Qualitative Analysis of Xylazine, Nitazenes and Common Opioids/Analogs by LC/MS/MS Following Collection with the M-Vac™ Wet-Vacuum System

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Abstract

The introduction of synthetic opioids like fentanyl and nitazene compounds along with a non-opioid sedative, xylazine, to the illicit drug supply have made it increasingly difficult to determine the true contents of drug products. These compounds are likely entering the U.S. at a rapid rate due to a lack of detection at the postal inspection level, as there is currently no method for detection in the increasingly small amount required to exhibit effects. The Marshall University Forensic Science Center sought to devise a new method for analysis of these compounds along with commonly encountered opioids found on packaging materials.

The M-Vac™ was used to recover a sample containing 34 compounds from the inside of a Tyvek® envelope. The sample then underwent solid phase extraction followed by qualitative analysis using LC/MS/MS, where all 34 compounds were successfully identified. The results from this initial study demonstrate the potential for the identification of drug residues from porous substrates and from a sample previously discarded following DNA collection.

Introduction

As the opioid epidemic continues to gain traction, dangerous new substances are being introduced to the illicit drug supply. Fentanyl is one of the highest contributors to drug-related overdoses with a potency100x greater than that of morphine, making it a popular cutting agent choice for drugs like heroin.[1] Nitazenes are around tenfold more potent than fentanyl and it is likely under-reported because laboratories may not be equipped with a method for detecting these drugs.[2] Xylazine, known by the street name 'Zombie Drug', causes CNS depression and severe necrotic skin ulcerations. Naloxone is ineffective for both nitazenes and xylazine.[2,3]

As of March 2023, the CDC estimated that overdose deaths related to xylazine have increased by about 1238% from 2018-2021, and the White House Office of National Drug Control Policy declared xylazine, or the use of fentanyl adulterated or associated with xylazine (FAAX), an emerging threat on April 12th, 2023.[3,4] The mixture of fentanyl with xylazine has been observed in drug seizures in 48 states.

Spectroscopy-based techniques are capable of penetrating through transparent packaging material with relatively pure samples.[5] Adulterants are likely to make up a very small portion of a sample, so this technique is unrealistic. Additionally, a wipe-based collection method for the outer side of packaging material is possible, however it yields a complex matrix containing dust, dirt, plasticizers, and other residues.[5]

The M-Vac™ is used to recover touch DNA (tDNA) where a low quantity of DNA is expected to be found.[6] As there is currently no use for M-Vac™ solution post-DNA recovery, this research will focus on a potential use for the discarded solution. The solution underwent solid phase extraction (SPE) followed by qualitative analysis with LC/MS/MS to detect and identify trace drug residue.

Materials and Methods

Table 1. 34 Compounds and Their Suppliers

Lipomed Pharmaceutical®	Cayman Chemical®	Sigma-Aldrich®	Krstenansky Lab®
Codeine	Protonitazene	Lidocaine, ≥ 98%	U-47700
Morphine	NDI	Quinine, 90%	AH-7921
Fentanyl	NPP	Diphenhydramine	
Cocaine	Isotonitazene-d_7	Dextromethorphan	
Fentanyl-d_5	Nitazene	Oxycodone	
Heroin-d_9	Xylazine	Hydrocodone	
6-Acetylmorphine	Heroin	Methamphetamine	
6-Acetylcodeine	Valeryl Fentanyl	Methadone	
6-Acetylmorphine-d_3	Isotonitazene	Buprenorphine	
6-Acetylcodeine-d_3	Metonitazene	Ketamine	
	Metonitazene-d_3		

NDI = N-desethyl Isotonitzene; NPP = N-Pyrrolidino Protonitazene

- 5 mg of a solid mixture of 34 compounds (**Table 1**) placed inside a Tyvek® packaging envelope
- 10 cm x 10 cm area vacuumed with the M-Vac™
- Solid phase extraction using a procedure for Novel Synthetic 2-Benzylbezimidazole Opioid Compounds "Nitazenes" (provided by United Chemical Technologies Inc.)
- Qualitative analysis of extracted sample with MassLynx software and the Waters Acquity™ Ultra Performance LC equipped with a UCT Selectra® C18 column (5 cm x 2.1 mm, 1.8 µm) coupled to a Waters Acquity™ TQ Detector utilizing electrospray ionization in positive mode (ESI+)
- Optimal gradient utilized mobile phase A, composed of 0.1% formic acid in water, and mobile phase B, composed of 0.1% formic acid in acetonitrile.

Table 2. LC/MS/MS Gradient Program

Time (min)	Flow Rate (ml/min)	% A	%B
Initial	0.3	90	10
1	0.3	90	10
4	0.3	80	20
6	0.3	5	95
7	0.3	90	10

Results

Table 3. Selected Opioid MRM Transitions and Retention Times

Compound Name	Retention Time (min)	Parent Ion (m/z)	Product Ion 1 (m/z)	Product Ion 2 (m/z)
Heroin	4.37	370	58	165
Morphine	0.61	286	44	58
Hydrocodone	2.17	300	128	171
Fentanyl	5.08	337	77	105
Valeryl Fentanyl	5.28	365	57	85
Carfentanil	5.19	395	105	113

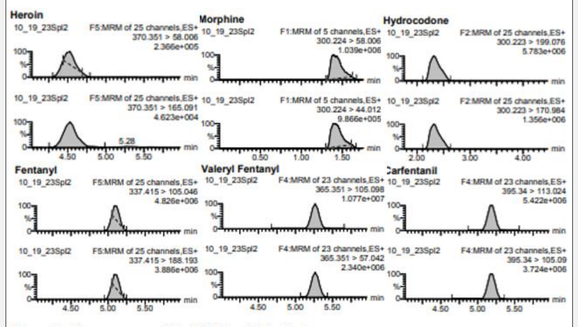

Figure 1. Chromatograms of Opioids Listed in **Table 3**

Table 4. Selected Nitazene MRM Transitions and Retention Times

Compound Name	Retention Time (min)	Parent Ion (m/z)	Product Ion 1 (m/z)	Product Ion 2 (m/z)
Nitazene	5.02	353	72	91
Metonitazene	5.01	383	100	121
Isotonitazene	5.19	411	44	72

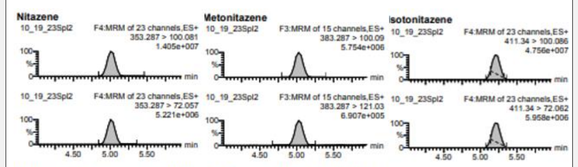

Figure 2. Chromatograms of Nitazene Compounds Listed in **Table 4**

Table 5. Other Selected MRM Transitions and Retention Times

Compound Name	Retention Time (min)	Parent Ion (m/z)	Product Ion 1 (m/z)	Product Ion 2 (m/z)
Xylazine	5.02	353	72	91
Cocaine	5.01	383	100	121
Ketamine	5.19	411	44	72

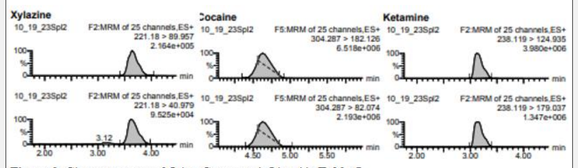

Figure 3. Chromatograms of Other Compounds Listed in **Table 5**

A report was generated for each identified drug using TargetLynx software. The chromatograms for certain selected drugs listed in **Table**(s) **3**, **4**, and **5** can be seen in **Figure**(s) **1**, **2**, and **3**, respectively.

Conclusions

- 34 compounds successfully recovered from a Tyvek® packaging envelope using the M-Vac™
- 34 compounds were successfully detected by LC/MS/MS following solid phase extraction
- Despite similar retention times among certain drugs, the method successfully separated and identified each ion to the correct analyte
- Demonstrates potential for M-Vac™ recovery of both DNA and trace drug residue in a single use
- Future research will include method validation and simultaneous recovery of a sample containing both DNA and trace drug residue

References

1. Comer SD, Cahill CM. Fentanyl: Receptor pharmacology, abuse potential, and implications for treatment. Neuroscience and Biobehavioral Reviews 2019;106:49–57. doi:10.1016/j.neubiorev.2018.12.00.
2. Pergolizzi Jr J, Raffa R, LeQuang JA, Breve F, Varrassi G. Old drugs and new challenges: A narrative review of nitazenes. Cureus 2023 June;15(6). doi:10.7759/cureus.40736.
3. Gupta R, Holtgrave DR, Ashburn MA. Xylazine — Medical and Public Health Imperatives. New England Journal of Medicine 2023 June; 388(24): 2209–2212. doi:10.1056/nejmp2303120.
4. Millennium Health/ September. Signals Report: Xylazine. Millennium Health LLC, 13 Sept. 2023, www.millenniumhealth.com/news/xylazine-signals-report/.
5. Sisco E, Robinson EL, Burns A, Mead R. What's in the Bag? Analysis of Exterior Drug Packaging by TD-DART-MS to Predict the Contents. Forensic Science International 2019; 304: 109939. doi:10.1016/j.forsciint.2019.109939.
6. Vickar T, Bache K, Daniel B, Frascione N. The use of the M-Vac® wet-vacuum system as a method for DNA recovery. Science & Justice: Journal of the Forensic Science Society 2018 Jan; 58(4): 282-286. doi:10.1016/j.scijus.2018.01.003.
7. United Chemical Technologies Inc. Solid Phase Extraction of Novel Synthetic 2-Benzylbenzimidazole Opioid Compounds "Nitazenes". UCT, LLC 2022. www.unitedchem.com/wp-content/uploads/2022/10/Nitazene-Application-Note-_Final-102122.pdf. Accessed 1 Aug 2023.

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