Long-Term Stability of Isotonitazene and Protonitazene in Whole Blood

Amanda L. Pacana[1], PhD; Britni N. Skillman[1], PhD

[1]*Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340*

Sam Houston State University

INTRODUCTION

For drug concentrations to be accurately interpreted in forensic matrices, the stability of the analyte(s) of interest should be considered, especially when samples are stored for long periods of time (e.g., months or years). Factors such as enzymatic activity, storage temperature, pH, analyte chemistry, and bacterial growth can all influence the stability of samples. While many of these factors can be mitigated using additives at the time of collection, other factors such as temperature can vary during instances of power loss, during specimen transport, or on the benchtop during analysis. Samples may also need to undergo analysis or re-analysis weeks or months after specimen receipt, so it is important to have stability information to properly inform standard operating procedures.

Since their emergence in 2019, the nitazene class of novel opioids has expanded in scope, with little information on their stability in forensic matrices. While most samples are generally stored frozen after analysis for long-term storage, a 2022 study found that refrigerated temperatures were best suited for nitazene storage with frozen temperatures demonstrating more inconsistency[1]. The current study sought to further qualitatively assess the stability of two forensically relevant nitazenes over a six-month period at two concentrations in five storage conditions.

REFERENCES

[1]Walton SE, Krotulski AJ, Logan BK (2022) A forward-thinking approach to addressing the new synthetic opioid 2-benzylbenzimidazole nitazene analogs by liquid chromatography–tandem quadrupole mass spectrometry (LC–QQQ-MS). J Anal Toxicol, 46:221–231. https://doi.org/10.1093/jat/bkab117

[2]Pacana, Skillman (2025) A novel screening workflow for nitazene analogs using LC-MS/MS precursor ion scan acquisition. J Anal Toxicol, online. https://doi.org/10.1093/jat/bkaf046

ACKNOWLEDGEMENTS & DISCLOSURES

Thank you to Sam Houston State University, including the Department of Forensic Science, for funding and support. The authors do not have any conflicts of interest to disclose.

RESULTS & DISCUSSION

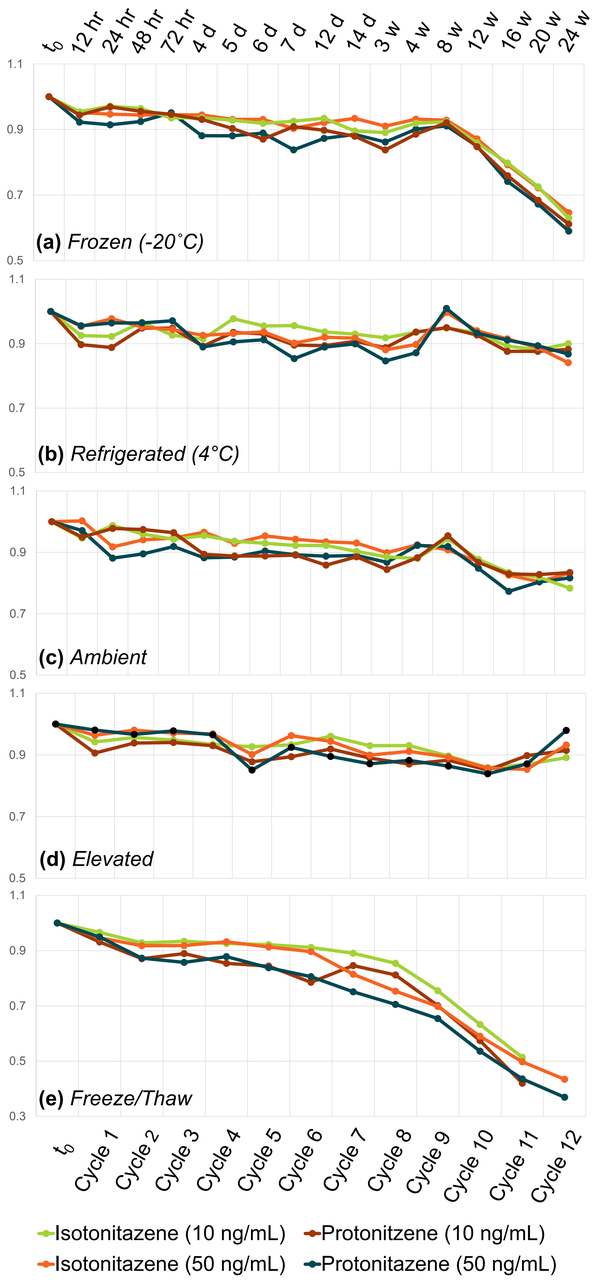

Figure 1 (a-e): Analyte relative peak areas over time for under different storage conditions (values normalized to t_0).

Table 1. Analyte stability in weeks (w) or cycles.

Analyte (ng/mL)		Condition				
		-20˚C	~4˚C	~20˚C	35˚C	Freeze/ Thaw
Isotonitazene	(10)	12-16 w	≥ 24 w	≥ 24 w	≥ 8 w	9 cycles
	(50)	12-16 w	≥ 24 w	≥ 20 w^	≥ 8 w	8 cycles
Protonitazene	(10)	12-16 w	≥ 24 w	≥ 24 w	≥ 8 w	9 cycles
	(50)	12-16 w	≥ 24 w	≥ 20 w^	≥ 8 w	7 cycles

^6-month time period sample not analyzed due to data file error

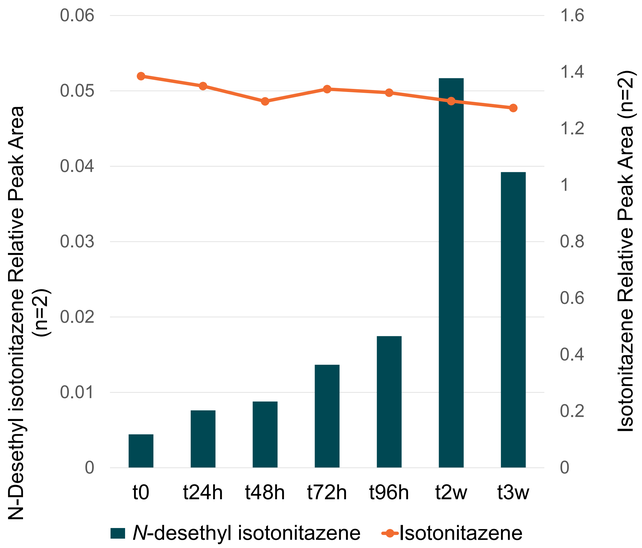

Figure 2: Relative peak area values for isotonitazene vs potential degradation product *N*-desethyl isotonitazene.

- Isotonitazene and protonitazene are **best stored under refrigerated** conditions
- Isotonitazene and protonitazene have **more extensive degradation** over several freeze/thaw cycles
- When analyzed individually, ***N*-desethyl metabolites were qualitatively identified** with their respective parent compound
- Isotonitazene and protonitazene positive samples stored for long periods of time could potentially undergo **degradation to *N*-desethyl compounds**

MATERIALS & METHODS

Sample Preparation:

- Batch preparation with both analytes in whole, preserved bovine blood (10 ng/mL and 50 ng/mL)
- Aliquots in red-top blood tubes for storage:
  - *Frozen* (-20˚C)
  - *Refrigerated* (~4˚C)
  - *Ambient* (~20˚C)
  - *Elevated* (35˚C)
  - *Freeze/thaw cycles* (-20˚C/ambient)
- Sampling performed in duplicate using 0.5 mL of sample and liquid-liquid extraction[2]:
  - *Initial* (t_0)
  - *Daily* (t_{1d}-t_{7d}, t_{12d})
  - *Weekly* (t_{2w}-t_{4w})
  - *Monthly* (t_{8w}-t_{24w})
- *Acceptance criteria*:
  - Within ± 20% peak area response relative to t_0, instable if difference was >20% for at least 2 consecutive points.

Instrumental:

- Agilent 1290 Infinity LC & Agilent 6530 Accurate-Mass Q-TOF (+ESI) in target MS/MS using previously optimized gradient[2] and source conditions

Degradation Analysis:

- Analytes individually prepared in blood (30 ng/mL) and stored at 40˚C
- Relative response and degradation product formation monitored using Auto MS/MS at initial (t_0), t_{24h}, t_{48h}, t_{72h}, t_{96h}, t_{2w}, and t_{3w} time points

CONCLUSIONS

Nitazenes may be more stable in samples when refrigerated rather than frozen (**Figure 1**). Samples potentially containing nitazenes should be stored under refrigeration, though short-term frozen storage is possible (**Table 1**). Multiple freeze/thaw cycles should be avoided and potential degradation into *N*-desethyl compounds under certain conditions may complicate metabolite interpretation (**Figure 2**). Further studies are needed to better define specific degradation pathways.

Scan here for electronic poster

Department of Forensic Science
College of Criminal Justice
forensics.shsu.edu