Identification of the Veterinary Sedative Medetomidine in Combination with Opioids and Xylazine in Maryland

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Abstract
Public health, public safety, and forensic science personnel continue to face the emergence of new compounds into the drug market. While focus is often put on the detection of new analogs of known illicit drugs, monitoring the changes in cutting agents and other compounds can be equally as important. Over the last year, near real-time monitoring of the drug supply in Maryland has been completed through a public health – public safety partnership whereby residue from suspected drug packaging or used paraphernalia is collected and analyzed. Through this project, we have recently detected the presence of the veterinary sedative medetomidine in a small number of samples. The presence of medetomidine has been identified in both public health and law enforcement samples and in the presence of fentanyl and xylazine – another veterinary sedative that has been widely observed over the last year. While the rate at which medetomidine has been detected remains low, it is concerning and worthy of continued monitoring.

Keywords: Drug Analysis; New Substance; Forensic Science; Public Health; DART-MS

Introduction
A constantly changing drug landscape continues to be a challenge for the public health, public safety, and forensic science communities. New and emerging compounds across multiple drug classes are continually being detected and identified – as evidenced by work from efforts like NPS Discovery [1] and the European Monitoring Centre for Drugs and Drug Addiction [2]. In addition to the detection of new compounds, there is also the need to monitor for known compounds that may be diverted or misused. The need to monitor for these types of compounds has become increasingly obvious with the introduction of the xylazine, a veterinary sedative, into the fentanyl and heroin drug supply. Detected in the drug supply as far back as 2006 [3], the presence of xylazine in heroin and fentanyl cases has seen a dramatic rise (up to 193 % between 2020 and 2021 [4]) in recent years, driving a polydrug overdose epidemic [5–8]. The co-presence of xylazine in opioid samples is believed to increase respiratory depression which drives the increased risk of overdose [9].

While the knowledge base around the proliferation of xylazine into the drug supply continues, and research into its effects when consumed with opioids continues to grow, there are a number of other veterinary sedatives that could play a similar role. These include compounds such as acepromazine [10], detomidine [11], and medetomidine [12]. All three of these compounds are routinely used in veterinary medicine and their effects with drugs like xylazine [13] and ketamine [14,15] have also been studied.

Detecting and identifying these types of compounds require early warning systems. While a number of these systems exist across the world, a new public health – public safety initiative was started a year ago in the state of Maryland. Through this initiative, residue samples are collected by law enforcement and public health personnel from suspected drug packaging or used drug paraphernalia which are then mailed to the laboratory and analyzed by direct analysis in real time mass spectrometry (DART-MS). This process has enabled the ability to detect changes in the drug supply in as little as 24 hours and has enabled detection and identification of drugs, cutting agents, and

diluents. Through this new partnership, another veterinary sedative, medetomidine, has been detected in several of the samples across the state of Maryland in the last six months.

Detection of medetomidine in residue from suspected drug packaging (from law enforcement agencies) or used drug paraphernalia (from public health agencies) was enabled through routine testing completed as part of a collaborative project between the National Institute of Standards and Technology (NIST) alongside public health agencies and law enforcement agencies across the state. The goals of the project are to i) provide public health and public safety officials with more timely information on the drug landscape in a particular region and ii) inform the community in a timely manner on the possible presence of new or emerging compounds.

Materials & Methods

The samples analyzed in this study consist of meta-aramid swabs (DSA Detection, North Andover, MA, USA) or cotton swabs (Puritan Medical Products Company LLC, Guilford, ME, USA) used to collect suspected drug residue on the exterior of drug paraphernalia. Once collected, wipes or cotton swabs are placed inside individual coin envelopes and mailed to the laboratory for analysis. It should be noted that samples are collected by public health and law enforcement personnel who have been trained to sample drug paraphernalia in their respective environments. All samples are de-identified prior to arriving at the laboratory. Additional information on the sampling and analytical protocols can be found elsewhere [16]. Once received, the wipe or swab is trimmed and extracted with 1 mL of acetonitrile (Omni-solv grade, Sigma-Aldrich, St. Louis, MO, USA) in a 2 mL amber glass vial. The trimmed wipe or swab is removed, leaving the extract ready for analysis.

Confirmation of medetomidine in the residues from used drug paraphernalia was completed using a previously published workflow for the project [16]. Briefly, extracted residue samples were analyzed using DART-MS – collecting full scan mass spectra at multiple in-source collision induced dissociation (is-CID) voltages to enable non-targeted screening. Samples were analyzed on a JEOL JMS-4G AccuTOF (JEOL USA, Peabody, MA, USA) coupled with a DART-SVP ion source (IonSense / Bruker, Saugus, MA, USA) using previously published methods [16]. The resulting mass spectra were searched against the NIST DART-MS Forensics Database (version Firefly) [17] using NIST/NIJ DART-MS Data Interpretation Tool (version 2.0d) [18]. Polyethylene glycol dissolved in methanol was used as mass calibration compound while AB-FUBINACA (Cayman Chemical, Ann Arbor, MI, USA) dissolved in acetonitrile was used as the mass drift compensation compound.

After preliminary identification by DART-MS, additional confirmation testing was completed, in line with the workflow, using liquid chromatography tandem mass spectrometry (LC-MS/MS). Product ion scans of *m/z* 201 (corresponding with the protonated molecule of medetomidine) were collected using an Sciex 4000QTrap (Ontario, CA) triple quadrupole mass spectrometer coupled with a Thermo UltiMate-3000 (Waltham, MA, USA) liquid chromatography system. Full method parameters can be found elsewhere [16]. The resulting product ion scans from the paraphernalia samples were compared to the product ion scans obtained by analyzing a standard of medetomidine (Cayman Chemical, purchased as dexmedetomidine hydrochloride) dissolved in methanol. No attempt was made to determine the optical isomer form (dex- versus levo-) in the samples.

Results & Discussion

The first identification of medetomidine in Maryland was from suspected drug packaging sampled by a law enforcement agency in July 2022. The submitted wipe was found to contain fentanyl, fluorofentanyl, caffeine, diphenhydramine, mannitol, quinine, and xylazine along with medetomidine

(Figure 1A) when analyzed by DART-MS. Medetomidine, like most drugs, produces a protonated molecule under low is-CID (low fragmentation) conditions in DART-MS (indicated by a peak at approximately *m/z* 201.1392), enabling presumptive identification. Since this compound had not been previously identified in a submitted sample, additional confirmation testing was completed to verify the presence of medetomidine. Confirmatory testing was completed by collecting a product ion scan of the presumed protonated molecule (*m/z* 201) from both the sample and a reference standard using LC-MS/MS. The resulting product ion scans (Figure 1B) were found to be nearly identical with retention times of 7.33 min and the formation of two major product ions at *m/z* 68 and *m/z* 95, both of which have been reported in literature [19]. Upon verification of medetomidine in the sample, both law enforcement and public health personnel were notified of its presence alongside its co-presence with fentanyl and xylazine (another dangerous veterinary sedative).

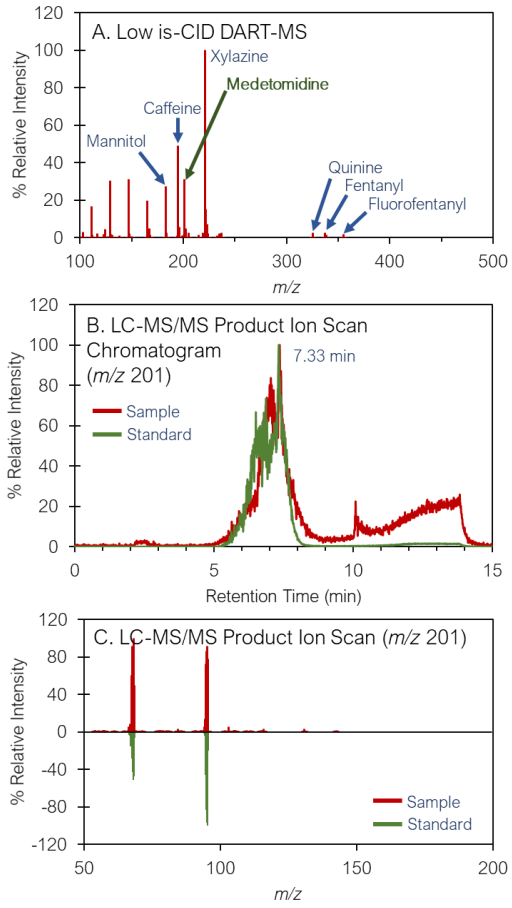

Figure 1. Low is-CID DART-MS mass spectrum of the first sample that was found to contain medetomidine (A.). The confirmatory LC-MS/MS results (B. and C.) showing the comparison of the chromatograms and product ion scans from the sample (blue) and a medetomidine standard (green) are also shown.

After the initial detection, medetomidine was not detected again until October 2022, where it was detected in two samples taken from used drug paraphernalia that were submitted by public health sources. One sample was residue collected from a syringe and the second was from a capsule. Both samples were found to be multi-component mixtures. The syringe sample was found to contain cocaine, fentanyl, and xylazine (Figure 2A) while the capsule was found to contain caffeine, fentanyl,

quinine, and xylazine (Figure 2B) – in addition to medetomidine. It is important to note that the analysis of residue from used paraphernalia (especially items like syringes) does not enable us to know whether all drugs were consumed together or whether the item was used multiple times.

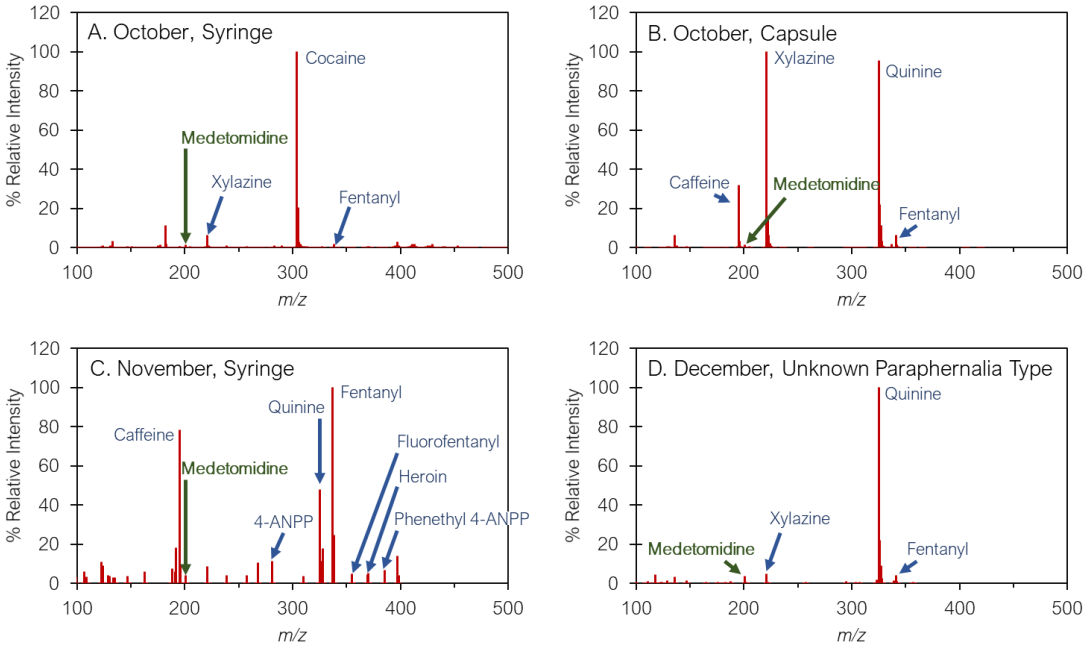

Figure 2. Low is-CID DART-MS spectra of the four samples where medetomidine was detected. The ion corresponding to the protonated molecule for medetomidine is identified in each sample as well as all other compounds identified in the sample.

Detection of medetomidine has since remained sparse, but consistent in samples submitted by public health agencies. In November 2022, it was identified in another syringe which also contained 4-ANPP, caffeine, fentanyl, fluorofentanyl, heroin, phenethyl 4-ANPP, quinine, and xylazine (Figure 2C). An additional sample from an unknown type of paraphernalia was received in December 2022 where medetomidine was found in combination with fentanyl, quinine, and xylazine (Figure 2D). While the frequency of detection of medetomidine is low, the presence of this compound in samples containing fentanyl and xylazine is concerning as it, like xylazine, is a sedative. While the DART-MS results are qualitative, the response from medetomidine has always been minor relative to the major component in the sample. Further, quantitative testing is the focus of ongoing work.

Conclusion

While significant focus has been placed on the continued presence of xylazine in the drug supply, another veterinary sedative, medetomidine, has also been encountered. Though it has only been detected five times in the state of Maryland over the last six months, it is being co-detected in samples containing fentanyl and xylazine. Unfortunately, there is limited information on the effects of medetomidine in humans and no information of its effect when combined with other sedatives or opioids. It is still too early to know if the presence of medetomidine in the drug supply will grow or subside, given the increasing awareness of xylazine in the drug supply, especially as new drugs containing this compound are released into the market [20]. The detection of other veterinary sedatives also brings into question whether or not calls to develop xylazine immunoassay test strips [21] should be expanded to include additional compounds, even if they have yet to be seen on a

large-scale. The presence of medetomidine in used drug samples, ultimately, highlights the increasing need for monitoring and early detection of the drug supply from multiple sample sources including public health, law enforcement, and forensics.

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Disclaimer

Certain commercial products are identified 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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