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PAPER

CRIMINALISTICS

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Detection of Brodifacoum and other Rodenticides in Drug Mixtures using Thermal Desorption Direct Analysis in Real Time Mass Spectrometry (TD-DART-MS)†

ABSTRACT: In recent months, there has been increased reporting of seized drug and toxicology cases containing rodenticides, the active ingredient in rat poisons. Seeing as rodenticides are not scheduled substances, they are not commonly screened for in seized drug analysis. This work investigates the use of TD-DART-MS for the simultaneous detection of rodenticides and drugs. Six rodenticides were evaluated, an optimal method was established, and limits of detection in the tens of nanograms were calculated. Additional studies highlight that detection at less than 1% by weight in mixtures with AB-FUBINACA, cocaine, heroin, or methamphetamine was possible. This work presents an optimized method for detection of these compounds, allowing for the simultaneous detection of drugs and rodenticides, providing drug chemists with a tool for rapid identification of these compounds for forensic or public health purposes.

KEYWORDS: forensic science, rodenticides, DART-MS, drug analysis, brodifacoum, synthetic cannabinoids, screening

Drug chemists are being asked to complete increasingly more complicated analyses, in part due to the ever-expanding world of novel psychoactive substances (NPS). However, as other noncontrolled, but toxic, compounds are added to street samples, the complications become even greater. One such class of toxic compounds that is being increasingly found in drug samples is rodenticides (also known as rat poisons or superwarfarins). The presence of these chemicals in street samples has prompted warnings from the Centers for Disease Control (CDC)(1,2) and the National Institute for Drug Abuse (NIDA)(3) in recent months.

Rodenticides are commonplace chemicals that can be found in many homes, hardware stores, and big box stores and can be purchased in many forms including blocks, pellets, and powders. More potent compounds are also used in commercial applications. These compounds can be divided into several classes, two of which are first-generation and second-generation rodenticides. First-generation rodenticides represent compounds such as chlorophacinone, diphacinone, and pindone which typically require higher doses and concentrations to achieve the desired outcomes. Second-generation rodenticides such as brodifacoum and bromadiolone are substantially more toxic, because of a higher binding affinity to anti-coagulant enzymes, achieving the desired outcomes in a single dose at a lower concentration (4). Of the available compounds, brodifacoum is the most commonly used commercial product because of its high mortality rate in rodents and lack of known resistance in pests (5) while diphacinone is the most common household compound. In addition to their use as rodenticides, these chemicals have the potential to be used as chemical warfare agents in a terrorist type attack (6).

Rodenticides in the drug supply are not a new issue, though recent reports (1,3) have increased awareness of these compounds in synthetic cannabinoids. Other reports and case studies have shown that poisoning by brodifacoum is not limited to this class of compounds. Rodenticides have also been reported in cocaine (7), heroin (8), methamphetamine, and marijuana (9,10). In the literature, detection of rodenticides has been primarily focused on toxicological samples or environmental samples, to identify potential threats to animal life or human life due to accidental or intentional release. For toxicological sampling, liquid chromatography tandem mass spectrometry (LC/MS/MS)(11–13) and gas chromatography mass spectrometry (GC/MS)(14) have been used to analyze blood samples from humans as well as animals (15,16). These techniques are extremely sensitive, being able to quantify single nanograms of rodenticide per milliliter of blood (16); however, they unfortunately require lengthy sample preparation and run times. Similar techniques, namely LC-UV or LC/MS/MS, have also been used for environmental samples to monitor brodifacoum levels in areas after a major release of the chemical (17–19). Detection of these compounds in toxicological samples of patients who were exposed during drug use has also been reported (20).

While analyzing toxicological samples to determine the presence of rodenticides in a drug overdose patient is critical, rapid detection of these compounds in the actual street sample, or

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†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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residue from the street sample, can provide information to investigators and public health informants that can aid in reducing the likelihood of additional exposures. One technique, that is currently deployed in some forensic laboratories, that has been shown to be able to rapidly detect low levels of compounds in complex matrices is direct analysis in real time mass spectrometry (DART-MS). DART-MS has been shown to readily detect a range of chemicals from drugs (21) to explosives (22,23) to chemical warfare agents (24) at low nanogram sensitivity. Furthermore, DART-MS has been shown to readily detect a number of pesticides off of foods and wipes of surfaces (25). This work focuses on investigating whether this technique can provide rapid, sensitive detection of rodenticides in the presence of drugs of abuse using a variation of this technique—thermal desorption DART-MS (TD-DART-MS). In addition to developing a method and understanding the sensitivity of the instrument for detection of these compounds, the ability to detect rodenticides in the presence of AB-FUBINACA (a synthetic cannabinoid), cocaine, heroin, and methamphetamine was also explored. TD-DART-MS was shown to be a sensitive, rapid tool for detection of rodenticides in drugs with minimal to no sample preparation, providing a viable tool for obtaining and relaying forensic and public health information.

Materials and Methods

Materials and Chemicals

A total of six rodenticides were examined in this work, brodifacoum, bromadiolone, chlorophacinone, difenacoum, diphacinone, and pindone, and were purchased as 0.1 mg/mL solutions in methanol or acetonitrile from AccuStandard (New Haven, CT, USA). Samples were further diluted, as required, using methanol (Chromasolv Grade, Sigma-Aldrich, St. Louis, MO, USA). For the competitive ionization studies, 1 mg/mL solutions of AB-FUBINACA, cocaine, heroin, and methamphetamine were used either neat or diluted in methanol. AB-FUBINACA was acquired from Cayman Chemical (Ann Arbor, MI, USA) while the remaining drug standards were acquired from Cerilliant (Round Rock, TX, USA). All samples were pipetted (1–5 μL) onto the center of the desorbed area of the PTFE-coated fiberglass wipes (DSA Detection, North Andover, MA, USA) for analysis. McCormick gourmet organic Oregano was purchased from a local grocery store. Seized plant material was provided by Maryland State Police Forensic Sciences Division and was known to contain 4-fluoro ADB, MMB-FUBINACA, and ADB-FUBINACA.

Instrumentation

All analyses were completed using a thermal desorption configuration of direct analysis in real time mass spectrometry (TD-DART-MS). The setup, which is described elsewhere in detail (26), consists of a JMS-T100LP time-of-flight mass spectrometer (JEOL USA, Peabody, MA, USA) coupled with a DART-SVP ion source (IonSense, Saugus, MA, USA) and an in-house built thermal desorption unit attached to the mass spectrometer with the Vapur® interface. The TD-DART-MS configuration was used, instead of the traditional DART-MS configuration, because it provided increased reproducibility, enhanced sensitivity, and lower exposure risks to the analyst while also allowing for wipe-based sample introduction.

Unless otherwise stated, the following parameters were used. DART specific parameters included a source gas of zero-air nitrogen, a gas stream temperature of 400°C, and an exit grid voltage of ±100 V. A thermal desorber temperature of 250°C and a Vapur flow rate of 4 L/min were employed. Mass spectrometer parameters included operation in positive ionization mode with an orifice temperature of 120°C, an orifice 2 and ring lens voltage of ±5 V, a peaks voltage of ±400 V, and a detector voltage of 2300 V. For the representative response section, an orifice 1 voltage switch between +20, +30, +60, and +90 V was employed; all other analyses used only the +20 V orifice 1 voltage. Data were collected from 60 to 700 *m/z* at 1 sec/scan.

Results and Discussion

Method Optimization

The first component of this study was focused on development of an optimized method for the detection of the rodenticide compounds, and comparison of that method to previous work for drug detection using the TD-DART-MS platform. Two analytes, difenacoum and diphacinone, were studied in-depth for the method optimization portion, as they represented the two base structures for 3 of the 4 remaining analytes. Using the previous parameters for drug detection (26) as a starting point, three parameters were optimized: thermal desorber temperature, Vapur® flow rate, and DART source gas. Since the goal of this work was simultaneous detection of rodenticides and drugs, analysis was completed in positive ionization mode.

The thermal desorber temperature and Vapur flow rate were optimized concurrently by evaluating a matrix of four temperatures (225, 250, 275, and 300°C) and three flow rates (4, 6, and 8 L/min). Optimization was completed by analyzing 50 ng/swipe deposits of difenacoum and diphacinone and integrating the area under the peak from the extracted ion chronographs (EIC) of the protonated molecule peaks. The results of the optimization are shown in Fig. 1. Consistent with previous work(26,27), a low flow rate (Fig. 1, blue circles) was found to produce the highest signal for both rodenticides across the range of thermal desorber temperatures. This was expected because higher flow rates contribute to a shorter residence time in the ionization region, which lowers the probability for ionization. Similarly, higher flow rates can cause cooling within the thermal desorber, because of the increased pull of room air through the desorber which can hinder thermal desorption of the analyte and contribute to condensation within the thermal desorber or glass tubing. An optimal thermal desorber temperature of 250°C was observed for both compounds, providing sufficient heat to desorb the molecule off the wipe without causing flash desorption or thermal decomposition, which can be observed at high heating temperatures. The optimal desorber temperature of 250°C was within the range (240–255°C) of temperatures that have been used for drug detection for the TD-DART-MS system (26,27). From the results of this portion, a desorber temperature of 250°C and a Vapur flow rate of 4 L/min were chosen and used for the remaining portions of the study.

The third component that was investigated in this study was the DART source gas, where both nitrogen and helium were examined. Helium was chosen as it is the most commonly used gas for DART-MS analysis (28), while nitrogen was chosen because, under the confined settings of the TD-DART-MS system, increased signal and sensitivity can be achieved (26). Investigation of the effect of these gases on difenacoum and diphacinone indicated a slight enhancement (approximately 25%) in signal for 50 ng/swipe deposits of difenacoum when using

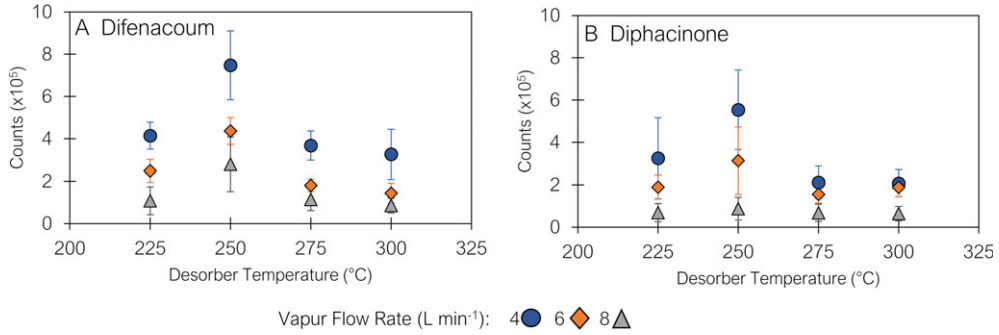

FIG. 1—*Optimization of the thermal desorber temperature (x-axis) and Vapur flow rate (different series) for difenacoum (A) and diphacinone (B). Integrated peak areas of the protonated molecule from the extracted ion chronographs (EICs) are plotted. Error bars represent the standard deviation of 5 replicate measurements.*

helium, while diphacinone showed an enhancement (approximately 40%) when using nitrogen. Expansion of this investigation to all six rodenticides highlighted marginal and nonuniform results for source gas preference for all compounds except pindone, which had a factor of ten improvement using helium. While pindone did have a higher response with helium, since all the prior drug work was completed using nitrogen as the source gas, nitrogen was used for further studies measuring limits of detection and the ability to detect rodenticides in drug mixtures.

Representative Responses & Limits of Detection

Once an optimized method was established, representative spectra were obtained for all six rodenticides. Positive ionization mass spectra were collected across a range of orifice voltages to examine the effects of increased in-source collision induced dissociation (CID), which typically leads to enhanced fragmentation for organic species. Four orifice voltages were chosen (+20, +30, +60, and +90 V), and the compounds were analyzed with both nitrogen and helium as the DART source gas in order to evaluate whether the spectral response was at all effected by the source gas. Figure 2 highlights the mass spectra of brodifacoum using nitrogen (A and C) and helium (B and D) at a low (+20 V) and high (+60 V) orifice 1 voltage. Additional spectra for the remaining compounds can be found in the supplemental information (Figs S1–S7), as well as in Table 1. At low fragmentation voltages (+20 V), the mass spectra for all rodenticides, except bromadiolone, were dominated by a protonated molecule. Bromadiolone produced a major $[M\text{-}OH]^+$ ion and a minor protonated molecule. Spectra between nitrogen and helium were nearly identical at the low fragmentation voltage. At higher voltages, both gases produced increased fragmentation and by +90 V, the protonated molecule was no longer the main ion, regardless of the gas used. Spectra collected using helium as the DART source gas did increase fragmentation (higher abundance fragment peaks relative to the protonated molecule peak), though the identity of the fragment ions was the same between the two gases.

Analysis of these compounds in negative ionization mode was also investigated at −20, −30, −60, and −90 V using both nitrogen and helium as DART source gases. All six rodenticides produced a strong deprotonated molecule in negative ionization mode, and spectra collected with nitrogen as the DART source gas also showed the formation of a nitrate adduct (Fig. 2*E*). Fragmentation under negative mode was minimal using both nitrogen and helium, with the deprotonated molecule remaining the base peak in all four orifice voltages.

In addition to understanding the representative responses from these compounds, the limits of detection (LOD) when using the optimized method were also established. Calculation of the LODs was completed in accordance with the guidelines from ASTM E2677 (29) as they provide a 90% confidence level of the LOD. To be able to calculate the LOD in this manner, ten replicates of between five and eight mass loadings were analyzed along with ten blank wipes, to obtain the background signal at the respective masses. Mass loadings ranged from 2.5 to 300 ng/wipe, depending on the relative sensitivity for the different compounds. Calculation of the LOD was completed using the integrated area from the EICs of the predominant peak in the low fragmentation (+20 V) spectra of each compound. The LODs, which are reported in Table 1, were found to be in the single to tens of nanograms per wipe, indicating sensitive detection of these compounds was achievable. The 90% confidence limit of the LODs was also in the tens of nanograms for all compounds except chlorophacinone, which had an upper limit of 130 ng/wipe. While single to tens of nanogram LODs were obtained, these values are higher than those for most drugs, which can be detected down to the single nanogram and subnanogram range (26,27). Because of the decreased sensitivity, studying detection of these compounds in drug mixtures was crucial.

Analysis of Binary Mixtures Containing Rodenticides and Drugs

While detection of individual rodenticides is important, and tens of nanogram detection limits can be achieved, the goal of this work was simultaneous detection of rodenticides and drugs. To determine whether this was possible, a series of ionization studies were completed. These studies measured the enhancement or degradation of the rodenticide signal as a function of relative drug concentration.

Two rodenticides, brodifacoum and diphacinone, were analyzed in the presence of increasing amounts of one of four drugs (AB-FUBINACA, cocaine, heroin, or methamphetamine) using the optimized method. For all analyses, a constant mass of 50 ng/wipe of brodifacoum or diphacinone was deposited onto the PTFE-coated wipe along with 0, 50, 250 ng, 1, and 5 μg of the drug to produce rodenticide : drug ratios of 1:0, 1:1, 1:5, 1:10, and 1:100. The integrated areas from the EICs of the predominant ion in the low fragmentation (+20 V) mass spectra were taken for each of these ratios and compared to the 1:0

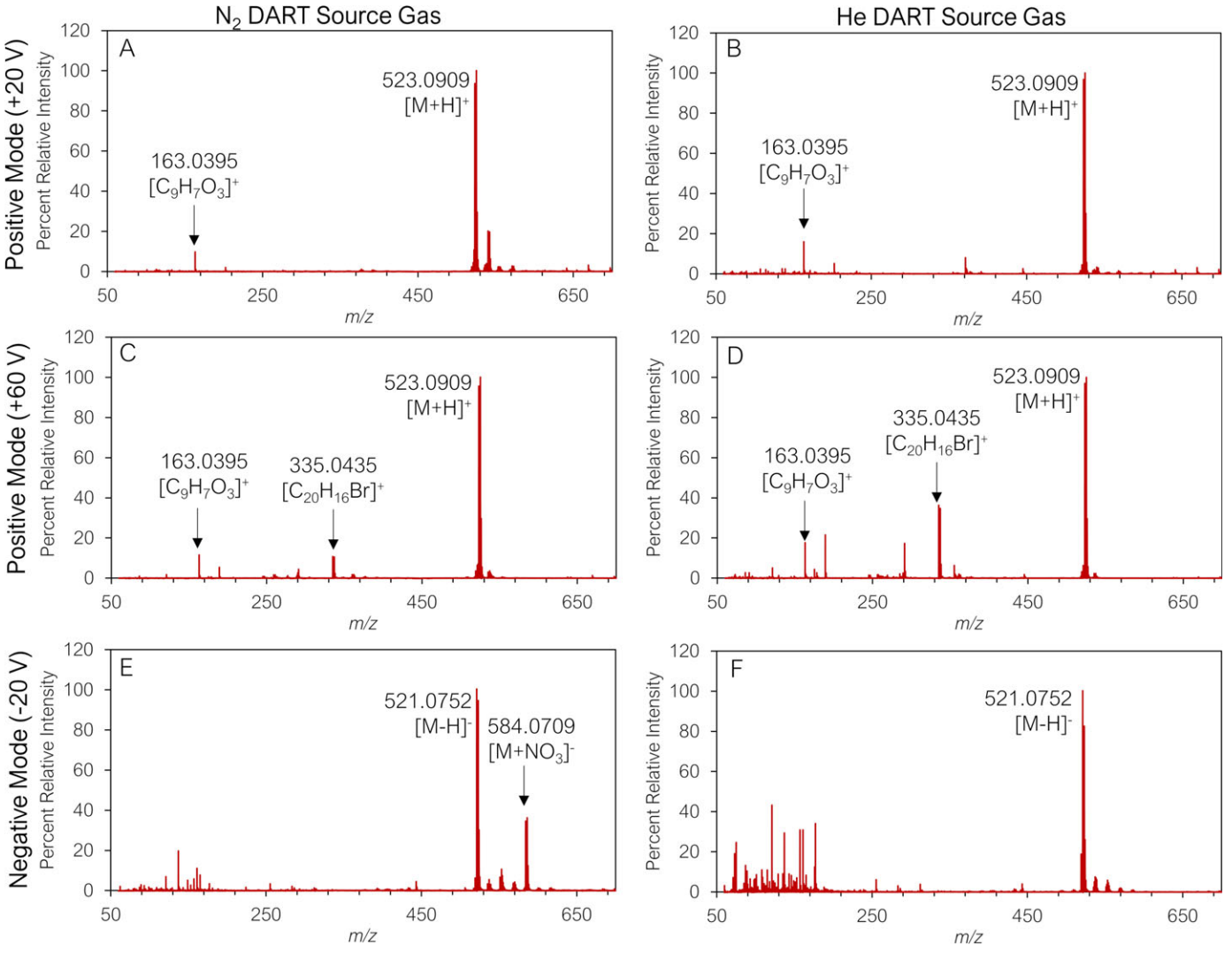

FIG. 2—*Representative mass spectra of brodifacoum using nitrogen (A, C, and E) and helium (B, D, and F) as the DART ionization gas in positive mode (A through D) and negative mode (E and F). For positive mode, both a low fragmentation orifice 1 voltage (A and B) and a higher fragmentation orifice 1 voltage (C and D) are shown. For negative mode, only the low fragmentation orifice 1 spectra (E and F) are shown. Additional representative mass spectra can be found in Figures S1–S7.*

samples to determine the response when a drug was present relative to the response when the drug was not present. For these analyses, a peaks voltage of 1300 V and a detector voltage of 2325 V were employed to enhance the sensitivity of the molecular ions.

Figure 3 presents the results of the competitive ionization studies for the two rodenticides and four drugs examined (representative mass spectra can be found in Figures S8 and S9). Minimal competitive ionization was observed, in all instances, for the 1:1 rodenticide : drug mixture ratio. However, as the relative amount of drug increased, a reduction in the rodenticide signal was observed in all but one instance (brodifacoum and methamphetamine, Fig. 3*A*). Reduction of the rodenticide response with increasing drug concentration is largely believed to be due to competitive ionization, as the drugs likely have higher proton affinities than brodifacoum or diphacinone.

While competitive ionization was believed to be the main driver in reduction of the rodenticide signal, it was not the only component that dictated the response. Comparison of the methamphetamine analysis (Fig. 3, orange squares), for instance, shows essentially no reduction in signal for brodifacoum at the 1:100 brodifacoum : methamphetamine mixture ratio, while complete reduction in the diphacinone signal was seen at this level with methamphetamine. Conversely, similar rates of signal decrease for diphacinone were observed in the presence of all drugs examined, while the rate at which the brodifacoum signal decreased in the presence of AB-FUBINACA was noticeably greater than for any of the other drugs studied. These differences in response are likely due to the contribution of thermal desorption profile differences between the two rodenticides and between the rodenticides and the drugs examined.

An example of the differences is shown in Fig. 4, which highlights the EICs for brodifacoum and diphacinone at 50 ng/wipe, in the presence of AB-FUBINACA and methamphetamine at 500 ng/wipe (1:10 rodenticide : drug mixture). It should be noted that the EICs are scaled to percent relative intensity to better highlight thermal desorption differences. Looking at the desorption profiles of brodifacoum (Fig. 4, blue traces) and diphacinone (Fig. 4, green traces), it can be observed that the desorption profile of brodifacoum is slower and broader than

TABLE 1—*Rodenticides that were studied in this work*

Compound	Molecular Weight (Da)	Formula	Base Peak +20 V (*m/z*)	Base Peak +90 V (*m/z*)	Base Peak −20 V (*m/z*)	LOD (ng)	90% Upper Confidence Limit (ng)
Brodifacoum	522.0831	$C_{31}H_{23}BrO_3$	523.0909 $[M+H]^+$	335.0435 $[C_{20}H_{16}Br]^+$	521.0752 $[M-H]^-$	14.8	22.2
Bromadiolone	526.0780	$C_{30}H_{23}BrO_4$	509.0752 $[M-OH]^+$	251.0708 $[C_{16}H_{11}O_3]^+$	525.0701 $[M-H]^-$	24.6	35.3
Chlorophacinone	374.0710	$C_{23}H_{15}ClO_3$	375.0787 $[M+H]^+$	235.0759 $[C_{16}H_{11}O_2]^+$	373.0631 $[M-H]^-$	81.0	130.0
Difenacoum	444.1725	$C_{31}H_{24}O_3$	445.1803 $[M+H]^+$	257.1330 $[C_{20}H_{17}]^+$	443.1647 $[M-H]^-$	4.7	7.2
Diphacinone	340.1099	$C_{23}H_{16}O_3$	341.1177 $[M+H]^+$	235.0759 $[C_{16}H_{11}O_2]^+$	339.1021 $[M-H]^-$	12.9	17.0
Pindone	230.0943	$C_{14}H_{14}O_3$	231.1021 $[M+H]^+$		229.0865 $[M-H]^-$	15.0	19.0

Also listed is the predominant ion observed at low (+20 V) and high (+90 V) fragmentation voltages in positive mode, the predominant ion observed at low (−20 V) fragmentation voltage in negative mode, and the limit of detection (LOD) for these compounds.

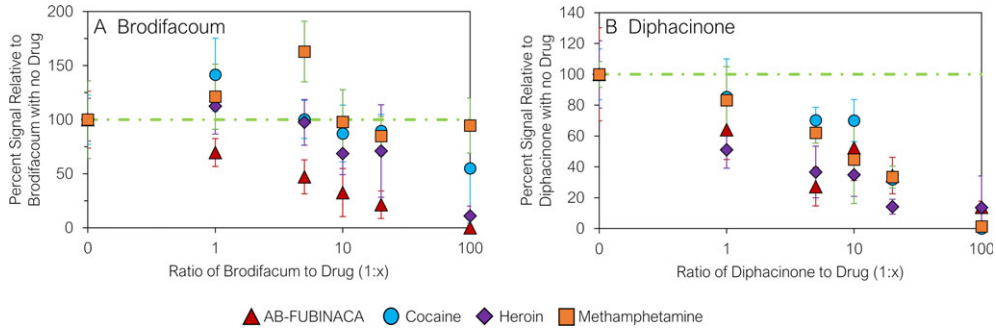

FIG. 3—*Competitive ionization studies of rodenticide/drug mixtures. The response for brodifacoum (A) and diphacinone (B) in the presence of increasing levels of AB-FUBINACA (red triangle), cocaine (blue circle), heroin (purple diamond), and methamphetamine (orange square). The green dotted line represents the level of the rodenticide with no drug present. Note that the* x*-axis is plotted on a log scale.*

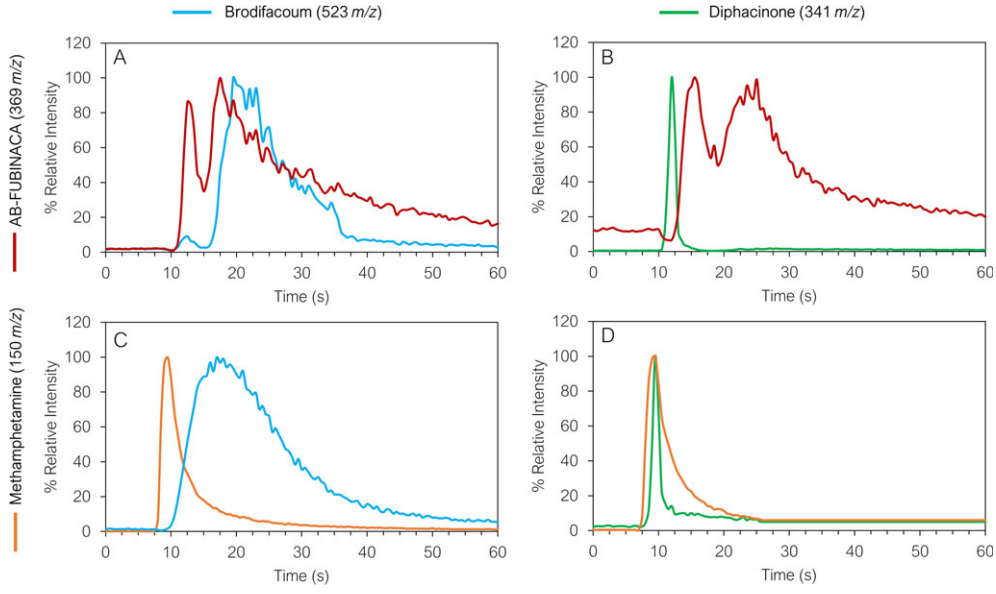

FIG. 4—*Extracted ion chronographs (EICs) of rodenticide drug mixtures. Mixtures of brodifacoum (blue) and AB-FUBINACA (red) (A), diphacinone (green) and AB-FUBINACA (red) (B), brodifacoum (blue) and methamphetamine (orange) (C), and diphacinone (green) and methamphetamine (orange) (D) are presented.*

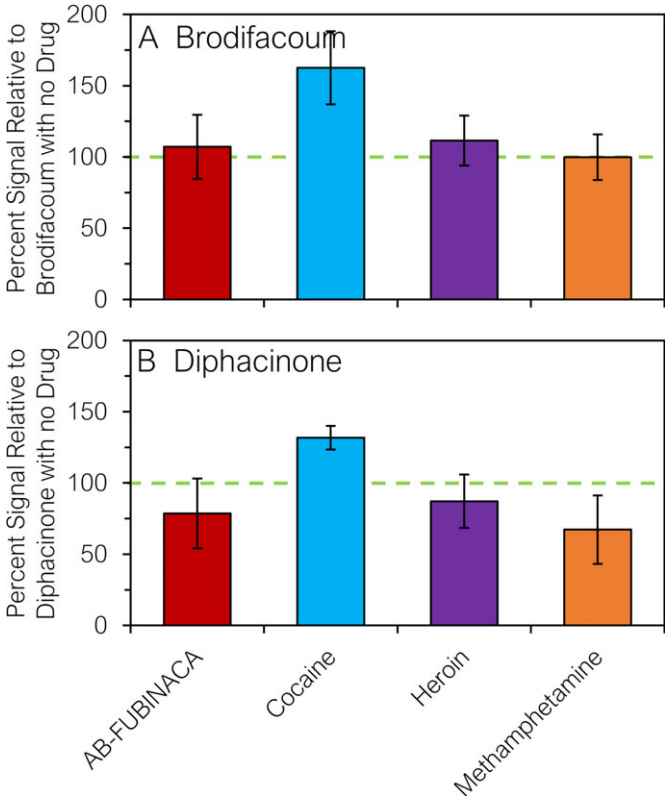

FIG. 5—*Response of brodifacoum (A) and diphacinone (B) at a 1:100 rodenticide : drug ratio in negative ionization mode. Error bars represent the standard deviation of three to five measurements.*

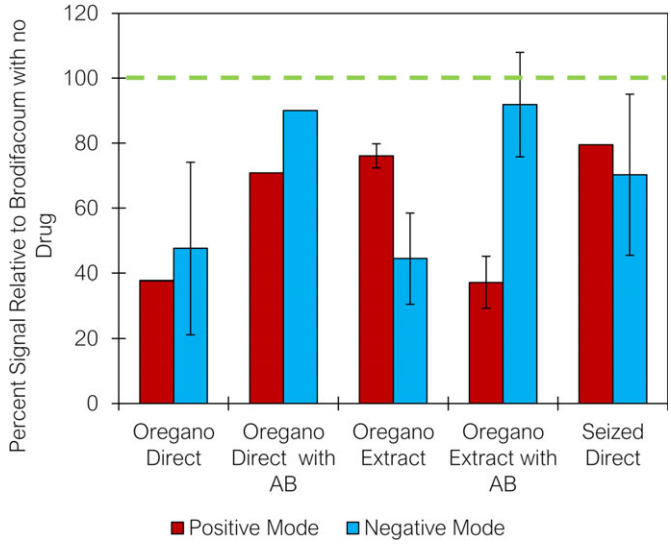

FIG. 6—*Response of brodifacoum in the presence of various plant material matrices. The red bar indicates the response in positive ionization mode with the blue bar indicated the response in negative ionization mode. "Direct" indicates the analysis of plant material direct placed on the wipe while "AB" indicates the presence of AB-FUBINACA. Error bars represent the standard deviation of two to three measurements.*

diphacinone, whose desorption is rapid and short-lived. Relative to sample introduction, maximum signal intensity occurs at 10 sec for brodifacoum, compared to 3 sec for diphacinone. Additionally, it takes nearly 3 sec after sample introduction for a response from brodifacoum to occur compared to a near instantaneous response from diphacinone. When these desorption profiles are compared to those of the drugs, the reason for the varying effects of the drugs becomes clearer. In the case of AB-FUBINACA (Fig. 4, red traces), a broad, and somewhat delayed (approximately 1.5 sec), desorption profile is observed, which nearly mimics that of brodifacoum. The near-simultaneous release of both compounds, combined with ten times greater mass of the drug, was what drove the response of brodifacoum much lower than other drugs. In the case of methamphetamine (Fig. 4, orange trace), however, desorption of the drug was nearly complete by the time maximum desorption of brodifacoum occurred, minimizing the likelihood of competitive ionization. The converse of these two circumstances happens with diphacinone, where a rapid desorption was observed. In this case, methamphetamine and diphacinone co-desorbed, greatly increasing the chances of competitive ionization, while in the presence of AB-FUBINACA, the majority of the diphacinone was desorbed prior to the drug being desorbed. Because of the differences in desorption profiles for the different compounds, understanding these types of trends is critical if simultaneous detection is desired under a single set of conditions.

While low levels (<5% w/w) of rodenticides in drug mixtures may be difficult to detect in positive ionization mode, leveraging the ability to also produce negative rodenticide ions was shown to be extremely valuable. An abbreviated competitive ionization study was completed comparing the pure rodenticide, in negative ionization mode, to a 1:100 rodenticide : drug mixture, to determine whether detection of the rodenticides at a level that was largely unobtainable in positive ionization mode could be attained. Figure 5 (Figures S10 and S11) shows the results of this study which indicate that in negative ionization mode, the presence of the drug has a much lower impact on the response of both brodifacoum and diphacinone. Because cocaine, heroin, and methamphetamine do not readily form negative ions, competitive ionization with these drugs was not observed, allowing for a strong response of the rodenticide. Even with AB-FUBINACA, which was the only drug examined to illicit a response in negative mode, the effects of competitive ionization were negligible (Fig. 5, red columns). Given the strong response of brodifacoum and diphacinone at the 1:100 level, detection at levels lower than this is also likely possible. Therefore, if a street sample is suspected of having rodenticides present and a typical screening analysis in positive mode does not produce a conclusive response, completing a second analysis in negative mode can lead to enhanced detection of these compounds, especially in the presence of high proton affinity compounds such as cocaine or methamphetamine.

Analysis of Simulated Case Samples Containing Plant Material

While samples of cocaine, heroin, and methamphetamine suspected of containing rodenticides would most commonly be encountered in powdered form, contaminated synthetic cannabinoids samples would likely be submitted as plant material. Detection of rodenticides on a plant material represents a potential challenge as it is a complex matrix that could significantly hinder ionization of the compound of interest. To understand whether competitive ionization in the presence of plant material would present difficulty in detection of these compounds, an additional set of competitive ionization studies was completed.

The ability to detect brodifacoum in the presence of oregano, a proxy for seized plant material (both with and without AB-FUBINACA), and actual seized plant material containing synthetic cannabinoids was investigated.

Competitive ionization studies were completed in a similar fashion to those described in the previous study. For the samples containing brodifacoum and oregano, both extracts (created by extracting approximately 1.5 mg of oregano in methanol) and actual plant material were examined. For the seized case sample (consisting of plant material containing 4-fluoro ADB, MMB-FUBINACA, and ADB-FUBINACA), only analysis of the actual plant material was completed, as this likely represented the preferable method of analysis for this type of sample. Analysis of the oregano extract was completed by pipetting 5 μL of extract along with 50 ng of brodifacoum onto the wipe. For analyses with AB-FUBINACA present, 50 ng of the drug was also added to the wipe. Analysis of the plant material, for both oregano and the seized material, was completed by pipetting 50 ng of brodifacoum (and 50 ng of AB-FUBINACA) onto the wipe then adding approximately 1 mg of plant material. The oregano samples were completed because they provided insight into the effects of just plant material itself, and plant material in the presence of a known mass of synthetic cannabinoid. The concentration of the synthetic cannabinoids in the seized plant material was unknown. Samples were analyzed in both positive and negative ionization mode.

Figure 6 shows the results of this study for the various plant matrices. In all instances, detection of brodifacoum was still possible with a relative signal strength of at least 37%. Unlike the previous section, which showed how analysis in negative mode eliminated competitive ionization, there was no clear indication that positive or negative ionization mode was better for analysis of plant material. This can likely be attributed to a combination of increasingly complex background matrices, which may have compounds present that compete in negative mode, as well as less control over sample deposition and composition due to the plant material. Representative mass spectra of the direct analysis oregano and seized plant material samples can be found in the supplemental information (Figures S12 and S13). The ability to readily detect brodifacoum in the presence of these complex background matrices, and in the presence of multiple synthetic cannabinoids, indicates that analysis of case samples should likely be possible. However, the extract concentration of rodenticides on the plant material of forensic samples still has not been established.

Conclusions

TD-DART-MS provides a rapid and sensitive method for the detection of rodenticides both individually and in the presence of drugs. Optimization of a method for the six rodenticides studied showed that the parameters needed to achieve sensitive detection were nearly identical to those previously reported for drug analysis. Low fragmentation spectra typically produced protonated molecules in positive mode, except for bromadiolone which produced an $[M\text{-}OH]^+$ ion. As the in-source CID voltage was increased, enhanced fragmentation of the compounds was observed, and in the case of pindone, no identifiable fragments were present at high voltages. These compounds also produced a strong deprotonated molecule in negative mode, which proved to be extremely useful in the analysis of drug mixtures. Limits of detection for these compounds were in the single to tens of nanograms range.

Detection of these compounds was possible in drug mixtures. While significant competitive ionization occurred when the rodenticide was less than 5% w/w and the sample was analyzed in positive ionization mode, the deleterious effects could be bypassed using negative ionization mode, thus unlocking low level detection of these compounds in complex mixtures.

The methods and work presented here provide forensic scientists and researchers with a rapid and sensitive method for the detection of rodenticides in drug mixtures. This type of analysis can be critical when there are suspected cases of drug tampering, from both a forensic and public health perspective. Additional ongoing work is looking to quantify the amount of rodenticide commonly found in street samples while also evaluating the use of this technique for detection of these compounds in the presence of other compounds and off abnormal surfaces such as plant material.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Positive ionization mass spectra of brodifacoum at +20 V (A), +30 V (B), +60 V (C), and +90 V (D) orifice 1 voltage with select peak assignments.

Figure S2. Positive ionization mass spectra of bromadiolone at +20 V (A), +30 V (B), +60 V (C), and +90 V (D) orifice 1 voltage with select peak assignments.

Figure S3. Positive ionization mass spectra of chlorophacinone at +20 V (A), +30 V (B), +60 V (C), and +90 V (D) orifice 1 voltage with select peak assignments.

Figure S4. Positive ionization mass spectra of difenacoum at +20 V (A), +30 V (B), +60 V (C), and +90 V (D) orifice 1 voltage with select peak assignments.

Figure S5. Positive ionization mass spectra of diphacinone at +20 V (A), +30 V (B), +60 V (C), and +90 V (D) orifice 1 voltage with select peak assignments.

Figure S6. Positive ionization mass spectra of pindone at +20 V (A), +30 V (B), +60 V (C), and +90 V (D) orifice 1 voltage with select peak assignments.

Figure S7. Low fragmentation voltage (−20 V) negative mode ionization spectra of all the rodenticides studied in this work with select peak assignments.

Figure S8. Low fragmentation voltage (+20 V) positive mode ionization spectra of brodifacoum in the presence of the four drugs studied.

Figure S9. Low fragmentation voltage (+20 V) positive mode ionization spectra of diphacinone in the presence of the four drugs studied.

Figure S10. Low fragmentation voltage (−20 V) negative mode ionization spectra of brodifacoum in the presence of the four drugs studied.

Figure S11. Low fragmentation voltage (−20 V) negative mode ionization spectra of diphacinone in the presence of the four drugs studied.

Figure S12. Representative mass spectra of oregano and brodifacoum experiments.

Figure S13. Representative mass spectra of seized plant material and brodifacoum experiments.