A Template for the Validation of DART-MS for Qualitative Seized Drugs Analysis

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Abstract
Direct analysis in real time mass spectrometry (DART-MS) is an increasingly employed tool for a wide range of forensic applications including seized drug analysis. A significant body of research surrounds DART-MS for the analysis of seized drugs and how it can be used to address many of the challenges caused by the increased presence of emerging drugs and novel psychoactive substances. A lack of available resources to help address validation, operation, training, and data interpretation needs is just one of the hurdles that laboratories face when adopting new technologies, such as DART-MS. To provide additional resources to assist in validation development, this work provides a template that can be adopted or adapted for DART-MS or other ambient ionization mass spectrometry techniques for qualitative seized drug analysis. The template, which was created as a result of recent implementation efforts, provides a description of validation studies with a focus on understanding the potential challenges and limitations caused by the prevalence of novel psychoactive substances and other emerging drugs. The studies address accuracy and precision, reproducibility, specificity, sensitivity, environmental factors, use in casework, and robustness. In addition to providing a template for validation, the results obtained from completing these studies on two high-resolution DART-MS systems are also presented. This work, and the corresponding supplemental information, was created to add to the available resources that laboratories can leverage to assist in overcoming the adoption hurdles of ambient ionization mass spectrometry methods such as DART-MS.

Keywords: DART-MS; Validation; Seized Drug; Screening; Qualitative Analysis; Ambient Ionization

Introduction

Forensic laboratories face a number of challenges with the introduction of new technologies including time, cost, and resource constraints. Even when cost is not a constraint, the need to develop a training plan, standard operating procedure, validation plan, and other documents can be overwhelming. When the technology is new to the laboratory it can add another layer of complexity as practitioners may be unfamiliar with the technique, its fundamentals, or even how to operate the system. To alleviate these obstacles, there is an increased need for resources that the community can leverage to lower the barriers for adoption.

One technology that laboratories are implementing with increasing frequency is direct analysis in real time mass spectrometry (DART-MS)[1–4]. DART-MS is one of the many ambient ionization mass spectrometry techniques that allow for rapid chemical analysis of samples with high sensitivity and minimal, if any, sample preparation. While DART-MS has been demonstrated for a wide range of forensic applications, qualitative seized drug analysis is the most widely researched and widely implemented[2]. DART-MS has been successfully demonstrated for the analysis of traditional drugs[5], novel psychoactive substances[6,7], steroids[8], pharmaceuticals[9], and other compounds of interest to a drug chemist[10]. Fortunately, the interest in this technology has led to a large base of scientific publications demonstrating various applications and data treatment approaches for the technique, but the prevalence of additional resources beyond that are few.

Some of the resources that do exist for laboratories that are considering implementing DART-MS or another ambient ionization mass spectrometry technique include the foundational validation work completed by Steiner *et al.* for the qualitative analysis of seized drugs[5] and for the confirmation of pharmaceutical samples using physical identifiers and DART-MS[9]. A freely-available spectral library also exists for seized drug analysis[11,12] as do a number of webinars and presentations that dive into fundamentals and real-world use of the technology[13,14]. Several textbooks also exist that can provide foundations for training practitioners who are new to the technique[15–18]. To supplement this body of existing resources, this work provides a template for the validation of DART-MS, or other ambient ionization mass spectrometry platforms, that laboratories can adapt for their own purposes. Given the change in the drug landscape since the foundational validation by Steiner *et al*, the validation described here emphasizes the need to ensure detection of novel psychoactive substances (NPSs) and other emerging drugs while also allowing for an understanding of the limitations of these techniques, specifically as it relates to isomer differentiation. Associated documentation to support implementation efforts has also been made available[19]. The results of this validation process from two instruments are also presented.

Materials & Methods

Instrument & Method

The two instruments used in this study were both JEOL AccuTOF 4G LC-Plus mass spectrometers (Peabody, MA, USA) coupled with DART-SVP ion sources (IonSense, Saugus, MA, USA). A suite of different software was used for data analysis and included msAxel (JEOL), MassMountaineer (Diablo Analytical, Antioch, CA, USA), and AnalyzerPro XD (SpectralWorks, Runcorn, Cheshire, UK). The NIST DART-MS Forensics Database was also used to assist in data analysis[11].

Both positive and negative ionization modes were investigated in all components of this validation except for non-probative casework (Study 6). All analyses used helium as the DART gas with a temperature of 400 ºC. For positive ionization mode a DART grid voltage of +150 V was employed. Mass spectrometer settings included an *m/z* scan range from *m/z* 80 to *m/z* 800 at 0.4 s per scan. An orifice 1 temperature of 120 ºC, ring lens voltage of +5 V, orifice 2 voltage of +5 V and ion guide voltage of +800 V were used. Orifice 1 was cycled, using the parameter switching between +20 V, +30 V, +60 V, and +90 V at 0.4 s per voltage. The negative mode method was identical to the positive mode, aside from the voltage polarity. Data acquisition was set to run for up to 180 min but was stopped whenever all samples for a particular study were collected. Polyethylene glycol (PEG-600) was sampled at the beginning and end of every run as well as approximately every 10 min throughout the run.

For the non-probative casework (Study 6) portion, only positive mode ionization was used, and the method was slightly modified. Instead of parameter switching for the orifice 1 voltage, a method with an orifice 1 voltage of +30 V and another with +60 V was used. In addition, instead of a 180 min acquisition time the method was shortened to 1 min and data was collected using the Sequence Table instead of the Single Run option, which was used for all other studies. A single non-probative case sample was analyzed in a single 1 min run along with a tetracaine verification. A +30 V orifice 1 datafile was collected for each sample and a +60 V datafile was collected when additional fragmentation was needed to assist in compound identification. This study also incorporated the use of tetracaine as an internal standard, which has been previously discussed elsewhere[20].

Data Processing

The *m/*z (mass) calibration was completed using PEG-600 which was sampled, at a minimum, at the beginning and end of each collected datafile for all studies except non-probative casework (Study 6). A full calibration was completed approximately weekly in msAxel using a PEG-600 spectrum. For each datafile, a mass drift compensation was also applied using the multi-point *m/z* drift compensation function within msAxel along with an *m/z* value corresponding to one of the major peaks in the PEG-600 spectrum

(typically *m/z* 415.2538 in positive ionization mode and *m/z* 295.1393 in negative ionization mode). For the non-probative casework study (Study 6), tetracaine (*m/z* 265.1916) was used as the mass drift compensation ion.

For studies where the accuracy of the *m/z* calibration was investigated, the mass spectra of interest were extracted in msAxel, saved as ".txt" files and searched against an in-house search list[19] in MassMoutaineer using the "Search From List" functionality. Constraints of ±5 mmu (±0.005 Da) for the mass tolerance and a minimum relative intensity threshold of 5 % were used. For studies where peak area was measured, extracted ion chronograms (EICs) corresponding to the base peak for the compound of interest were extracted and integrated using the msAxel default integration settings.

For studies where comparison to a spectral library was required, raw datafiles were converted to ".netCDF" datafiles in msAxel and then opened in AnalyzerPro XD. The mass spectra were extracted using the peak detection functionality in AnalyzerPro and then searched against the NIST DART-MS Forensics Database (for positive ionization mode) or an in-house created library (for negative ionization mode). While this was completed using AnalyzerPro, it can also be done by extracting the spectra in msAxel and then using the "NIST Search" functionality in MassMountaineer.

Chemicals & Consumables

Both single- and multi-component standard solutions were used in this work. Individual standards were purchased from Cayman Chemical (Ann Arbor, MI, USA) as either powders or 1 mg/mL methanolic solutions. For analyses that required a single-component solution, 50 µg/mL solutions were created by either dissolving powder in methanol or diluting 1 mg/mL stock solutions in methanol. For sensitivity measurements, solutions were diluted, gravimetrically, in methanol to concentrations as low as 0.5 µg/mL. Two multi-component solutions were used in this validation to study accuracy, precision, reproducibility, robustness, and environmental effects. A 15-component solution, comprised of the compounds listed in Table 1, was used for positive mode analysis. A three-component solution, comprised of the compounds listed in Table 2, was used for negative mode analysis. Solutions were prepared so that the concentration of all compounds in the mixture was approximately 50 µg/mL.

Polyethylene glycol 600 (PEG-600) (Sigma-Aldrich, St. Louis, MO, USA) was used as the mass spectrometer tuning compound and tetracaine (Sigma-Aldrich) was used as an internal standard. All sampling was completed using glass microcapillaries (Corning, Corning, NY, USA).

Table 1. Compounds present in the 15-component solution that was used for the accuracy and precision, reproducibility, environmental, and robustness components of the validation study in positive ionization mode. The monoisotopic molecular masses are listed. All compounds readily formed a protonated molecule, $[M+H]^+$, listed as the DART-MS base peak.

Compound	Formula	Molecular Mass (Da)	DART-MS Base Peak (*m/z*)
Methamphetamine	$C_{10}H_{15}N$	149.120	150.128
α-Pyrrolidinobutiophenone	$C_{14}H_{19}NO$	217.147	218.154
Butylone	$C_{12}H_{15}NO_3$	221.105	222.113
Ethylone	$C_{12}H_{15}NO_3$	221.105	222.113
α -Pyrrolidinovalerophenone	$C_{15}H_{21}NO$	231.162	232.170
Phencyclidine	$C_{17}H_{25}N$	243.199	244.207
Tenocyclidine	$C_{15}H_{23}NS$	249.155	250.163
Cocaine	$C_{17}H_{21}NO_4$	303.147	304.155
Alprazolam	$C_{17}H_{13}ClN_4$	308.083	309.091
Stanozolol	$C_{21}H_{32}N_2O$	328.251	329.259
Heroin	$C_{21}H_{23}NO_5$	369.158	370.165
Furanyl Fentanyl	$C_{24}H_{26}N_2O_2$	374.199	375.207
Furanyl Fentanyl 3-Furancarboxamide	$C_{24}H_{26}N_2O_2$	374.199	375.207
5-Fluoro ADB	$C_{20}H_{28}FN_3O_3$	377.211	378.219
Nandrolone Decanoate	$C_{28}H_{44}O_3$	428.328	429.336

Table 2. Compounds present in the three-component solution that was used for the accuracy and precision, reproducibility, environmental, and robustness components of the validation study in negative ionization mode. The monoisotopic molecular masses are listed. All compounds readily formed a deprotonated molecule, $[M-H]^-$, listed as the DART-MS base peak.

Compound	Formula	Molecular Mass (Da)	DART-MS Base Peak (*m/z*)
Gamma Hydroxy-Butyrate (GHB)	$C_4H_8O_3$	104.047	103.039
Secobarbital	$C_{12}H_{18}N_2O_3$	238.132	237.124
AB-FUBINACA	$C_{20}H_{21}FN_4O_2$	368.165	367.157

Overview of the Validation Process

Study 1. Accuracy and Precision

To measure accuracy, the 15-component solution (positive mode, Table 1) and three-component solution (negative mode, Table 2) were analyzed ten times over the span of one day to evaluate the accuracy of the *m/z* calibration. The *m/z* assignments for the base peaks in the low orifice 1 voltage (±20 V) spectra were evaluated to determine if they consistently fell within a ±0.005 Da tolerance of the calculated theoretical masses. To process this data, spectra were extracted in msAxel and processed in MassMountaineer using the Search From List functionality.

In addition to evaluating mass tolerance of the base peaks, the ability to consistently produce accurate fragment peaks was also evaluated. Single-component solutions of each of the components (Table 1 and Table 2) were run ten times over the span of one day. The base peak or secondary base peak produced in the higher orifice 1 voltage (±30 V, ±60 V, and ±90 V) spectra for each compound was then compared to

ensure the instrument produced repeatable fragment ions and accurately calibrated ions for each compound. Data was processed in the same manner as above.

Study 2. Reproducibility

To measure reproducibility, the 15-component solution (positive mode, Table 1) and three-component solution (negative mode, Table 2) were analyzed five times per a set on seven separate days over a three-week period to evaluate the reproducibility of the system. The *m/z* assignments for the base peaks in the low orifice 1 voltage (±20 V) spectra were monitored to determine if they consistently fell within a ±0.005 Da tolerance of the calculated theoretical mass for all compounds. In addition to measuring the reproducibility of the *m/z* values, the ability to reproducibly calibrate and mass drift compensate the mass spectrometer using PEG-600 was also examined. To monitor calibration, the "1-R" value obtained during the calibration process in msAxel was noted and was considered passing if it was less than $9.9x10^{-12}$ with the removal of up to one peak at the beginning or end of the calibration range. Mass drift compensation was considered passing if the process was successfully completed. Finally, a methanol blank was analyzed in-between samplings of the multi-component solutions to monitor the potential for carryover or false positive identification of peaks in a blank spectrum. This was completed by extracting and searching the methanol spectra in the same manner as the solution spectra.

Study 3. Specificity

Specificity of the system was evaluated through two separate studies for each ionization mode. In the first study, single-component solutions for all compounds listed in Table 1 and Table 2 were analyzed five times each and the resulting mass spectra from each of the four fragmentation voltages were searched against the NIST DART Forensics library[11,12] using NIST MS Search[21] to identify how well spectra from different compounds could be differentiated. An in-house library was created due to the lack of a publicly available negative ionization mode library. The resulting reverse match factor (obtained using NIST MS Search) was noted as were instances where the compound analyzed was not returned as the top match.

Given that isomer differentiation is a known limitation of DART-MS analysis, a second study was completed to understand the limitations of the system in differentiating commonly seen isomer sets. Single-component solutions of the compounds listed in Table 3 were analyzed across the four orifice 1 voltages and searched against the NIST DART Forensics library in the same manner as above.

Table 3. Compounds used for the isomer specificity study (Study 3) which focused on understanding the ability to differentiate commonly seen isomers.

Positive Mode Sets		
Set 1	**Set 3**	**Set 5**
Methamphetamine	Cyclopropyl Fentanyl	6-APDB
Phentermine	Crotonyl Fentanyl	5-APDB
	Methacryl Fentanyl	Buphedrone
		Dimethylcathinone
Set 2	**Set 4**	Ethcathinone
Butylone	m-FBF	Mephedrone
Dimethylone	o-FBF	2-MMC
Ethylone	p-FBF	MMAI
3,4-EDMC	m-FiBF	
3,4-MDPA	o-FiBF	
	p-FiBF	
Negative Mode Set		
AB-FUBINACA	AB-FUBINACA 2'-indazole isomer	AB-FUBINACA 2-fluorobenzyl isomer
AB-7-FUBAICA	AB-FUBINACA isomer 1	

Study 4. Sensitivity

Sensitivity was evaluated in accordance with ASTM Method E2677[22], which provided a statistically calculated limit of detection (LOD) with an assigned confidence interval. The sensitivity was measured for each compound in Table 1 and Table 2, individually. Solutions of known concentration were created, gravimetrically, from solid standards or stock solutions. Nominal solution concentrations of 0.5 µg mL^{-1}, 1 µg mL^{-1}, 5 µg mL^{-1}, 10 µg mL^{-1}, and 25 µg mL^{-1}, along with pure methanol (0 µg mL^{-1}) were used. A template for the creation of the gravimetric solutions is provided elsewhere[19]. For each compound, ten replicates of each solution concentration were analyzed by pipetting 1 µL aliquots directly onto the glass microcapillary. Once analyzed, integrated peak areas from the extracted ion chronograms (EICs) of the base peak for each compound were obtained. Calculation of the LOD was completed by entering the concentrations and peak areas into the ASTM E2677 LOD calculator[23] using a confidence limit of 0.10 (90 % confidence).

Study 5. Environmental (Solvent) Effects

The effects of solvent on instrument response were investigated by creating the 15-component (positive mode) and three-component (negative mode) solutions at the same concentration (50 µg/mL) used in the accuracy and precision studies in three additional solvents (acetone, chloroform and hexane). These solutions, along with the methanolic solution, were analyzed, in triplicate. Once analyzed, the ±20 V orifice 1 voltage mass spectra were extracted and searched using MassMountaineer to determine if solvent would

have an effect on compound detection or compound identification. In addition, the integrated peak areas from the EICs of the base peaks for each compound were extracted and plotted to understand the effect of solvent on the response (intensity) for each compound.

Study 6. Non-Probative Casework

In order to establish the use of this technique in a real-world implementation, a blind sampling study was completed. A total of 50 adjudicated or mock case samples were analyzed with the DART-MS. For this study, the use of a tetracaine internal standard was incorporated into the case extract, but analysis could be done without an internal standard. Samples were prepared by dissolving 1 mg to 2 mg of powder into 1 mL of methanol containing tetracaine at 0.1 mg/mL. A more in-depth explanation of this approach, and the work involved in developing and evaluating tetracaine as an internal standard compound can be found elsewhere[20]. Analysis was completed using a sequence-based run approach with 1 min analyses. In the 1 min run, the tetracaine internal standard was first analyzed, followed by three analyses of the sample. The resulting mass spectra were extracted and analyzed in the same manner as the specificity study and compared to the GC-MS results. A successful identification was defined as a positive search result (greater than 5% relative abundance and within ±5 mmu of the theoretical mass) of all detectable controlled substances found in the GC-MS analysis along with identification of the internal standard in the DART-MS mass spectra.

Study 7. Method Robustness

To establish robustness of the technology, a second practitioner completed the reproducibility study (Study 2) using the same solutions from Study 2. This allowed for investigation of any analyst-dependent parameters.

A template for compiling the data for each of the above listed studies is provided elsewhere[19].

Results & Discussion

Study 1. Accuracy and Precision

Results for the accuracy and precision studies for both instruments and both ionization modes produced peaks with *m/z* values that were within the ±0.005 Da tolerance for all compounds. Figure 1 shows the drift (difference between measured and theoretical *m/z* values), in Da, from the theoretical monoisotopic mass for all compounds in the 15-component solution analyzed in positive mode. Supplemental Figure 1 shows the results for all compounds in the three-component solution in negative mode.

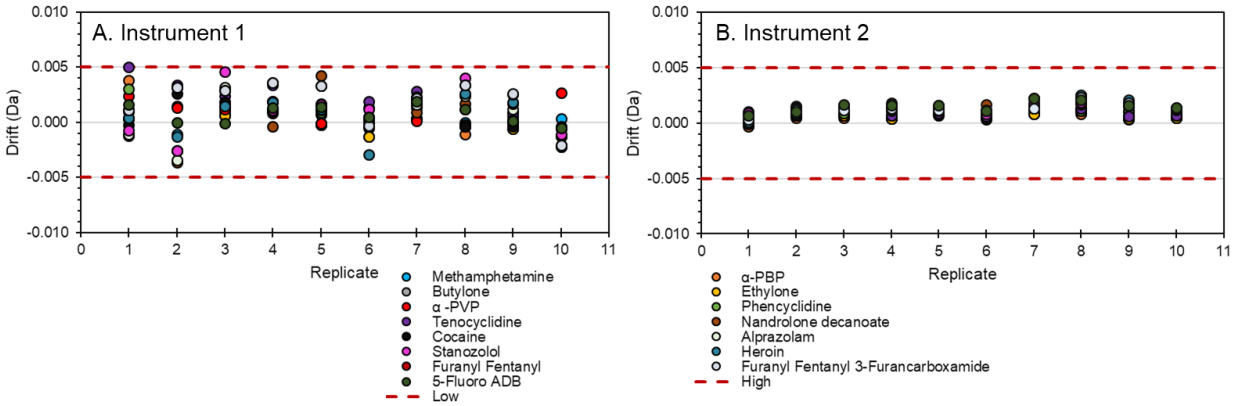

Figure 1. Calculated *m/z* drifts from theoretical values for the ten replicate analyses for the positive mode accuracy and precision study (Study 1) for Instrument 1 (A., left) and Instrument 2 (B., right). The theoretical *m/z* values corresponding to this data can be found in (Table 1). The red dotted lines indicate the high and low bounds of the allowable drift.

Analysis of the higher orifice 1 fragmentation voltage spectra for both positive and negative ionization modes produced *m/z* values that were consistently within the ±0.005 Da drift window for all compounds at all orifice 1 voltages examined. Supplemental Figures 2, 3, and 4 show the drift for the major peaks observed in the +30 V, +60 V, and +90 V orifice 1 voltages for all 15 compounds analyzed individually in positive mode. Supplemental Figures 5, 6, and 7 show the same data obtained from the -30 V, -60 V, and -90 V orifice 1 voltages for all 3 compounds analyzed individually in negative mode. Supplemental Tables 1 and 2 show the theoretical *m/z* values used to generate the data for comparison for the ±30 V, ±60 V, and ±90 V spectra.

Study 2. Reproducibility

As a result of the reproducibility studies, the *m/z* values corresponding to all compounds were found to be within the ±0.005 Da tolerance specified for all replicates from all seven days of analysis. The standard deviations from the theoretical *m/z* values ranged from ±0.0005 Da to ±0.0012 Da. The coefficient of variation was found to be at or below 0.006 % for all compounds. Table 4 shows the summary results for the positive and negative reproducibility studies for Instrument 1. Supplemental Table 3 shows the summary results for Instrument 2. Supplemental Figure 8 shows the calculated drift from the theoretical monoisotopic mass for all compounds in the 15-componentsolution in positive mode across the seven days while Supplemental Figure 9 shows the results for the three-component solution in negative mode.

Table 4. Summary results for the reproducibility studies for Instrument 1.

	Theoretical *m/z*	Minimum *m/z*	Maximum *m/z*	Average *m/z*	Standard Deviation	Coefficient of Variation (%)
Positive Ionization Mode						
Methamphetamine	150.1277	150.1258	150.1289	150.1277	0.0008	0.0006
α-PBP	218.1539	218.1520	218.1551	218.1537	0.0008	0.0004
Butylone	222.1124	222.1107	222.1135	222.1122	0.0008	0.0004
Ethylone	222.1124	222.1107	222.1135	222.1122	0.0008	0.0004
α -PVP	232.1695	232.1681	232.1707	232.1694	0.0007	0.0003
Phencyclidine	244.2059	244.2047	244.2077	244.2062	0.0008	0.0003
Tenocyclidine	250.1624	250.1602	250.1648	250.1630	0.0012	0.0005
Nandrolone decanoate	429.3363	429.3342	429.3395	429.3370	0.0011	0.0003
Cocaine	304.1543	304.1533	304.1569	304.1547	0.0008	0.0003
Alprazolam	309.0901	309.0892	309.0927	309.0908	0.0009	0.0003
Stanozolol	329.2587	329.2580	329.2623	329.2598	0.0010	0.0003
Heroin	370.1649	370.1629	370.1678	370.1652	0.0011	0.0003
Furanyl Fentanyl	375.2067	375.2055	375.2107	375.2075	0.0012	0.0003
Furanyl Fentanyl 3-Furancarboxamide	375.2067	375.2055	375.2107	375.2075	0.0012	0.0003
5-Fluoro ADB	378.2187	378.2164	378.2219	378.2193	0.0011	0.0003
Negative Ionization Mode						
AB-FUBINACA	103.0390	103.0388	103.0406	103.0395	0.0005	0.0005
GHB	237.1234	237.1217	237.1247	237.1236	0.0006	0.0003
Secobarbital	367.1565	367.1536	367.1582	367.1565	0.0010	0.0003

PEG calibration residuals were found to fall below 10^{-12} for all runs in the reproducibility study for both positive and negative modes. Residual values for the PEG calibrant ranged from $6.3x10^{-13}$ to $1.6x10^{-12}$ for positive mode and $1.7x10^{-13}$ to $1.8x10^{-12}$ for negative mode. The multi-point *m/z* drift compensation function was also employed, in lieu of calibration, for some of the datafiles and was found to work as well as traditional single point calibration.

Analysis of the methanol blanks from the positive mode runs on multiple days on both instruments produced instances where a peak with a similar *m/z* value to cocaine was found. Given that there are no intense peaks in the methanol spectra, the 5 % threshold used for searching was lower than the intensity of background noise peaks. The peaks that were identified as cocaine were found to be at a similar level to the background noise, and therefore not related to carryover. In negative mode, no peaks were detected in the methanol blanks that corresponded to *m/z* values of interest.

Study 3. Specificity

The results of the first specificity study for Instrument 1 are presented in Table 5 (Instrument 2 data is shown in Supplemental Table 4). As expected, isomer differentiation (i.e. butylone and ethylone or furanyl fentanyl and furanyl fentanyl 3-furancarboxamide) was not always possible, especially at low (±20 V and ±30 V) orifice 1 voltages. This is a known limitation of DART-MS analysis that utilizes a time-of-flight mass spectrometer[2]. Six of the 15 compounds in positive mode had at least one other isomer or related compound returned as a higher hit in MS Search which, again, is expected given the spectral similarity of compounds with identical molecular masses of fragmentation. There were no instances where the compound of interest was not identified in the top five hits. MS Search scores were above 720 a.u. for all compounds across all voltages (at or above 698 a.u. for Instrument 2), indicating good agreement with library spectra. Results across the two instruments were comparable, though Instrument 2 did have fewer instances where the top hit returned was not the compound of interest. In negative ionization mode (Supplemental Tables 5), significantly higher search scores (compared to positive mode) were observed for both instruments and may be an artifact of the mass spectral library being created in-house.

Table 5. Average reverse search scores and lists of other compounds that produced reverse search scores higher than the compound of interest for the positive mode study on Instrument 1. The number in parentheses next to the compounds indicates how many times, out of the five replicate spectra, that the compound returned a reverse search score greater than the compound of interest.

	Average Reverse Search Score				**Other Compounds That Produced Scores Higher Than Compound**			
	20 V	**30 V**	**60 V**	**90 V**	**20 V**	**30 V**	**60 V**	**90 V**
Methamphetamine	809	827	821	848	Phentermine (1)	Phentermine (1)	Amphetamine (3)	Amphetamine (3) Benzphetamine (1) n-Ethylamphetamine (2)
α-PBP	874	887	865	918	MePPP (2)	None	None	None
Butylone	840	787	870	865	Ethylone (5) Metaxalone (3)	Ethylone (4) Metaxalone (1)	None	None
Ethylone	863	906	890	859	Butylone (1) Metaxalone (1)	None	None	Butylone (1)
α -PVP	838	771	813	846	None	None	None	None
Phencyclidine	838	810	862	851	None	None	None	None
Tenocyclidine	938	915	979	793	None	None	None	None
Nandrolone decanoate	752	720	760	760	None	None	None	None
Cocaine	767	839	875	890	None	None	None	None
Alprazolam	928	927	920	928	None	None	None	None
Stanozolol	922	914	905	890	None	None	None	None
Heroin	772	763	767	868	None	None	None	None
Furanyl Fentanyl	817	838	830	871	3-Furanyl Fent (3)	3-Furanyl Fent (2)	3-Furanyl Fent (3)	3-Furanyl Fent (3)
Furanyl Fentanyl 3-Furancarboxamide	847	855	793	837	2-Furanyl Fentanyl (1)	None	2-Furanyl Fentanyl (1)	2-Furanyl Fentanyl (1)
5-Fluoro ADB	968	975	958	860	None	None	None	None

Abbreviation: "3-Furanyl Fent" is Furanyl Fentanyl 3-Furancarboximide.

As expected in the second specificity study, isomer differentiation was also not always possible, especially at low (±20 V and ±30 V) orifice 1 voltages. Table 6 shows the results of the second specificity study on Instrument 1 (Supplemental Table 6 shows the results for Instrument 2). For isomer set 1, differentiation of methamphetamine and phentermine was not possible at low fragmentation voltages but was possible at high fragmentation voltages, though methamphetamine and other amphetamines produce similar spectra at high fragmentation voltages. For isomer Set 2 and isomer Set 5, the synthetic cathinones, differentiation was not possible at low fragmentation voltages, as expected, as the spectra were dominated by the protonated molecule. Higher orifice 1 voltage spectra, however, did allow for a greater degree of differentiation than expected. This trend was also observed for the synthetic cannabinoid set in negative mode (Supplemental Table 7). For the fentanyl isomer sets, Set 3 and Set 4, differentiation across the fragmentation voltages was not possible due to both identical protonated molecules and similar fragmentation spectra within each set. The results of these studies highlight that leveraging the higher fragmentation voltages may assist in isomer differentiation for some cases. While this is true for pure compounds, the added benefit of the higher fragmentation spectra may not be realized for multi-component solutions unless advanced search algorithms are employed.

Table 6. Average reverse search scores and lists of other compounds that produced reverse search scores higher than the compound of interest for the positive mode study in Instrument 1. The number in parentheses next to the compounds indicates how many times, out of the five replicate spectra, that compound returned a reverse search score greater than the compound of interest.

	Average Reverse Search Score				**Other Compounds That Produced Scores Higher Than Compound**			
	20 V	**30 V**	**60 V**	**90 V**	**20 V**	**30 V**	**60 V**	**90 V**
Set 1								
Methamphetamine	809	827	821	848	Phentermine (1)	Phentermine (1)	Amphetamine (3)	Amphetamine (3) Benzphetamine (1) n-Ethylamphetamine (2)
Phentermine	848	795	880	857	None	None	None	None
Set 2								
Butylone	840	787	870	865	Ethylone (5) Metaxalone (3)	Ethylone (4) Metaxalone (1)	None	None
Dimethylone	939	942	966	986	EDMC (1) MDPA (4)	MDPA (2)	None	None
Ethylone	863	906	890	859	Butylone (1) Metaxalone (1)	None	None	Butylone (1)
3,4-EDMC	920	915	758	981	Dimethylone (2) MDPA (2)	None	None	None
3,4-MDPA	971	958	944	989	None	None	None	None
Set 3								
Cyclopropyl Fent.	791	821	863	880	Crotonyl (3) Methacryl (3)	Crotonyl (4) Methacryl (4)	Crotonyl (1) Methacryl (1)	None
Crotonyl Fent.	833	859	837	775	Methacryl (4)	Methacryl (5)	Cyclopropyl (4) Methacryl (1)	Cyclopropyl (5) Methacryl (5)

Methacryl Fent.	804	858	837	836	None	Crotonyl (3) Cyclopropyl (2)	Crotonyl (2) Cyclopropyl (3)	Cyclopropyl (5)
Set 4								
m-FBF	787	859	851	872	o-FBF (3) p-FBF (3) p-FiBF (5)	o-FBF (1) p-FBF (5) p-FiBF (1)	m-FiBF (1) o-FiBF (5) p-FBF (5) p-FiBF (4)	m-FiBF (2) o-FiBF (4) p-FBF (3) p-FiBF (5)
o-FBF	805	859	873	869	o-FiBF (2) p-FiBF (5)	m-FBF (4) p-FBF (5) p-FiBF (3)	p-FBF (4)	m-FBF (1) m-FiBF (5) p-FBF (1) p-FiBF(5)
p-FBF	771	910	891	947	o-FiBF (2) p-FiBF (5)	None	None	None
m-FiBF	822	850	822	904	m-FBF (4) p-FBF (4) p-FiBF (5)	m-FBF (4) o-FBF (2) o-FiBF (1) p-FBF (4) p-FiBF (5)	m-FBF (3) o-FBF (1) o-FiBF (3) p-FBF (4) p-FiBF (5)	m-FBF (1) o-FiBF (2) p-FBF (2)
o-FiBF	843	826	887	864	m-FBF (1) o-FBF (4) o-FiBF (4) p-FBF (5) p-FiBF (5)	m-FiBF (1) p-FBF (2)	m-FBF (1) m-FiBF (1) p-FiBF (2)	m-FBF (1) p-FBF (1)
p-FiBF	848	941	919	960	m-FiBF (1) p-FBF (1)	None	None	None
Set 5								
6-APDB	964	938	963	987	None	None	5-APDB (1)	None
5-APDB	908	931	966	992	None	None	None	None
Buphedrone	940	940	983	975	Ethcathinone (2)	Dimethylcath. (1)	None	None
Dimethylcathinone	923	949	985	989	Buphedrone (2) Ethcathinone (1)	Buphedrone (1) Ethcathinone (1)	None	None
Ethcathinone	896	892	931	997	Buphedrone (4) Dimethylcath. (4)	Buphedrone (5) Dimethylcath. (4) 2-MMC (1)	None	None
Mephedrone	945	877	969	984	Buphedrone (1) Ethcathinone (2)	Buphedrone (4) Dimethylcath (3) Ethcathinone (1)	None	None
2-MMC	901	870	982	984	Buphedrone (3) Dimethylcath. (2) Mephedrone (2)	Buphedrone (5) Dimethylcath. (4)	None	None
MMAI	881	902	974	988	None	None	None	None

Abbreviations: "Cyclopropyl" is cyclopropyl fentanyl. "Crotonyl" is crotonyl fentanyl. "Methacryl" is methacryl fentanyl. "Dimethylcath." is dimethylcathinone.

Study 4. Sensitivity

The calculated limits of detection for all compounds were found to range from 0.12 ng to 4.41 ng which are in line with published LOD values for DART-MS[2]. The specific LODs for each compound are given in Tables 7. LODs are reported in ng since a 1 µL volume of solution was used for all experiments.

Table 7. Calculated limits of detection using a 90 % confidence for both instruments.

Compound	LOD (ng)	
	Instrument 1	Instrument 2
Methamphetamine	0.87	0.38
α-Pyrrolidinobutiophenone	0.21	1.82
Butylone	0.12	1.40
Ethylone	4.41	1.10
α - Pyrrolidinovalerophenone	1.14	1.59
Phencyclidine	1.02	1.73
Tenocyclidine	1.07	2.71
Nandrolone	1.79	1.46
Cocaine	0.27	1.05
Alprazolam	1.24	0.54
Stanozolol	0.41	0.47
Heroin	0.54	1.74
Furanyl Fentanyl	1.01	0.99
Furanyl Fentanyl 3-Furancarboxamide	1.03	2.02
5-Fluoro ADB	2.37	1.61
Negative Mode		
GHB	1.48	0.36
Secobarbital	1.21	1.42
AB-FUBINACA	2.10	0.44

Study 5. Environmental (Solvent) Effects

For positive mode, compound detection was possible for all three replicates in chloroform, hexane, and methanol. Six compounds (nandrolone decanoate, alprazolam, stanozolol, heroin, furanyl fentanyl, and furanyl fentanyl 3-furancarboxamide) were not detectable in one of the acetone replicates for Instrument 1. Methanol and hexane typically produced the highest abundance peaks with acetone consistently performing worst, as shown in Figure 2. No solvent-related *m/z* drift or formation of adduct species was observed. Chloroform and acetone were found to be largely detrimental to analyte signal, though there are some compound-specific dependences. Similar results were observed in negative mode (Supplemental Figure 10), with detection of all three compounds possible in all solvents (except GHB in chloroform for Instrument 2), and lowest signals obtained in an acetone or chloroform solution.

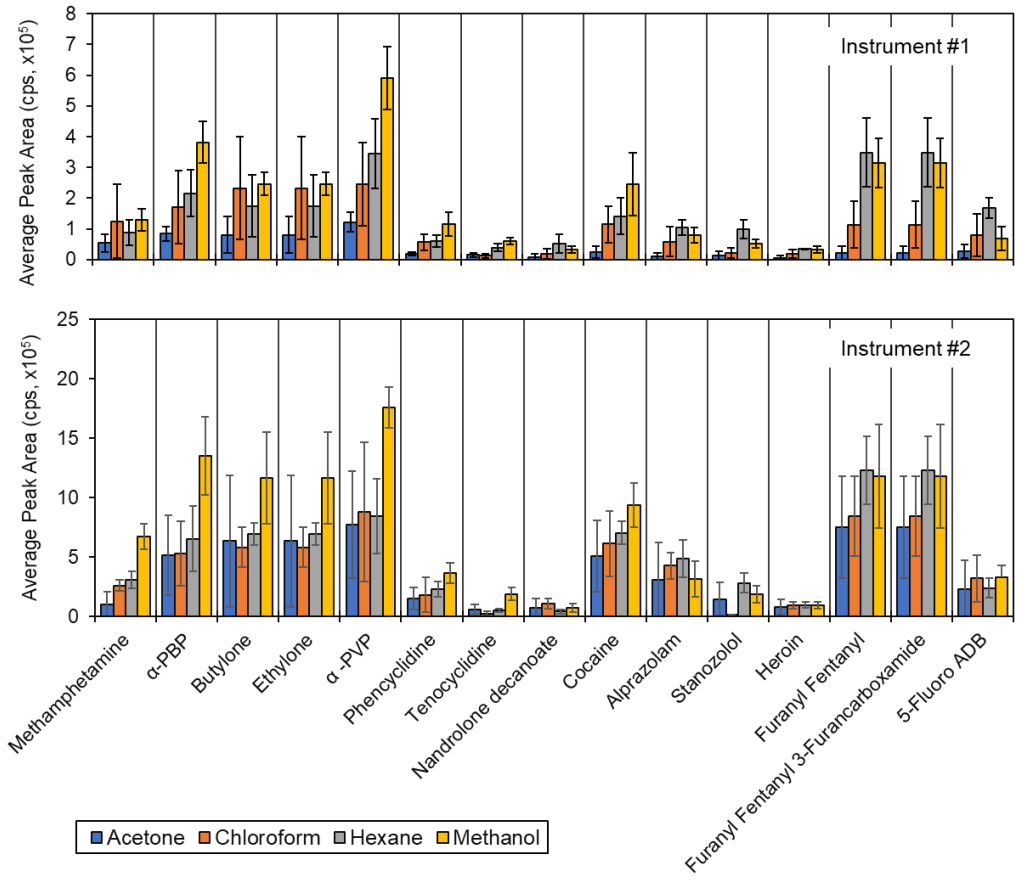

Figure 2. Average peak area as a function of solvent for all compounds analyzed in the positive mode environmental study on Instrument 1 (top) and Instrument 2 (bottom). Error bars represent the standard deviation of three replicate measurements.

Study 6. Non-probative Casework

During the non-probative casework studies, several opportunities for change were identified that led to modifications in the analytical protocols. The first modification was brought about because it was established that the mass spectra obtained using +20 V and +30 V orifice 1 voltages were nearly identical except for an increased dimer presence in the +20 V spectra. To minimize the dimer contribution, the +30 V orifice 1 spectra were used for analysis of case samples. Second, to minimize issues with the false identification in low intensity spectra, the use of an internal standard was incorporated, as discussed above. Inclusion of tetracaine as an internal standard ensured that the 5 % relative abundance threshold did not cause false identification of noise peaks, which was observed in Study 2, and also provided a mass calibration check standard in each sample. In order for a sample to have a positive identification, the peak corresponding to tetracaine had to also be within the ±0.005 Da tolerance and present at or above 5 % relative intensity.

As demonstrated in the specificity portion of the validation study it was demonstrated that while DART-MS is incapable of providing differentiation of positional isomers, use of the fragment ions can allow for the differentiation of some structural isomers. To assist in the identification process, a series of fragment ion search lists were created for instances where differentiation was possible and were employed in this portion of the study, where appropriate. When the main search list provided multiple results for the same *m/z* value, the fragmentation search lists were loaded concurrently and the sample was re-searched to identify which, if any, of the fragment ions of interest were detected. For some compounds this required the acquisition and searching of the +60 V spectra, in addition to the +30 V spectra, in order to obtain the necessary fragment ion.

Using the above modifications, a total of 43 samples containing a controlled substance and seven samples containing no controlled substances were analyzed by DART-MS. A summary of these results is shown in Table 8. In 39 of the 43 samples containing a controlled substance, DART-MS was able to correctly identify all substances that were identified by GC-MS. Of the four samples (1, 3, 14, and 42) where not all controlled substances were detected, three correctly identified at least some of the controlled substances and one did not identify any of the controlled substances, a false negative. For the three incomplete results (Samples 3, 14, and 42), detection of low-concentration compounds that have poorer ionization efficiencies were not obtained at the 5 % threshold. Given the lack of chromatography in DART-MS, competitive ionization can prohibit detection of low concentration compounds when those compounds are more poorly ionized than the major constituents. This is a phenomenon that has been previously documented [6,10] and is something that drug chemists should be aware of, especially for heroin / fentanyl mixtures when heroin is the minor component. For Sample 1, where a false negative was obtained, insufficient amount of material was found to be the likely cause of the missed compound identification. Sample 1 was analyzed as if it was a powder but originated from a counterfeit pharmaceutical tablet. Due to the small amount of material sampled (<2 mg) the controlled substances were likely below the detection limit of the instrument.

In all seven samples that did not contain controlled substances, no controlled substances were detected by DART-MS. Use of tetracaine as an internal standard was found to assist in correctly identifying negative samples and eliminating false identification of background or noise peaks. The *m/z* for tetracaine was found to fall within tolerance for all samples. A number of excipients were also able to be identified in the samples. As expected, limitations due to the inability to differentiate isomers precluded definitive identification by DART-MS in some instances. This does not present any limitations in the analysis, but instead, highlights the complementarity of data obtained by DART-MS and GC-MS.

Table 8. Results of the non-probative casework study (Study 6). For samples where multiple items were identified, individual compounds are listed on separate lines. For the DART-MS result, when multiple potential compounds could be assigned to the same *m/z* value, they are listed with a vertical line "|" between them. The controlled substances identified by GC-MS are also provided, for comparison.

Sample	DART-MS Result	GC-MS Result
1	No Compounds Identified	Fentanyl Alprazolam Etizolam
2	Methamphetamine (Frag ID)	Methamphetamine
3	-- MDMA **Excipients:** Caffeine, Quinine	Heroin MDMA
4	Fentanyl | methyl Acetyl Fentanyl | Isofentanyl Levamisole Tramadol **Excipients:** Phenylpropanamide, Mannitol[†], Procaine, Pindolol	Fentanyl Levamisole Tramadol[INS]
5	4-methyl-α-PHP (Frag ID) **Excipients:** Dextrorphan	4-methyl-α-PHP
6	MDMA (Frag ID)	MDMA
7	No Controlled Substances **Excipients:** Mannitol[ϟ]	No Controlled Substances
8	Heroin 6-Monoacetylmorphine **Excipients:** Papaverine[ϟ]	Heroin
9	methyl Norfentanyl (Frag ID)	methyl Norfentanyl
10	Cathinone *m/z* 178 (Frag ID)	4-Ethylmethcathinone
11	Cathinone *m/z* 236 **Excipient:** Caffeine	Dibutylone
12	Cathinone *m/z* 192[†] Cathinone *m/z* 220 Fentanyl | methyl Acetyl Fentanyl | Isofentanyl	4-Ethylmethcathinone 4-Methyl-α-ethylaminopentiophenone Fentanyl
13	MMB-FUBINACA | MEP-FUBINACA (Frag ID)	MMB-FUBINACA
14	--- Cyclopropyl Fentanyl | Crotonyl Fentanyl | Methacrylfentanyl Phenyl Fentanyl --- **Excipients:** Mannitol, Caffeine	Heroin Cyclopropyl Fentanyl Phenyl Fentanyl Acetylmorphine
15	AB-FUBINACA (isomer) | AB-7-FUBAICA (Frag ID)	AB-FUBINACA 2-fluorobenzyl isomer
16	No Controlled Substances	No Controlled Substances
17	Cathinone *m/z* 236	Dibutylone
18	Acetyl fentanyl | Benzyl fentanyl Fentanyl | methyl Acetyl fentanyl | isofentanyl **Excipients:** Quinine, Mannitol	Acetyl fentanyl Fentanyl
19	Heroin Acetyl fentanyl | Benzyl fentanyl Fentanyl | methyl Acetyl fentanyl | isofentanyl	Heroin Acetyl fentanyl[INS] Fentanyl[INS]

	Fluorobutyryl fentanyl (iso) | Fluoroisobutyryl fent. (iso) **Excipients:** Caffeine, Mannitol, Quinine[ϟ]	FIBF[INS]
20	No Controlled Substances **Excipients:** Guaidenesin, Quinine	No Controlled Substances
21	No Controlled Substances **Excipients:** Acetaminophen, Xylitol	No Controlled Substances
22	Fentanyl | methyl Acetyl fentanyl | isofentanyl XLR11 (isomer)	Fentanyl XLR11
23	JWH-201 | JWH-250 | JWH-302	JWH-250
24	JWH-018	JWH-018
25	α-PVP	α-PVP
26	Cathinone *m/z* 236	Eutylone
27	No Controlled Substance **Excipients:** Caffeine	No Controlled Substance
28	Cathinone *m/z* 192 (Frag ID)	Methylethcathinone
29	α-PBP | 4-Me-α-PPP | Deschloro-N-ethyl ketamine 5-Fluoro-AKB48 **Excipients:** Mannitol	α-PBP 5-Fluoro-AKB48
30	Cathinone *m/z* 236 JWH-201 | JWH-250 | JWH-302 Fentanyl | methyl Acetyl fentanyl | isofentanyl	Dibutylone JWH-250 Fentanyl
31	Tramadol	Tramadol
32	JWH-201 | JWH-250 | JWH-302	JWH-250
33	Heroin Fentanyl | methyl Acetyl fentanyl | isofentanyl Fluorobutyryl fentanyl (iso) | Fluoroisobutyryl fent. (iso) Acetyl fentanyl | Benzyl fentanyl **Excipients:** Caffeine	Heroin Fentanyl FIBF Acetyl Fentanyl[INS]
34	Cathinone *m/z* 236 **Excipients:** Caffeine	Eutylone
35	Fentanyl | methyl Acetyl fentanyl | isofentanyl Tramadol **Excipients:** Phenylpropanamide, Mannitol, Caffeine, Levamisole, Procaine, Pindolol, Methoxpropamine	Fentanyl Tramadol[INS]
36	methyl AP-237 | AP-238	AP-238
37	Heroin 6-Monoacetylmorphine[†]	Heroin
38	Methyl Fentanyl (Frag ID) JWH-201 | JWH-250 | JWH-302	α-Methyl Fentanyl JWH-250
39	Fentanyl | methyl Acetyl fentanyl (iso) | Isofentanyl **Excipient:** Quinine, Caffeine, Xylazine	Fentanyl
40	Cyclopropyl Fentanyl | Crotonyl Fentanyl | Methacrylfentanyl Cathinone *m/z* 212	Cyclopropyl Fentanyl 4-Chloroethcathinone
41	No Controlled Substances **Excipients:** Mannitol	No Controlled Substances
42	Heroin Noscapine[ϟ] Fentanyl | methyl Acetyl fentanyl (iso) | Isofentanyl Acetyl fentanyl | Benzyl fentanyl --- Fluorobutyryl fentanyl (iso) | Fluoroisobutyryl fent. (iso) 6-Monoacetylmorphine	Heroin Noscapine Fentanyl[INS] Acetyl Fentanyl[INS] Cocaine[INS] FIBF[INS]

	Excipients: Caffeine	
43	Methylone (isomer) (Frag ID)	Methylone
44	N-methyl Cyclopropyl norfentanyl	Methyl Cyclopropyl norfentanyl
45	No Controlled Substances **Excipients:** Lidcocaine, Quinine	No Controlled Substances
46	Cathinone *m/z* 192	Methylethcathinone
47	JWH-018 MDPV	JWH-018 MDPV
48	Ethylpentylone | Dimethylpentylone | Tertylone (Frag ID)	N-Ethyl pentylone
49	MMB-FUBINACA | MEP-FUBINACA (Frag ID)	FUB-AMB
50	α-PVP	α-PVP

† Detected only on Instrument 1.
ϟ Detected only on Instrument 2.
INS GC-MS result had compound present but at an insufficient level to report.
(Frag ID) Required the use of an additional fragment ion search list to differentiate from other compounds at same *m/z* value.
-- Compound was detected in GC-MS but not by DART-MS.
(iso) Indicates there are multiple isomeric species present that DART-MS cannot distinguish.

Study 7. Method Robustness

After completing the method robustness study with additional chemists on both instruments, no analyst-dependent issues were identified. The *m/z* values corresponding to all components were found to be within the ±0.005 Da tolerance specified for all replicates from all seven days of analysis. The standard deviations from the theoretical *m/z* values ranged from ±0.0006 Da to ±0.0024 Da (or ±0.6 mDa to ±2.4 mDa). The coefficient of variation was found to be at or below 0.007 % for all compounds. Supplemental Tables 8 and 9 show the summary results for the positive mode while Supplemental Table 10 shows the results for negative mode. PEG calibration residuals were again found to be acceptable and ranged from 6.0×10^{-13} to 1.2×10^{-12} for positive mode. For negative mode, all method robustness datafiles were calibrated using multi-point *m/z* drift compensation against a calibration file with a residual of 1.7×10^{-13}. As in Study 2, methanol blanks in positive mode for several days produced low-intensity peaks with a similar *m/z* value to cocaine but were at or below the level of background noise. No peaks at *m/z* values of interest were present in the negative mode methanol blanks.

Conclusions

This work provides a template validation plan that can be adapted by other laboratories who are bringing DART-MS or other ambient ionization mass spectrometry tools online. In addition to the template, the Supplemental Information provides worksheets that laboratories can leverage to assist in the processing and collation of data. Completion of the validation studies on two newly delivered instruments showed they were fit for casework. As expected, isomeric differentiation is not always possible by DART-MS given the lack of chromatography. In addition, it was found that the utilization of an internal standard for casework analysis eliminated the false identification of low-intensity noise peaks in spectra without detectable compounds. The goal of this study was to provide an additional resource to chemists that are focusing on

adopting new technology into their workflow. Current efforts are looking at the validation of a variation of DART-MS, thermal desorption (TD-DART-MS), for the qualitative analysis of seized drugs.

Disclaimers

Certain commercial products are identified in order 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.

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

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Supplemental Information for:
A Template for the Validation of DART-MS for Qualitative Seized Drugs Analysis

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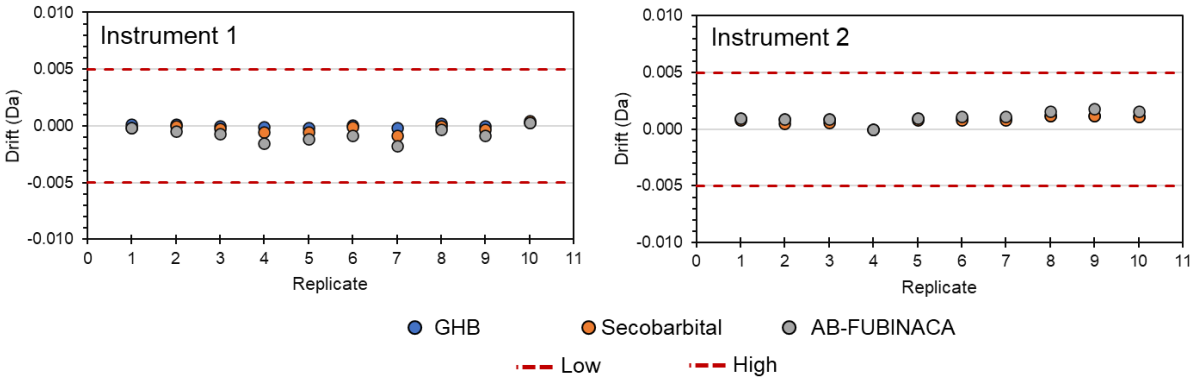
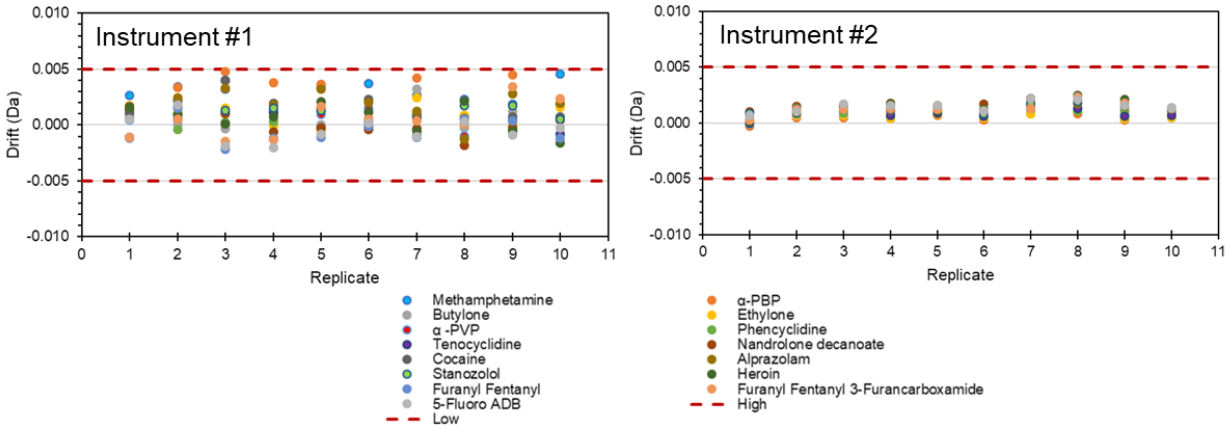
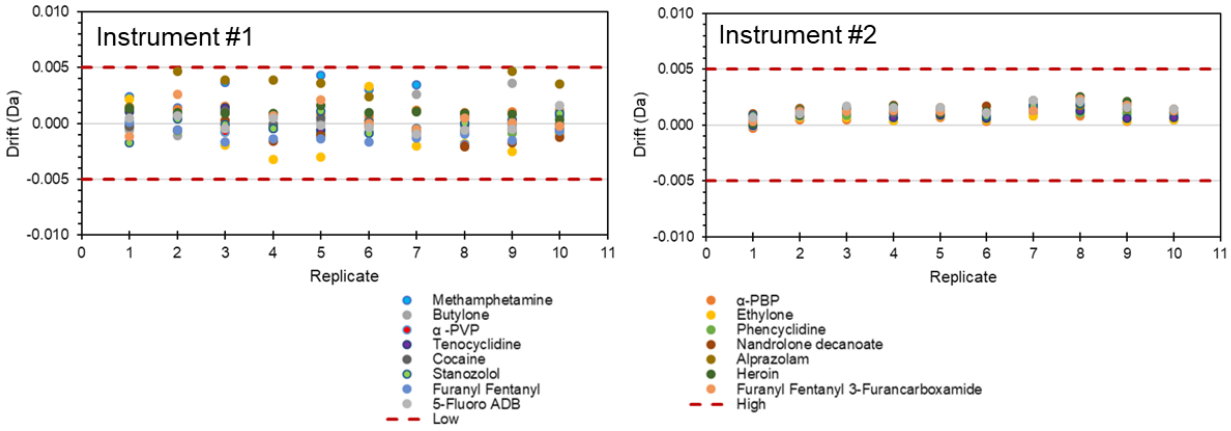
For Submission To: Forensic Chemistry



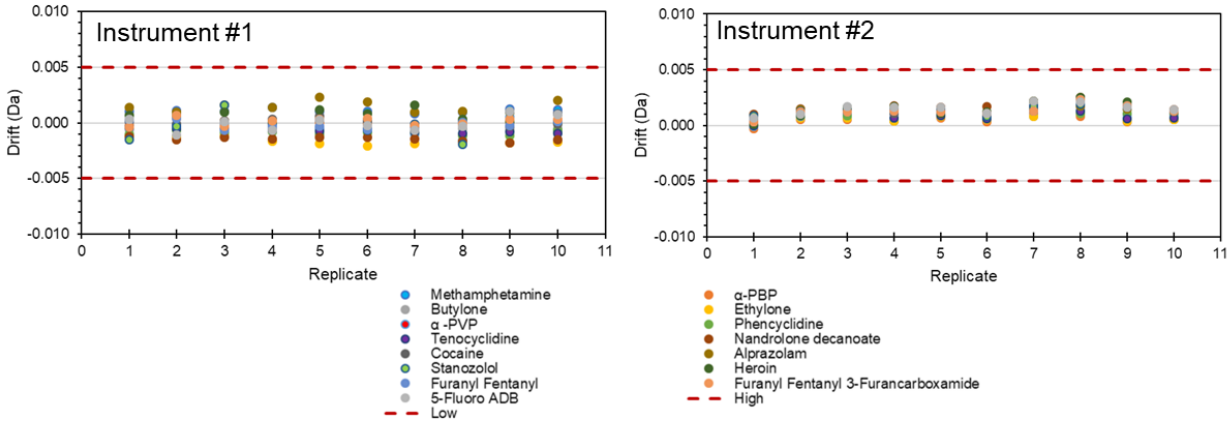
Supplemental Figure 1. Results from the negative mode accuracy and precision study (Study 1) for Instrument 1 (left) and Instrument 2 (right). The theoretical *m/z* values corresponding to this data can be found in Table 2 of the manuscript. The red dotted lines indicate the high and low bounds of the allowable drift from the theoretical *m/z* values.



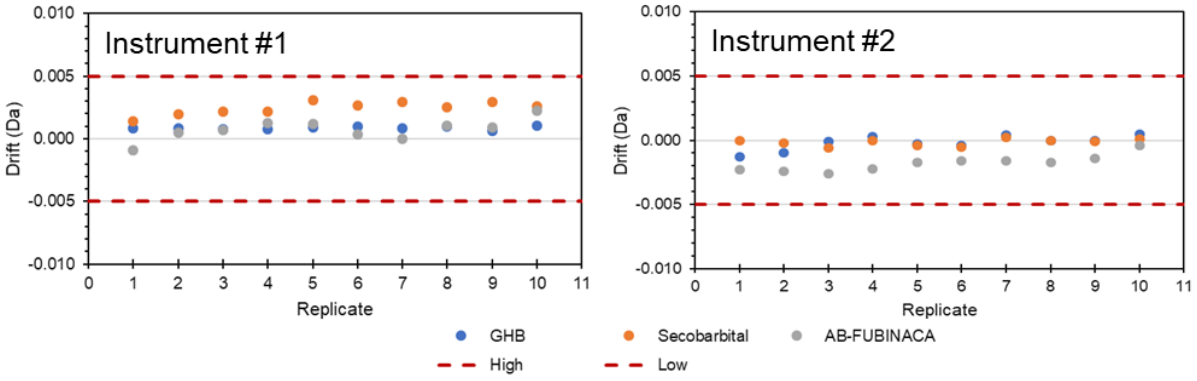
Supplemental Figure 2. Results from the +30V spectra for the single component accuracy study (Study 1) in positive mode for Instrument 1 (left) and Instrument 2 (right). The theoretical *m/z* values corresponding to this data can be found in Supplemental Table 1. The red dotted lines indicate the high and low bounds of the allowable drift from the theoretical *m/z* values.



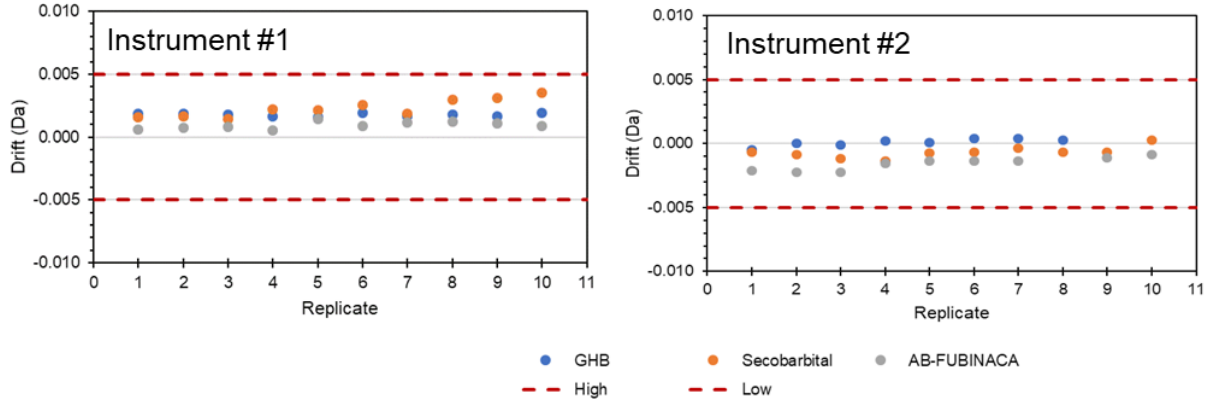
Supplemental Figure 3. Results from the +60V spectra for the single component accuracy study (Study 1) in positive mode for Instrument 1 (left) and Instrument 2 (right). The theoretical *m/z* values corresponding to this data can be found in Supplemental Table 1. The red dotted lines indicate the high and low bounds of the allowable drift from the theoretical *m/z* values.



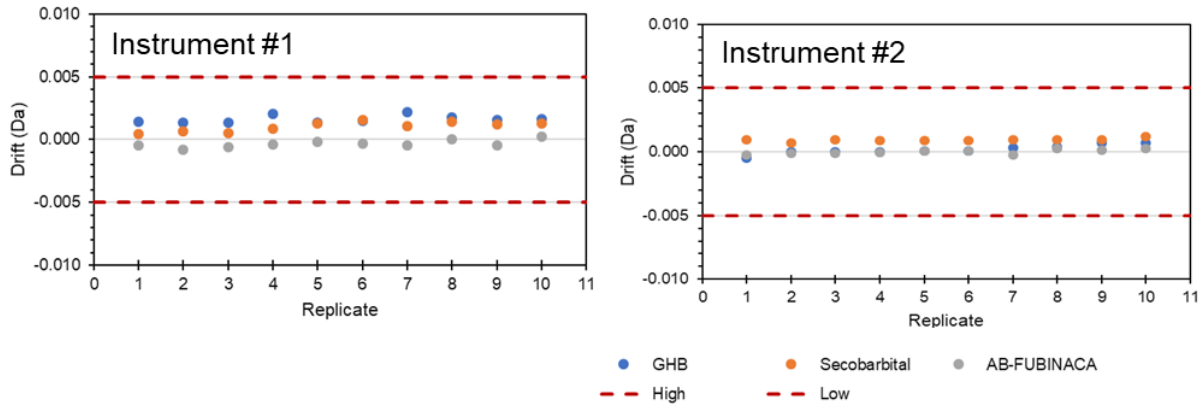
Supplemental Figure 4. Results from the +90V spectra for the single component accuracy study (Study 1) in positive mode for Instrument 1 (left) and Instrument 2 (right). The theoretical *m/z* values corresponding to this data can be found in Supplemental Table 1. The red dotted lines indicate the high and low bounds of the allowable drift from the theoretical *m/z* values.



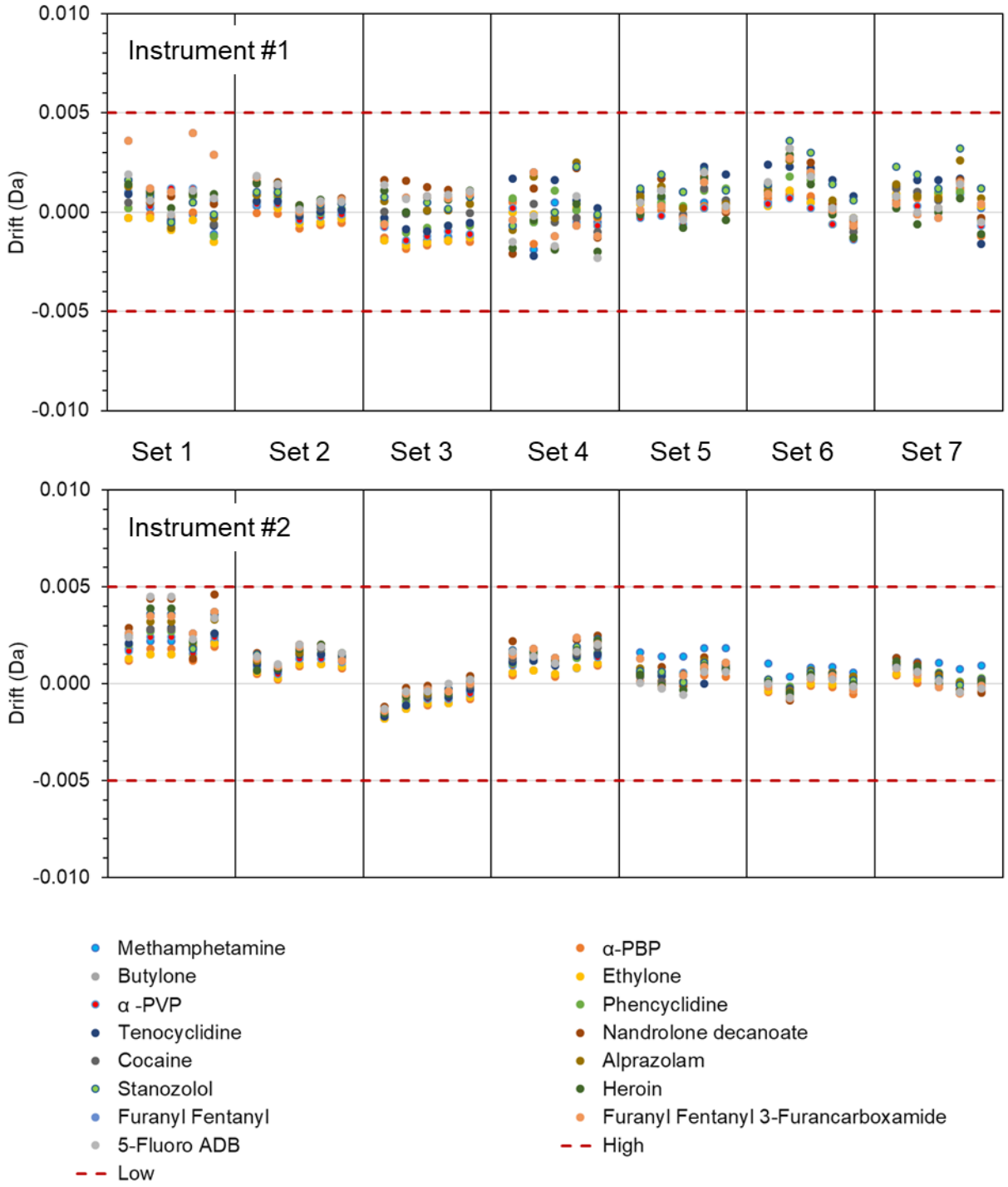
Supplemental Figure 5. Results from the -30V spectra for the single component accuracy study (Study 1) in negative mode for Instrument 1 (left) and Instrument 2 (right). The theoretical *m/z* values corresponding to this data can be found in Supplemental Table 2. The red dotted lines indicate the high and low bounds of the allowable drift from the theoretical *m/z* values.



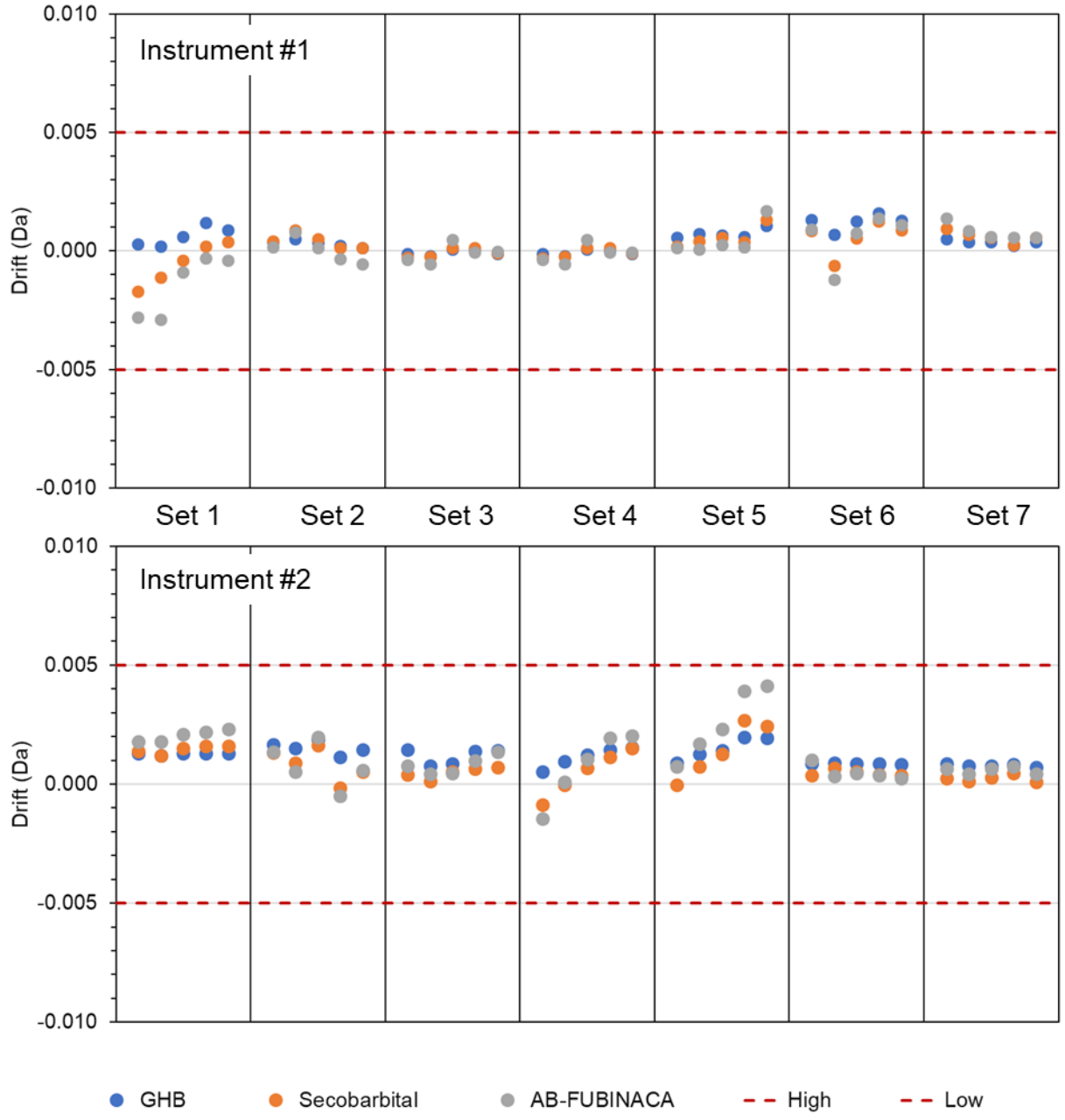
Supplemental Figure 6. Results from the -60V spectra for the single component accuracy study (Study 1) in negative mode for Instrument 1 (left) and Instrument 2 (right). The theoretical *m/z* values corresponding to this data can be found in Supplemental Table 2. The red dotted lines indicate the high and low bounds of the allowable drift from the theoretical *m/z* values.



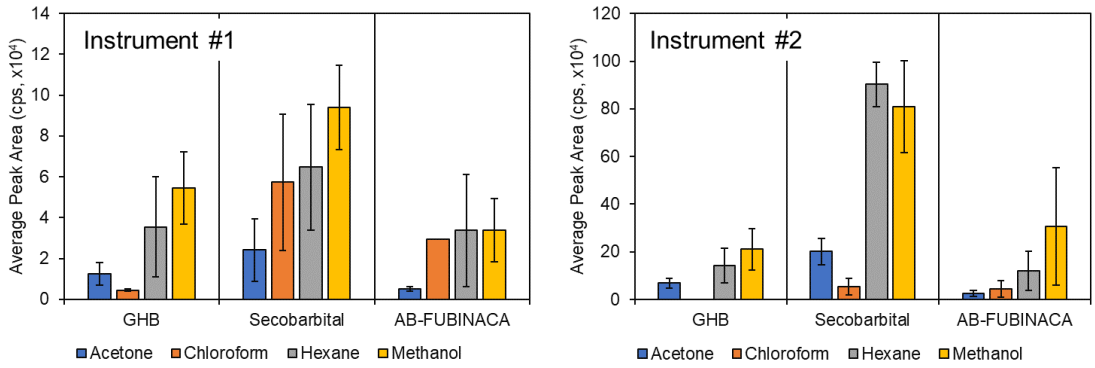
Supplemental Figure 7. Results from the -90V spectra for the single component accuracy study (Study 1) in negative mode for Instrument 1 (left) and Instrument 2 (right). The theoretical *m/z* values corresponding to this data can be found in Supplemental Table 2. The red dotted lines indicate the high and low bounds of the allowable drift from the theoretical *m/z* values.



Supplemental Figure 8. Results from the positive mode reproducibility study (Study 2) for Instrument 1 (top) and Instrument 2 (bottom). The red dotted lines indicate the high and low bounds of the allowable *m/z* drift from the theoretical *m/z* values.



Supplemental Figure 9. Results from the negative mode reproducibility study (Study 2) for Instrument 1 (top) and Instrument 2 (bottom). The red dotted lines indicate the high and low bounds of the allowable *m/z* drift from the theoretical *m/z* values.



Supplemental Figure 10. Average peak area as a function of solvent (colored bars) for all compounds analyzed in the negative mode study (Study 5) on Instrument 1 (left) and Instrument 2 (right). Error bars represent the standard deviation of triplicate measurements

Supplemental Table 1 List of theoretical *m/z* values used for +30, +60, +90 V positive mode accuracy and precision studies (Study 1).

	+30 V *m/z* Value	**+60 V *m/z* Value**	**+90 V *m/z* Value**
Methamphetamine	150.1277	91.0542	65.0386
α-PBP	218.1539	147.0804	91.0542
Butylone	222.1124	174.0913	105.0699
Ethylone	222.1124	174.0931	91.0542
α -PVP	232.1695	161.0961	91.0542
Phencyclidine	244.2059	159.1168	86.0964
Tenocyclidine	250.1624	166.1590	86.0964
Nandrolone decanoate	429.3363	257.1900	91.0542
Cocaine	304.1543	182.1176	119.0491
Alprazolam	309.0901	309.0901	281.0714
Stanozolol	329.2587	311.2482	311.2482
Heroin	370.1649	328.1543	268.1332
Furanyl Fentanyl	375.2067	188.1434	105.0699
Furanyl Fentanyl 3-Furancarboxamide	375.2067	188.1434	105.0699
5-Fluoro ADB	378.2187	318.1976	233.1085

Supplemental Table 2 List of theoretical *m/z* values used for -30, -60, -90 V negative mode accuracy and precision studies (Study 1).

	+30 V *m/z* Value	**+60 V *m/z* Value**	**+90 V *m/z* Value**
GHB	103.0390	85.0284	85.0284
Secobarbital	237.1234	197.0921	110.0964
AB-FUBINACA	367.1565	225.0822	141.0659

Supplemental Table 3. Summary results for the reproducibility studies (Study 2) for Instrument 2.

	Theoretical ***m/z***	**Minimum** ***m/z***	**Maximum** ***m/z***	**Average** ***m/z***	**Standard Deviation**	**Coefficient of Variation (%)**
Positive Ionization Mode						
Methamphetamine	150.1277	150.1262	150.1299	150.1287	0.0009	0.0006
α-PBP	218.1539	218.1521	218.1558	218.1541	0.0009	0.0004
Butylone	222.1124	222.1106	222.1145	222.1127	0.0009	0.0004
Ethylone	222.1124	222.1106	222.1145	222.1127	0.0009	0.0004
α -PVP	232.1695	232.1678	232.1719	232.1701	0.0010	0.0004
Phencyclidine	244.2059	244.2043	244.2086	244.2067	0.0010	0.0004
Tenocyclidine	250.1624	250.1607	250.1653	250.1631	0.0010	0.0004
Nandrolone decanoate	429.3363	429.3351	429.3409	429.3374	0.0014	0.0003
Cocaine	304.1543	304.1528	304.1577	304.1551	0.0011	0.0004
Alprazolam	309.0901	309.0887	309.0934	309.0911	0.0011	0.0004
Stanozolol	329.2587	329.2573	329.2623	329.2597	0.0012	0.0004
Heroin	370.1649	370.1634	370.1688	370.1658	0.0013	0.0004
Furanyl Fentanyl	375.2067	375.2053	375.2104	375.2076	0.0013	0.0003
Furanyl Fentanyl 3-Furancarboxamide	375.2067	375.2053	375.2104	375.2076	0.0013	0.0003
5-Fluoro ADB	378.2187	378.2174	378.2232	378.2196	0.0014	0.0004
Negative Ionization Mode						
AB-FUBINACA	103.0390	103.0395	103.0410	103.0402	0.0004	0.0004
GHB	237.1234	237.1225	237.1261	237.1241	0.0007	0.0003
Secobarbital	367.1565	367.1551	367.1606	367.1576	0.0011	0.0003

Supplemental Table 4. Average reverse search scores and lists of other compounds that produced reverse search scores higher than the compound of interest for the positive mode study (Study 3) on Instrument 2. The number in parentheses next to the compounds indicates how many times, out of the five replicate spectra, that compound returned a reverse search score greater than the compound of interest.

	Average Reverse Search Score				Other Compounds That Produced Scores Higher Than Compound			
	20 V	**30 V**	**60 V**	**90 V**	**20 V**	**30 V**	**60 V**	**90 V**
Methamphetamine	840	782	830	927	None	None	Amphetamine (5)	None
α-PBP	901	919	897	881	None	None	None	None
Butylone	899	848	941	966	None	Ethylone (5)	None	None
Ethylone	869	875	857	876	None	None	None	None
α -PVP	859	867	822	874	None	None	None	None
Phencyclidine	881	852	878	890	None	None	None	None
Tenocyclidine	897	892	967	785	None	None	None	None
Nandrolone decanoate	737	711	799	771	None	None	None	None
Cocaine	897	873	915	911	None	None	None	None
Alprazolam	916	918	945	894	None	None	None	None
Stanozolol	877	868	878	891	None	None	None	None
Heroin	767	698	824	857	None	None	None	None
Furanyl Fentanyl	862	845	876	879	3-Furanyl Fent (5)	3-Furanyl Fent (5)	None	None
Furanyl Fentanyl 3-Furancarboxamide	878	878	874	897	None	None	2-Furanyl Fentanyl (2)	2-Furanyl Fentanyl (1)
5-Fluoro ADB	947	975	945	894	None	None	None	None

Abbreviation: 3-Furanyl Fent is Furanyl Fentanyl 3-Furancarboxamide.

Supplemental Table 5. Average reverse search scores and lists of other compounds that produced reverse search scores higher than the compound of interest for the negative mode study (Study 3) on Instruments 1 and 2. The number in parentheses next to the compounds indicates how many times, out of the five replicate spectra, that compound returned a reverse search score greater than the compound of interest.

Instrument 1								
	Average Reverse Search Score				Other Compounds That Produced Scores Higher Than Compound			
	20 V	**30 V**	**60 V**	**90 V**	**20 V**	**30 V**	**60 V**	**90 V**
AB-FUBINACA	972	991	985	968	None	None	None	None
GHB	970	971	933	776	None	None	None	None
Secobarbital	938	956	939	906	None	None	None	None
Instrument 2								
	Average Reverse Search Score				Other Compounds That Produced Scores Higher Than Compound			
	20 V	**30 V**	**60 V**	**90 V**	**20 V**	**30 V**	**60 V**	**90 V**
AB-FUBINACA	925	928	939	936	None	None	None	None

GHB	854	941	970	819	None	None	None	None
Secobarbital	945	962	953	953	None	None	None	None

Supplemental Table 6. Average reverse search scores and lists of other compounds that produced reverse search scores higher than the compound of interest for the positive mode study (Study 3) in Instrument 2. The number in parentheses next to the compounds indicates how many times, out of the five replicate spectra, that compound returned a reverse search score greater than the compound of interest.

	Average Reverse Search Score				**Other Compounds That Produced Scores Higher Than Compound**			
	20 V	**30 V**	**60 V**	**90 V**	**20 V**	**30 V**	**60 V**	**90 V**
Set 1								
Methamphetamine	840	782	830	927	None	None	Amphetamine (5)	None
Phentermine	876	895	855	831	None	None	Benzylpiperazine (3)	None
Set 2								
Butylone	899	848	941	966	None	Ethylone (5)	None	None
Dimethylone	939	943	960	977	EDMC (1) MDPA (5)	Dimethylone (1) MDPA (2)	None	None
Ethylone	869	875	857	876	None	None	None	None
3,4-EDMC	867	895	715	939	Dimethylone (4) MDPA (5)	Dimethylone (1) MDPA (2)	None	None
3,4-MDPA	949	937	929	966	None	None	None	None
Set 3								
Cyclopropyl Fent.	827	806	847	879	Crotonyl (5) Methacryl (5)	Crotonyl (5) Methacryl (5)	None	None
Crotonyl Fent.	828	856	855	748	Methacryl (5)	Methacryl (5)	Cyclopropyl (1) Methacryl (5)	Cyclopropyl (5) Methacryl (5)
Methacryl Fent.	841	849	842	826	None	Crotonyl (1) Cyclopropyl (1)	Crotonyl (1)	Cyclopropyl (4)
Set 4								
m-FBF	844	833	837	890	m-FiBF (5) o-FiBF (4) p-FBF (3) p-FiBF (5)	m-FiBF (2) o-FiBF (4) p-FBF (4) p-FiBF (5)	m-FiBF (2) o-FiBF (5) p-FBF (5) p-FiBF (5)	o-FiBF (2) p-FBF (2) p-FiBF (5)
o-FBF	767	826	749	712	m-FiBF (2) p-FBF (2) p-FiBF (2)	m-FiBF (2) p-FiBF (3)	m-FBF (4) m-FiBF (4) o-FiBF (4) p-FBF (4) p-FiBF (4)	m-FBF (4) m-FiBF (4) o-FiBF (4) p-FBF (4) p-FiBF (4)
p-FBF	852	830	875	799	None	m-FBF (1) m-FiBF (1) o-FiBF (5) p-FiBF (5)	o-FiBF (5) p-FiBF (5)	m-FBF (5) o-FiBF (5) p-FBF 95) p-FiBF (5)
m-FiBF	858	848	858	879	m-FBF (1) m-FiBF (1) o-FiBF (3) p-FBF (3) p-FiBF (4)	m-FiBF (5) o-FiBF (5) p-FBF (4) p-FiBF (5)	o-FiBF (2) p-FBF (2) p-FiBF (2)	m-FBF (1) o-FiBF (5) p-FBF (5) p-FiBF (5)
o-FiBF	890	871	887	929	m-FiBF (1) p-FBF (2) p-FiBF (5)	m-FiBF (2) p-FBF (1) p-FiBF (5)	p-FiBF (1)	p-FiBF (5)
p-FiBF	909	889	890	967	None	None	None	None

Set 5								
6-APDB	949	926	940	984	5-APDB (1) Ethcathinone (1) Mephedrone (1)	None	5-APDB (3)	None
5-APDB	936	890	934	989	None	None	None	None
Buphedrone	948	921	972	952	Ethcathinone (3) Mephedrone (2)	Dimethylcath. (4)	None	None
Dimethylcathinone	945	914	970	970	Ethcathinone (2) Mephedrone (1)	Ethcathinone (4) Mephedrone (3)	Ethcathinone (2) Mephedrone (1)	None
Ethcathinone	928	895	903	980	Buphedrone (4) Mephedrone (3)	Buphedrone (4) Dimethylcath. (4) Mephedrone (4) 2-MMC (2)	None	None
Mephedrone	926	872	951	967	Buphedrone (3) Dimethylcath. (2) Ethcathinone (3) 2-MMC (1)	Buphedrone (2) Dimethylcath (5) Ethcathinone (1) 2-MMC (1)	2-MMC (3)	2-MMC (3)
2-MMC	903	845	944	953	Buphedrone (5) Dimethylcath. (3) Ethcathinone (2) Mephedrone (5)	Buphedrone (5) Dimethylcath. (4) Ethcathinone (2) Mephedrone (4)	Mephedrone (1)	None
MMAI	920	886	936	978	Dimethylcath. (1) Ethcathinone (2)	Dimethylcath. (4)	None	None

Supplemental Table 7. Average reverse search scores and lists of other compounds that produced reverse search scores higher than the compound of interest for the negative mode study (Study 3). The number in parentheses next to the compounds indicates how many times, out of the five replicate spectra, that compound returned a reverse search score greater than the compound of interest.

Instrument 1								
	Average Reverse Search Score				**Other Compounds That Produced Scores Higher Than Compound**			
	20 V	**30 V**	**60 V**	**90 V**	**20 V**	**30 V**	**60 V**	**90 V**
AB-FUBINACA	972	991	985	968	None	None	None	None
AB-7-FUBAICA	979	935	985	980	AB-FUB. 2'-indazole (2)	AB-FUBINACA (5) AB-FUB. 2'-indazole (4) AB-FUB. 2-fluorobenzyl (2)	None	None
AB-FUBINACA 2'-indazole isomer	992	987	967	987	None	None	None	None
AB-FUBINACA isomer 1	926	919	965	969	AB-FUBINACA (5) AB-FUB. 2'-indazole (5)	AB-FUBINACA (5) AB-FUB. 2'-indazole (5)	None	None
AB-FUBINACA 2-fluorobenzyl isomer	917	896	989	989	AB-FUBINACA (4) AB-FUB. 2'-indazole (5) AB-FUB. isomer 1 (2)	AB-FUBINACA (5) AB-FUB. 2'-indazole (5) AB-FUB. isomer 1 (4)	None	None
Instrument 2								
	Average Reverse Search Score				**Other Compounds That Produced Scores Higher Than Compound**			
	20 V	**30 V**	**60 V**	**90 V**	**20 V**	**30 V**	**60 V**	**90 V**
AB-FUBINACA	925	928	939	936	None	None	None	None
AB-7-FUBAICA	942	927	969	920	AB-FUB. 2'-indazole (5) AB-FUBINACA (1) AB-FUB. isomer 1 (1)	AB-FUB. 2'-indazole (4) AB-FUBINACA (3) AB-FUB. isomer 1 (3)	None	None
AB-FUBINACA 2'-indazole isomer	973	978	959	983	None	AB-7-FUBINACA (1)	None	None
AB-FUBINACA isomer 1	954	943	961	966	AB-FUB. 2'-indazole (5) AB-FUBINACA (5) AB-FUB. 2-fluorobenzyl (3)	AB-FUB. 2'-indazole (5) AB-FUBINACA (3) AB-7-FUBINACA (5)	None	None
AB-FUBINACA 2-fluorobenzyl isomer	914	866	956	957	AB-FUB. 2'-indazole (5) AB-FUBINACA (5) AB-FUB. isomer 1 (5) AB-7-FUBINACA (5)	AB-FUB. 2'-indazole (5) AB-FUBINACA (5) AB-FUB. isomer 1 (5) AB-7-FUBINACA (5)	None	None

Abbreviations: "AB-FUB. 2'-indazole" is AB-FUBINACA 2'-indazole isomer. "AB-FUB. isomer 1" is AB-FUBINACA isomer 1. "AB-FUB. 2-fluorobenzyl" is AB-FUBINACA 2-fluorobenzyl isomer.

Supplemental Table 8. Summary results for the positive mode reproducibility studies (Study 7) for the second examiner for Instrument 1.

	Theoretical *m/z*	**Minimum *m/z***	**Maximum *m/z***	**Average *m/z***	**Standard Deviation**	**Coefficient of Variation (%)**
Methamphetamine	150.1277	150.12589	150.1293	150.1279	0.0006	0.0004
α-PBP	218.1539	218.15181	218.155	218.1540	0.0007	0.0003
Butylone	222.1124	222.10951	222.11467	222.1125	0.0008	0.0004
Ethylone	222.1124	222.10951	222.11467	222.1125	0.0008	0.0004
α -PVP	232.1695	232.16827	232.1709	232.1696	0.0007	0.0003
Phencyclidine	244.2059	244.20479	244.20934	244.2068	0.0009	0.0004
Tenocyclidine	250.1624	250.16205	250.1654	250.1637	0.0008	0.0003
Nandrolone decanoate	429.3363	429.3319	429.34076	429.3364	0.0015	0.0004
Cocaine	304.1543	304.151	304.15845	304.1547	0.0012	0.0004
Alprazolam	309.0901	309.08804	309.0925	309.0904	0.0010	0.0003
Stanozolol	329.2587	329.25568	329.26178	329.2593	0.0012	0.0004
Heroin	370.1649	370.16251	370.16959	370.1649	0.0012	0.0003
Furanyl Fentanyl	375.2067	375.20428	375.2115	375.2071	0.0014	0.0004
Furanyl Fentanyl 3-Furancarboxamide	375.2067	375.20428	375.2115	375.2071	0.0014	0.0004
5-Fluoro ADB	378.2187	378.2149	378.22354	378.219	0.0014	0.0004

Supplemental Table 9. Summary results for the positive mode reproducibility studies (Study 7) for the second examiner for Instrument 2.

	Theoretical *m/z*	**Minimum *m/z***	**Maximum *m/z***	**Average *m/z***	**Standard Deviation**	**Coefficient of Variation (%)**
Methamphetamine	150.1277	150.1264	150.1306	150.1287	0.0010	0.0007
α-PBP	218.1539	218.1511	218.1567	218.1540	0.0014	0.0006
Butylone	222.1124	222.1097	222.1153	222.1126	0.0014	0.0006
Ethylone	222.1124	222.1097	222.1153	222.1126	0.0014	0.0006
α -PVP	232.1695	232.1668	232.1728	232.1700	0.0015	0.0006
Phencyclidine	244.2059	244.2034	244.2093	244.2065	0.0015	0.0006
Tenocyclidine	250.1624	250.1596	250.1658	250.1629	0.0016	0.0006
Nandrolone decanoate	429.3363	429.3326	429.3412	429.3370	0.0024	0.0006
Cocaine	304.1543	304.1516	304.1582	304.1549	0.0018	0.0006
Alprazolam	309.0901	309.0875	309.0944	309.0908	0.0019	0.0006
Stanozolol	329.2587	329.256	329.2629	329.2594	0.0020	0.0006
Heroin	370.1649	370.1617	370.1692	370.1655	0.0021	0.0006
Furanyl Fentanyl	375.2067	375.2036	375.2110	375.2072	0.0021	0.0006
Furanyl Fentanyl 3-Furancarboxamide	375.2067	375.2036	375.2110	375.2072	0.0021	0.0006
5-Fluoro ADB	378.2187	378.2154	378.2232	378.2192	0.0022	0.0006

Supplemental Table 10. Summary results for the negative mode reproducibility studies (Study 7) for the second examiner for both instruments.

	Theoretical *m/z*	**Minimum *m/z***	**Maximum *m/z***	**Average *m/z***	**Standard Deviation**	**Coefficient of Variation (%)**
Instrument 1						
AB-FUBINACA	103.0390	103.0387	103.0406	103.0397	0.0006	0.0006
GHB	237.1234	237.1227	237.1266	237.1248	0.0011	0.0005
Secobarbital	367.1565	367.1555	367.1610	367.1584	0.0017	0.0005
Instrument 2						
AB-FUBINACA	103.0390	103.0397	103.0412	103.0402	0.0003	0.0003
GHB	237.1234	237.1235	237.1266	237.1245	0.0007	0.0003
Secobarbital	367.1565	367.1567	367.1614	367.1582	0.0011	0.0003