 1. Sampling materials for the wiping/swabbing of paraphernalia. The desired collection area on the wipe (Panel A) and swab (Panel B) is shown inside the red square. Areas outside the red squares were removed prior to analysis.

2.2. DART-MS analysis

Once samples were extracted, they were analyzed by DART-MS to identify what compounds were present. Extracts were analyzed by dipping the closed end of a melting point capillary tube (VWR International, LLC., Randor, PA, USA) into the solution, then introducing the capillary into the open-air gap of the DART gas stream. Samples were analyzed using a DART-SVP ion source (IonSense, Saugus, MA, USA) coupled to a JEOL AccuTOF LC-4G mass spectrometer (JEOL USA, Peabody, MA, USA). The DART source was operated in positive ionization mode using helium as the ionization gas at a temperature of 400 °C and a grid voltage of +50 V. The mass spectrometer was operated in positive ionization mode with an orifice temperature of 120 °C, an

orifice 2 voltage of +5 V, a ring lens voltage of +5 V, an RF ion guide voltage of +700 V, and a mass scan range of m/z 80 to m/z 800. In-source collision induced dissociation (is-CID) mass spectra were collected at three orifice 1 voltages: +30 V, +60 V, and +90 V using the parameter switching function with a cycle time of 0.2 s / scan.

A methanolic solution of the synthetic cannabinoid AB-FUBINACA (Cayman Chemical, Ann Arbor, MI, USA) was used as both a positive control and a mass drift compensator, within every analysis, while a methanolic solution of polyethylene glycol (PEG-600) was used for daily mass calibration. Data was collected using a 1 min analysis time with an introduction method of AB-FUBINACA, blank capillary tube, sample replicate 1, sample replicate 2, sample replicate 3. A single-point mass drift compensation was completed using the base peak of AB-FUBINACA (m/z 352.1456). A single averaged, centroided, and background subtracted mass spectrum of the sample was extracted for each is-CID voltage using the averaged spectrum for the blank capillary tube as background. Compounds present in the extracted mass spectra were detected and preliminarily identified using version 2 of the NIST/NIJ DART-MS Data Analysis Tool (DIT)[34, 35] and the NIST DART-MS Forensics Database (v.4 Firefly[36]). Search parameters within the DIT included a minimum relative intensity of 3 % and a mass tolerance of ±0.005 Da. Identifications were made using the +30 V and +60 V spectra, taking into consideration the average fraction of peak intensity explained (FPIE), average reverse match factor (RevMF), mass difference ($\Delta m/z$) and low fragmentation protonated molecule isotope ratio difference (LFPM IRD).

2.3. Additional analysis by tandem mass spectrometry

Due to limitations associated with DART-MS analysis, a subset of samples required further investigation using either DART-MS/MS or LC-MS/MS. DART-MS/MS was used to confirm the presence or absence of a non-isomeric compound that had either (i) not previously been seen in this study and was novel, (ii) was present at a low level in the DART-MS spectra, or (iii) produced low mass spectral match scores when analyzed using the DIT. Confirmation was completed by manually comparing the product ion scan of the questioned m/z value in the sample to that of a known standard.

If a compound presumptively identified by the DIT using DART-MS spectra either had multiple isomers or was believed to be novel, the extract was also analyzed by LC-MS/MS. For instances

where the compound had isomers, the retention time and product ion scan of the sample were compared to that of standards of all available isomers because there were no available spectral databases to search against. Several samples required both DART-MS/MS and LC-MS/MS analyses. Detailed methods and parameters for DART-MS/MS and LC-MS/MS are outlined in the supplementary information (Supplementary Section 1; Methods for Analysis by Tandem Mass Spectrometry).

3. Results and Discussion

3.1. Sample breakdown

A total of 496 samples were collected from SSPs throughout the state of Maryland between October 2021 and August 2022. Figure 2 shows the breakdown of each paraphernalia type sampled. Empty plastic bags were the most frequently sampled type of paraphernalia (n = 271, 54.6 %) followed by cookers/caps (n = 82, 16.5 %) then capsules (n = 58, 11.7 %). Monitoring the type of paraphernalia sampled can not only be useful for understanding and further developing best practices for sampling, it can also provide information on paraphernalia trends to public health and public safety officials.
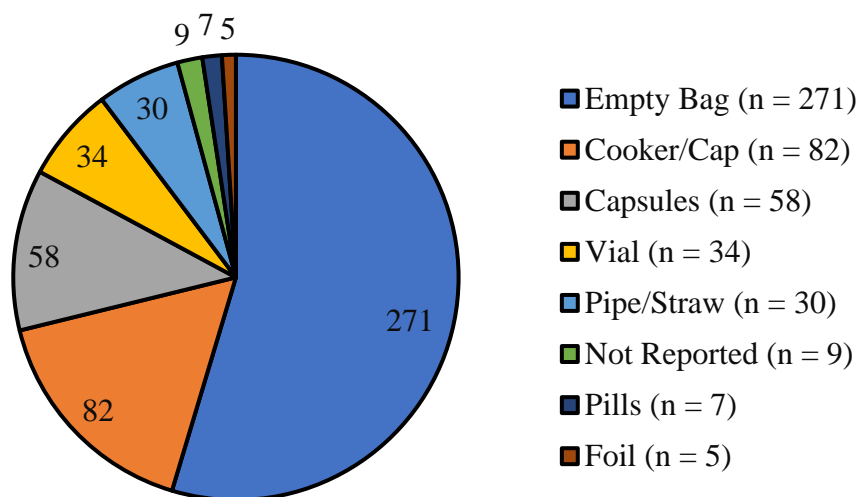
Figure 2. Breakdown of the types of paraphernalia samples in this study (total = 496).

3.2. DART-MS

The overarching goal of this project was to develop an analytical platform that provides comprehensive and near real-time drug and cutting agent information for most samples. A comprehensive and real-time method would be one that identifies all components present including novel compounds and can be completed rapidly ideally within 24 to 48 hours. This would require the implementation of multiple analytical techniques due to their various limitations. Given that real-world samples are expected to contain an unknown number of compounds of unknown classes, a non-targeted technique was utilized as the first step. Ambient ionization coupled with high resolution mass spectrometry was determined to be most appropriate for the initial non-targeted screen as it can detect a wide range of compounds at varying levels of relative concentrations. Ambient ionization techniques, such as DART, are also soft enough to ionize molecules with limited to no fragmentation, enabling detection of intact protonated molecules. The use of is-CID provided non-discriminate fragmentation that, when coupled to protonated molecule information, gave more specific information of the chemical makeup. This contrasts with MS/MS approaches where CID requires a list of precursor *m/z* values to target. Use of DART with high resolution mass spectrometry was also chosen because of the increased specificity over unit mass resolution systems, the presence of existing databases[37] and search software[34, 38] as well as the substantial literature background proving its capability.[28, 39]

All samples were analyzed with DART-MS and searched with the DIT to preliminarily determine the compounds present. The version of the NIST DART-MS Forensics Database used for compound identification contained spectra for over 1,100 compounds to compare against. The process of analysis, calibration, and data interpretation was completed in less than 5 min per sample. A breakdown of the overall number of occurrences for each compound identified is shown in Table 1 with the ten most commonly encountered drugs displayed in Figure 3. Of the compounds identified, fentanyl was encountered most often, being found in 363 samples (73.3 %). Other frequently encountered compounds included xylazine (n = 318, 64.1 %), caffeine (n = 158, 31.9 %), quinine (n = 98, 19.8 %), and cocaine (n = 77, 15.5 %). In total, 55 different compounds were detected during the pilot study including synthetic cathinones, cannabinoids, opioids, opiates, benzodiazepines, arylcyclohexylamines, nitazenes, and cutting agents. Fourteen of the 55 compounds were only encountered in a single sample and many compounds (n = 37) were present

in fewer than ten samples. Heroin, being detected in only six samples (1.2 %), was always detected in combination with fentanyl. These results show the capability of DART-MS analysis and the DIT to detect and identify a wide range of compounds.
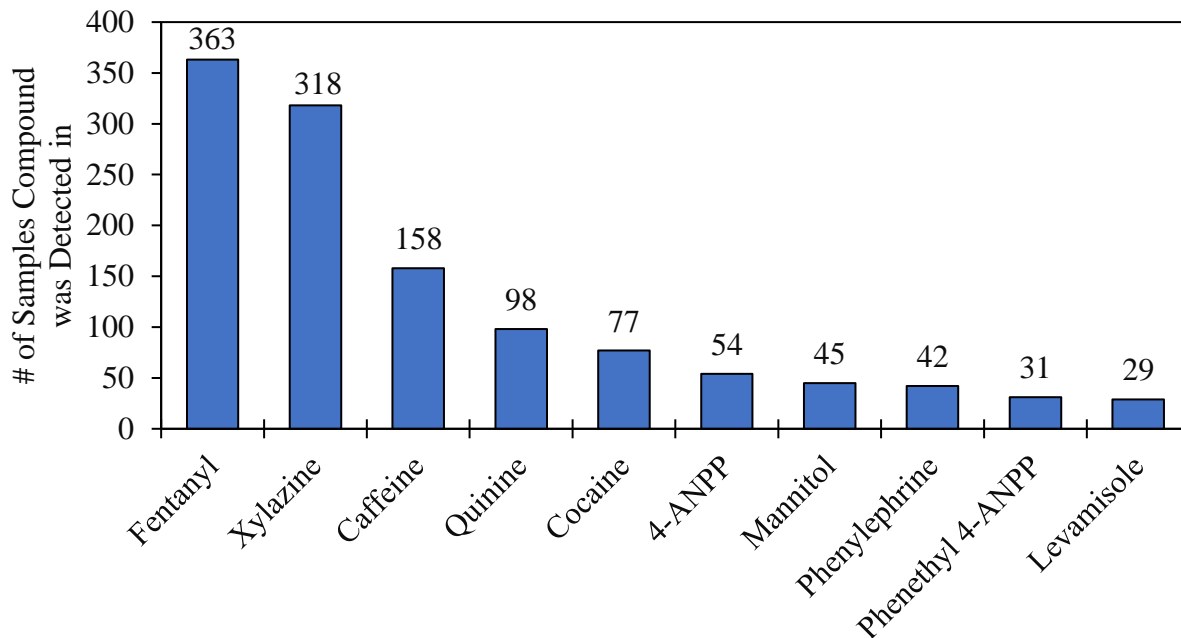
Figure 3. Plot showing the 10 most detected compounds from paraphernalia samples.

A breakdown of the number of compounds detected in each sample is shown in Table 2. Samples contained up to ten compounds with most containing either two or three compounds (n = 267, 53.8 %). Approximately 2.2 % of samples (n = 11) had no compounds detected. Originally, it was thought that this could be a result of the type of paraphernalia sampled; however, no trends were observed to support this conjecture. Reasons for the lack of detection could include no compounds of interest present, sampling issues, or concentrations below the limit of detection.

A major benefit of using a technique such as DART-MS, compared to techniques like FTIR or LFIs, is that identification of active ingredients and cutting agents can occur simultaneously. This data can provide critical information to public health and public safety officials to better ensure the safety of drug users through informed wound care and to track drug distribution networks from importation of materials to its spread across the United States. In this pilot study, 20 different

cutting agents and adulterants were identified in the sample set (Table 1, asterisks) with xylazine (n = 318, 64.1 %), caffeine (n = 158, 31.9 %), and quinine (n = 98, 19.8 %) being the three most frequently encountered. Aspirin, benzocaine, hordenine, piracetam, piroxicam, and procaine were found in single samples, and an additional six cutting agents were present in fewer than ten samples.

In terms of poly-compound combinations, there were 174 unique combinations across the sample set. Of those, 156 of the combinations were observed in fewer than five samples, and 128 were unique. Fentanyl and xylazine along with fentanyl, xylazine, and caffeine were the most frequently encountered combinations, accounting for 97 (19.6 %) and 69 (13.9 %) of samples, respectively. Variations on fentanyl and xylazine in the presence of other cutting agents or precursors accounted for the next five most frequently observed poly-compound combinations. A breakdown of the various compound combinations and their frequencies is provided in the Supplemental Information. It should be noted that although there were a large number of unique combinations, it is likely that the actual number of unique combinations is lower. This is due to variations in compound concentrations (from sampling, heterogeneity of the sample, or competitive ionization) that may cause minor differences in compound detection. Likewise, since these samples were collected from paraphernalia, it is possible that differences in drug makeup could occur if the paraphernalia was used in multiple instances (*e.g.*, a cooker was used for fentanyl and cocaine on separate occasions).

The data collected from this study could potentially be used to understand changes in fentanyl manufacturing by looking at the presence of precursors and by-products from synthesis such as 4-anilino-N-phenethylpiperidine (4-ANPP), N-phenethyl-4-piperidone (NPP), and phenethyl 4-ANPP. Changes in the frequency with which these compounds are detected may indicate changes in the synthesis route[40] and even purity of the products,[41] although it is important to take into consideration the limit of detection for these compounds before reaching these conclusions. Additional research on these points is the focus of future work.

3.3. Additional analyses by tandem mass spectrometry

DART-MS was implemented as the first line technique to provide prompt but comprehensive results to public health and public safety officials. In utilizing this technique, however, a number

of samples were identified that would benefit from additional analysis to determine if the presence of a questioned compound could be confirmed or to identify the exact substance when DART-MS could not distinguish between compounds with identical masses (*e.g.*, isomers). In total, 37 samples were subject to additional analysis which consisted of DART-MS/MS and/or LC-MS/MS.

3.3.1. DART-MS/MS

The limitations of DART-MS were centrally focused around the reduced confidence in identifying compounds due to an identification that (i) was the first occurrence of a novel compound, (ii) was associated with a peak in the low energy (+30 V) is-CID mass spectra present at a low abundance, or (iii) had a resulting identification with a low DIT match score (FPIE and/or RevMF scores less than 0.6 a.u., on a scale of 0 a.u. to 1 a.u. with 1 a.u. being a perfect spectral match)[38]. To address these, DART-MS/MS was leveraged as it is a rapid, targeted technique that can confirm the presence (rule in) or absence (rule out) of a compound by comparing the product ion scan of a given ion in the sample to that of a known standard.

An example of the utility of DART-MS/MS is highlighted in Figure 4. In this sample, DART-MS analysis of the extract (Figure 4A.) resulted in preliminary DIT compound identifications of fentanyl, etizolam, fentanyl carbamate, meclonazepam, quinine, xylazine, and caffeine. The analysis also resulted in a potential match for desomorphine at nominal *m/z* 272 with scores of 0.660 a.u. and 0.929 a.u. for FPIE and RevMF, respectively. Since desomorphine had not been previously observed in the pilot study, and given the high toxicity of desomorphine and the potential for harm in unknowing users, it was prudent to analyze the sample using DART-MS/MS. The product ion scans of nominal *m/z* 272 for the sample and a desomorphine standard, shown in Figure 4B., were sufficiently different allowing us to rule out the presence of this compound in the sample at a detectable level. Completing this additional testing required no extra sample preparation and the analysis was able to be completed in less than 5 min.
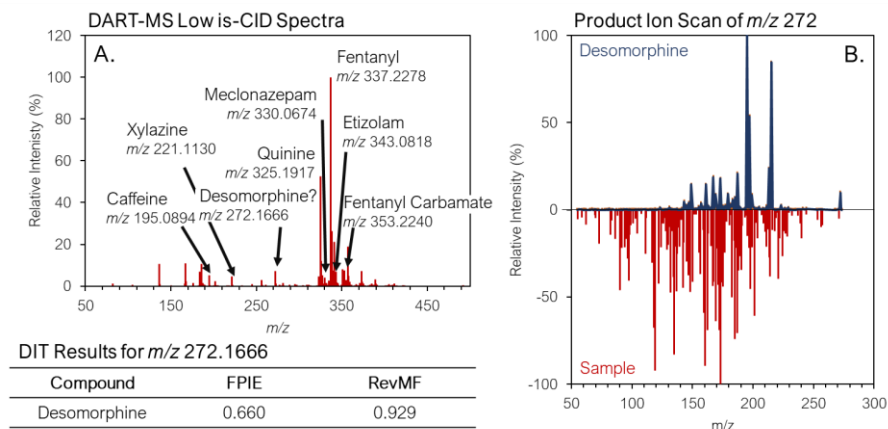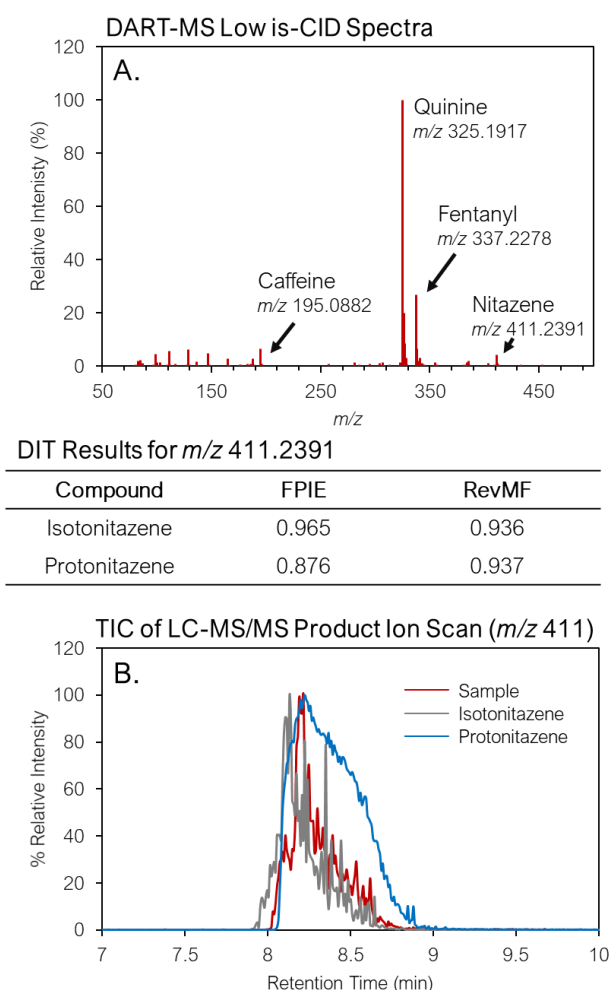
Figure 4. (A.) Low fragmentation is-CID DART-MS mass spectra of sample 038 with compounds of interest identified. Below the spectra are the search results, obtained using the DIT, for *m/z* 272.1666. (B.) DART-MS/MS product ion scan of *m/z* 272 from the desomorphine standard (top, blue) and the sample (bottom, red).

In total, 32 of the 496 samples were analyzed by DART-MS/MS to determine the presence or absence of at least one compound. Table S1 lists the samples that were analyzed (with arbitrary numbers assigned) by DART-MS/MS along with the peak of interest, the compound(s) preliminarily identified by the DIT using the DART-MS spectra, and the conclusion of the DART-MS/MS analysis. There were three possible conclusions: (1) *Confirmed*, (2) *Ruled out*, and (3) *Confirmed but further analysis needed*. *Confirmed* indicated that the product ion scans for the nominal *m/z* of interest of the sample and standard were visually similar and the presence of the compound was reported. *Ruled out* indicated that the product ion scans for the nominal *m/z* of interest of the sample and standard were not visually similar and the presence of the compound was not reported. *Confirmed but further analysis needed* indicated that the product ion scans were visually consistent, but there were multiple possible compounds (typically isomers) that may have the same product ion scans. For these samples, a broad preliminary identification (*e.g.*, synthetic cathinone) was initially reported and LC-MS/MS was then used to identify the specific compound. A total of 16 compounds were confirmed using DART-MS/MS in this pilot study, including nicotine, methadone, methamphetamine, gabapentin, and tramadol, while 19 were ruled out. Three additional samples required further analysis by LC-MS/MS.

3.3.2. LC-MS/MS

When DART-MS and DART-MS/MS were insufficient for the differentiation of isomers or the identification of novel compounds, LC-MS/MS was leveraged. For example, DART-MS analysis of sample 078 resulted in DIT identification of fentanyl, quinine, caffeine, and either isotonitazene or protonitazene (Figure 5A.). Prior to this sample, neither isotonitazene nor protonitazene had been observed. DART-MS/MS was used to rule in both options (data not shown), but LC-MS/MS was needed to differentiate between the two isomers. Based on slight retention time differences (Figure 5B.) it was determined that protonitazene was present.

DIT Results for *m/z* 411.2391

Compound	FPIE	RevMF
Isotonitazene	0.965	0.936
Protonitazene	0.876	0.937

Figure 5. (A.) Low fragmentation is-CID DART-MS mass spectra of sample 078 with compounds of interest identified. Below the spectra are the search results, obtained using the DIT, for *m/z* 411.2391. (B.) LC-MS/MS total ion chromatograph (TIC) of the product ion scan of *m/z* 411 from the sample (red) as well as the isotonitazene (grey) and protonitazene (blue) standards.

In total, nine samples benefited from additional LC-MS/MS analysis. The method time was 19 min per sample and standard. Table S2 lists the samples (with arbitrary numbers assigned) that were analyzed by LC-MS/MS along with the peaks of interest, the standards used for targeted analysis, and the LC-MS/MS conclusion. There were two possible conclusions: (1) *Confirmed*, meaning that the retention time and product ion scan from the sample were consistent with those of the standard, or (2) *Unable to confirm*, meaning that the sample and standard had either retention times or product ion scans that were not consistent. The compounds that were confirmed by LC-MS/MS, in addition to protonitazene, included despropionyl fluorofentanyl, ephedrine, eutylone, and phenylephrine. Three samples contained synthetic cathinones that could not be definitively identified and would require additional studies and standards to identify. These results were reported as unknowns along with their most probable drug class (*i.e.*, unknown synthetic cathinone).

4. Conclusions

The focus of this project was to develop an analytical platform using DART-MS, and if needed, additional mass spectral techniques, to provide near real-time qualitative analysis of collected trace drug residues from paraphernalia which was demonstrated and supported by the successful analysis of real-world samples collected from Syringe Service Programs throughout the state of Maryland. This approach was found to overcome many of the analytical and practical challenges of common laboratory and field-based techniques, providing rapid, high-quality detection and identification of drugs, cutting agents, and other compounds of interest. The speed for individual sample collection and screening would allow for rapid analysis of large sample quantities providing information on drug landscapes. Leveraging trace residues increased safety of personnel while also simplifying sample collection and transportation. A centralized site for sample analysis also enabled uniform reporting across multiple collection sites throughout the state, enabling direct comparison of data. While this approach was demonstrated in a public health setting, it could easily be applied to other areas where rapid drug screening is desired, such as the point of drug seizure, fatal and non-fatal overdose scenes, prison systems, and triaging in drug units of forensic laboratories.

While the sole use of DART-MS to obtain a near-complete chemical profile was successful in the vast majority of samples (92.5 %), there were several important limitations that necessitated additional analysis based on the information needed. If confidence in the identification of new and emerging compounds is necessary (*e.g.*, in a public health setting where an overdose prevention response may be triggered) additional analysis, using rapid techniques like DART-MS/MS or more traditional techniques like LC-MS/MS, can be beneficial. Also, if definite compound identification is necessary for isomeric species, additional analysis will be required. This may be less important for public health settings, where simply knowing the presence of a compound or its isomer is sufficient, but it may be invaluable for public safety and drug scheduling efforts.

Given that the majority of samples encountered in this work were fentanyl-based, further investigation of this analytical platform is currently underway via expansion into other geographical regions where the drug landscape is likely different. Also, qualitative analysis is sufficient at this time as it allows for rapid and accurate identification, but additional research will focus on comparing quantitative results from trace residues and bulk material. There are also several open research questions currently under investigation. These include understanding the utility, if any, of negative ionization mode spectra to identify compounds of interest, better understanding the relationship of drug residues to the actual drug used or stored in the paraphernalia, best practices for sampling other types of paraphernalia, and improving data interpretation tools. In addition to added analytical experiments, future work will also focus on utilizing the data to visual drug, cutting agent, and paraphernalia trends and their impact on society, intelligence and public health.

5. Disclaimer

Certain commercial products are identified to adequately specify the procedure; this does not imply endorsement or recommendation by NIST, nor does it imply that such products are necessarily the best available for the purpose.

This research was reviewed by the NIST Internal Review Board and was deemed to not be human subjects research.

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Table 1. The compounds identified by the DIT in this study using DART-MS spectra along with their frequency of detection (total = 496). Cutting agents have an asterisk (*) after the name.

Compound	% of Samples	Compound	% of Samples
4-ANPP	10.9 % (n = 54)	Levamisole*	5.8 % (n = 29)
4-Piperidone	1.0 % (n = 5)	Lidocaine*	2.6 % (n = 13)
6-Monoacetylmorphine	0.6 % (n = 3)	Mannitol*	9.1 % (n = 45)
Acetaminophen*	1.6 % (n = 8)	MDA	0.4 % (n = 2)
Amphetamine	0.4 % (n = 2)	MDMA	1.4 % (n = 7)
Aniline	4.2 % (n = 21)	MDPHP	0.2 % (n = 1)
Aspirin*	0.2 % (n = 1)	Meclonazepam	0.4 % (n = 2)
Benzocaine*	0.2 % (n = 1)	Methadone	0.4 % (n = 2)
Bromazolam	0.2 % (n = 1)	Methamphetamine	2.4 % (n = 12)
Caffeine*	31.9 % (n = 158)	Nicotine	1.0 % (n = 5)
Cannabidiol	0.4 % (n = 2)	Noscapine*	0.6 % (n = 3)
Cannabinol	1.4 % (n = 7)	NPP	2.0 % (n = 10)
Clonazepam	0.2 % (n = 1)	Papaverine*	0.4 % (n = 2)
Cocaine	15.5 % (n = 77)	Phenacetin*	2.4 % (n = 12)
Despropionyl fluorofentanyl	0.8 % (n = 4)	Phenethyl 4-ANPP	6.3 % (n = 31)
Diphenhydramine*	0.6 % (n = 3)	Phenylephrine*	8.5 % (n = 42)
Ephedrine*	0.6 % (n = 3)	Piracetam*	0.2 % (n = 1)
Etizolam	0.6 % (n = 3)	Piroxicam*	0.2 % (n = 1)
Eutylone	1.8 % (n = 9)	Procaine*	0.2 % (n = 1)
Fentanyl	73.3 % (n = 363)	Protonitazene	0.2 % (n = 1)
Fentanyl carbamate (or isomer)	1.2 % (n = 6)	Quinine*	19.8 % (n = 98)
Flubromazepam	0.2 % (n = 1)	Salicylamide*	0.8 % (n = 4)
Fluorofentanyl	4.8 % (n = 24)	THC (or isomer)	2.4 % (n = 12)
Fluoxymesterone	0.2 % (n = 1)	Tramadol	1.2 % (n = 6)
Gabapentin	0.6 % (n = 3)	Tropacocaine	0.2 % (n = 1)
Heroin	1.2 % (n = 6)	Unidentified Cathinone	4.8 % (n = 24)
Hordenine*	0.2 % (n = 1)	Xylazine*	64.1 % (n = 318)
Ketamine	0.2 % (n = 1)		

†Abbreviations used in the table include: 4-ANPP (4-anilino-N-phenethylpiperidine), MDA (3,4-methylenedioxyamphetamine), MDMA (3,4-methylenedioxymethamphetamine), MDPHP (3',4'-methylenedioxy-α-pyrrolidinohexiophenone), NPP (N-phenethyl-4-piperidone), and THC (Δ9-tetrahydrocannabinol).

‡ Note: For some compounds specific isomeric identifications were not made.

Table 2. Breakdown of the number of compounds detected in each sample (total = 496)

Number of Compounds	Percentage of Samples
1	15.3 % (n = 76)
2	27.8 % (n = 138)
3	26.0 % (n = 129)
4	13.5 % (n = 67)
5	8.3 % (n = 41)
6	3.0 % (n = 15)
7	1.6 % (n = 8)
8	0.8 % (n = 4)
9	1.0 % (n = 5)
10	0.4 % (n = 2)
0	2.2 % (n = 11)

Supplementary Information

Parameters for the DART-MS/MS and LC-MS/MS methods and tables displaying samples and compounds that required further examination by DART-MS/MS and/or LC-MS/MS are included in the supplemental information. An Excel table that displays the unique compound combinations and their frequencies is also provided.

1. Methods for Analysis by Tandem Mass Spectrometry

1.1. DART-MS/MS

DART-MS/MS was completed using a DART-SVP ion source (IonSense) coupled with a TSQ Quantis triple quadrupole mass spectrometer (Thermo Electron NA, West Palm Beach, FL, USA). DART parameters were identical to those used for DART-MS analysis (Section 2.2) and included operation in positive ionization mode with helium as the source gas heated to 400 °C. To lower the helium flow going into the mass spectrometer, a Vapur interface (IonSense) was used with an auxiliary rough pump metered to pull at ~4.5 L/min. The mass spectrometer was operated in positive ion mode and product ion scans of the compounds of interest were collected. The protonated molecule m/z was selected as the precursor ion and the product ion mass scan range was set to scan from m/z 50 to five m/z above the precursor ion at a rate of 500 Da/s. A collision energy ramp 10 V to 45 V was used along with a CID gas of 2 mTorr. Other relevant mass spectrometer parameters included a source fragmentation of 30 a.u., a sweep gas of 2 a.u., no sheath gas or auxiliary gas, and an ion transfer tube temperature of 350 °C. Samples were introduced into the DART gas stream using the same procedure as the DART-MS analysis. Individual product ion scans were collected for the sample and the known standard, and the resulting spectra were visually compared to determine if the sample contained the suspected compound of interest.

1.2. LC-MS/MS

LC-MS/MS was occasionally used as an additional confirmation technique for samples where DART-MS identified compounds with multiple isomers. A Thermo Ulti-Mate3000 liquid chromatography system (Waltham, MA, USA) coupled to a Sciex 4000 QTrap mass spectrometer (Framingham, MA, USA) was used for analysis. Standards of potential matches were analyzed

alongside samples for comparison. Separation was achieved using a Restek (Bellefonte, PA, USA) Raptor Biphenyl column (150 mm x 4.6 mm x 2.7 µm). A volume of 50 µL was injected and the total analysis time was 18 min with a flow rate of 0.75 mL min^{-1}. During the analysis, a 12 min solvent gradient was used (95 % water/5 % methanol with 0.1 % formic acid to 100 % methanol with 0.1 % formic acid) followed by a 3 min isocratic period (100 % methanol with 0.1 % formic acid). Zero-air nitrogen was used for both the desolvating and nebulizing gases within the electrospray ionization (ESI) source of the MS. Additional ESI source parameters included an operating temperature of 550 °C and a spray voltage of +5500 V. A product ion scan was used for the entire run, collecting data at 0.5 s/scan.

Table S1. Summary of the DART-MS/MS analysis, including the peak of interest (precursor ion (PI) for the product ion scan), the compound(s) preliminarily identified by the DIT, and the conclusion *Confirmed* (C), *Ruled out* (X), or *Confirmed but further LC-MS/MS analysis required* (C*).

Sample Number	PI (*m/z*)	Preliminary Compound Identified	DART-MS/MS Conclusion
011	194	MDMA	X
015	383	fluoro Valeryl fentanyl	X
018	397	dimethoxy Fentanyl (isomer)	X
	425	N-(2C-E) Fentanyl	X
019	315	THC	C
	163	Nicotine	C
020	330	XLR-11	X
023	417	Alfentanil; isopropyl Furanyl fentanyl	X
	383	fluoro Valeryl fentanyl	X
	236	Eutylone or isomer	C*
	310	Methadone	C
038	272	Desomorphine	X
049	150	Methamphetamine	C
058	196	Tropacocaine	C
070	395	methoxy Valeryl fentanyl	X
074	370	Heroin	X
076	337	Fentanyl	C
077	385	Phenethyl 4-ANPP	C
078	411	Isotonitazene; Protonitazene	C*
105	341	Fluoroacetyl fentanyl	X
107	233	Norfentanyl; 4-hydroxy DET; 4-hydroxy MiPT; 4-hydroxy MPT; 5-methoxy MET	X
119	172	Gabapentin	X
121	172	Gabapentin	C
125	264	Tramadol	C
	337	Fentanyl	C
140	299	Despropionyl Fluorofentanyl	C*
155	328	6-Monoacetylmorphine	C
201	208	Methylone	X
209	339	2,3 seco-Fentanyl	X
261	194	MDMA	X
289	194	MDMA	C
290	194	MDMA	X
312	290	Benzoylecgonine	C
318	208	MMDPPO	X
329	328	Naloxone	X
463	353	Bromazolam	C
474	166	Benzocaine	C
503	290	Benzoylecgonine	C

†Abbreviations used in the table include: MDMA (3,4-methylenedioxymethamphetamine), THC (Δ^9-tetrahydrocannabinol), 4-ANPP (4-anilino-N-phenethylpiperidine), 4-hydroxy DET (4-hydroxy diethyltryptamine), 4-hydroxy MiPT (4-hydroxy-N-methyl-N-isopropyltryptamine); 4-hydroxy MPT (4-hydroxy-N-methyl-N-propyltryptamine); 5-methoxy MET (5-methoxy-N-methyl-N-ethyltryptamine), MMDPPO (α-methyl-3,4-methylenedioxyphenylpropanaldoxime).

Table S2. Summary of the LC-MS/MS analysis, including the peak of interest (precursor ion (PI) *m/z* used for the product ion scan), the standards used for targeted analysis, and the conclusion *Confirmed* (C), *Unable to confirm* (U).

Sample Number	PI	Standards used for Targeted Analysis	LC-MS/MS Conclusion
011	192	2,3-Dimethylmethcathinone; 3,4-Dimethylmethcathinone; N-Ethylbuphedrone; 3-Ethylmethcathinone; 4-Ethylmethcathinone; N-ethyl-N-Methylcathinone; Isopentedrone; 4-methyl-N,N-Dimethylcathinone; 2-Methylbuphedrone; 3-Methylbuphedrone; 4-Methylbuphedrone; 3-Methylethcathinone; 4-Methylethcathinone; 2-NMC; Pentedrone; Phenetrazine; Propylcathinone	U
023	236	Dibutylone; N-Ethylmethylone; 2,3-Eutylone; Eutylone; iProne; 5-Methylethylone; 2,3-Pentylone; Pentylone; Propylone	C - Eutylone
066	192	2,3-Dimethylmethcathinone; 3,4-Dimethylmethcathinone; N-Ethylbuphedrone; 3-Ethylmethcathinone; 4-Ethylmethcathinone; N-ethyl-N-Methylcathinone; Isopentedrone; 4-methyl-N,N-Dimethylcathinone; 2-Methylbuphedrone; 3-Methylbuphedrone; 4-Methylbuphedrone; 3-Methylethcathinone; 4-Methylethcathinone; 2-NMC; Pentedrone; Phenetrazine; Propylcathinone	U
075	168	Metaraminol; Phenylephrine; Synephrine	C - Phenylephrine
078	411	Isotonitazene; Protonitazene	C - Protonitazene
140	299	Despropionyl Fluorofentanyl	C
194	168	Metaraminol; Phenylephrine; Synephrine	C - Phenylephrine
	166	Ephedrine; Hordenine; Pseudoephedrine	U
268	166	Ephedrine; Hordenine; Pseudoephedrine	C - Ephedrine
502	250	Dimethylpentylone; 3',4'-Methylenedioxy-a-dimethylamino-isovalerophenone	U

†Abbreviations used in the table include: 2-NMC (N,2-dimethyl-N-(4-methylphenyl)-propanamide).