Detection of nitazenes in vape juice

Daniel McMillan[1]; Toyosi Akanji[2]; Karl Oetjen[2]
[1]SCIEX, UK; [2]SCIEX, USA

Background and Aim

The continuous emergence of novel synthetic opioids (NSO) on the recreational drug market is creating an additional challenge for drug tracking agencies and laboratories to meet. In recent years, a class of synthetic opioid originally developed in the 1950s as opioid analgesics called the nitazenes has been detected in the illicit drug supply and implicated in overdose mortality. Nitazenes vary greatly in potency and purity and thus often require only a small amount to cause acute intoxications. Their recent detection in illicit vape juice which resulted in accidental and fatal drug overdoses poses a major challenge for public health officials.

In this study, a collection of vape juice products was purchased from vaping stores and screened for a panel of 15 nitazenes. A quantitative LC-MS/MS method was developed to accurately detect low-levels of nitazenes in the vape juice products analyzed.

Methods

Sample collection and calibration curve preparation. Three vape juice samples were purchased from vaping stores. The samples were diluted 1000 times in methanol and directly injected for LC-MS/MS analysis. These samples were used as the matrix to establish the calibration curve and were not intended for the identification of nitazene adulteration.

Liquid chromatography. Liquid chromatography was performed using a Shimadzu LC-40 with a Phenomenex Kinetex 2.6 µm Phenyl-hexyl 100 Å, 50 x 4.6 mm column. The flowrate was 0.700 mL/min, the column oven was set to 40°C, and the injection volume was 10 µL. Mobile phase A was 10mM ammonium formate in Optima grade water, and mobile phase B was Optima grade methanol with 0.05% (v/v) formic acid. The total run time was 10 min and gradient conditions are described in **Table 1**.

Table 1. LC gradient for the analysis of nitazenes in vape juice.

Time (min)	Flow rate (mL/min)	Mobile phase A%	Mobile phase B%
0.0	0.700	90	10
7.0	0.700	2	98
8.5	0.700	2	98
8.6	0.700	90	10
10.0	0.700	90	10

Mass spectrometry. Samples were analyzed using the SCIEX QTRAP 4500 system and data was acquired using the scheduled MRM algorithm with positive electrospray ionization. A single acquisition method was used for the analysis of all 15 nitazene analytes as well as the internal standard, isotonitazene-D17. At least 2 transitions per analyte were monitored, except for Clonitazene which only had one stable transition **(Table 2)**. Optimized compound-specific parameters were used. The optimized gas & source conditions used were, ion spray voltage: 2500 V, source temperature: 600°C., gas 1: 60 psi, gas 2: 60 psi, curtain gas: 30 psi, CAD gas: 7 psi. The base structure for nitazene with the substitution for the specific analogues monitored is shown in **Table 3**.

Table 2. Compound-specific parameters for the analysis of nitazenes using the SCIEX QTRAP 4500 system. First value represents the quantifier MRM parameters, second value represents the qualifier MRM parameters.

Name	Q1	Q3	DP	CE	CXP
4'-Hydroxy nitazene	369.2	100.1/107	106	29/71	8/16
Metonitazene	383.2	100.1/72.1	81	31/57	6/4
Metodesnitazene	338.2	100/120.9	96	29/43	16/14
Etonitazene	397.2	100.1/72.1	76	27/59	8/6
Etodesnitazene	352.2	100.1/72	81	39/57	8/10
Protonitazene	411.2	100.1/107	111	33/79	8/16
Butonitazene	425.3	100.1/72.1	111	29/67	14/8
Isotonitazene	411.2	100.1/71.9	101	29/65	10/6
Isotodesnitazene	366.3	100.1/106.9	81	27/61	10/16
N-Desethyl isotonitazene	383.2	72.1/312.1	96	37/25	10/24
N-Desethyl etonitazene	369.2	72/298	81	35/27	12/18
Clonitazene	387.2	100	121	31	16
Flunitazene	371.2	100.1/109.1	116	33/79	8/8
N-Pyrrolidino etonitazene (Etonitazepyne)	395.2	98.1/135.1	75	33/33	10/10
N-Piperidinyl etonitazene (Etonitazepipne)	409.2	112.1/106.9	101	31/77	8/10
Isotonitazene-D7	*418.3*	*100.1/72*	*56*	*23/59*	*8/4*

SCIEX QTRAP 4500 system

Chromatography

- Good separation was achieved for the panel of the 15 nitazenes **(Figure 1)**
- Protonitazene and isotonitazene isobars were chromatographically separated using the LC conditions **(Figure 1 insert)**

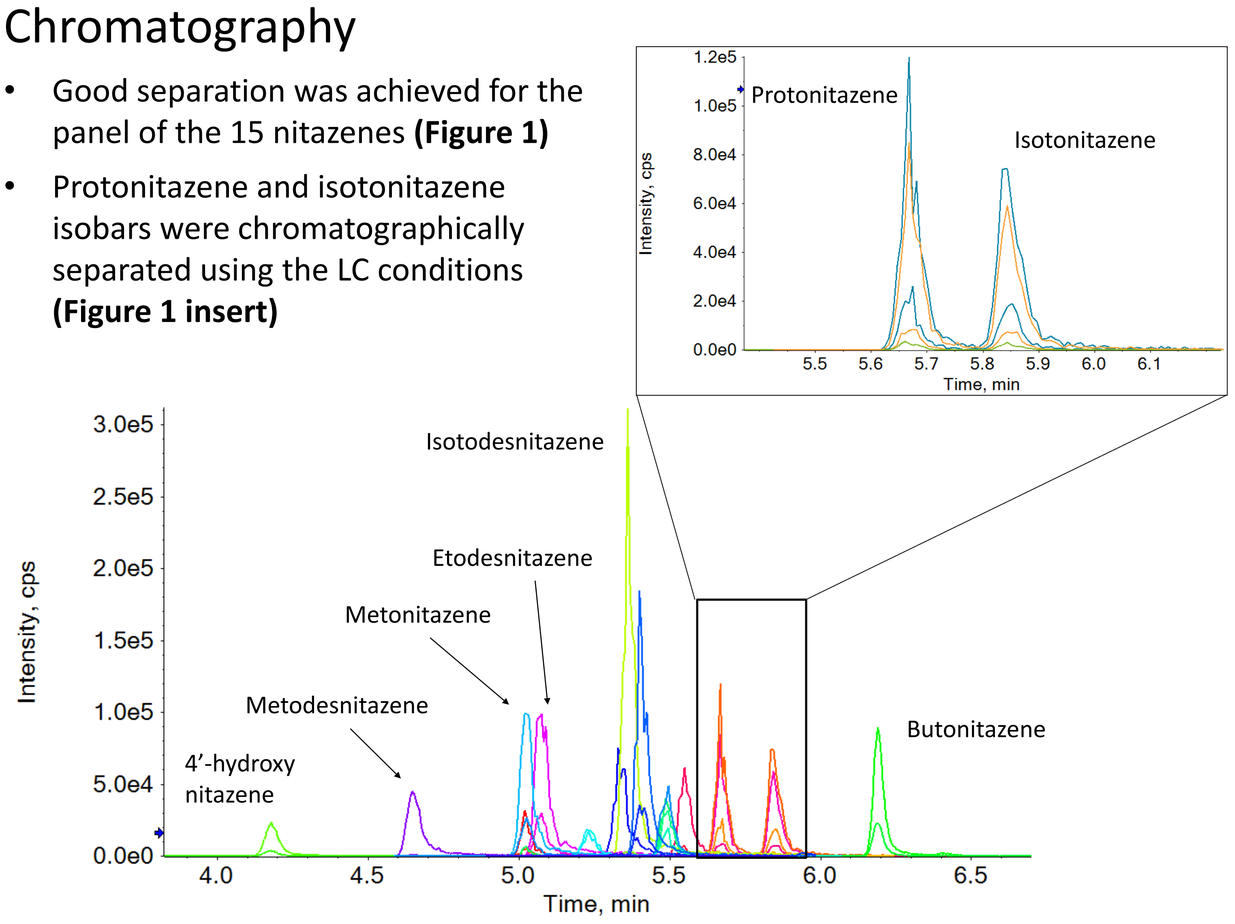


Figure 1. Overlaid extracted ion chromatogram (XIC) of a mixed 10 ppb nitazene solvent standard. Chromatogram zoomed to show region from 4 to 6.5 min. Insert shows chromatographic separation of protonitazene and isotonitazene isobars

Vape juice matrix spikes

- Three vape juice samples (A, B, C) were spiked with the nitazene standard mix, diluted 1000-fold with methanol and analyzed
- All quantifier transitions were detected at 1 ppb **(Figure 2);** qualifier transitions were detected at 10 ppb

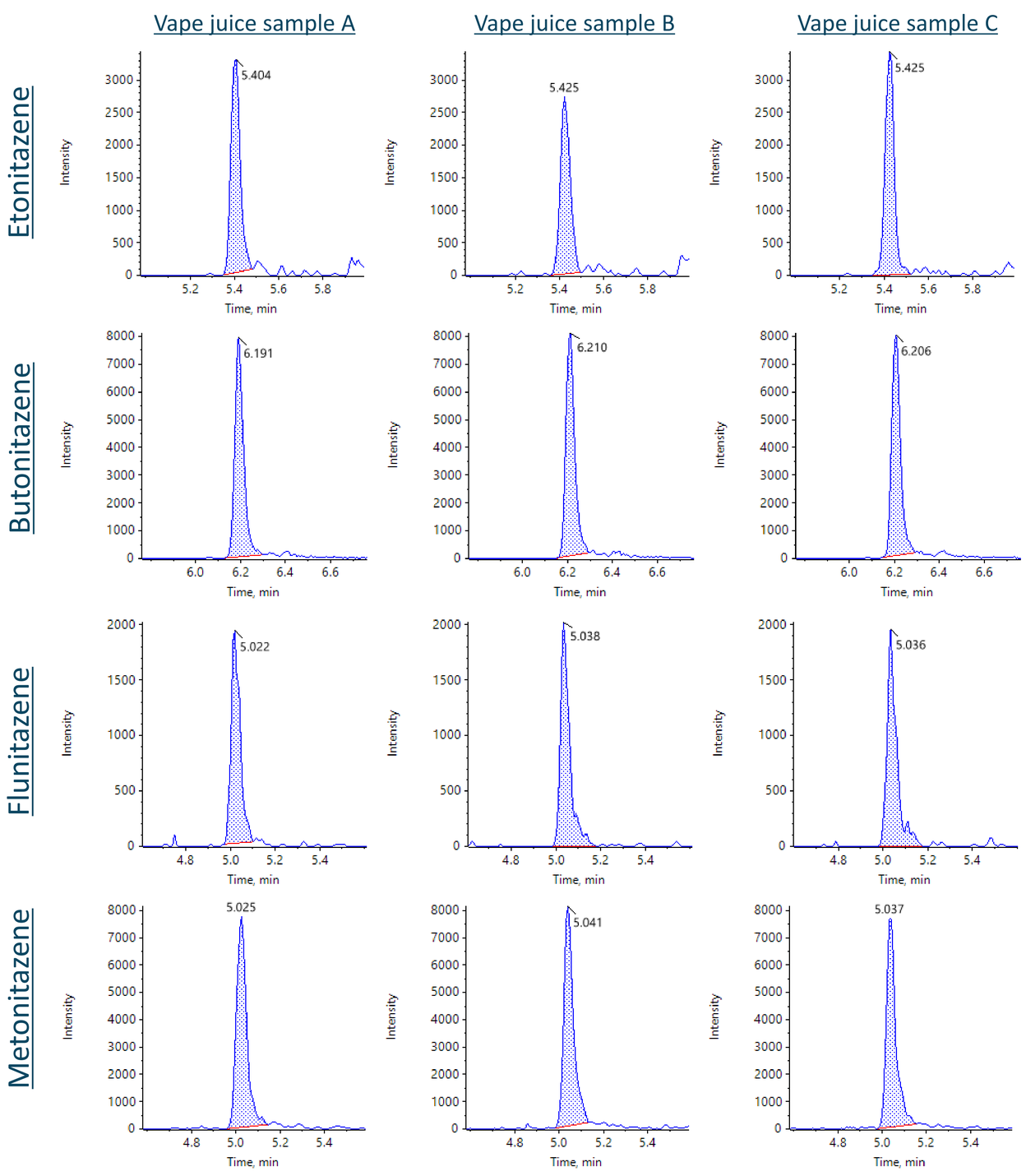
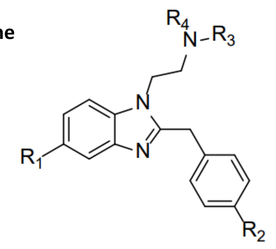


Figure 2. XICs of quantifier MRMs for etonitazene, butonitazene, flunitazene and metonitazene spiked in vape juice samples A, B, and C at 1 ppb.

Table 3. Nitazene analytes monitored in vape juice showing the base structure with substitutions.

Name	R1	R2	R3	R4
4'-Hydroxy nitazene	NO_2	OH	CH_2CH_3	CH_2CH_3
Metonitazene	NO_2	OCH_3	CH_2CH_3	CH_2CH_3
Metodesnitazene	H	OCH_3	CH_2CH_3	CH_2CH_3
Etonitazene	NO_2	OCH_2CH_3	CH_2CH_3	CH_2CH_3
Etodesnitazene	H	OCH_2CH_3	CH_2CH_3	CH_2CH_3
Protonitazene	NO_2	$OCH_2CH_2CH_3$	CH_2CH_3	CH_2CH_3
Butonitazene	NO_2	$OCH_2CH_2CH_2CH_3$	CH_2CH_3	CH_2CH_3
Isotonitazene	NO_2	$OCH(CH_3)_2$	CH_2CH_3	CH_2CH_3
Isotodesnitazene	H	$OCH(CH_3)_2$	CH_2CH_3	CH_2CH_3
N-Desethyl isotonitazene	NO_2	$OCH(CH_3)_2$	H	CH_2CH_3
N-Desethyl etonitazene	NO_2	OCH_2CH_3	H	CH_2CH_3
Clonitazene	NO_2	Cl	CH_2CH_3	CH_2CH_3
Flunitazene	NO_2	F	CH_2CH_3	CH_2CH_3
N-Pyrrolidino etonitazene (Etonitazepyne)	NO_2	OCH_2CH_3	$CH_2CH_2CH_2CH_2$	
N-Piperidinyl etonitazene (Etonitazepipne)	NO_2	OCH_2CH_3	$CH_2CH_2CH_2CH_2CH_2$	
Isotonitazene-D7	*NO_2*	*$OCD(CD_3)_2$*	*CH_2CH_3*	*CH_2CH_3*

Base structure of nitazene with substitutions.



Calibration curve linearity

- Calibrator concentrations ranged from 0.5 to 50 ppb
- r^2 values >0.992 for all nitazenes **(Figure 3)**

Figure 3. Solvent-based calibration curves for metonitazene and isotonitazene

Conclusions

- A comprehensive method for the analysis of 15 nitazenes in vape juice was successfully developed
- The 10 min LC gradient method enabled baseline separation of protonitazene and isotonitazene isobars
- Matrix spikes showed good sensitivity down to 1 ppb