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Analysis of benzodiazepines by thermal desorption direct analysis in real time mass spectrometry (TD-DART-MS)†

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One of the several classes of novel psychoactive substances (NPSs) that present analytical challenges for forensic chemists is benzodiazepines. Like other NPS classes, the emergence of new compounds within this class continues, creating a need for the development of new techniques and methods that allow for rapid detection and identification of these compounds in forensics laboratories. This work investigates the use of thermal desorption direct analysis in real time mass spectrometry (TD-DART-MS) as a tool for the rapid and sensitive detection of benzodiazepines. A suite of 19 benzodiazepines were investigated to determine their representative responses. The limits of detection (LODs) for these compounds were found to range from 0.05 ng to 8 ng. Competitive ionization studies highlighted that the detection of these compounds in the presence of cutting agents and low amounts of heroin was possible. Additionally, the presence of three complex background matrices that are common in trace detection applications (artificial fingerprint residues, dirt, and plasticizers) was investigated and was shown to have a minimal effect on the detection of these compounds. TD-DART-MS was demonstrated as a potentially powerful tool for rapid on-site or laboratory-based screening.

Introduction

According to the National Institute on Drug Abuse, 70237 Americans died from drug overdose in 2017. Amongst those deaths, 11537 (or roughly 16%) involved the use of one or more benzodiazepines (BZDs).[1] BZDs are a part of a widespread class of drugs used primarily as anxiolytics, sedatives, hypnotics, anticonvulsants, and muscle relaxants[2] that have been prescribed in the United States over the last two decades. The increased prescription of BZDs is coupled with a rise in non-medical use and abuse among adolescents and adults,[3–5] driven by a marked growth of counterfeit pharmaceutical tablets.[6]

Like synthetic opioids and other classes of novel psychoactive substances (NPSs), BZDs have a range of structural and functional analogs that are being clandestinely produced. These designer BZD analogs are often marketed as "legal highs" and sold as research chemicals. They are sold in many different forms: tablets, powders, pellets, blotters, and capsules. Many of the tablets are designed and manufactured to be visually indistinguishable from genuine pharmaceutical products.[7] These tablets often include one or more designer BZDs, at inconsistent concentrations, and have also been found to contain synthetic opioids, leading to numerous fatalities in unsuspecting users.[8] The rise of BZD use and the increased prevalence of BZD analogs have brought about the need for analytical methods and tools that can accurately detect and identify known BZD analogs and that will be able to identify new BZD analogs when they are encountered.

The forensic analysis of drug evidence often requires the use of two different types of techniques. The primary technique is a confirmatory technique that provides a definitive identification of the exact drug, or drugs, present in a sample. Analysis of BZDs, both traditional and designer, has been demonstrated across a number of different confirmatory techniques including liquid chromatography mass spectrometry (LC-MS),[9–11] high-performance liquid chromatography,[9,12,13] gas chromatography (GC)-MS,[9,14,15] GC-ion trap tandem-MS,[16] Fourier transform infrared (FTIR) and Raman spectroscopy.[17–20] The second type of analysis, which precedes confirmatory analyses, is screening techniques. Techniques in this category are used to provide chemists with an idea of what type of drug is present in the sample in a rapid and sensitive manner. Ideal screening techniques are rapid, able to detect a wide range of drug classes, and are as sensitive as the confirmatory technique(s), to minimize the chance of false negatives. A number of screening techniques have been investigated for BZD detection, though many have limitations which present difficulties or uncertainties in detecting emerging BZD analogs. Colorimetric tests for BZDs exist but there is not a universally applicable test for the entire

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class of compounds.[21–23] The use of microcrystalline tests has been shown but has not been extensively studied for BZD analogs.[24] Thin Layer Chromatography (TLC) has been demonstrated but the approach is time consuming, requiring at least 2 to 3 solvent systems to obtain sufficient separation.[21,25] Ion mobility spectrometry has been used for the rapid detection of benzodiazepines,[26] but poor resolution can lead to a high degree of false positives with benign compounds. Portable Raman systems have been used to analyze pharmaceutical BZDs, but the presence of cutting agents can cause difficulties in the detection of BZDs.[17]

Another screening technique that has been demonstrated is direct analysis in real time (DART)-MS.[27] DART involves an ambient ionization source that is capable of quickly (less than 10 s) desorbing and ionizing a sample with minimal sample preparation.[7,28,29] Compared to other screening tools, DART-MS is relatively new but is seeing increased use in forensic laboratories due to its ability to accurately screen for a wide range of compounds.[30] One of the downsides to DART-MS is the need to manually introduce the sample in the sampling region, commonly *via* a glass microcapillary, which can result in poor sample-to-sample reproducibility.[29] In recent years a variation of DART-MS, thermal desorption (TD)-DART-MS, has been developed to help address sample reproducibility issues.[29,31,32] Unlike traditional DART-MS, TD-DART-MS utilizes an independent thermal desorber and glass T-junction configuration along with wipe-based sample introduction, to allow samples to be reproducibly introduced and desorbed, thereby increasing sample-to-sample repeatability. This variation also minimizes potential exposure of the analyst *via* aerosolization and/or vaporization because of the confined configuration and active pull of vapors towards the mass spectrometer.[29,31] The use of TD-DART-MS has been demonstrated for the detection of drugs of abuse,[29] including synthetic opioids,[31] as well as other compounds of interest, such as rodenticides, which can be spiked into drugs.[33] It has been demonstrated as a viable tool for the presumptive analysis of drug evidence, through collection and analysis of trace residues on the exterior of drug packaging,[34] in addition to more traditional forensic analyses. Additionally, because it is a mass spectrometry-based technique, detection and identification of previously unseen BZDs is more easily achieved than with other screening techniques.

This study investigates the ability of TD-DART-MS to detect a range of BZD analogs. Like other NPS classes, a number of challenges in BZD detection must be understood, including the ability to detect BZDs that are structurally similar as well as structurally different, the ability to detect BZDs in the presence of common cutting agents or excipients, and, if trace residue applications are desired, the ability to detect these compounds at low concentrations in complex background matrices. The use of a technique such as this could provide substantial progress in reducing case backlogs and turnaround times.

Experimental

Materials

A complete list of the 19 BZDs investigated in this work is shown in Table 1. All compounds were purchased from Cerilliant (Round Rock, TX, USA) as 1 mg mL^{-1} methanolic solutions. The compounds were further diluted in methanol (Chromasolv grade, Sigma-Aldrich, St. Louis, MO, USA) to 100 μg mL^{-1}, 10 μg mL^{-1}, and 1 μg mL^{-1} to allow for easy deposition of the desired masses. Solutions were either inkjet printed (for optimization studies) or pipetted (for all remaining studies) onto polytetrafluoroethylene (PTFE)-coated fiberglass wipes (DSA Detection, North Andover, MA, USA) for analysis. All samples were allowed to dry prior to analysis. The four cutting agents examined (stearic acid, mannitol, lactose, and caffeine) were purchased in a powder form (Sigma-Aldrich, St. Louis, MO, USA) and dissolved in methanol. Heroin was purchased as a 1 mg mL^{-1} methanolic solution from Cerilliant. Materials used as background simulants included artificial fingerprint materials,[35] a standard reference material sediment (NIST SRM 1944, New York/New Jersey Waterway Sediment) to mimic dirt, and a polypropylene bag. To test the ability of the technique to analyze real samples, nine adjudicated case samples, consisting of powder from crushed tablets, were obtained from the Maryland State Police Forensic Sciences Division (MSP-FSD), and dissolved in methanol. Polyethylene glycol – 600 (PEG-600) (Sigma-Aldrich, St. Louis, MO, USA) diluted in methanol was used as the calibration compound for the mass spectrometer.

Inkjet printing parameters

A custom drop-on-demand Jetlab 4 XL-B (MicroFab Technologies, Plano, TX) inkjet printer was used to deposit precise

Table 1 List of benzodiazepines examined in this study. Molecular weights listed are the monoisotopic molecular weights. Compounds with an asterisk (*) are Food and Drug Administration (FDA) approved

Compound name	Formula	MW (Da)	Compound name	Formula	MW (Da)
Alprazolam*	$C_{17}H_{13}ClN_4$	308.083	Flunitrazepam*	$C_{16}H_{12}FN_3O_3$	313.086
Bromazepam*	$C_{14}H_{10}BrN_3O$	315.001	Lorazepam*	$C_{15}H_{10}Cl_2N_2O_2$	320.012
Clonazepam*	$C_{15}H_{10}ClN_3O_3$	315.041	Medazepam*	$C_{16}H_{15}ClN_2$	270.092
Clonazolam	$C_{17}H_{12}ClN_5O_2$	353.068	Midazolam*	$C_{18}H_{13}ClFN_3$	325.078
Deschloroetizolam	$C_{17}H_{16}N_4S$	308.110	Nimetazepam*	$C_{16}H_{13}N_3O_3$	295.096
Diazepam*	$C_{16}H_{13}ClN_2O$	284.072	Oxazepam*	$C_{15}H_{11}ClN_2O_2$	286.051
Diclazepam	$C_{16}H_{12}Cl_2N_2O$	318.033	Quazepam*	$C_{17}H_{11}ClF_4N_2S$	386.027
Estazolam*	$C_{16}H_{11}ClN_4$	294.067	Temazepam*	$C_{16}H_{13}ClN_2O_2$	300.067
Etizolam	$C_{17}H_{15}ClN_4S$	342.071	Zolazepam	$C_{15}H_{15}FN_4O$	286.123
Flubromazolam	$C_{17}H_{12}BrFN_4$	370.023			

amounts of select BZDs onto wipes from methanolic solutions. Detailed printing parameters are provided elsewhere.[31] A printed mass of 10 ng per wipe was achieved using a two by two deposition array.

Thermal desorption direct analysis in real time mass spectrometry (TD-DART-MS) parameters

TD-DART-MS, a variant of traditional DART-MS which includes a thermal desorption unit independent of the DART ionization source was used. In-depth details of this configuration have been previously reported.[31,33] Briefly, the configuration utilizes an on-axis DART-SVP source (IonSense, Saugus, MA, USA), in-line with a Vapur interface (IonSense) that is mounted to a mass spectrometer. An insulated glass T-junction is placed between the source and the interface. The thermal desorber (Morpho Detection, Newark, CA, USA) is press-fit directly onto the arm of the bottom junction and provides temperature control up to 300 °C. In this study, the parameters of the system included (unless otherwise noted) a 400 °C DART gas stream temperature, a 200 °C thermal desorber temperature, and a Vapour flow rate of 4.0 L min^{-1}.

The TD-DART system was interfaced to a JEOL JMS-T100LP time-of-flight mass spectrometer (JEOL USA, Peabody, MA, USA). Relevant MS parameters included operation in positive ionization mode, a +400 V peak voltage, a 100 °C orifice temperature, a +5 V orifice 2 and ring lens voltage, a 2300 V detector voltage, and a scan range of *m/z* 60 to *m/z* 800 at 1 scan s^{-1}. Four different orifice 1 voltages were used (+20 V, +30 V, +60 V, and +90 V) to evaluate is-CID fragmentation spectra. Unless otherwise stated, the data presented herein are from an orifice 1 voltage of +20 V.

Gas chromatography-mass spectrometry (GC-MS) parameters

GC-MS was used to both confirm the presence of BZDs in the nine adjudicated case samples that were analyzed and to quantify the amount of alprazolam present in the samples. The GC-MS system used was a Thermo Trace 1310 GC combined with a TSQ 8000evo triple quadrupole MS (Waltham, MA, USA). A Restek RTX-5 column (20 m × 0.25 mm × 0.25 μm) was used. A generic screening method was employed to confirm the presence of BZDs in the adjudicated case samples. Parameters included a 10 : 1 split ratio, an inlet temperature of 300 °C, a constant flow rate of 1.8 mL min^{-1}, and a temperature program of 150 °C held for 1.5 min followed by a ramp at 10 °C min^{-1} to 300 °C which was then held for 6 min. A transfer line and MS source temperature of 300 °C were used along with a scan range of *m/z* 50 to *m/z* 400 at 0.2 s per scan. A second method was used for quantification of alprazolam. This method used SRM mode, monitoring transitions for both alprazolam and flubromazolam, (used as an internal standard). The MS/MS transitions were *m/z* 222 → *m/z* 175 and *m/z* 341 → *m/z* 261 for flubromazolam and *m/z* 273 → *m/z* 245 and *m/z* 308 → *m/z* 273 for alprazolam. Additional parameters for this method included a 10 : 1 split ratio, an inlet temperature of 300 °C, a constant flow of 1.9 mL min^{-1}, an isothermal oven temperature of 300 °C for a total runtime of 5 min, and a 300 °C transfer line and MS source temperature. The response from the case samples was interpolated on a calibration curve (100 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 20 μg mL^{-1}, 15 μg mL^{-1}, 10 μg mL^{-1}, and 5 μg mL^{-1}), which, was used to calculate the weight percent of alprazolam in each tablet. Case samples were prepared by weighing out 3 mg to 5 mg of powder and dissolving in 1.5 mL of methanol. The LOD of the method was approximately 1 μg mL^{-1}.

Results and discussion

TD-DART-MS method optimization

A method for BZD analysis was optimized using the lower (medazepam), approximate mean (clonazepam), and highest (quazepam) molecular weight compounds investigated. Based on previous experience with TD-DART-MS method optimization for several classes of drugs,[29,31,34] four instrumental parameters were optimized: the DART ionization gas, Vapur flow rate, thermal desorber temperature, and exit grid voltage. These parameters were studied in a parametric fashion by investigating the change in signal intensity of the base peak of the three representative BZDs across the range of possible values for that parameter. For all optimizations the mass of BZD deposited on each wipe was 10 ng.

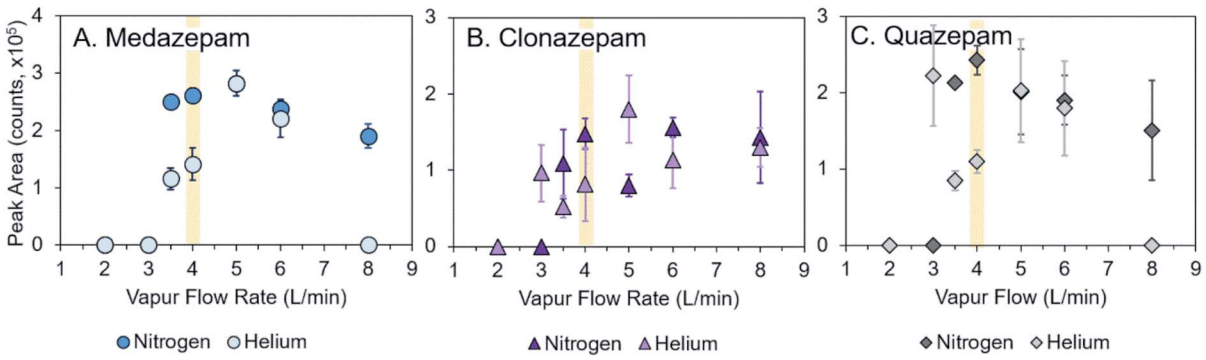

Fig. 1 Effect of the DART ionization gas (series) and Vapur flow rate (*x*-axis) on the peak area for medazepam (A), clonazepam (B), and quazepam (C). Datapoints represent the average area of the protonated molecule across five replicate analyses. Uncertainties are one standard deviation of the replicate measurements. The datapoints highlighted in yellow show the value chosen for the optimized method.

The use of nitrogen or helium as the DART ionization gas was compared across a range of Vapur flow rates to simultaneously optimize both parameters. The Vapur flow, which controls the rate at which a gas is pulled towards the MS inlet, was varied from 2 L min^{-1} to 8 L min^{-1} with a constant desorber temperature of 275 °C. Fig. 1 shows the effect of Vapur flow rate on the integrated peak areas of the protonated molecule. Nitrogen (darker shaded datapoints) was found to produce a more consistent response across the different Vapur flow rates for the three representative compounds. Nitrogen also provided better reproducibility in signal, with generally lower standard deviations. Better signal reproducibility with nitrogen is believed to be caused by enhanced mixing between the DART ionization gas and analyte vapors within the T-junction.[33] An optimal Vapur flow rate of 4 L min^{-1} was chosen as it provided the maximum, or close to the maximum, signal with good reproducibility. The signal intensity decreased as the flow rate decreased from 4 L min^{-1}, likely due to insufficient flow to actively pull analyte vapor towards the MS inlet. At higher Vapur flow rates the residence time of analyte molecules in the T-junction is likely not sufficient for ionization.

The thermal desorber temperature is a critical parameter to optimize as it controls the rate and efficacy of analyte molecules desorbed off the wipe material. Using nitrogen as the DART gas and a Vapur flow rate of 4 L min^{-1}, the thermal desorber temperature was studied across the range of 150 °C to 300 °C, in 25 °C increments (Fig. 2). Medazepam produced a consistent response across the temperature range. The Clonazepam signal was found to decrease significantly at temperatures below 200 °C, likely due to poor desorption, and at temperatures higher than 250 °C, likely due to thermal degradation. The signal for quazepam decreased at desorber temperatures above 225 °C, and was undetectable at 150 °C. This resulted in an optimal temperature range of 200 °C to 225 °C. A desorber temperature of 200 °C was chosen as it provided a slightly higher signal for quazepam, compared to 225 °C, and a near equivalent signal for medazepam and clonazepam.

The last parameter optimized was the exit grid voltage, which prevents ion–ion recombination. Fig. 3 presents the response of medazepam, clonazepam, and quazepam at exit grid voltages ranging from 0 V to 300 V, in 50 V increments. Minimal variation in signal was observed across the range of exit grid voltages, which is consistent with previous work. An optimal exit grid voltage of 250 V was chosen as it provided acceptable reproducibility and spectral responses for all three compounds. The final parameters for the optimized method included the use of nitrogen as the DART ionization gas, a Vapur flow rate of 4 L min^{-1}, a thermal desorber temperature of 200 °C, and an exit grid voltage of 250 V.

Representative response and sensitivity

Once established, the optimized parameters were used to collect representative spectra and create a library for the suite of 19 BZDs. Spectra were obtained using a deposited mass of 25 ng. All compounds were analyzed at four orifice 1 voltages (+20 V, +30 V, +60 V, and +90 V) in order to be consistent with previous DART-MS work[27] and gain insight into the types of fragment ions that are formed during is-CID fragmentation.

Fig. 4 shows the representative mass spectra of alprazolam at the four investigated orifice 1 voltages. The spectra of all other compounds can be found in the ESI.† As with most studied BZDs, the response of alprazolam was dominated by the protonated molecule at low orifice 1 voltages of +20 V and +30 V while increasing fragmentation was observed at the highest (+90 V) orifice 1 voltage. Both the protonated molecule and the

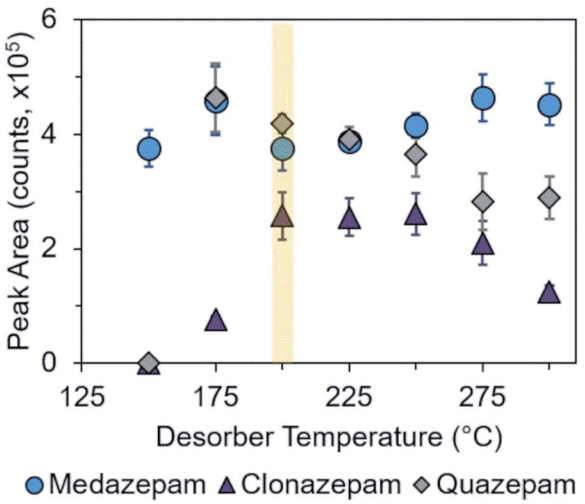

Fig. 2 Effect of the desorber temperature on the response of medazepam (blue circle), clonazepam (purple triangle), and quazepam (grey diamond). Datapoints represent the average area of the protonated molecule across five replicate analyses. Uncertainties show the standard deviation of the replicate measurements. The datapoints highlighted in yellow show the value chosen for the optimized method.

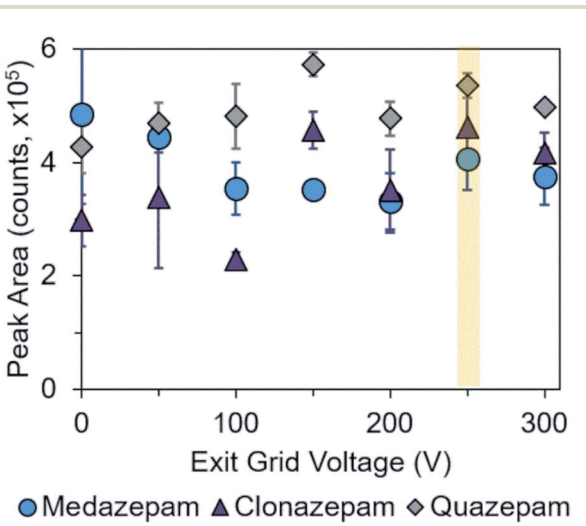

Fig. 3 Effect of the exit grid voltage on the response of medazepam (blue circle), clonazepam (purple triangle), and quazepam (grey diamond). Datapoints represent the average area of the protonated molecule across five replicate analyses. Uncertainties are one standard deviation of the replicate measurements. The datapoints highlighted in yellow show the value chosen for the optimized method.

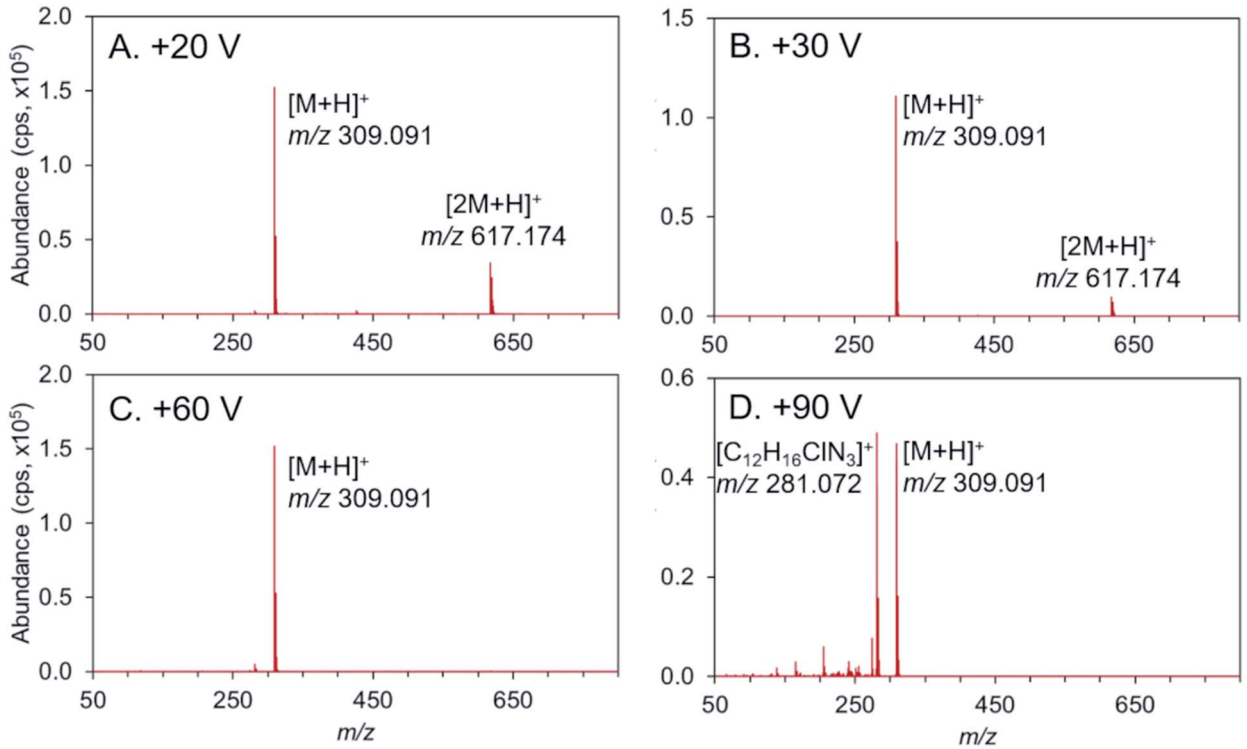

Fig. 4 Representative TD-DART-MS mass spectra of 25 ng of alprazolam at orifice 1 voltages of +20 V (A), +30 V (B), +60 V (C), and +90 V (D).

protonated dimer were observable at the +20 V and +30 V orifice 1 voltages, with the dimer being completely dissociated at the +60 V level. Significant fragmentation of alprazolam occurred at +90 V with *m/z* 281.072 being the base peak, corresponding to the loss of CH_2N. While most BZDs followed this trend, there were some outliers. Lorazepam (Fig. S11†) and oxazepam (Fig. S15†) readily underwent loss of a hydroxide, even at low orifice 1 voltages.

To obtain an accurate measure of the limit of detection (LOD), five compounds (clonazepam, diazepam, flubromazolam, medazepam, and quazepam) were analyzed in accordance with procedures outlined in ASTM E2677.[36] Using this approach, ten replicates of each compound at four masses (1 ng, 5 ng, 10 ng, and 25 ng) were analyzed along with ten blank wipes. The integrated peak areas at *m/z* values corresponding to the protonated molecule were then obtained from the +20 V orifice 1 spectra. The calculated LODs, at a 90% confidence interval (LOD_{90}), are shown in Table 2. In all cases, LODs below 10 ng were obtained. For the remaining BZDs, approximate LODs were established by analyzing decreasing masses of the compounds until a signal to noise (S/N) ratio near, but not below, 3 : 1 for triplicate samples was obtained. Table 3 shows the approximate LODs, which were found to be in the range of 0.05 ng per wipe to 1 ng per wipe. The average S/N ratios of the triplicate samples are also shown.

Table 2 Calculated limits of detection (LOD_{90}) for a select set of compounds. Also presented is the 90% upper confidence limit for the reported LOD_{90}s

Compound name	LOD_{90} (ng)	90% Upper confidence limit (ng)
Clonazepam	8.03	11.50
Diazepam	1.64	2.23
Flubromazolam	1.74	2.48
Medazepam	1.87	2.78
Quazepam	1.54	4.91

Competitive ionization of benzodiazepines in binary mixtures

While sensitive detection of these compounds is important, if the technique is to be used in a trace detection application, such as for the analysis of drug packaging residues, it is necessary to understand the effect of other compounds on detection capabilities. A series of studies were completed to measure the competitive ionization effect that common cutting agents (caffeine, lactose, mannitol, and stearic acid) and heroin has on BZDs. The competitive ionization studies were completed by creating binary mixtures containing a static amount (20 ng) of the representative BZD along with increasing amounts of the competing compound (cutting agent or heroin). The levels of the competing compound were 0 ng (just the BZD analyzed), 20 ng, 100 ng, 200 ng, and 1000 ng to obtain the competing compound : BZD ratios of 1 : 1, 5 : 1, 10 : 1 and 50 : 1. The extent of competitive ionization occurring was measured by normalizing the response of the BZD in the binary mixture to the pure BZD signal using peak areas from data collected at an orifice 1 voltage of +20 V.

Fig. 5 highlights the effect of the competing compounds on the signal of the three representative BZDs. In the figures, the

Table 3 Approximate LODs for 14 of the studied compounds. The reported average S/N ratio is the mean of triplicate measurements. Uncertainties are one standard deviation of the measurements

Compound name	Approximate LOD (ng)	Average S/N ratio	Compound name	Approximate LOD (ng)	Average S/N ratio
Alprazolam	0.20	23.4 (±11.5)	Flunitrazepam	0.10	18.3 (±15.5)
Bromazepam	0.50	20.3 (±7.2)	Lorazepam	1.00	19.8 (±8.4)
Clonazolam	1.00	25.1 (±16.2)	Midazolam	0.10	22.3 (±4.0)
Deschloroetizolam	0.10	26.9 (±5.8)	Nimetazepam	0.10	18.1 (±12.7)
Diclazepam	0.10	11.3 (±6.1)	Oxazepam	0.50	10.4 (±3.1)
Estazolam	0.20	7.2 (±3.0)	Temazepam	0.20	7.6 (±3.5)
Etizolam	0.10	11.4 (±4.4)	Zolazepam	0.05	22.5 (±30.4)

dotted red line represents the normalized average response of 20 ng deposits of the BZD. If points fall below the dotted line it indicates that suppression of the BZD through competitive ionization is occurring whereas if points fall above the line it indicates an enhancement in ionization from the competing compound. All of the studied cutting agents (Fig. 5A–D) showed some degree of competitive ionization with the BZDs, though the BZD was still detectable in all cases. At most, an approximately 60% reduction in signal was observed.

Possibly more concerning than the cutting agent mixture response is that of BZD in the presence of heroin (Fig. 5E). While the medazepam signal stayed relatively constant with increasing amounts of heroin, there was a rapid decrease in the BZD peak area for quazepam and clonazepam. At a 50 : 1 ratio detection of quazepam or clonazepam was not possible. While detection at low relative weight percentages was hindered, for screening purposes this may not be problematic. Even though detection of the BZD was not possible, detection of heroin at these higher levels was readily achieved and therefore a controlled substance would be identified in the mixture.

Detection of benzodiazepines in background matrices

If a technique were to be used with trace detection capacity, detection of BZDs must be achieveable not only in the presence of competing compounds but also in the presence of background matrices. In scenarios encountered by law enforcement or forensic scientists, samples could be collected off materials that were not stored under ideal conditions, and therefore would likely contain phthalates and plasticizers from plastic

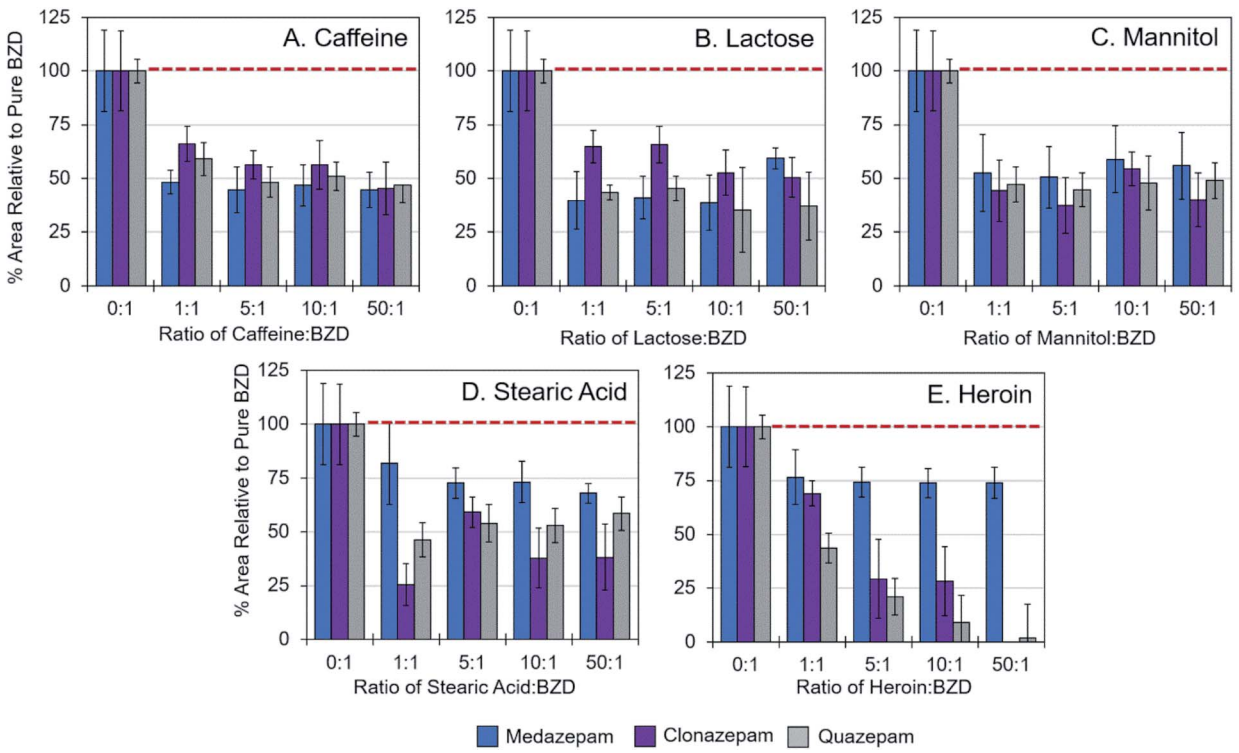

Fig. 5 Competitive ionization studies for medazepam (blue), clonazepam (purple), and quazepam (grey) in the presence of increasing amounts of (A) caffeine, (B) lactose, (C) mannitol, (D) stearic acid, and (E) heroin. Data represent the average of five measurements with uncertainties representing the standard deviation of that measurement. The dotted red line indicates the normalized average signal of the pure BZD.

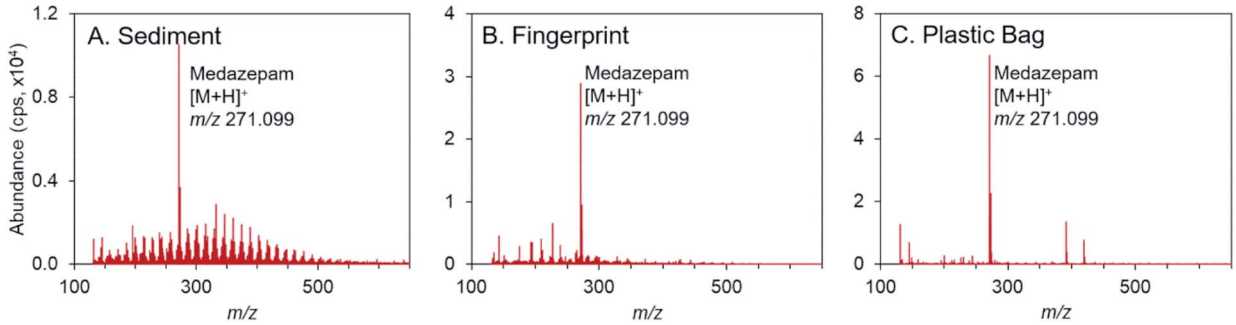

Fig. 6 Representative mass spectra of medazepam in the presence of SRM-dirt (A), an artificial fingerprint material (B), and off a plastic bag (C) at +20 V.

bags, fingerprint residues, and/or dirt and dust. To evaluate whether these background matrices would complicate the detection of BZDs, analysis of trace levels of BZDs in the presence of matrices was examined. The background matrices used included the standard reference material sediment to simulate dirt (NIST SRM 1944), artificial fingerprint residues,[35] and a polypropylene bag.

Dirt presents several challenges for trace detection, namely the presence of trace organics, trace inorganics, and particulates which can clog the instrumentation. NIST SRM 1944 contains a number of organic and inorganic constituents, as well as particulates with a mean diameter of approximately 150 μm. The organic fraction of this SRM contains a range of polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, polychlorinated dibenzo-*p*-dioxins, and dibenzofurans. The effect of dirt on the detection of BZDs was examined by depositing approximately 50 μL of an aqueous suspension of NIST SRM 1944 (New York/New Jersey Waterway Sediment) onto a wipe along with 20 ng of one of the three representative BZDs. The wipe was allowed to dry before being directly analyzed. Fig. 6A highlights the detection of medazepam in this matrix using TD-DART-MS, with a strong signal for the protonated molecule.

Along with dirt or dust, it is likely that a surface containing trace narcotics may also contain fingerprints. An artificial fingerprint material containing over forty compounds commonly found in fingerprint residues at biologically relevant concentrations was used as the second matrix. Approximately 3 μg of the fingerprint material and 20 ng of the representative BZDs were deposited directly onto PTFE wipes and analyzed. The resulting spectrum of medazepam is shown in Fig. 6B, which highlights the detection of medazepam in the presence of this complex matrix.

To study the effect of phthalates and plasticizers on BZD detection a wipe was used to collect BZD deposits from a plastic bag. The representative BZDs were deposited (20 ng) onto a polypropylene bag and allowed to dry. The entirety of the bag was then wiped and analyzed. The BZD was rapidly detected by TD-DART-MS after being collected off of the plastic bag, as shown in Fig. 6C. Medazepam exhibited the highest signal in all of the spectra.

Analysis of adjudicated case samples

While analysis and detection of standards is important to understand how the instrument is expected to perform, it is critical to also evaluate it through the use of real-world samples. To do this, nine adjudicated case samples obtained from MSP-FSD were analyzed. The samples were prepared for TD-DART-MS by depositing 2 μL of a methanolic solution obtained using a small amount (<5 mg) of powder directly onto a wipe. Using the optimized TD-DART-MS method, all nine case samples were screened and searched against the library that was created using the representative spectra. All samples were found to have alprazolam, a finding which was confirmed using gas chromatography mass spectrometry (GC-MS). Fig. 7 shows the TD-DART-MS mass spectrum and corresponding gas

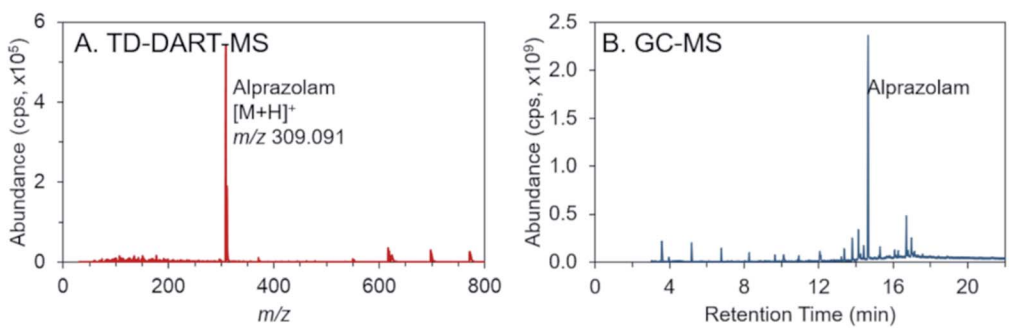

Fig. 7 Representative TD-DART-MS mass spectrum of an adjudicated case sample (A) and the corresponding GC-MS chromatogram (B).

chromatogram for one of the case samples. Quantification of the alprazolam in the case samples was also completed by GC-MS and resulted in a weight percent ranging from a mass fraction of 0.54% to 1.20%.

Conclusions

Rapid detection of BZDs with nanogram sensitivity is achievable using TD- DART-MS. While detection of pure compounds is simple, competitive ionization can occur in the presence of mixtures and matrices. The presence of common cutting agents and other drugs, such as heroin, was found to cause varying degrees of signal suppression due to competitive ionization. Complex matrices, including artificial fingerprint residues, dirt, and plastic bag residues did not prevent detection of BZDs.

While the use of TD-DART-MS in this manner is not quantitative, it does prove to be a qualitative tool to help screen for BZDs in bulk evidence and in trace samples obtained from wiping evidence or an environment. This work highlights the ability of TD- DART-MS to rapidly detect BZDs. The detection of low sample quantities can be advantageous to forensic scientists as it provides a sensitive and rapid analysis of target analytes, using wipes, off evidence packaging, evidence itself, or extracts. This instrument provides potential avenues for rapid on-site or laboratory-based screenings from mobile units to forensics laboratories as well as improved confidence in compound identification when fragmentation is used.

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.

Conflicts of interest

There are no conflicts to declare.

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