Evaluation of an Internal Standard for Qualitative DART-MS Analysis of Seized Drugs

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Highlights

- Implementation of tetracaine as an internal standard for qualitative DART-MS analysis of seized drugs was completed.
- The internal standard allowed for tunable DART-MS sensitivity when processing data using peak intensity thresholds.
- Use of an internal standard eliminated false identifications in negative spectra caused by identification of noise peaks.
- Competitive ionization was observed with some drugs but did not completely suppress signal.

Abstract

Rapid and accurate screening tools for seized drug analysis continue to be needed due to the complexities associated with the emerging drug landscape. Direct analysis in real-time mass spectrometry (DART-MS) is one technique that has been used for this purpose and is seeing increased implementation due to its ability to provide high-fidelity information rapidly. As with any analytical technique, ensuring data integrity with DART-MS results is critical. To further enhance data integrity and eliminate some of the challenges associated with qualitative analysis of seized drugs using DART-MS, the incorporation of an internal standard was investigated. After evaluating a number of candidate compounds, tetracaine was chosen because of its desirable characteristics. An appropriate concentration of tetracaine was established that provided similar sensitivity to GC-MS for a panel of drugs. The presence of tetracaine in a drug extract was found to cause a reduction in signal for some common drugs due to competitive ionization but did not cause complete suppression of signal at relevant concentrations. Evaluation of a set of 60 representative case samples with and without internal standard found that the presence of internal standard did not negatively impact the results and that its presence eliminated the false identification of noise peaks in negative samples. The use of an internal standard also provided within-sample mass calibration and analyte concentration checks. It also allowed for automated mass drift compensation, removing a time-consuming process for high resolution DART-MS data processing.

Keywords: DART-MS; Seized Drug; Internal Standard; Qualitative Analysis; Competitive Ionization

Introduction

Direct analysis in real time mass spectrometry (DART-MS) is one of several screening techniques that is seeing increased prevalence in seized drug analysis due its speed, simple sample preparation, and ability to provide accurate, high-fidelity information. Since the invention of the technique, the ability to analyze controlled substances has been shown[1]. Of the forensic applications where DART-MS has been demonstrated, seized drugs has the prevalent footprint[2]. The large body of research highlights a range of applications from traditional qualitative seized drug analysis[3] to quantitative analysis of psychoactive substances in plant materials[4] to identification of emerging drugs[5] to analysis of drug residues for predictive screening[6].

As with any technique, maintaining data integrity is of utmost importance. For DART-MS analysis, especially when equipped with high-resolution mass spectrometers, multiple-layers of data integrity are employed. These include calibration of the mass spectrometer, use of a positive control to demonstrate mass accuracy or fragmentation consistency, and use of a negative control to demonstrate lack of carryover or contamination. An additional layer of data integrity that has not been adopted in qualitative analysis is the use of an internal standard. Internal standards are commonplace in quantitative analysis[2,4,5] and have been validated for qualitative gas chromatography mass spectrometry (GC-MS) analysis of drugs[7]. The use of an internal standard as an additional layer of data integrity in qualitative analysis has several appeals: inclusion of an internal standard provides in-spectrum mass calibration verification for samples, demonstrates that the instrument is working when negative results are obtained, and provides an estimate of analyte concentration. Also, given that DART-MS data is typically processed by looking for peaks above a pre-defined relative intensity in the mass spectrum, the use of an internal can minimize the false identification of noise peaks – which occurs regularly in spectra where a peak significantly above background does not exist. There are potential pitfalls to inclusion of an internal standard given the lack of chromatography and possibility of competitive ionization. Prior research has shown that the signal of low proton affinity compounds can be degraded or even eliminated in mixtures[8,9].

In this work, the use of an internal standard for qualitative analysis of seized drugs was investigated. Through a series of studies, the utility and potential challenges of including an internal standard in the sample analysis routine was identified. These studies encompassed identifying the internal standard compound, tuning its concentration, and measuring competitive ionization against a suite of commonly seen drugs. To evaluate real-world use of the internal standard, a suite of 60 case extracts from mock or adjudicated samples with and without internal standard were analyzed. Ultimately, it was found that the benefits of incorporating an internal standard into qualitative analysis outweighed the potential challenges and provided an increased level of data integrity.

Materials and Methods

For this work a JEOL AccuTOF 4G LC-Plus mass spectrometer (JEOL USA, Peabody, MA, USA) coupled with a DART-SVP ion source (IonSense, Saugus, MA, USA) was used. Relevant DART settings included operation in

positive ionization mode with helium as the source gas at a temperature of 400 °C with an exit grid voltage of +150 V. Mass spectrometer parameters included operation in positive ionization mode with an orifice 1 voltage of +30 V, a ring lens and orifice 2 voltage of +5 V, and an ion guide voltage of +800 V. Mass spectra were collected from m/z 80 to m/z 800 at a rate of 2 scans s^{-1} . For some of the casework samples an additional run was required to obtain higher fragmentation spectra to assist in compound differentiation. This method was identical except that it used a +60 V orifice 1 voltage. Calibration of the mass spectrometer was completed using polyethylene glycol (PEG-600) on a weekly basis. For each run, a multi-point mass drift compensation corresponding to the protonated molecule of tetracaine (m/z 265.1916) was completed. Sampling was completed using glass microcapillaries that were either dipped into the solution or, for the sensitivity and competitive ionization studies, had solution pipetted onto them.

Data was collected using one-minute analysis runs set up in the sequence table of msAxel (JEOL USA). A post-processing method was implemented that automatically applied the multi-point mass drift compensation and automatically centroided the data. In each one-minute analysis, the tetracaine internal standard solution was analyzed once followed by three samplings (for casework analysis) or five samplings (for sensitivity and competitive ionization studies) of the solution. An example of the resulting chronogram is shown in Figure 1. An identical approach was used for the samples analyzed without internal standard except a single-point mass drift compensation was used in the post-processing. For the casework evaluation portion of this study, spectra were extracted and analyzed using Mass Mountaineer (Diablo Analytical, Antioch, CA, USA). A single composite spectrum from the three samplings was extracted and searched against an in-house search list with a ± 5 mmu (± 0.005 Da) tolerance and a 2.5 % or 5 % relative abundance threshold. For the competitive ionization studies, the integrated peak areas were obtained from extracted ion chronograms of the protonated molecules of the drugs.

Tetracaine was used as the internal standard compound and was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanolic solutions of alprazolam, cocaine, fentanyl, heroin, methamphetamine, and MPP-PICA were purchased from Cayman Chemical (Ann Arbor, MI, USA) and used as is or diluted, volumetrically, in methanol. Samples for the casework study were either adjudicated samples or training samples. All samples were also analyzed by gas chromatography mass spectrometry (GC-MS) to confirm the identification of controlled substances.

Results & Discussion

Identifying an Internal Standard Compound

Identification of an appropriate internal standard was completed by evaluating potential compounds that met several qualifiers given the current DART-MS workflow used at the laboratory. The workflow uses only positive ionization mode and uses methanolic extracts for sample analysis, therefore the internal standard needed to easily produce a positive ion and be readily soluble in methanol. Since the internal standard was to be used as a gauge for the approximate concentration of samples (to assist analysts in identifying an appropriate amount of material to be used for creating samples for GC-MS) the compound could not be a commonly observed diluent. Another purpose of the

internal standard is to act as a mass calibration check and therefore a compound that had a protonated molecule within the typical m/z range of most drugs (approximately m/z 150 to m/z 450) was needed. The compound could not have a molecular formula that was identical to any compound of interest or, given the mass accuracy of the mass spectrometer in ± 5 mDa, could not be within ± 10 mDa of any compound of interest. The compound could not heavily fragment when using low orifice voltages and form predominantly a protonated molecule. It needed to be stable in methanol over a prolonged time (at least one month), not be prone to carryover, and be cost-effective.

Initial efforts looked at a number of mass check, or mass drift compensation, compounds used in literature to see if they could be potential candidates for an internal standard. Reserpine ($C_{33}H_{40}N_2O_9$) has been reported in a number of papers as a mass check compound[10,11], but its high molecular mass (608 Da) made it undesirable for this application. Sugar alcohols, such as xylitol, mannitol, and sorbitol, have also been analyzed[12] and were appealing because of their capability to produce strong positive and negative ions. However, they are poorly soluble in methanol and would require a water-methanol mixture to reach the desired concentrations. These compounds are also common diluents in casework, which could affect data processing approaches. Other compounds such as caffeine, acetaminophen, and quinine also fell into this category. Many quantitative methods leverage the use of deuterated or carbon-labeled internal standards[4,13,14], but these types of materials would be cost prohibitive for high-throughput qualitative drug screening by DART-MS. Tetracosane is a commonly used internal standard for GC-MS analysis[7], but is not easily ionized by DART-MS.

Figure 1. Representative chromatogram (A.) and mass spectra (B. and C.) for data collection using the sequence-based approach for Case 18 (Table 2). Example chromatogram (A.) shows the single analysis of the tetracaine solution (used as a positive control) as well as the triplicate analysis of the sample. Extracted mass spectra of the tetracaine solution (B.) and sample (C.) are also shown with peak labeling associated with the assignments in the bottom left quadrant. The dotted lines in (B.) and (C.) represent the 5 % (red) and 2.5 % (blue) relative intensity thresholds.

After investigation and analysis, it was found that tetracaine was a compound that met the needs describe above. The compound had an acceptable molecular weight ($C_{15}H_{24}N_2O_2$, MW = 264.1878) and produced, predominantly, an intact molecular ion (as shown in Figure 1). Searching the NIST DART-MS Forensics Database[15,16] and the SWGDRUG Monographs[17] revealed two potential compounds of interest at the same nominal mass as tetracaine – etaqualaone ($C_{17}H_{16}N_2O$) and Udes03 ($C_{15}H_{21}FN_2O$) which is also known as 4-fluoro U-47931E. The accurate m/z values for the protonated molecules of both compounds (m/z 265.1335 and m/z 265.1711 respectively) were sufficiently different from that of tetracaine (m/z 265.1910) that misidentification was not a concern. Analysis of tetracaine did produce a small fragment ion at m/z 176.1075 (corresponding to $[C_{11}H_{13}NO]^+$) in addition to the protonated molecule, but it was not found to cause unwanted false identifications of compounds of interest. Using tetracaine was found to be cost-effective and it easily dissolved in methanol. Stability and carryover, which are discussed below, were also found to be acceptable.

Establishing an Appropriate Internal Standard Concentration

Once chosen, an appropriate concentration of tetracaine was established. For qualitative analysis of seized drugs, GC-MS is the most commonly used instrument for confirmation and, therefore, it is critical that screening tools such as DART-MS be at least as sensitive. Traditionally DART-MS data is analyzed by looking for agreement between peaks in a spectrum above a pre-selected threshold (commonly between 1 % and 10 % relative intensity to the base peak) and a list of m/z values corresponding to known compounds. Using an internal standard, therefore, guarantees a peak with a roughly known intensity will be present in every spectrum and therefore can be used to “tune” the sensitivity of the result by changing its concentration. Given this, an appropriate internal standard concentration was defined as one that provides detection of compounds of interest above the threshold level that is used by the laboratory at a level equal to or lower than the reporting level of the GC-MS systems used for confirmation.

Prior to establishing the internal standard concentration, the limit of detection and reporting limit for three drugs (cocaine, fentanyl, and methamphetamine) was established on eight of the GC-MS systems used for casework at the Maryland State Police Forensic Sciences Division (MSP-FSD). Details on how these values were obtained are provided in Supplemental Information. At MSP-FSD a reportable peak is defined as a peak present in a chromatogram with an abundance of at least 200,000 counts. Across the eight instruments, the average concentration required to produce a reportable peak for cocaine, fentanyl, and methamphetamine (Table 1) ranged from 66 $\mu\text{g/mL}$ to 128 $\mu\text{g/mL}$. Given differences in instrument age and sensitivity, the reporting limit varied across GC-MS systems, with a minimum concentration of 10 $\mu\text{g/mL}$ which roughly corresponded to the average limit of detection for the drugs examined.

Table 1. Limits of detection and estimated reporting limits for eight of the GC-MS systems used for casework.

Drug	Limit of Detection Range ($\mu\text{g/mL}$)	Average Limit of Detection ($\mu\text{g/mL}$)	Reporting Limit Range ($\mu\text{g/mL}$)	Average Reporting Limit ($\mu\text{g/mL}$)
Cocaine	0.5 to 30.1	10.4	10.4 to 109.8	66.0
Fentanyl	0.7 to 40.6	14.9	14.3 to 197.0	123.0
Methamphetamine	4.9 to 48.2	9.0	17.0 to 198.4	128.0

Given a minimum drug concentration of 10 $\mu\text{g/mL}$ was needed to obtain a reportable peak, a series of experiments were completed to establish the concentration of tetracaine necessary to allow for drugs of interest at 10 $\mu\text{g/mL}$ to be reliably detected by DART-MS and to produce peaks above a given relative intensity threshold. Initial studies showed that a tetracaine concentration of approximately 100 $\mu\text{g/mL}$ (0.1 mg/mL) was required to achieve the desired detection when using a 2.5 % relative abundance threshold. This was verified by analyzing a panel of six drugs (alprazolam, cocaine, fentanyl, heroin, methamphetamine, and MPP-PICA) individually at three concentrations (1 mg/mL, 100 $\mu\text{g/mL}$, and 10 $\mu\text{g/mL}$) each containing tetracaine at 100 $\mu\text{g/mL}$. The relative intensity of the protonated molecule peak for each drug was obtained and the cumulative results are shown in Figure 2. Using a tetracaine concentration of 100 $\mu\text{g/mL}$, detection of all six drugs at 10 $\mu\text{g/mL}$ was achieved. At this concentration, peaks corresponding to cocaine (Figure 2, blue) and fentanyl (Figure 2, yellow) were present above 5 % relative intensity while the remaining four drugs produced peaks with relative intensities between 2.5 % and 5 %. Given these results, a tetracaine concentration of 100 $\mu\text{g/mL}$ was chosen.

Figure 2. Peak intensity, relative to tetracaine, for six drugs. Three commonly used thresholds (1 %, 2.5 %, and 5 %) are also provided. Uncertainties represent the standard deviation of five replicates. Points above 100 % show that the drug peak was more intense than the tetracaine peak.

Understanding Competitive Ionization Effects

With chromatography-free mass spectrometry techniques such as DART-MS, competitive ionization is a concern. Because of the high frequency in which multi-component mixtures are observed in seized drug analysis, the effect of including tetracaine was examined by measuring the degree of competitive ionization across a range of concentrations for the six drugs previously examined. Individual drugs were analyzed at three concentrations (10 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, and 1000 $\mu\text{g/mL}$) in solutions of both pure methanol and methanol containing tetracaine (100 $\mu\text{g/mL}$). Integrated peak areas of the protonated molecules for each drug were then obtained and the ratios of peak areas for runs containing the internal standard to those without were plotted. The results of this experiment are shown in Figure 3 where points below a ratio of 1 indicate that the presence of internal standard caused reduction in the drug signal due to competitive ionization while a ratio above 1 indicated the presence of internal standard had no effect or an advantageous effect on the ionization of the drug. An alternative representation of this data can be found in Supplemental Figure 1.

At low concentrations, competitive ionization was readily observed for four (alprazolam, heroin, methamphetamine, and MPP-PICA) of the six drugs. Signal suppression was greatest for methamphetamine which demonstrated a ~90 % reduction in peak area at 10 $\mu\text{g/mL}$ when tetracaine was present. A similar level of suppression was observed at a concentration of 100 $\mu\text{g/mL}$. For alprazolam, heroin, and MPP-PICA signal reduction of 50 % to 70 % was observed at 10 $\mu\text{g/mL}$ and a reduction between 40 % and 60 % was observed at 100 $\mu\text{g/mL}$. It is important to note that while competitive ionization did occur, detection of all compounds was possible at all levels and the respective peaks were consistently present at or above the 2.5 % relative intensity threshold.

For cocaine and fentanyl, competitive ionization was not observed at any concentration studied, likely due to the high proton affinity of these two compounds. For all drugs except methamphetamine, competitive ionization was not observed when the drug was in excess relative to tetracaine (1000 $\mu\text{g/mL}$ datapoints). Methamphetamine exhibited a minor reduction in signal at this concentration.

Figure 3. Ratio of the integrated peak area from the protonated molecule of the drug with and without the presence of the internal standard, as a function of concentration. A ratio of 1 (red dotted line) indicates no competitive ionization. Points below a ratio of 1 indicate competitive ionization occurred in the presence of tetracaine while points above a ratio of 1 indicate an increase in drug signal due to the presence of tetracaine. Uncertainties represent the standard deviation of five replicates.

Internal Standard Stability

Stability of the internal standard over time was evaluated over a two-month period by (1) investigating if the peak intensity of tetracaine dropped over time and (2) monitoring the full scan mass spectrum for the presence of degradation products. The intensity of the tetracaine protonated molecule remained consistent (some variation of signal was observed due to the sample introduction procedure). The presence of the fragment ion at nominal m/z 176 was observed in the freshly made solution and was not believed to be a degradation product. Over the two-month period the peak did not increase in intensity. A separate tetracaine solution that was aged over 3 months did show the presence of a suspected degradation peak at m/z 208.1332 ($[C_{12}H_{18}NO_2]^+$). Given these results, it was determined that the tetracaine solution is stable for short-term use but should be replaced on a monthly or bi-monthly basis.

Analysis of Case Samples

The final component of the evaluation involved the analysis of extracts from 60 case samples. Extracts were prepared by dissolving a 1 mg to 2 mg aliquot of powder into methanol with the tetracaine internal standard (100 µg/mL) and a separate aliquot into methanol without internal standard. Both extracts were analyzed using identical instrument parameters which were outlined above. The spectra of the tetracaine solution without sample (used as a positive control) and the composite sample spectra were extracted and searched using MassMountaineer. Both a 2.5 % and 5 % threshold were used to identify if there was a benefit to using one threshold over the other.

The summary results are shown in Table 2 along with the controlled substances that were identified by GC-MS. All tetracaine solution without sample spectra (positive control spectra) (120 in total) produced results only for the tetracaine molecular ion (m/z 265.1916) and its minor fragment ion (m/z 176.1074). The sample spectra from extracts with and without internal standard were largely identical. Slight differences between the GC-MS results and one or both the DART-MS results were observed for eight of the sixty samples. Of the extracts containing internal standard, there was one case (Case 42) where identification of all drugs in the mixture was not achieved. Noscapine, which is poorly ionized by DART, and cocaine, which was present at an insufficient amount for reporting by GC-MS, were not identified by DART. The remaining four controlled substances in that mixture were readily identified. For the casework extracts, the relative intensities of the peaks corresponding to controlled substances were above 5 % relative intensity to the base peak for all but three compounds that were present at a low concentration in the sample. These were two instances of heroin (Cases 3 and Case 14) and one instance of ketamine (Case 60). All three of these peaks were, however, present above 2.5 % relative intensity. Importantly, there were no false-positive identifications of controlled substances in any of the samples, including the eight cases that were negative.

For the extracts created in methanol without internal standard, there were no instances where identification of a controlled substance was missed. In four instances (Case 33, Case 40, and Case 42 – two substances) controlled substances in the sample were identified but were between 2.5 % and 5 % relative intensity to the base peak. Unlike the extracts containing internal standard there were ten instances where a hit for a controlled substance was obtained but was not identified by GC-MS (false-positive) (Case 1 – three instances, Case 7, Case 16 – two instances, Case 29, Case 36, Case 40, and Case 41). For two of these (Cases 36 and 40), identification of a false positive occurred in the presence of actual controlled substances, and in one of these instances the peak was below 5 % relative intensity to the base peak. The remaining four cases contained no controlled substances and the hit occurred because noise peaks were present above the 2.5 % or 5 % threshold that fell within the m/z tolerance on an entry in the compound search list used in MassMountaineer. While it is simple to explain the presence of these false-positive peaks, and conformational analysis will further demonstrate that they are not attributable to actual analytes, the use of an internal standard provides a peak with sufficient intensity that noise peaks are not above the threshold in the searching process.

Overconcentration is another concern when analyzing samples by DART-MS, especially high-resolution mass spectrometers, as it can lead to drifts in m/z values that can push peaks outside of the tolerance window. The presence of the internal standard in the mass spectra provides an indication of whether the sample is overly concentrated. In this work it was observed that when the internal standard peak was not the base peak in the spectrum and fell below 5 % relative abundance, the dominant peak(s) in the spectra were outside the m/z tolerance window. While the dominant peak(s) were outside of the tolerance window, the tetracaine peak was within the window, indicating there

was not a mass calibration issue. Dilution of the sample in methanol containing tetracaine brought the m/z value(s) of the dominant peak(s) into tolerance.

Table 2. Results of analysis of 60 case samples by DART-MS using extracts that contained the tetracaine internal standard (left column) and did not contain the tetracaine internal standard (center column) using a 2.5 % and 5 % threshold for searching. The controlled substances identified using GC-MS are provided in the right column. Excipient results are listed for the DART-MS analyses, but were not used in comparing to the GC-MS results.

#	Results for DART-MS Analysis with Tetracaine Internal Standard	Results for DART-MS Analysis without Tetracaine Internal Standard	GC-MS Results
1	No Compounds Detected	Androsterone [†] , Methoxy-PCP [†] , MMB-FUBINACA [†] Excipients: Levamisole	No Controlled Substances
2	Methamphetamine	Methamphetamine	Methamphetamine
3	Heroin* MDMA Excipients: Caffeine, Mannitol*, Quinine	Heroin MDMA Excipients: Caffeine, Mannitol, Quinine	Heroin MDMA
4	Fentanyl Tramadol Excipients: Levamisole, Mannitol, Phenylpropanamide, Pindolol, Procaine	Fentanyl Tramadol Excipients: Levamisole, Mannitol, Pindolol, Procaine	Fentanyl Tramadol ^{INS}
5	4-methyl- α -PHP Excipients: Dextrophan	4-methyl- α -PHP	4-methyl- α -PHP
6	MDMA	MDMA	MDMA
7	No Controlled Substances Excipients: Mannitol	GBL [†] Excipients: Mannitol	No Controlled Substances
8	Heroin 6-Monoacetylmorphine ^A	Heroin	Heroin
9	methyl Norfentanyl	methyl Norfentanyl	methyl Norfentanyl
10	4-Ethylmethcathinone	4-Ethylmethcathinone	4-Ethylmethcathinone
11	Dibutylone Excipients: Caffeine	Dibutylone Excipients: Caffeine	Dibutylone
12	Fentanyl 4-Methyl- α -ethylaminopentiofenone	Fentanyl 4-Methyl- α -ethylaminopentiofenone	Fentanyl 4-Methyl- α -ethylaminopentiofenone
13	MMB-FUBINACA	MMB-FUBINACA	MMB-FUBINACA
14	Cyclopropyl Fentanyl Heroin* Phenyl Fentanyl Excipients: Caffeine, Mannitol	Cyclopropyl Fentanyl Heroin Phenyl Fentanyl Excipients: Caffeine, Mannitol	Cyclopropyl Fentanyl Heroin Phenyl Fentanyl
15	AB-FUBINACA 2-fluorobenzyl isomer Excipients: Inositol*	AB-FUBINACA 2-fluorobenzyl isomer	AB-FUBINACA 2-fluorobenzyl isomer
16	No Controlled Substances	GBL [†] Cathinone at m/z 192 [†]	No Controlled Substances
17	Dibutylone	Dibutylone	Dibutylone
18	Acetyl fentanyl Fentanyl Excipients: Quinine, Mannitol	Acetyl Fentanyl Fentanyl Excipients: Quinine, Mannitol	Acetyl fentanyl Fentanyl
19	Heroin Acetyl fentanyl Fentanyl FIBF 6-Monoacetylmorphine* ^A Excipients: Caffeine, Mannitol	Heroin Acetyl Fentanyl Fentanyl FIBF Excipients: Caffeine, Mannitol, Lidocaine*	Heroin Acetyl fentanyl (indication) Fentanyl (indication)
20	No Controlled Substances Excipients: Guaifenesin, Quinine	No Controlled Substances Excipients: Guaifenesin, Quinine, 4-Fluorophenibut	No Controlled Substances
21	No Controlled Substances Excipients: Acetaminophen, Xylitol	No Controlled Substances Excipients: Acetaminophen, Xylitol	No Controlled Substances
22	Fentanyl	Fentanyl	Fentanyl

	XLR11	XLR11	XLR11
23	JWH-250	JWH-250	JWH-250
24	JWH-018	JWH-018	JWH-018
25	α -PVP	α -PVP	α -PVP
26	Eutylone	Eutylone Excipients: Caffeine	Eutylone
27	No Controlled Substances Excipients: Caffeine	No Controlled Substances Excipients: Caffeine	No Controlled Substances
28	Methylethcathinone	Methylethcathinone	Methylethcathinone
29	α -PBP 5-Fluoro-AKB48 Excipients: Mannitol	α -PBP 5-Fluoro-AKB48 Stanozolol* [†] Excipients: Mannitol	α -PBP 5-Fluoro-AKB48
30	Dibutylone Fentanyl JWH-250	Dibutylone Fentanyl JWH-250	Dibutylone Fentanyl JWH-250
31	Tramadol Excipients: Sibutramine*	Tramadol Excipients: Mannitol*	Tramadol
32	JWH-250	JWH-250	JWH-250
33	Acetyl fentanyl Fentanyl FIBF Heroin 6-Monoacetylmorphine ^Δ Excipients: Caffeine, Quinine*	Acetyl Fentanyl Fentanyl FIBF Heroin* Excipients: Caffeine, Diphenhydramine*, Lidocaine, Procaine*	Acetyl Fentanyl ^{INS} Fentanyl FIBF Heroin
34	Eutylone Excipients: Caffeine	Eutylone Excipients: Caffeine	Eutylone
35	Fentanyl Tramadol Excipients: Caffeine, Levamisole, Mannitol, Methoxpropamine, Phenylpropanamide, Pindolol, Procaine	Fentanyl Tramadol Excipients: Caffeine*, Levamisole, Mannitol, Methoxpropamine, Phenylpropanamide, Pindolol, Procaine	Fentanyl Tramadol ^{INS}
36	AP-238	AP-238 methyl Norfentanyl [†]	AP-238
37	Heroin 6-Monoacetylmorphine ^Δ	Heroin 6-Monoacetylmorphine ^Δ	Heroin
38	JWH-250 α -Methyl Fentanyl	JWH-250 α -Methyl Fentanyl	JWH-250 α -Methyl Fentanyl
39	Fentanyl Excipients: Caffeine, Lidocaine*, Quinine, Xylazine	Fentanyl 6-Monoacetylmorphine* ^Δ Excipients: Caffeine, Lidocaine, Mannitol*, Phenacetin*, Quinine, Xylazine	Fentanyl
40	4-Chloroethcathinone Cyclopropyl Fentanyl	4-Chloroethcathinone* Cyclopropyl Fentanyl Deschloroketamine* [†]	4-Chloroethcathinone Cyclopropyl Fentanyl
41	No Controlled Substances Excipients: Mannitol	Cyclopropyl or Crotonyl Fentanyl* [†] Excipients: Mannitol	No Controlled Substances
42	Acetyl fentanyl --- Fentanyl FIBF Heroin --- 6-Monoacetylmorphine ^Δ Excipients: Caffeine, Mannitol*	Acetyl Fentanyl Cocaine* Fentanyl FIBF Heroin Noscapine* 6-Monoacetylmorphine* ^Δ Excipients: Caffeine, Mannitol*, Quinine	Acetyl Fentanyl ^{INS} Cocaine ^{INS} Fentanyl ^{INS} FIBF ^{INS} Heroin Noscapine
43	Methylone	Methylone	Methylone
44	N-methyl Cyclopropyl norfentanyl	N-methyl Cyclopropyl norfentanyl 4-Acetoxy-DET* [†]	N-methyl Cyclopropyl norfentanyl
45	No Controlled Substances Excipients: Lidocaine, Quinine	No Controlled Substances Excipients: Lidocaine, Quinine	No Controlled Substances
46	Methylethcathinone	Methylethcathinone	Methylethcathinone

47	JWH-018 MDPV	JWH-018 MDPV	JWH-018 MDPV
48	N-Ethyl pentylone	N-Ethyl pentylone	N-Ethyl pentylone
49	MMB-FUBINACA	MMB-FUBINACA	MMB-FUBINACA
50	α -PVP	α -PVP	α -PVP
51	Cocaine Excipients: Levamisole	Cocaine Excipients: Levamisole	Cocaine
52	Cocaine Excipients: Levamisole	Cocaine Excipients: Levamisole	Cocaine
53	Cocaine Excipients: Levamisole	Cocaine Excipients: Levamisole	Cocaine
54	Cocaine	Cocaine	Cocaine
55	Cocaine Excipients: Phenacetin	Cocaine Excipients: Phenacetin	Cocaine
56	Cocaine Excipients: Levamisole	Cocaine Excipients: Levamisole	Cocaine
57	Eutylone Methamphetamine Excipients: Caffeine	Eutylone Methamphetamine Excipients: Caffeine	Eutylone Methamphetamine
58	Fentanyl Excipients: Mannitol, Quinine, Caffeine	Fentanyl Excipients: Mannitol, Quinine, Caffeine	Fentanyl
59	Etizolam Excipients: Caffeine, Quinine, Diphenhydramine, Mannitol	Etizolam Excipients: Caffeine, Quinine, Diphenhydramine, Mannitol*	Etizolam
60	MDA Ketamine* Excipients: Phenacetin	MDA Ketamine Excipients: Phenacetin	MDA Ketamine ^{INS}

* Denotes compounds that had relative intensities below 5 % but above 2.5 %.

† Denotes hits for controlled substances obtained in the DART-MS search that were not present in the sample.

^Δ6-Monoacetylmorphine was not noted as an additional identified substance because it is a breakdown of heroin.

^{INS} Denotes compounds that were identified but were insufficient to report in the GC-MS result (defined as a peak in the chromatogram with less than 200,000 counts).

Conclusions

In this work, tetracaine was identified and evaluated as a candidate internal standard for the qualitative analysis of seized drugs by DART-MS. Tetracaine was found to be inexpensive, soluble in methanol at the necessary concentration, and did not interfere with detection of any commonly observed compounds of interest. Studies were conducted to determine an appropriate concentration for the internal standard to allow for identification of the same compounds as GC-MS while also minimizing the risk of false positive assignment of noise peaks. Competitive ionization due to the presence of tetracaine was observed for four of the six drugs investigated, but it did not cause complete suppression of the drug signal and the drug signals remained easily detectable. Analysis of case samples highlighted some of the benefits of incorporating an internal standard, including having a built-in mass calibration check, elimination of false positive identifications and having an indicator of analyte concentration. Using the internal standard allowed for automated mass drift compensation of data, removing a time-consuming step in high-resolution DART-MS analysis. Inclusion of tetracaine as an internal standard does lead to the possibility of missing low-concentration components in drug mixtures. This is especially true for compounds with poorer ionization efficiencies such as heroin and noscapine. While this was an issue in only one of the case samples, it is something that chemists should be aware of. It can be mitigated by adding additional sample to the extract if desired. Current efforts are focused on further understanding the benefits and drawbacks of including an internal standard for

qualitative analysis of seized drugs while also evaluating if tetracaine is a viable compound to also use as an internal standard for GC-MS analysis.

Disclaimer

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.

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Supplemental Information

Additional Information for the Measurement of Limits of Detection and Reporting Limits for GC-MS Systems.

In order to establish the limits of detection (LOD) and reporting limits for the GC-MS systems, a series of multi-component solutions consisting of cocaine, fentanyl, and methamphetamine were created with concentrations ranging from 1 $\mu\text{g/mL}$ to 300 $\mu\text{g/mL}$. Solutions were prepared gravimetrically from 1 mg/mL stock solutions of the three compounds. Each concentration was then analyzed ten times on each GC-MS with the appropriate confirmation method. Peak heights for the three compounds were extracted from each data file. For determination of limit of detection, the peak heights were entered into the ASTM E2677 Limit of Detection Web Portal (https://www-s.nist.gov/loda/loa_description.htm), and the LOD was calculated using a 10 % confidence limit. To determine the reporting limit, the peak height was plotted as a function of concentration and the concentration corresponding to peak height of 200,000 counts was extrapolated.

Supplemental Figure 1. Plots of integrated peak area of protonated molecules for alprazolam (A.), cocaine (B.), fentanyl (C.), heroin (D.), methamphetamine (E.) and MPP-PICA (F.) when analyzed with (blue) and without (orange) internal standard. Uncertainties represent the standard deviation of five replicates.