Solid Phase Extraction of Novel Synthetic 2-Benzylbenzimidazole Opioid Compounds "Nitazenes"

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INTRODUCTION

A new class of synthetic opioids is emerging called benzimidazole-opoids also known as "nitazenes". First synthesized in the 1950s by a Swiss pharmaceutical company, these compounds were initially investigated as potential analgesics.[1] They were not approved for medical use but are reappearing as a new threat to the on-going opioid epidemic. Nitazenes are reported to range from three to twenty times more potent than fentanyl.[2] Medical officials warn that multiple doses of naloxone (Narcan) may be necessary to reverse an overdose.[3]

In July of 2019, isotonitazene was the first nitazene compound identified in a biological sample in the United States.[3] In August 2021, the US Drug Enforcement Administration (DEA) temporarily placed isotonitazene in Schedule I.[4] Seven additional nitazene compounds have been temporarily placed in Schedule I.[4] The number of cases across the country continues to rise. Due to the novelty and potency of these compounds it was crucial to develop an extraction method with a low level of quantitation.

Nine nitazene compounds were extracted from human urine and blood utilizing UCT's Clean Screen® DAU (PN: CSDAU133) solid phase extraction (SPE) cartridges. Using a Shimadzu Nexera LC-30AD with MS-8050 analytes were separated on a SelectraCore® C18 core-shell column (100 x 2.1 mm, 2.7 µm).

[1] Diversion Control Division, Benzimidazole-Opioids Other Name: Nitazenes (2022).
[2] Vandeputte, M.M., Krotulski, A.J., Walther, D. et al. Pharmacological evaluation and forensic case series of N-pyrrolidino etonitazene (etonitazepyne), a newly emerging 2-benzylbenzimidazole 'nitazene' synthetic opioid. Arch Toxicol 96, 1845–1863 (2022). https://doi.org/10.1007/s00204-022-03276-4
[3] (2020). (rep.). Isotonitazene.
[4] Seven Benzimidazole-Opioids: Butonitazene, Etodesnitazene, Flunitazene, Metodesnitazene, Metonitazene, N-Pyrrolidino Etonitazene, and Protonitazene, 86 Fed. Reg. 69183-69186 (December 7, 2021)

INSTRUMENT PARAMETERS

LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050
UHPLC Column	SelectraCore® C18 Column 100 x 2.1 mm, 2.7 µm (PN: SCS27-C181021)
Guard Column	SelectraCore® C18 Guard Column 5 x 2.1 mm, 2.7 µm (PN: SCS27-C18GDC21)
Column Temperature	40°C
Flow Rate	0.45 mL/min
Injection Volume	5 µL
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in methanol
Gradient	Conc. B 10% (0 min) - 43% (2.5 to 3.5 min) - 70% (7 min) - 100% (8 to 11 min) - 10% (11.3 to 15 min)

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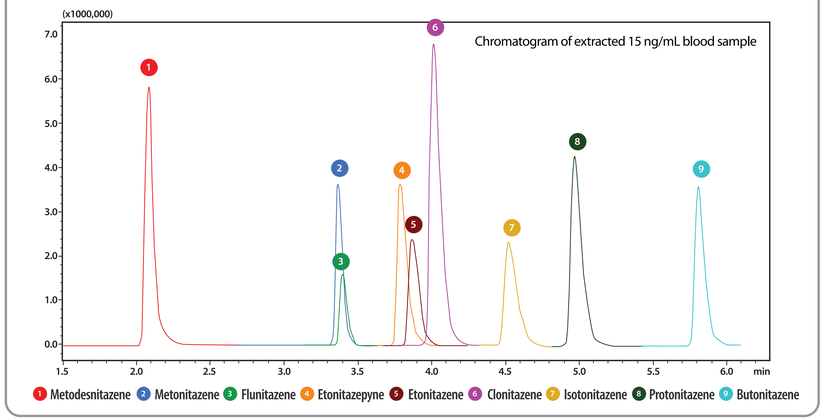
Analyte	Parent Ion (m/z)	Product Ion 1 (m/z)	CE (eV)	Product Ion 2 (m/z)	CE (eV)	RT (min)
Butonitazene	425.5	100.1	-23	72.1	-45	5.83
Clonitazene	386.5	100.1	-26	125.1	-36	4.03
Etonitazene	397.4	100.1	-21	72.0	-36	3.88
Etonitazepyne	395.6	98.1	-23	56.1	-55	3.80
Flunitazene	371.3	100.1	-23	73.1	-26	3.41
Isotonitazene	411.5	100.1	-21	72.2	-45	4.53
Metodesnitazene	339.2	100.1	-21	72.1	-40	2.09
Metonitazene	383.5	100.1	-22	72.2	-39	3.38
Protonitazene	411.7	100.1	-24	72.1	-39	4.98

CHROMATOGRAM



SPE PROCEDURE

SAMPLE PREP
0.5 mL sample + ISTD + 200 µL ACN + 1.3 mL 100 mM phosphate buffer pH 7. Vortex and centrifuge

CONDITION
1 x 3 mL MeOH
1 x 3 mL 100 mM phosphate buffer pH 7

LOAD
Load at 1 to 2 mL/min

WASH
1 x 3 mL deionized water
1 x 3 mL 50:50 MeOH:H_2O

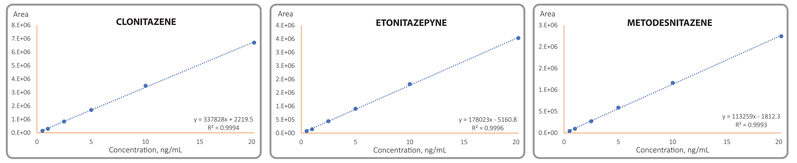
DRY
Dry column for at least 10 minutes under full pressure or vacuum

ELUTE
1 x 3 mL of MeOH:NH_4OH (98:2)

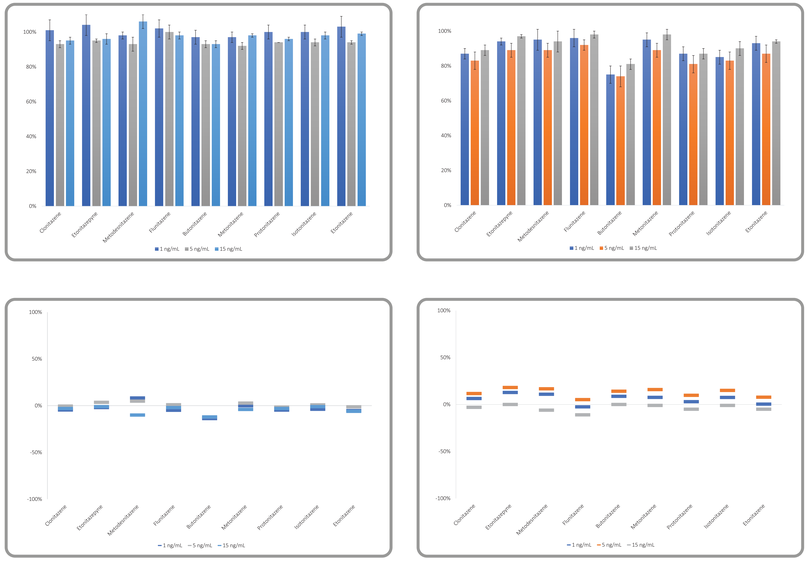
EVAPORATE
Add 250 µL of 10% HCl in MeOH and vortex. Evaporate eluate at 10 psi, 35°C

RECONSTITUTE
1 mL of 50:50 MeOH:H_2O

CALIBRATION CURVES



RESULTS



Clean Screen® DAU was utilized to extract analytes from urine and blood samples at three concentrations. The extraction recoveries of analytes from urine ranged from 93-106% with relative standard deviation less than 10%. Matrix effects for all analytes were within ± 25%. Extraction recoveries of analytes from blood ranged from 74 - 96% with relative standard deviations less than 10%. Matrix effects for all analytes in blood were also within ± 25%.

CONCLUSION

Questions / Comments: **methods@unitedchem.com**

Due to their novelty and presence at low concentrations in biological samples, working with nitazenes proved to be challenging. Some notable discoveries emerged during development of the extraction procedure. First, a sizable amount of the non-polar analytes, particularly butonitazene and isotonitazene, remain in the test tube after loading the sample onto the SPE cartridge resulting in low recoveries. Acetonitrile was added during sample preparation to increase the amount of analyte loaded onto the cartridge. The second challenge was the volatility of the free-base nitazene compounds. It is difficult to avoid the evaporation step after extraction as these compounds are present at low concentrations in biological samples. Utilizing the nitrogens in the chemical structure, 10% hydrochloric acid was added to the eluate to form more stable salt forms to prevent evaporation.

Disclosure: The speaker, author, moderator, planning member and/or presenter/s do have financial relationships with UCT, Inc. , as defined in the AACC policy on potential bias or conflict of interest. The specific product/s : Clean Screen® DAU and the SelectraCore® column will be mentioned and/or discussed.