Rapid Screening of Benzimidazole Opioids (Nitazenes) in Suspect Counterfeit Tablets using SERS, FT-IR and DART-TD-MS

Martin M. Kimani[1], Sara Kern[1], Adam Lanzarotta[1], Michael Thatcher[1], Skyler Smith[1], Lisa M. Lorenz[1], Melissa Collins[1], Gregory W. Howe[2], Anthony E. Wetherby Jr.[3]

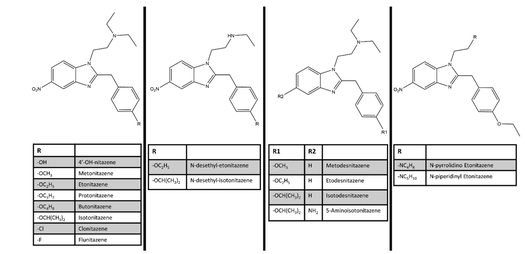
[1]FDA/ORA/ORS/FCC; [2]FDA/ORA/ORS/PSMPL; [3]FDA/ORA/ORS/WEAC

Abstract

Developing methods to rapidly screen for novel synthetic 2-benzylbenzimidazole opioids, also known as nitazenes, has become increasingly important due to their high potency. These compounds have potency comparable or exceeding that of fentanyl by up to 10 times and have been implicated in approximately 5% of all drug overdose deaths in the US in 2021. This work details the authenticity determination of suspect tablets and the identification of three nitazene analogs (*N*-pyrrolidino etonitazene, isotonitazene and etodesnitazene) in suspect tablets seized at a mail facility using Raman and surface enhanced Raman scattering (SERS) with a handheld device, a portable Fourier transform infrared spectrometer (FT-IR) and a direct analysis in real time Ambient ionization coupled to a thermal desorption unit and a mass spectrometer (DART-TD-MS). These methods are rapid and excellent for screening opioids in suspect tablets but could not fully determine the exact structure of some of the nitazene analogs present due to spectral similarities or similar fragmentation patterns. Liquid chromatography mass spectrometry (LC-MS) confirmed the presence of these nitazene compounds in addition to other opioids/drugs that were in trace quantities. The quantitative high performance liquid chromatography coupled with ultra-violet (HPLC-UV) detection experiments determined that the suspect tablets contained an average of 0.817 mg of *N*-pyrrolidine etonitazene per tablet. The results obtained reveal that the simultaneous deployment of these complementary and orthogonal portable analytical techniques as part of a workflow allows suspect tablets to be screened and nitazene-type drugs to be identified in suspect counterfeit tablets at remote sampling sites.

Introduction

- In the last three years, there have been increased public health alerts from the forensic community and published reports on the emergence and abuse of several 2-benzylbenzimidazole compounds that are structurally different from opioids such as morphine and fentanyl but are thought to have similar or elevated potency relative to fentanyl[1].
- A published report by Vandeputte *et al.,* outline the high potency of *N*-pyrrolidino etonitazene [EC_{50} =0.348 nM (0.137—0.8765)] in a μ-opioid receptor-β-arrestin 2 activation assay relative to both fentanyl [EC_{50} =14.9 nM (10.6—21.0)] and morphine [EC_{50} = 290 nM (132-668)][2].
- Many nitazene drugs have been seen pressed into counterfeit pills and falsely marketed as pharmaceutical medication (like Dilaudid "M-8" tablets, alprazolam "G3722" tablets, and oxycodone "M30" tablets).
- The present work details the authenticity determination of suspect counterfeit tablets and the analysis of 2-benzylbenzimidazole analogs (Figure 1) in "M30" tablets using portable devices and a combination of complementary and orthogonal techniques, including Raman, SERS, FT-IR, and DART-TD-MS.



R	
-OH	*K*'-OH-nitazene
$-OCH_3$	Metonitazene
$-OC_2H_5$	Etonitazene
$-OC_3H_7$	Protonitazene
$-OC_4H_9$	Butonitazene
$-OCH(CH_3)_2$	Isotonitazene
-Cl	Clonitazene
-F	Flunitazene

R	
$-OC_2H_5$	N-desethyl-etonitazene
$-OCH(CH_3)_2$	N-desethyl-isotonitazene

R1	R2	
$-OCH_3$	H	Metodesnitazene
$-OC_2H_5$	H	Etodesnitazene
$-OCH(CH_3)_2$	H	Isotodesnitazene
$-OCH(CH_3)_2$	NH_2	5-Aminoisotonitazene

R	
$-NC_4H_8$	N-pyrrolidino Etonitazene
$-NC_5H_{10}$	N-piperidinyl Etonitazene

Figure 1. Structures of the 16 studied 2-benzylbenzimidazoles

Materials and Methods

Figure 2. Representative authentic and suspect counterfeit tablets used in this study.

- All standard reference materials (Figure 1) were acquired from the Cayman Chemical Co. (Ann Arbor, MI). Their mass spectral data, Raman or IR vibrational data were collected and stored in the respective portable device libraries[3].
- **Raman/SERS**: The Raman spectra of three authentic tablets were collected in triplicate and stored in the devices' in-house developed user libraries. All suspect tablets were initially analyzed once for authenticity by placing the tablets in the device tablet holder accessory, collecting a spectrum, and searching against the appropriate authentic drug library. The individual tablets were prepared for analysis by crushing and grinding approximately ½ tablet into a fine powder using a mortar and pestle, which was transferred to a 4 mL glass vial. To each vial was added 750 μL of 10% aqueous methanol and the suspension was mixed on a vortex mixer for approximately 30 seconds to extract the analyte(s) into the solvent. The mixture was filtered into a new and clean glass vial using a 0.45-μm nylon filter to remove any particulate material. The Ag colloid solution (500 μL) and 1 M LiCl aqueous solution (5 μL) were transferred to the vial, the mixture was vortexed for 30 seconds and the sample was left to equilibrate for 30 seconds prior to conducting Raman analysis.
- **FT-IR**: For neat analyses, portions of one individual tablet core from each research sample were separately scraped onto the IRE surface using a razor blade. Pressure was applied to the diamond prism and a spectrum was collected. Extraction analyses required approximately half of the ground tablet cores which were added to a 4-mL glass scintillation vial along with 0.5 mL of deionized water and 1 mL of chloroform.[41] The vial was then thoroughly vortexed. The chloroform layer was removed using a glass disposable pipette and added dropwise to a clean glass microscope slide or, if necessary, filtered using an all-plastic syringe attached to a 0.45-μm pore size filter, and then transferred to a clean glass microscope slide. The chloroform extract was allowed to air dry, and the resulting residue was transferred to the IRE surface using a razor blade and fine-pointed probe. Pressure was applied, and a spectrum was collected.
- **DART-MS**: Analysis of solid samples was accomplished by lightly pressing an individual tablet core from each research sample against a sample trap. The sample trap was then wiped first with a dry laboratory wipe and then with a laboratory wipe that was moistened with methanol prior to analysis.

Figure 3. Hand-held and portable analytical devices used in this study.

Results and Discussion

- Analysis of authentic and the suspect counterfeit tablets using Raman and FT-IR resulted in either a pass (consistent with authentic (CWA)) or fail (not consistent with authentic (NCWA)). A Fail result (NCWA) was obtained for all the suspect tablets analyzed and as expected significant differences are observed throughout the Raman and FT-IR spectrum of the authentic tablet spectrum (Figures 4IA, 4IIA, and 4IIIA) relative to that of the suspect tablet spectrum (Figures 4IB, 4IIB, and 4IIIB).

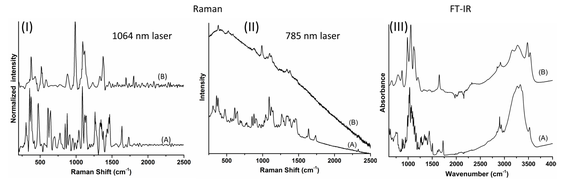


Figure 4. I and 2II. Neat Raman spectra of the authentic tablet core (a) and suspect tablet core from RS-1 (b). Figure 4III. Neat Fourier transform infrared spectrometer (FT-IR) spectra of the authentic tablet core (a) and suspect tablet core from RS-1 (b).

- Raman and FT-IR analysis of the suspect counterfeit tablets only revealed the presence of calcium hydrogen phosphate (Figures 5IA and 5IIA). Consequently, the analyte of interest was extracted from these tablets using 10% aqueous MeOH and analyzed using the SERS procedure. The APIs present in these samples were also extracted using a 50/50 water/chloroform micro-liquid-liquid extraction procedure and the dried chloroform residue analyzed using FT-IR.

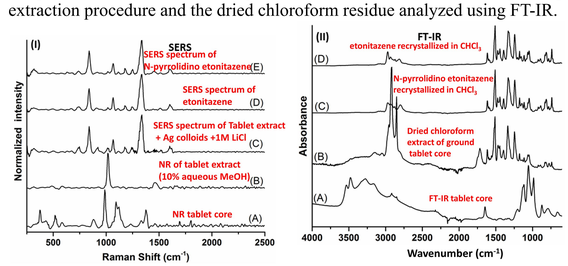


Figure 5. SERS and FT-IR results obtained.

- Analysis using DART-TD-MS requires minimal sample preparation and allows quick (20 second) run times. Many of the nitazene compounds shared common fragment ions at m/z 100 and 72. In each of the solid suspect samples RS-1 through RS-6, the correct nitazene compound present was identified 100% of the time (no false negatives), although in some cases false positives were observed in RS-3 and RS-4 (Table 1).

Figure 6. Mass spectral database match of sample RS-1 to *N*-pyrrolidino etonitazene

Table 1. Showing LC-MS, SERS, FT-IR, and DART-TD-MS results of analyzed samples.

	Observed results by…..					Overall results
Suspect Sample	**LC-MS**	**SERS TruScan**	**SERS Progeny**	**FT-IR**	**DART-TD-MS**	
RS-1, 2, 5, and 6	N-pyrrolidino etonitazene	N-pyrrolidino etonitazene, Etonitazene, N-piperidinyl etonitazene	N-pyrrolidino etonitazene	N-pyrrolidino etonitazene, N-desethyl etonitazene, Etonitazene, N-piperidinyl etonitazene	N-pyrrolidino etonitazene	N-pyrrolidino etonitazene
RS-3	Etodesnitazene, etizolam, 4-ANPP, fentanyl	Etodesnitazene	Etodesnitazene, metodesnitazene	Etodesnitazene	Etodesnitazene, N-desethyl etonitazene	Etodesnitazene
RS-4	Isotonitazene	Isotonitazene	Isotonitazene	Isotonitazene	Isotonitazene, Protonitazene	Isotonitazene

- In some suspect samples, SERS and FT-IR spectra indicated the presence of a nitazene-type compound, but the exact structure of some of the nitazene compounds could not be determined using these techniques due to spectral similarities from band broadening.
- LC–MS was able to detect additional compounds in RS-3 sample that were not detected using SERS, FT- IR, and DART-TD-MS. These additional analytes were likely in very low concentrations compared to etodesnitazene. Additionally, one main limitation of SERS, FT-IR, and DART-TD-MS is that these techniques do not employ a front-end separation prior to analysis and will thus only typically detect compounds higher in concentration or those soluble under the experimental conditions used.
- Overall, using orthogonal techniques such as MS and FT-IR [or orthogonal techniques MS and Raman] the analysts were able to identify the exact nitazene analog present in each sample.

Conclusion

- The complementary and/or orthogonal techniques evaluated in this study proved successful for determining that the suspect tablets were not consistent with authentic products and for the rapid detection of nitazene analogs.
- The SERS and FT-IR spectra of 16 different nitazene standards were collected, added to the handheld Raman devices and FT-IR libraries and successfully employed for identifying 2-benzylbenzimidazoles in suspect counterfeit tablets.
- The DART-TD-MS method was rapid and sensitive for detecting and identifying different nitazene analogs in the analyzed samples. Its main shortcoming was in the differentiation of nitazene isomers that have the same monoisotopic mass or compounds with similar fragmentation patterns, due to the lack of chromatographic separation.
- The simultaneous deployment of these devices as part of a workflow at a remote sampling site with limited laboratory space allows for the screening of suspect counterfeit tablets and increased confidence in the detection and identification of nitazene analogs.

References:

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2. Vandeputte et al., Chemical Characterization, and μ-Opioid Receptor Activity Assessment of the Emerging Group of "Nitazene" 2-Benzylbenzimidazole Synthetic Opioids. *ACS Chem Neurosci. 2021;* 12(7):1241-1251. Doi.org/10.1021/acschemneuro.1c00064
3. Kimani et al., Rapid Screening of 2-benzylbenzimidazole nitazene analogs in suspect counterfeit tablets using Raman, SERS, DART-TD-MS, and FT-IR. Drug Test Anal. 2023;1-12