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Screening of seized drugs utilizing portable Raman spectroscopy and direct analysis in real time-mass spectrometry (DART-MS)

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ABSTRACT

The continuous change of the drug landscape in the United States demands adaptation and incorporation of emerging analytical methods that preferably allow onsite screening but are also capable of supporting the analysis of seized drugs received at forensic laboratories across the country. Current methods employing color testing require the interaction of chemical reagents with powdered and liquid materials and can often yield inconclusive results, especially when the material is impure.

Implementation of portable Raman spectroscopy can remove the need for direct interaction with solid and liquid specimens. In this study a portable, 785 nm, Raman spectroscopy system was employed for screening of seized drug samples, including mixtures. First, a panel of analytes comprised of 15 common drugs of abuse, 15 diluents, and 64 different mixtures comprising various ratios of analytes were used to measure bias, precision, and repeatability in accordance with United Nations Office on Drugs and Crime (UNODC) guidelines for handheld Raman field identification devices for seized material. Accuracy and precision through glass packaging was 91% and 90%; and through plastic was 89% and 88%, respectively for the diluents examined. A subset of the pure and mixture samples was then analyzed using direct analysis in real time mass spectrometry (DART-MS). Identification of analytes was performed manually by observing the $[M+H]^+$ protonated molecule and conducting a library search of an in-house database. Using DART-MS, the drug analyte present in the sample was correctly identified 92% of the time using the library search feature. The presence of dimers and –OH losses were also observed for many of the analyte drugs of abuse. The combination of portable Raman spectroscopy and DART-MS data resulted in an overall accuracy of 96% for the detection of both drugs and diluents. The combined accuracy when analyzing authentic case samples was 83%, providing a rapid and accurate method for seized drug screening within drug chemistry laboratories.

1. Introduction

Forensic chemists rely on an assortment of analytical techniques and instrumentation to reach conclusions when dealing with unknown seized compounds. However, every year forensic laboratories in the United States are burdened by over one million submissions of suspected drugs [1], requiring significant time and resources despite often limited budgets. To alleviate these problems and improve the speed of analysis, rapid screening of samples is a logical first step. Current screening practices often involve the use of color tests. This approach is prone to subjective, visual, judgments from the chemist [2,3] and requires the use of different chemicals, some of which are toxic [4]. Furthermore, sensitivity and selectivity problems are common, especially for impure or low concentration samples and with novel substances [4,5]. Also of issue is the collection and submission of unknown samples to the forensic laboratory that, upon analysis, turn out to be harmless or legal substances. To address these concerns, innovative, safer, and more cost-effective methods for screening unknown seized substances are needed both within the laboratory and in the field.

Raman spectroscopy, a well-established, nondestructive technique is attractive because it can provide high discrimination between drug structures. The selectivity of Raman spectroscopy is superior to chemical

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Table 1
Analyte panel for drugs and diluents. Abbreviations or alternate, common, names are shown in parenthesis next to the name. Compounds with an asterisk (*) were purchased as hydrochloride salts. For the diluents, superscript letters indicate chemical supplier.

Drugs	Diluents
4-Methylethcathinone (4-MEC)	Acetaminophen[b]
4-Methylmethcathinone (Mephedrone)	Benzocaine[b]
Alprazolam	Boric Acid[e]
Buprenorphine*	Caffeine[e]
Cocaine*	Diltiazem*[c]
Codeine	Hydroxyzine*[d]
Fentanyl	Levamisole*[c]
Heroin	Lidocaine*[b]
Methamphetamine*	Maltose[f]
Mitragynine	myo-Inositol[h]
Morphine	Phenacetin[a]
Naltrexone*	Phenolphthalein[g]
PB-22	Procaine*[c]
Sufentanil	Sorbitol[d]
Δ9-Tetrahydrocannabinol (THC)	Starch[i]

Suppliers: [a]TCI Chemicals (Portland, OR), [b]Millipore-Sigma (St. Louis, MO), [c]Acros Organics (Geel, Belgium), [d]Spectrum Chemical MFG (New Brunswick, NJ), [e]Baker (Radnor, PA), [f]MPBio (Salon, OH), [g]Fisher Chemical (Fairlawn, NJ), [h]Alfa Aesar (Ward Hill, MA), [i]Kroger (Morgantown, WV).

color tests, increasing the ability to reliably differentiate and identify a wider range of compounds. The use of Raman spectroscopy for the identification of drugs of abuse has been well documented using both conventional desktop models [6–8] and portable instrumentation [7,9–12]. The implementation of low-cost, battery powered, portable Raman spectrometers in forensic drug chemistry casework has harnessed their versatility as a fast and safe option [13], simplifying the testing process, eliminating the need for sample preparation, and opening the door to a wider range of materials and packaging types [12,14,15].

Another means of improving drug screening has been the use of mass spectrometry techniques such as high-resolution mass analyzers coupled with ambient ionization. Direct analysis in real time mass spectrometry (DART-MS) has been shown to provide rapid, and sensitive analysis of a wide range of materials, including drugs of abuse, through direct introduction of small sample amounts with minimal to no sample preparation [16–19]. In addition, recent literature reports have demonstrated the ability of DART-MS to detect trace drug residues on the outside of packaging, allowing prediction of the internal contents prior to opening the packaging [20,21]. The combination of the DART ionization source with high-resolution mass spectrometry results in accurate mass measurements, providing more confident screening of drug compounds.

Combining the results from the orthogonal techniques for the detection of 15 common drugs of abuse and 15 diluents is presented herein. After establishing bias, precision, and reproducibility of portable Raman spectroscopy, a suite of pure and binary mixture samples was analyzed to determine the accuracy of this approach. A subset of these samples and mixtures was analyzed using DART-MS to establish the accuracy of the technique by itself and when these results were combined with portable Raman. To demonstrate real-world utility, this combination of screening techniques was used to analyze a set of authentic samples provided by the Maryland State Police Forensic Sciences Division (MSP-FSD).

2. Materials and methods

2.1. Reagents and materials

A total of 15 drugs of abuse and 15 diluents were purchased, as neat materials with a minimum purity of 99% from a number of chemical suppliers. Drug purity was verified using GC–MS. All drugs were purchased from Cayman Chemical (Ann Arbor, MI), and the identities of the compounds and the suppliers for the diluents are listed in Table 1.

Table 2
DART-MS parameters for analysis.

DART Temperature	400 °C
DART Gas	He
Orifice 1 Voltage	30 V, 60 V, 90 V switching at 0.2 s/scan
Ring Voltage	5 V
Orifice 2 Voltage	20 V
Ion Guide	500 V
m/z Scan Range	*m/z* 50 – *m/z* 800

2.2. Instrumentation

Raman spectra were obtained using a TacticID portable 785 nm laser Raman instrument from B&W Tek (Newark, DE). The unit was operated at either 20%, 60%, or 90% laser power. Spectra were acquired between the range of 176 cm^{-1} and 2900 cm^{-1} with 9 cm^{-1} resolution. Spectra were automatically compared with the stored instrument library, as well as an in-house library created using the same instrument. Assessment of spectral similarity was determined by the hit-quality-index (HQI) with the low-end cut-off set to the instrument's default of 85%. A polystyrene reference material was utilized daily to verify the performance of the instrument before any further measurements.

DART-MS spectra were acquired in positive ionization mode using an IonSense DART-SVP ion source (Saugus, MA) with a JEOL AccuTOF 4G LC-plus mass spectrometer (Peabody, MA). DART analysis was performed using the parameters outlined in Table 2. Direct sampling was implemented by first placing the closed end of a capillary tube within the DART gas stream for several seconds. Following brief cooling, the capillary was dipped and swirled into the powdered sample before being introduced to the ion source. To perform drift compensation, polyethylene glycol (PEG) was used. Resulting mass spectra were extracted and background subtracted using an area of the chronogram where samples were not analyzed in msAxel. Spectra were assessed manually, as well as through use of Mass Mountaineer (Fineview, NY) software with an in-house library of over 600 compounds provided by the National Institute of Standards and Technology (NIST). Search parameters for mixture analysis included a minimum peak height of 5% relative intensity, to minimize the potential for false positive identification, and an *m/z* agreement of ±0.005 Da, based on the MS manufacturers tolerance. DART-MS is a well-established technique in forensic seized drug analysis and therefore a validation of the technique was not required [22,23].

2.3. Establishing Bias, Precision, & reproducibility for the portable Raman

Establishment of bias, precision, and reproducibility of the portable Raman instrument was performed following ASTM E1683-02 [24], ASTM E1840-96 [25], and United Nations Office on Drugs and Crime (UNODC) guidelines [26] by investigating interference from different types of packaging, variability between analysts, mixture analysis, and verification of libraries within the instrument. For these studies only a diluent panel was used for testing. Pure diluents were analyzed inside glass vials and 2 mil plastic bags. The point-and-shoot adapter was used for analysis through plastic bags and no adaptor for analysis though glass. Spectra were acquired in triplicate at both 60% and 90% laser power. Reproducibility and repeatability were established through triplicate analysis performed by a total of three different operators. Analysis of variance (ANOVA) was used to evaluate within and between operator variability. The instrument's accuracy when analyzing pure drugs and diluents was reported.

Table 3
Mixtures of drugs and diluents investigated in this study. Ratios with a checkmark were analyzed using the portable Raman system (n = 64). Samples with an asterisk (*) were also analyzed using DART-MS (n = 25).

Mixture	Mass Ratio (Drug: Diluent)			
	1:4	1:7	1:10	1:20
Heroin HCl / acetaminophen	✓*	✓		✓
Fentanyl HCl / caffeine				✓
Fentanyl HCl / methamphetamine HCl	✓			
Cocaine HCl / levamisole	✓*			
Fentanyl HCl / cocaine HCl	✓			
Methamphetamine HCl / levamisole	✓*	✓		
Methamphetamine HCl / caffeine	✓*			
Cocaine HCl / benzocaine	✓*			
Alprazolam / caffeine	✓*	✓*		
Alprazolam / levamisole	✓	✓		
4-MMC HCl / maltose	✓*			✓
4-MMC HCl / lidocaine		✓*	✓	
4-MEC HCl / maltose	✓*	✓		
4-MEC HCl / benzocaine		✓*	✓	
PB-22 / lidocaine	✓			
Sufentanil / caffeine	✓			
Codeine / acetaminophen	✓	✓	✓	✓
Codeine / maltose	✓*	✓*	✓*	✓*
Morphine / maltose	✓*	✓*	✓*	✓
Naltrexone HCl / maltose	✓*	✓*	✓*	✓*
Buprenorphine HCl / starch	✓*	✓*	✓*	✓
Cocaine HCl / caffeine	✓	✓	✓	✓
Cocaine HCl / diltiazem	✓	✓	✓	✓
Cocaine HCl / hydroxyzine	✓		✓	
Cocaine HCl / lidocaine	✓	✓		✓
Cocaine HCl / maltose		✓		✓
Cocaine HCl / procaine	✓	✓	✓	
Cocaine HCl / boric acid			✓	

2.4. Assessment of mixtures

A total of 64 mixtures of target drugs and common diluents were created to simulate street samples and are shown in Table 3. Mixtures and ratios were selected based on published literature [27–32]. As an example, a 1:4 ratio was prepared by mixing 10 mg of target drug with 40 mg of diluent. All mixtures were analyzed via Raman through the plastic bags in triplicate at different areas to account for variability in the sample. The mixture analysis setting was used for all mixtures, to allow for identification of multiple compounds, with the number of hits—high spectrally correlated compounds, set to 5 and the ratio threshold set to 15%.

Previous studies have shown that DART-MS is an established technique for drugs of abuse analysis [16,20,33,34]. Therefore, a subset of 25 samples of the original 64 mixtures, highlighted in Table 3, was selected to demonstrate the applicability of DART-MS for mixture analysis. The accuracy of DART-MS, the TacticID instrument, and the orthogonal combination of both techniques were determined. The combined accuracy was determined when the compounds were correctly reported by either DART-MS or Raman. For example, if the drug was only reported from the DART-MS results and the diluent reported with Raman, a correct identification of both drug and diluent resulted for that particular mixture.

2.5. Authentic samples

Fifteen adjudicated case samples were provided by the Maryland State Police Forensic Sciences Division and analyzed via both the portable Raman system and DART-MS. Samples were assessed in triplicate using both methods and compared against their respective libraries. The Raman laser power was altered based on the color of the test material—20% or 60% for colored samples and 90% power for white powders. Analysis of the authentic samples by DART was performed as described previously in Table 2. Samples were prepared following MSP-

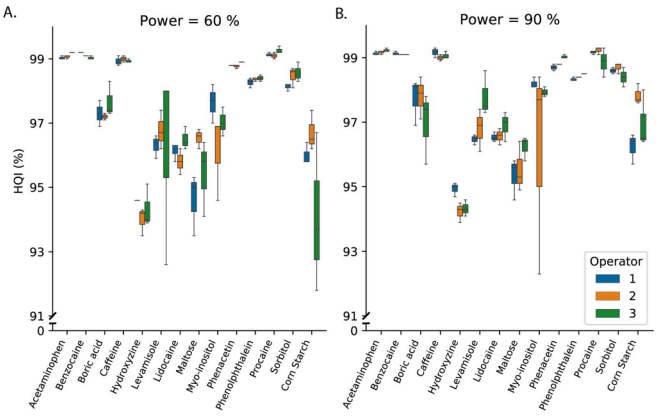

Fig. 1. Boxplots showing the distribution of the HQI (%) between three operators when the portable Raman was operated at 60% (A) and 90% (B) power. All diluents were powders and analyzed through plastic. Results for diltiazem are not shown because it was not present in the instrument library. Each box and whisker plot represents nine total measurements.

FSD protocols by dissolving 1 mg to 2 mg of powder in ≈1 mL of methanol. The averaged mass spectrum was obtained for each sample from the triplicate analyses and used for identification in MassMountaineer with a tolerance of ±0.005 Da and threshold of 5%, which was lowered to 1% for differentiation of isomers. A multi-point drift compensation with tetracaine was used for calibration to serve as a positive control.

3. Results and discussion

3.1. Portable Raman

3.1.1. Laser power and operator reproducibility

The hit quality index (HQI)—a common spectral comparison method [35,36], is a measure of the spectral correlation between the known library spectrum and the unknown test spectrum. Rodriguez et al. described HQI by Eq. (1) [37]. The Raman system reports the HQI as a percentage where a value closer to 100% represents higher similarity and a value closer to 0% represents poor similarity. Validation of the instrument was performed with diluents only as a cost saving option. Fig. 1 shows the distribution of the HQI for the diluents at 60% (Fig. 1A) and 90% (Fig. 1B) power for three operators. All HQI values were greater than 90% although there was higher variation with Operator 3. ANOVA results showed myo-inositol with the highest variation in the HQI value—2% coefficient of variation (CV) observed between and within operators. The percent CV for all other compounds was less than 2%.

$$HQI = \frac{(Library^{*}Test)^{2}}{(Library^{*}Library)(Test^{*}Test)} \quad (1)$$

3.1.2. Packaging container

Fig. 2 shows the distribution of HQIs when the diluents were measured through glass (Fig. 2A) and plastic (Fig. 2B) at 60% and 90% power as part of the instrument validation. Although all HQI values were greater than 85%, there was higher variation when the packaging material was glass at both laser powers. Analysis of corn starch through glass only returned a result using the mixture setting on the instrument and was not plotted in Fig. 2. However, the portable Raman instrument returned all the pure diluents tested as the top hit through both glass and plastic. The instrument is designed to analyze compounds through transparent glass vials <5 mm diameter thickness, as used in this study. The thickness of the plastic bags used in this study was 2 mil (0.0508 mm), which provided more consistent spectral intensities, and therefore typically higher HQIs compared to glass. Most of the drugs analyzed in

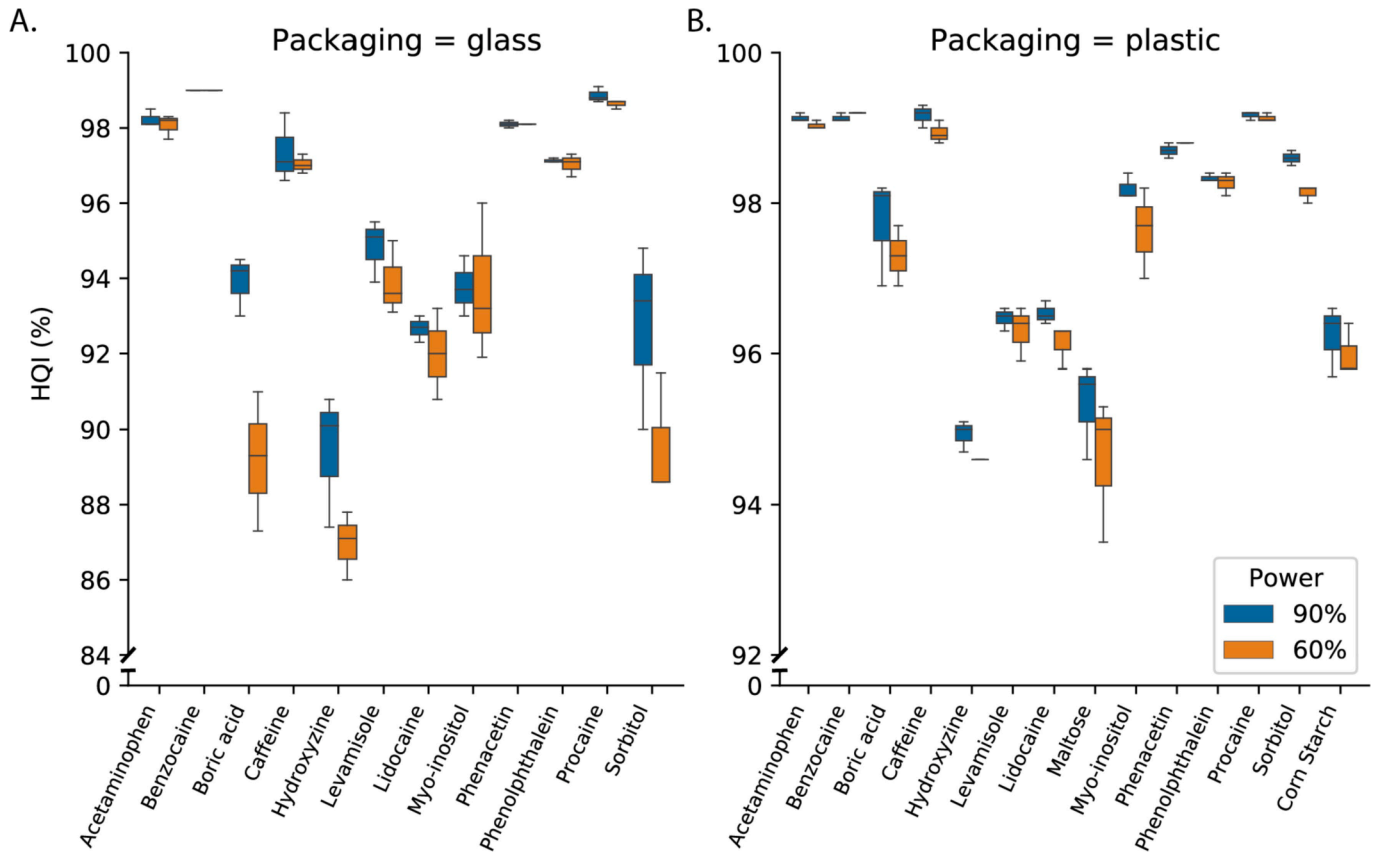

Fig. 2. Boxplot comparing the type of packaging—glass (A), and plastic (B), through which the diluents were analyzed when the instrument was operated at 60% and 90% power, by three operators. Diltiazem is not plotted as it was not present in the instrument's library and returned a "no match" result. Corn starch and maltose are not shown for glass (A) since the mixture setting was used to get a hit and the mixture setting provides a spectral weight percentage instead of an HQI. Note the differences between the y-axes, where (A) is from 84% to 100% and (B) is from 92% to 100%.

this study were white powders and the laser power selected for subsequent analysis was 90% because of the lower variation in the observed HQIs.

3.1.3. Performance measures

The performance of an instrument in relation to a particular purpose is important to understand, especially the false identification rates within a forensic context. Given that portable Raman systems can be used for field applications or laboratory case work, the ability to correctly identify compounds through glass or plastic packaging was investigated. A true positive (TP) was defined as the instrument correctly associating the spectrum of the drug with the spectrum of the drug in its library; a true negative (TN) was defined as the instrument returning a "no match" result when the drug was absent from the library or no drug was present in the sample; a false positive (FP) was defined as the instrument erroneously returning a match for a drug that was not present; and a false negative (FN) was defined as the instrument returning a "no match" result or failing to detect a drug when it was present and its spectrum was in the library. Eqs. (2)–(5) were used to calculate the accuracy, sensitivity, specificity, and precision for pure target drugs and diluents. When the compounds listed in Table 1 (with the exception of THC) were analyzed through plastic, the accuracy was 89%, the true positive rate (TPR) was 100%, the true negative rate (TNR) was 23% and the precision was 88%. When analysis was performed through glass, these values were 91%, 100%, 38%, and 90%, respectively. Although the portable Raman instrument demonstrated high accuracy and TPR, the high false positive rate is one reason it is regarded as a preliminary method. For this reason, we explored the potential of combining the portable Raman technique with DART-MS.

$$Accuracy = \frac{(TP + TN)}{(TP + FN + FP + TN)} \tag{2}$$

$$Sensitivity(TPR) = \frac{TP}{(TP + FN)} \tag{3}$$

$$Specificity(TNR) = \frac{TN}{(TN + FP)} \tag{4}$$

$$Precision = \frac{TP}{(TP + FP)} \tag{5}$$

When binary diluent–diluent mixtures were analyzed, both compounds were correctly identified in 17% of the samples as shown in Fig. 3(A). For drug-diluent mixtures as shown in Fig. 3(B), both compounds were correctly identified in 19% of the samples. In one instance, the drug-diluent mixture of naltrexone-maltose (1:7 ratio), both compounds were incorrectly identified (Supplemental Table S1). The mixtures at 1:4 and 1:7 ratios produced greater success in observing both compounds, possibly due to the more equal proportions of each compound (Supplemental Table S1).

Cocaine is one of the most prevalent drugs of abuse. A study conducted in Austria reported 10% of seized samples analyzed contained cocaine as the active ingredient [38] with purities ranging from 30% to 60% based on the geographic location [28,39,40]. Cocaine seizures in the European Union increased by more than 42 tonnes in 2018 from the previous year, the highest level recorded [41]. A 2020 midyear report in the US ranked cocaine as the third most popular drug of abuse comprising 13% of drug seizures [42]. To gain a better understanding of the ability to identify binary mixtures involving cocaine using portable

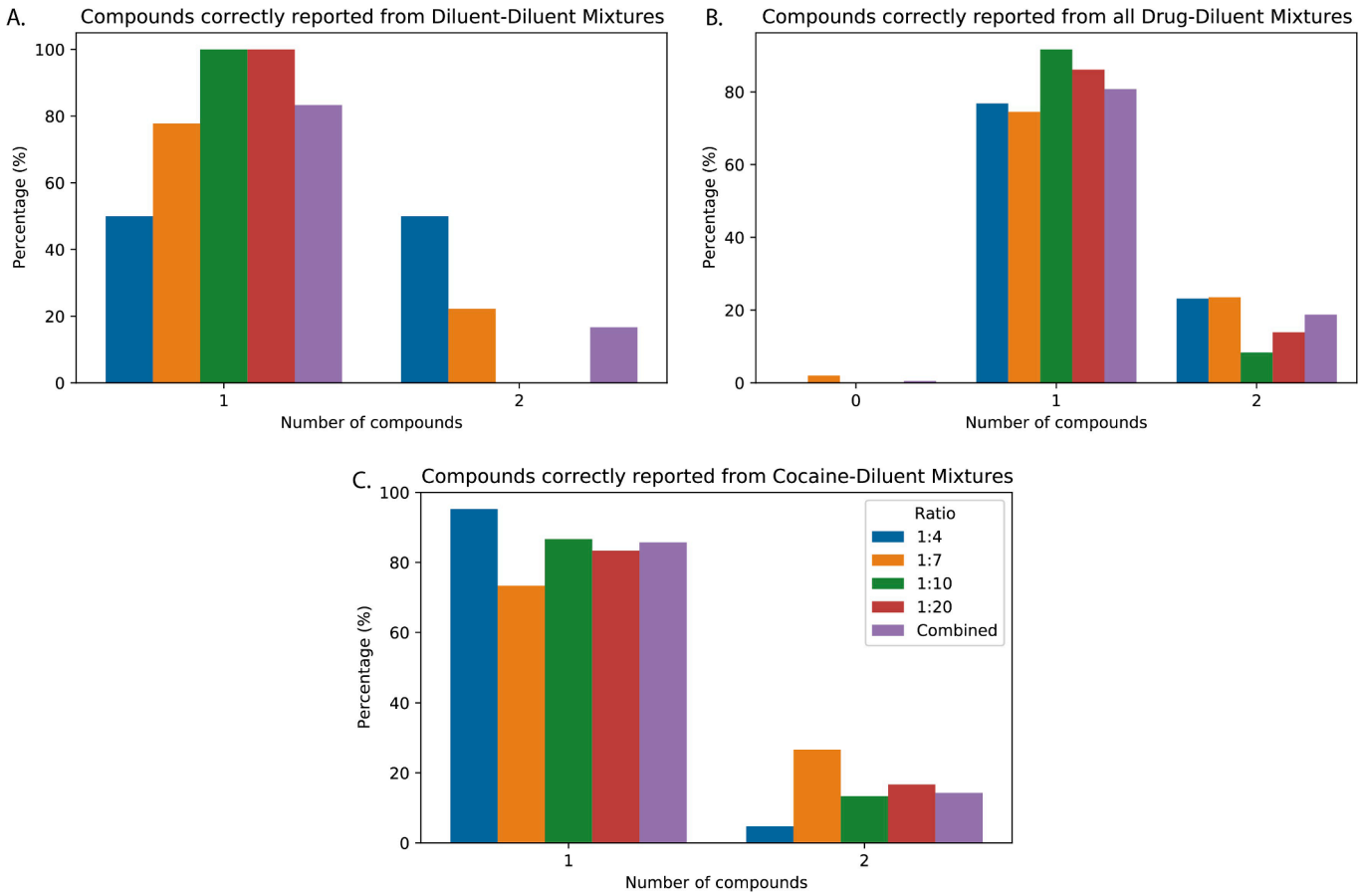

Fig. 3. (A)— Percent of correctly reported compounds for each ratio and the total dataset (purple) by the portable Raman system for diluent-diluent mixtures (ratios 1: 4 and 1:20, n = 6; ratios 1:7 and 1:10, n = 9). 83% of correctly identified substances matched 1 compound and 17% matched for both compounds as shown by the purple bars; (B)—Percent of correctly reported compounds by the portable Raman for drug-diluent mixtures (ratio 1: 4, n = 69; ratio 1:7, n = 51; ratios 1:20 and 1:10, n = 36). 81% of the identified substances returned a hit for 1 compound, and 19% for both compounds in binary mixtures; (C)—Percent of correctly identified compounds in cocaine- diluent mixtures (ratio 1:4, n = 21; ratios 1:10 and 1:7, n = 21; ratio 1:20, n = 12). All mixtures were analyzed with 90% laser power. The combined ratio is the overall percentage for the number of identified compounds calculated from the total number of samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Raman, the 90% laser power data was analyzed separately. Fig. 3(C) shows both cocaine and the diluent were correctly identified in 14% of the tested mixtures. However, cocaine was reported as the detected drug in only 24% of the samples while the diluent was correctly identified 90% of the time. Fig. 4 presents the Raman spectra obtained using the TacticID for cocaine, levamisole, and a ratio 1:4 cocaine-levamisole, demonstrating areas of congruence for both compounds within the mixture. The peaks at 1000 cm^{-1}, 1024 cm^{-1}, 1276 cm^{-1}, 1600 cm^{-1}, and 1716 cm^{-1} are attributed to symmetric stretching of aromatic ring breathing, asymmetric stretching of the aromatic ring, C-N stretching, C═C stretching of the aromatic ring, and C═O symmetric stretching, respectively in cocaine HCl [43]. The levamisole spectrum is marked by the absence of a peak at 1716 cm^{-1} present in the mixture and the cocaine spectra. Although cocaine and levamisole have a peak at 1260–1276 cm^{-1}, representing CN stretching, it is weaker in levamisole. Similarly, cocaine has a stronger peak at 1600 cm^{-1} than levamisole.

Fentanyl remains a drug of interest especially in the United States due to the ongoing opioid crisis. Three mixtures containing fentanyl were investigated in this study, but the portable Raman system was only able to detect fentanyl in one sample. Possible reasons for the missed detections include the limited amount of sample used in preparing the mixtures due to the high exposure risk associated with fentanyl, and fluorescence. Surface-enhanced Raman spectroscopy (SERS) was used by Haddad, Green and Lombardi to detect fentanyl in binary cocaine mixtures at 65 ppm [44], overcoming the low concentrations of fentanyl found in street samples [28]. Green et al. also compared the sensitivity of immunoassay based fentanyl testing strips, a TruNarc™ Raman spectrometer and a Bruker Alpha™ Fourier-Transform Infrared (FTIR) spectrometer for detecting fentanyl in street samples [45]. The TruNarc system resulted in an overall sensitivity of 25.7%, and 81.9% sensitivity with FTIR for all test compounds including fentanyl. Although the immunoassay test strips produced a higher sensitivity than both TruNarc and FTIR, they do not discriminate between fentanyl and its analogues.

Several portable Raman instruments are currently on the market for forensic applications. For a comparison of the specifications between some of these instruments, refer to the Forensic Technology Center of Excellence report [46]. Although the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) lists Raman spectroscopy as a category A technique indicating it has the highest discriminatory power [47], some laboratories consider portable Raman spectroscopy as category B due to the challenge in detecting all components in mixture samples. For example, Spicher et al. reported an accuracy of 97.6% when certified reference materials were analyzed with a portable Raman, but 76.9% accuracy for case samples [48] which usually contain several compounds and have the controlled substance as the minor ingredient. The overall accuracy of the portable Raman system in our study was 32% in detecting the target drug, 89% in detecting the diluent, and 19% in detecting both compounds in the binary mixtures analyzed above, highlighting the need for complementary techniques that also provide results just as fast as Raman and with minimal sample

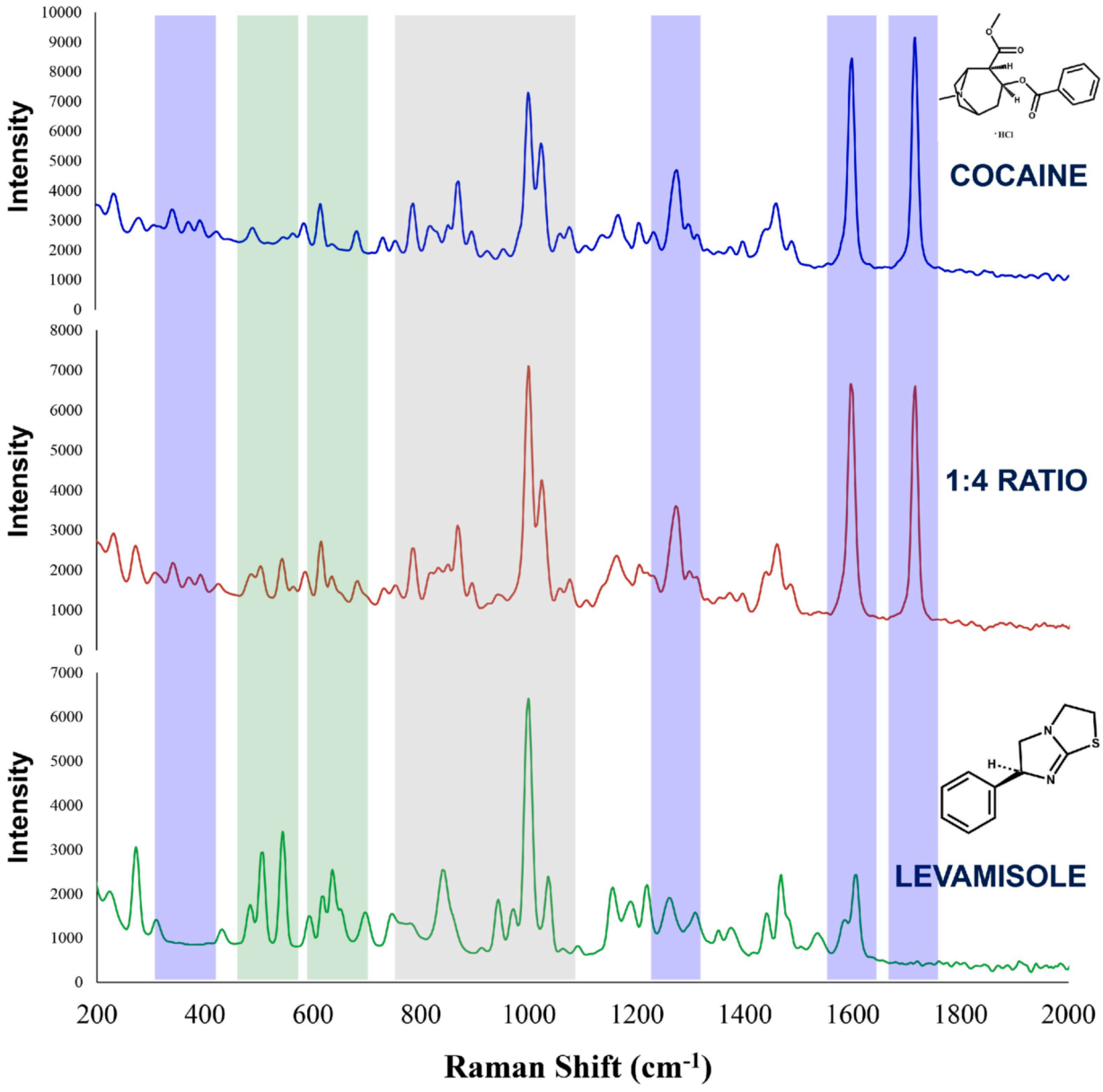

Fig. 4. Raman spectra of solid powders within plastic bags for cocaine, levamisole, and a mixture ratio of 1:4, cocaine-levamisole. Areas of congruence with levamisole are highlighted in green and with cocaine are highlighted in blue to demonstrate spectral regions for each analyte compared to the mixture. The area highlighted in gray represents contributions from both levamisole and cocaine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

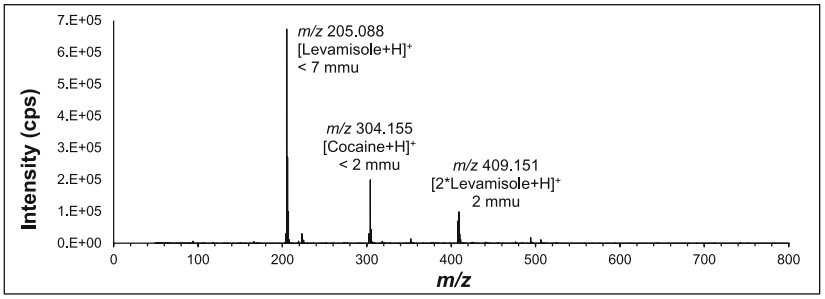

Fig. 5. Representative DART mass spectrum of a 1:4 mixture ratio of cocaine to levamisole. Peaks of interest are labeled based on MassMountaineer identification along with the difference in milli-mass units (mmu) between the library and the spectrum. Due to high concentration, the levamisole peak fell outside of the ±5 mmu window, which was widened to encompass this peak. For simplicity, only the spectrum collected at the 30 V voltage is shown.

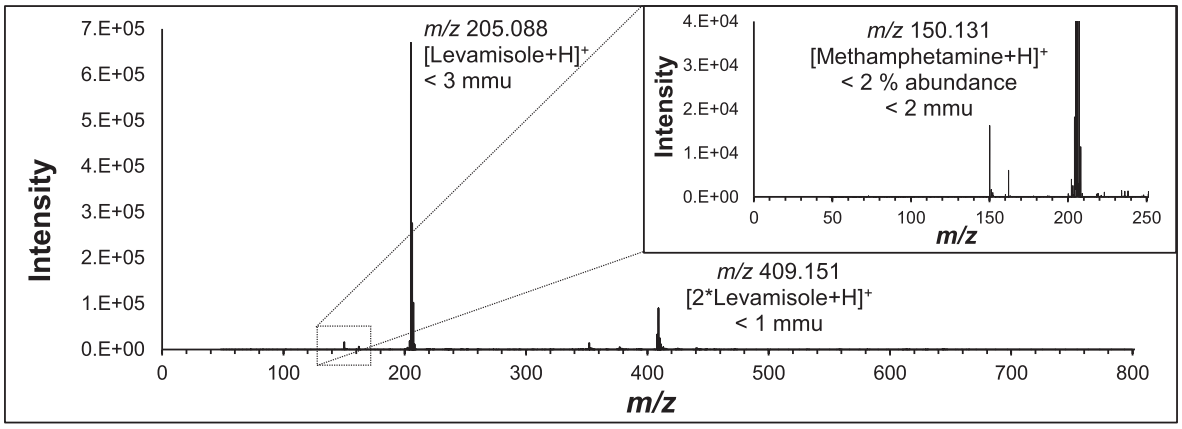

Fig. 6. Demonstration of the lack of identification of the methamphetamine peak in the MassMountaineer software due to the peak threshold value of 5%. MassMountaineer identifications are shown along with the milli-mass unit difference between the library and the spectrum. Methamphetamine *m/z* 150 can be seen when zooming into the group of peaks present near *m/z* 150. (abundance of less than 2%. The relative abundance window was widened to observe the methamphetamine peak. For simplicity, only the spectrum collected at the 30 V voltage is shown.

preparation.

3.2. DART-Ms

DART-MS was utilized as an orthogonal detection method for the samples tested by portable Raman. SWGDRUG lists mass spectrometry as a category A technique [47], but like portable Raman, DART-MS is used as a screening method. A polyethylene glycol standard was run on the instrument to account for drift compensation. For analysis by traditional DART sampling, *m/z* 283.17513 was chosen for drift compensation by the software. Fig. 5 shows a representative DART-MS spectrum for a 1:4 mixture ratio of cocaine-levamisole mixture analyzed using traditional microcapillary sampling.

Identification was based on manual inspection of the mass spectrum for each sample run in triplicate and using MassMountaineer data analysis software (Rev: 5.0.7.0) with an in-house library as demonstrated. Due to the structural properties of maltose and starch, these molecules do ionize easily and were therefore not observed in the DART-MS spectra. Previous work has demonstrated the ability to analyze carbohydrates via DART-MS; however, the authors utilized an in situ permethylation step to allow positive mode analysis [49]. In our proposed protocol, a generic drug screening method was used with direct analysis and minimal sample preparation. When considering positive identification of both the drug and diluent, the samples where the diluent was not identified contained either maltose or starch, while the remaining samples had positive identifications for the diluent, representing the loss of carbohydrate information due to the ionization mode rather than instrument ability. As such, performance of the DART analysis was judged by positive identification of the drug of abuse. The true positive rate was determined to be 93% with false negative rate of 7%.

In many cases, peaks not corresponding to the protonated molecule were present in the mass spectrum. Upon analysis, the majority of these peaks were easily explained through the presence of dimers and loss of water. Codeine, acetaminophen, naltrexone, caffeine, levamisole, and alprazolam demonstrated the formation of dimers while –OH losses were observed for buprenorphine, morphine, and codeine. In one instance, methamphetamine was not identified in the sample due to the 5% peak threshold set by the search algorithm. Manual examination of the mass spectrum easily revealed the methamphetamine peak of *m/z* 150 (Fig. 6).

3.3. Orthogonal detection

Analytical schemes which leverage orthogonal techniques to provide complimentary identification data have demonstrated improved reliability and accuracy, and therefore the data from the portable Raman and DART-MS were combined to compare the performance rates of the orthogonal approach. It is important to note that although Raman spectroscopy and mass spectrometry are considered SWGDRUG category A techniques, these instruments are being assessed as rapid screening techniques. Although the portable Raman initially struggled to identify the drug analyte in dilute mixture ratios, the diluent was correctly identified 100% of the time in the subset of mixtures used for the orthogonal detection study. In contrast, DART-MS excelled at detecting both drug and diluent compounds; however, many diluents were not identified due to analysis in positive mode. Therefore, the combination of both techniques yielded high accuracy for both drug and diluent compounds in all the analyzed samples, demonstrating the combined strength and enhanced reliability through orthogonal combination. Table 4 presents the comparison of overall performance rates for the samples assessed orthogonally, first by portable Raman and followed by DART-MS analysis. Specificity does not apply because the instruments always returned a match based on the library search. The full data set can be found in Supplementary Table S2. Performance measures were determined using Eqs. (2)–(5). Accuracy for both analytes (drug +

Table 4
Comparison of accuracy between Raman, DART-MS, and the orthogonal combination when mixtures were analyzed. The accuracy of the Raman shown below is only for the 25 mixtures that were also analyzed by DART-MS. Specificity is not applicable as there were no true negatives in this data set.

	Raman			DART-MS			Combined
	Drug	Diluent	Both Analytes	Drug	Diluent*	Both Analytes*	Both Analytes
Accuracy	48%	100%	56%	85%	33%	26%	96%
Sensitivity	56%	100%	56%	92%	36%	28%	96%
Specificity	NA	NA	NA	NA	NA	NA	NA
Precision	78%	100%	100%	92%	82%	78%	100%

*Diluents measured by DART-MS were acetaminophen, benzocaine, caffeine, levamisole, lidocaine, maltose, and starch.

Table 5
Summary of authentic samples analyzed through Raman and DART-MS and ground truth as observed from GC–MS. An explanation is provided for compounds detected via DART-MS but not observed via GC–MS analysis.

Case	GC–MS Results (Ground Truth)	Portable Raman Results	DART-MS Result
1	Heroin	–	Heroin
	Mannitol	Mannitol	–
	Quinine	–	Quinine
	6-Monoacetylmorphine	–	6-Monoacetylmorphine
		Additional Hits: Hydrogen peroxide Sodium azide JWH-122	
2	Cocaine	Cocaine HCl	Cocaine
	Levamisole	Levamisole HCl	Levamisole
3	Fentanyl	–	Fentanyl
	Caffeine	Caffeine	Caffeine
	Diphenhydramine	–	–
	Quinine	–	Quinine
		Additional Hits: Erythromycin Mannitol Sodium azide	Additional Hits: Levamisole Mannitol
4	MDMA	MDMA HCl	MDMA
		Additional Hits: Centrophenoxine Buprenorphine HCl 2-*N,N*-diethylamino-1-(4-methoxyphenyl)-1-propanone	
5	Fentanyl	–	Fentanyl
	Acetaminophen	Acetaminophen	Acetaminophen
			Additional Hits: Xylitol
6	Cocaine	Cocaine base	Cocaine
	Levamisole	Levamisole	Levamisole
	Phenacetin	Phenacetin	Phenacetin
7	Caffeine	Caffeine	Caffeine
		Starch	
8	Caffeine	–	Caffeine
	Mannitol	Mannitol	Mannitol
	Quinine	–	Quinine
9	No Drugs of Abuse	Maleic anhydride Hexobarbitone	Caffeine
10	Fentanyl	–	Fentanyl
	Acetylsalicylic Acid	Acetylsalicylic Acid	Acetylsalicylic Acid
	Benzocaine	–	–
	Caffeine	–	Caffeine
	N-Phenylpropamide	–	–
	Quinine	–	Quinine
11	Cocaine	Cocaine base	Cocaine
	Levamisole	–	Levamisole
	Phenacetin	Phenacetin	Phenacetin
	Inositol	–	–
		Additional Hits: Thebaine	
12	Phentermine	Phentermine HCl	Phentermine
13	Methamphetamine	–	Methampehtamine
	Ketamine	Ketamine HCl	Ketamine
	Phenacetin	Phenacetin	
		Additional Hits: Dimethyl sulfone	
14	Heroin	–	Heroin
	6-Monoacetylmorphine	–	6-Monoacetylmorphine
	Mannitol	Mannitol	Mannitol
	Quinine	–	Quinine
	6-Acetylcodeine	–	–
		Additional Hits: Sorbitol Hydrogen peroxide Hydroxyzine pamoate Codeine	
15	Cocaine	Cocaine HCl	Cocaine
	Benzoylecgonine	–	–

Table 6
The accuracy results for the authentic case samples. The calculation of the accuracy was performed in similar fashion as described above in Section 3.5. Sample #9 was not included since it was a true negative sample.

Performance measure	Raman	DART-MS	Combined
Drug accuracy	41%	82%	82%
Diluent accuracy	45%	68%	83%
Accuracy for all analytes	44%	74%	83%

diluent) was determined by the sum of the samples producing identifications for both the drug and diluent divided by the total number of samples. Lastly, the accuracy of the combination of the two instruments was assessed as the sum of the samples producing the respective identifications by either portable Raman and/or DART-MS divided by the total number of samples (Table 4).

3.4. Authentic sample results

To investigate how the orthogonal approach worked for real samples, fifteen authentic adjudicated case samples were obtained from the Maryland State Police Forensic Sciences Division. The majority of the samples were white powders or white crystalline samples and several samples were off-white to gray-brown. All samples were analyzed by portable Raman through plastic bags or through capsules. Table 5 provides the results of the portable Raman and DART-MS analyses along with the ground-truth results which were obtained using GC–MS analysis. Accuracy was defined as the ability of the instrument to detect those compounds assigned as ground truth for each respective group (drug, diluent, or all analytes). For example, if the ground truth contained two diluents, both needed to be detected for a positive result for diluent accuracy. In this manner, detection of all ground truth compounds was required. The overall accuracy of the portable Raman was 44% for all analytes, whereas the accuracy of the DART-MS analysis was 74% for all analytes (Table 6). The failure of the portable Raman instrument to detect some controlled substances due to their low proportion, was compensated for with DART-MS as the combination of the two techniques resulted in 83% accuracy in the detection of all ground truth compounds for the authentic samples. It is important to note that while both instruments performed well, in one instance, both instruments were needed to yield a full profile of the unknown substance as demonstrated by case #1. Some diluents can foul the GC–MS source, therefore most drug chemistry laboratories screen samples for controlled substances but do not always report diluents. In one case, #3, a diluent was detected by both Raman and DART-MS but not observed by GC–MS. Given that the diluent was mannitol it is expected as GC–MS is not sensitive to sugar alcohols.

4. Conclusions

On-site drug testing can help reduce drug backlogs, but the safety of personnel conducting the tests is important due to the increasing potency of illicitly manufactured substances. Portable Raman analysis allows for testing without opening certain types of packing, thereby reducing potential drug exposures. It produces high confidence in results when analyzing pure substances, but accuracy suffers when mixtures are present, as demonstrated in this study. The use of orthogonal techniques such as DART-MS can help resolve some of the challenges encountered in Raman analysis.

In this study, a portable Raman spectrometer was validated according to the UNODC guidelines on a panel of 15 commonly encountered drugs of abuse and 15 diluent compounds. The HQI for pure diluents through plastic was higher than that for glass, >90% and >86%, respectively. The between-operator precision was low at ≤2%. Analysis through plastic resulted in an accuracy of 89% and precision of 88%, while analysis through glass resulted in an accuracy of 91% and

precision of 90%. The system excelled at identification of analytes in their pure form and in higher percent ratio but demonstrated some difficulty in detection the analyte at low concentrations. In comparison, DART-MS demonstrated high accuracy and sensitivity for the drug analytes of interest and many of the diluent compounds. However, DART-MS struggled with diluent compounds that perform better in negative mode (only positive mode was used). Although these techniques are strong on their own, the combination of both instruments resulted in a drug accuracy of 96%, diluent accuracy of 100%, and overall accuracy for two-part mixtures of 96%. Analysis of authentic case samples using both techniques resulted in 44% accuracy by Raman, 74% by DART-MS, and 83% accuracy when both techniques were combined. This combination of orthogonal data demonstrates the improved reliability and accuracy possible when both techniques are used in screening. The ability to detect both drug and diluent analytes provides useful information for drug intelligence operations that can be performed rapidly for improved investigative leads and real-time decision making.

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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.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2021.100352.

References

[1] U.S. Drug Enforcement Administration Diversion Control Division (2019) National Forensic Laboratory Information System:NFLIS-Drug 2019 Annual Report.
[2] D. Lieblein, M.E. McMahon, P.E. Leary, et al., A comparison of portable infrared spectrometers, portable Raman spectrometers, and color-based field tests for the on-scene analysis of cocaine, Spectroscopy 33 (2018) 20–28.
[3] K.E. Toole, S. Fu, R.G. Shimmon, et al., Color tests for the preliminary identification of methcathinone and analogues of methcathinone, Microgram J. 9 (2012) 27–32.
[4] M. Philp, S. Fu, A review of chemical 'spot' tests: A presumptive illicit drug identification technique, Drug Test. Anal. 10 (1) (2018) 95–108, https://doi.org/10.1002/dta.v10.110.1002/dta.2300.
[5] R.S. Das, Y.K. Agrawal, Raman spectroscopy: Recent advancements, techniques and applications, Vib. Spectrosc. 57 (2) (2011) 163–176, https://doi.org/10.1016/j.vibspec.2011.08.003.
[6] J. Omar, B. Slowikowski, C. Guillou, F. Reniero, M. Holland, A. Boix, Identification of new psychoactive substances (NPS) by Raman spectroscopy, J. Raman Spectrosc. 50 (1) (2019) 41–51, https://doi.org/10.1002/jrs.v50.110.1002/jrs.5496.
[7] E.M.A. Ali, H.G.M. Edwards, Screening of textiles for contraband drugs using portable Raman spectroscopy and chemometrics, J. Raman Spectrosc. 45 (2014) 253–258, https://doi.org/10.1002/jrs.4444.
[8] V.L. Brewster, H.G.M. Edwards, M.D. Hargreaves, T. Munshi, Identification of the date-rape drug GHB and its precursor GBL by Raman spectroscopy, Drug Test. Anal. 1 (2009) 25–31, https://doi.org/10.1002/dta.11.
[9] M.D. Hargreaves, K. Page, T. Munshi, R. Tomsett, G. Lynch, H.G.M. Edwards, Analysis of seized drugs using portable Raman spectroscopy in an airport environment—a proof of principle study, J. Raman Spectrosc. 39 (7) (2008) 873–880, https://doi.org/10.1002/jrs.v39:710.1002/jrs.1926.
[10] A.D. Burnett, H.G.M. Edwards, M.D. Hargreaves, et al., A forensic case study: the detection of contraband drugs in carrier solutions by Raman spectroscopy, Drug Test. Anal. 3 (2011) 539–543, https://doi.org/10.1002/dta.169.
[11] A. Hakonen, K. Wu, M. Stenbæk Schmidt, P.O. Andersson, A. Boisen, T. Rindzevicius, Detecting forensic substances using commercially available SERS substrates and handheld Raman spectrometers, Talanta 189 (2018) 649–652, https://doi.org/10.1016/j.talanta.2018.07.009.
[12] K. Dégardin, A. Guillemain, Y. Roggo, Comprehensive study of a handheld raman spectrometer for the analysis of counterfeits of solid-dosage form medicines, J. Spectrosc. 2017 (2017) 1–13, https://doi.org/10.1155/2017/3154035.
[13] A. Kudelski, Analytical applications of Raman spectroscopy, Talanta 76 (1) (2008) 1–8, https://doi.org/10.1016/j.talanta.2008.02.042.
[14] C.A.F. de Oliveira Penido, M.T.T. Pacheco, I.K. Lednev, L. Silveira, Raman spectroscopy in forensic analysis: identification of cocaine and other illegal drugs of abuse, J. Raman Spectrosc. 47 (2016) 28–38, https://doi.org/10.1002/jrs.4864.
[15] E.L. Izake, Forensic and homeland security applications of modern portable Raman spectroscopy, Forensic Sci. Int. 202 (1-3) (2010) 1–8, https://doi.org/10.1016/j.forsciint.2010.03.020.
[16] E. Sisco, J. Verkouteren, J. Staymates, J. Lawrence, Rapid detection of fentanyl, fentanyl analogues, and opioids for on-site or laboratory based drug seizure screening using thermal desorption DART-MS and ion mobility spectrometry, Forensic Chem. 4 (2017) 108–115, https://doi.org/10.1016/j.forc.2017.04.001.
[17] E. Sisco, T.P. Forbes, M.E. Staymates, G. Gillen, Rapid analysis of trace drugs and metabolites using a thermal desorption DART-MS configuration, Anal. Methods 8 (35) (2016) 6494–6499, https://doi.org/10.1039/C6AY01851C.
[18] A.D. Lesiak, J.RE. Shepard, Recent advances in forensic drug analysis by DART-MS, Bioanalysis 6 (6) (2014) 819–842.
[19] H. Brown, B. Oktem, A. Windom, V. Doroshenko, K. Evans-Nguyen, Direct Analysis in Real Time (DART) and a portable mass spectrometer for rapid identification of common and designer drugs on-site, Forensic Chem. 1 (2016) 66–73, https://doi.org/10.1016/j.forc.2016.07.002.
[20] E. Sisco, E.L. Robinson, A. Burns, R. Mead, What's in the bag? Analysis of exterior drug packaging by TD-DART-MS to predict the contents, Forensic Sci. Int. 304 (2019) 109939, https://doi.org/10.1016/j.forsciint.2019.109939.
[21] A.H. Grange, G.W. Sovocool, Detection of illicit drugs on surfaces using direct analysis in real time (DART) time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 25 (9) (2011) 1271–1281, https://doi.org/10.1002/rcm.5009.
[22] E. Sisco, T.P. Forbes, Forensic applications of DART-MS: a review of recent literature, Forensic Chem. 100294 (2020).
[23] R.R. Steiner, R.L. Larson, Validation of the direct analysis in real time source for use in forensic drug screening, J. Forensic Sci. 54 (2009) 617–622, https://doi.org/10.1111/j.1556-4029.2009.01006.x.
[24] ASTM International (2014) E1683-02 Standard Practice for Testing the Performance of Scanning Raman Spectrometers.
[25] ASTM International (2014) E1840-96 Standard Guide for Raman Shift Standards for Spectrometer Calibration.
[26] UNODC (2017) Guidelines on handheld Raman field identification devices for seized material.
[27] T.R. Fiorentin, A.J. Krotulski, D.M. Martin, T. Browne, J. Triplett, T. Conti, B. K. Logan, Detection of cutting agents in drug-positive seized exhibits within the United States, J. Forensic Sci. 64 (3) (2019) 888–896, https://doi.org/10.1111/1556-4029.13968.
[28] Administration DE (2018) National drug threat assessment (2018). US Department of Justice.
[29] Administration DE (2019) National Drug Threat Assessment 2019.
[30] Drug Enforcement Administration D.O.J (2018) 2016 Heroin Domestic Monitor Program.
[31] B. Miserez, O. Ayrton, J. Ramsey, Analysis of purity and cutting agents in street mephedrone samples from South Wales, Forensic Toxicol. 32 (2) (2014) 305–310, https://doi.org/10.1007/s11419-014-0232-y.
[32] A. Guirguis, J.M. Corkery, J.L. Stair, S.B. Kirton, M. Zloh, F. Schifano, Intended and unintended use of cathinone mixtures, Hum. Psychopharmacol. Clin. Exp. 32 (3) (2017) e2598, https://doi.org/10.1002/hup.2598.
[33] R. Lian, Z. Wu, X. Lv, Y. Rao, H. Li, J. Li, R. Wang, C. Ni, Y. Zhang, Rapid screening of abused drugs by direct analysis in real time (DART) coupled to time-of-flight mass spectrometry (TOF-MS) combined with ion mobility spectrometry (IMS), Forensic Sci. Int. 279 (2017) 268–280, https://doi.org/10.1016/j.forsciint.2017.07.010.
[34] Gwak S, Almirall JR (2015) Rapid screening of 35 new psychoactive substances by ion mobility spectrometry (IMS) and direct analysis in real time (DART) coupled to quadrupole time-of-flight mass spectrometry (QTOF-MS). https://doi.org/10.1002/dta.1783.
[35] J.-K. Park, A. Park, S.K. Yang, S.-J. Baek, J. Hwang, J. Choo, Raman spectrum identification based on the correlation score using the weighted segmental hit quality index, Analyst 142 (2) (2017) 380–388, https://doi.org/10.1039/C6AN02315K.
[36] C.M. Gryniewicz-Ruzicka, J.D. Rodriguez, S. Arzhantsev, L.F. Buhse, J. F. Kauffman, Libraries, classifiers, and quantifiers: A comparison of chemometric methods for the analysis of Raman spectra of contaminated pharmaceutical

materials, J. Pharm. Biomed. Anal. 61 (2012) 191–198, https://doi.org/10.1016/j.jpba.2011.12.002.
[37] J.D. Rodriguez, B.J. Westenberger, L.F. Buhse, J.F. Kauffman, Standardization of Raman spectra for transfer of spectral libraries across different instruments, Analyst 136 (20) (2011) 4232, https://doi.org/10.1039/c1an15636e.
[38] O. Kudlacek, T. Hofmaier, A. Luf, F.P. Mayer, T. Stockner, C. Nagy, M. Holy, M. Freissmuth, R. Schmid, H.H. Sitte, Cocaine adulteration, J. Chem. Neuroanat. 83-84 (2017) 75–81.
[39] J. Broséus, N. Gentile, F. Bonadio Pont, J.M. Garcia Gongora, L. Gasté, P. Esseiva, Qualitative, quantitative and temporal study of cutting agents for cocaine and heroin over 9 years, Forensic Sci. Int. 257 (2015) 307–313, https://doi.org/10.1016/j.forsciint.2015.09.014.
[40] S. Schneider, F. Meys, Analysis of illicit cocaine and heroin samples seized in Luxembourg from 2005–2010, Forensic Sci. Int. 212 (1-3) (2011) 242–246, https://doi.org/10.1016/j.forsciint.2011.06.027.
[41] European Monitoring Centre for Drugs and Drug Addiction (2020) European Drug Report: Trends and Developments.
[42] System NFLI (2021) NFLIS-Drug 2020 Midyear Report.
[43] C.A.F.O. Penido, M.T.T. Pacheco, R.A. Zángaro, L. Silveira, Identification of different forms of cocaine and substances used in adulteration using near-infrared Raman spectroscopy and infrared absorption spectroscopy, J. Forensic Sci. 60 (2015) 171–178, https://doi.org/10.1111/1556-4029.12666.
[44] A. Haddad, O. Green, J.R. Lombardi, Detection of fentanyl in binary mixtures with cocaine by use of surface-enhanced Raman spectroscopy, Spectrosc. Lett. 52 (8) (2019) 462–472, https://doi.org/10.1080/00387010.2019.1671871.
[45] T.C. Green, J.N. Park, M. Gilbert, M. McKenzie, E. Struth, R. Lucas, W. Clarke, S. G. Sherman, An assessment of the limits of detection, sensitivity and specificity of three devices for public health-based drug checking of fentanyl in street-acquired samples, Int. J. Drug Policy 77 (2020) 102661, https://doi.org/10.1016/j.drugpo.2020.102661.
[46] Forensic Technology Center of Excellence (2018) Landscape Study of Field Portable Devices for Presumptive Drug Testing.
[47] SWGDRUG (2016) Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations.
[48] Spicher C, Yeatman T, Alford I, Waugh L (2016) The Evaluation of Portable Handheld Raman Systems for the Presumptive Identification of Narcotics: Thermo Scientific TruNarc® and Chemring Detection Systems PGR-1064®. Marshall Univ.
[49] E.S. Roberts, B.A. Boudreau, D.W. Brown, K.L. McQuade, E.E. Remsen, Analysis of carbohydrates in Fusarium verticillioides using size-exclusion HPLC – DRI and direct analysis in real time ionization – time-of-flight – mass spectrometry (DART-MS), Anal. Methods 8 (3) (2016) 673–681, https://doi.org/10.1039/C5AY01666E.