Development and Validation of a Combined Selected Ion Monitoring-Scan GC-MS Method for Nitazene Analogs

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ABSTRACT

Nitazene analogs are the latest class of novel synthetic opioids (NSOs) that have become prevalent in seized drug casework. Due to their potency and increasing prevalence, it is imperative that seized drug laboratories have sufficient methods of detection for commonly used instruments like gas chromatography-mass spectrometry (GC-MS). To address this need, this study develops and validates a selected ion monitoring-scan (SIM-scan) method to identify 20 nitazene analogs using traditional GC-MS instrumentation.

INTRODUCTION

The scheduling of fentanyl-related substances into Schedule I of the U.S. Controlled Substances Act has caused a shift towards non-fentanyl NSOs [1]. Nitazene analogs are one class of NSOs that have become prevalent due to their heroin-like effects and high potencies [2]. The increasing prevalence of nitazene analogs in seized drug casework poses a challenge for seized drug analysts, as common methods of analysis may struggle to detect the small quantities of nitazene analogs present in seized drug samples. For example, GC-MS instrumentation operated in full scan mode may not possess sufficient sensitivity to reliably detect nitazene analogs [3]. In comparison, SIM mode provides enhanced sensitivity by only monitoring specific ions but does not enable mass spectral library searching. The combination of SIM and scan detection provides a sensitive method that also enables the use of mass spectral library searching for unknown compound identification.

This study utilizes a combined SIM-scan method for the identification of 20 nitazene analogs using GC-MS. Chromatographic separation of the nitazene analogs was maximized while also enabling the identification of other controlled substances and cutting agents that may be present. The method was validated in terms of selectivity, limit of detection (LOD), repeatability, reproducibility, carryover, and processed sample stability, and applied to the analysis of 35 blind simulant samples, as well as two authentic samples. The developed SIM-scan GC-MS method provides a potential solution for seized drug laboratories to address the increasing presence of nitazene analogs in seized drug casework, which could easily be missed by routine scan data acquisition given the potency of nitazene analogs.

MATERIALS & METHODS

Chemicals and Sample Preparation

The 20 nitazene analogs analyzed in this study were 3'-methoxy metodesnitazene, 4'-hydroxy nitazene, 5-aminoisotonitazene, 5-methyl etodesnitazene, ethyleneoxynitazene, iso-butonitazene, isotodesnitazene, menitazene, metodesnitazene, N-desethyl etonitazene, N-desethyl isotonitazene, N-piperidinyl 4'-hydroxy nitazene, N-piperidinyl etonitazene, N-piperidinyl protonitazene, N-pyrrolidino 4'-hydroxy nitazene, N-pyrrolidino isotonitazene, N-pyrrolidino metonitazene, nitazene, propylnitazene, and protodesnitazene. Mixtures were prepared with PCP for method optimization at 25 ppm.

RESULTS & DISCUSSION

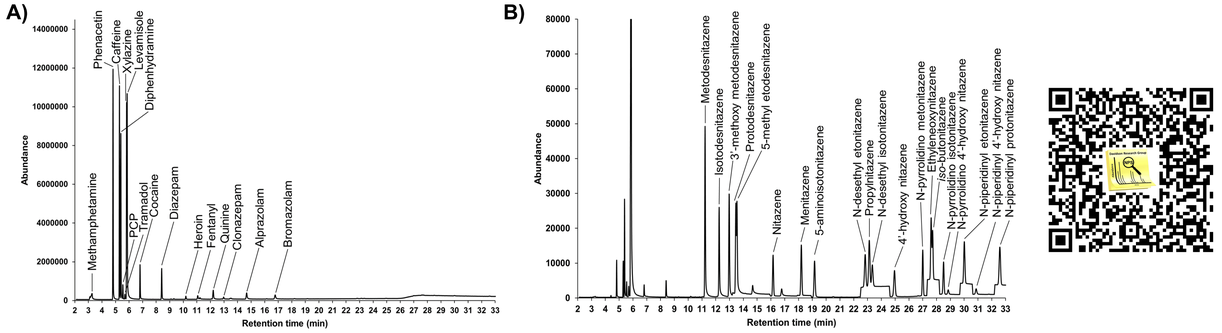

Figure 1. Comparison of A) scan acquisition data for the interferences and B) SIM acquisition data for targeted nitazene analogs.

- SIM provides better sensitivity and selectivity for the 20 nitazene analogs as compared to the full scan data.
- The LOD was compound-dependent but varied between 5 and 10 ppm.

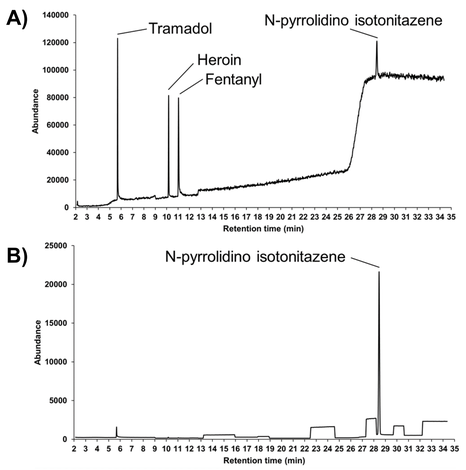

Figure 2. Exemplar results for Blind sample 20 for A) scan acquisition data and B) SIM acquisition data.

Table 1. Targeted SIM ions and dwell times within each SIM group.

Compound	SIM Ion Group (min)	Target Ion (*m/z*)	Ion 1 (*m/z*)	Ion 2 (*m/z*)	Dwell Time (ms)
PCP	2.00	200.0	91.0	242.1	100
Metodesnitazene	9.00	86.1	121.1	337.2	100
Isotodesnitazene	12.01	86.1	365.2	131.0	100
3'-methoxy metodesnitazene	12.73	86.1	151.1	367.2	100
Protodesnitazene	13.20	86.1	365.2	149.1	50
5-methyl etodesnitazene		86.1	135.0	365.2	50
Nitazene	15.91	86.1	91.0		100
Menitazene	17.90	86.1	105.1	103.0	100
5-aminoisotonitazene	18.90	86.1	380.3	169.1	100
N-desethyl etonitazene		135.1	311.1	89.0	50
Propylnitazene	22.49	86.1	91.0	104.0	50
N-desethyl isotonitazene		107.0	149.1	325.1	50
4'-hydroxy nitazene	24.60	86.1	107.0	58.0	100
N-pyrrolidino metonitazene	26.69	84.1	121.0	55.0	100
Ethyleneoxynitazene	27.30	86.1	133.0	79.0	50
Iso-butonitazene		86.1	107.0		50
N-pyrrolidino isotonitazene	28.19	84.1	55.0		100
N-pyrrolidino 4'-hydroxy nitazene		84.1	55.0		100
N-piperidinyl etonitazene	29.70	98.1	55.0		100
N-piperidinyl 4'-hydroxy nitazene	30.59	98.1	55.0	77.0	100
N-piperidinyl protonitazene	32.19	98.1	55.0		100

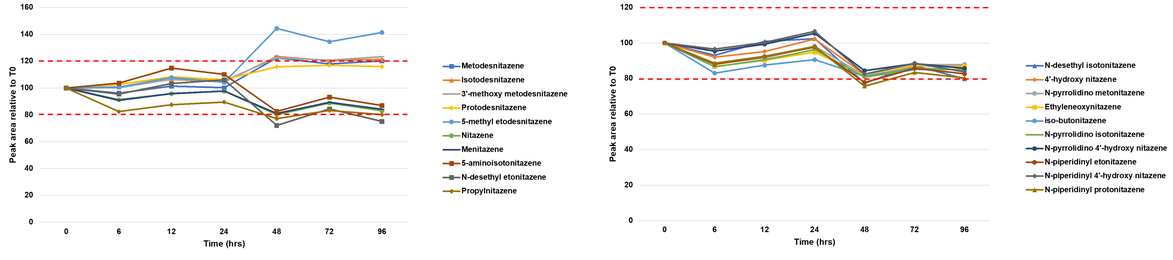

Figure 3. Relative peak areas when compared to time zero (t_0) for each nitazene analog over 96 hours. Analogs are split based on their elution order for ease of visualization.

- Although some unusual trends were observed for the processed sample stability, all compounds were stable for 24 hours, and 11 compounds were stable for the full 96 hours.
- The only blind simulant samples to be incorrectly identified were due to ion ratios outside of tolerance.

MATERIALS & METHODS

Instrumentation

An Agilent 8890 GC-5977B MS was used with an HP-5MS column. The carrier gas was helium with a 1.5 mL/min flow rate. A 250 °C inlet temperature was used with a 1 µL injection volume and a 10:1 split ratio. The temperature programming began at 100 °C (1 min hold) then a 40 °C/min ramp rate to 255 °C (1 min hold), followed by a 3 °C/min ramp rate to 260 °C (1 min hold), a 30 °C/min ramp rate to 265 °C (1 min hold), a 1 °C/min ramp rate to 280 °C (1 min hold), and finally, a 30 °C/min ramp rate to 300 °C (8 min hold). The total length of the method was 34.38 minutes.

Method Validation

Acceptance criteria for all validation parameters were S/N > 3, a retention time within 1%, and ion ratios within the appropriate tolerance. The LOD was assessed by analyzing a mixture of all 20 nitazene analogs at 50, 10, 5, and 1 ppm. Carryover was monitored by analyzing each nitazene analog individually at 100 ppm and assessing the subsequent blanks. Selectivity was evaluated by monitoring the retention times of other common interferences. Repeatability was assessed by analyzing the nitazene mixture 10 times within one day, and reproducibility was assessed by analyzing the nitazene mixture once a day for 10 days. Processed sample stability was evaluated using pooled samples that were left on the autosampler at T_0, and assessed at 0, 6, 12, 24, 48, 72, and 96 hours. Blind samples were identified using the same acceptance criteria.

CONCLUSIONS

- ❖ The SIM-scan method provided enhanced sensitivity for all 20 nitazene analogs, with an LOD between 5 and 10 ppm.
- ❖ The method was deemed selective, repeatable, reproducible, and no carryover was observed.
- ❖ All nitazene analogs are stable for at least 24 hours at room temperature.
- ❖ 33/35 blind simulant samples and both authentic samples were correctly identified.
- ❖ Potential solution to address identification of nitazene analogs in seized drug casework samples.

REFERENCES

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