

The Identification of the Novel Non-Opioid Analgesic Suzetrigine in Whole Blood and Urine using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Isabelle Parry, B.S.*, Britni Skillman, PhD, F-ABFT

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

INTRODUCTION

The long-term effects of the opioid epidemic have led to a search for alternatives for acute pain management. Suzetrigine (Figure 1) is a novel analgesic with no effect on the opioid receptors that was approved by the Food and Drug Administration (FDA) in January 2025¹. Suzetrigine's mechanism of action involves binding to and closing Na_v1.8 sodium channels, preventing the transmission of pain signals. While its abuse potential has not been fully researched, it is likely that it will begin to appear in casework as prescriptions increase. Its potential polydrug toxicity and co-morbidities with other health issues are unknown, so the presence of suzetrigine in casework samples could complicate interpretation of results if it cannot be identified. It is important that forensic toxicologists are prepared with a validated method to identify and quantify it in common toxicology matrices. This study sought to modify an existing liquid-liquid extraction method² and validate an LC-MS/MS method for suzetrigine's quantification in blood and qualitative identification in urine.

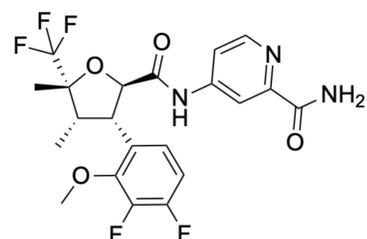


Figure 1: Structure of suzetrigine

MATERIALS & METHODS

This method was validated according to ANSI/ASB 036. The liquid-liquid extraction used is detailed in Figure 2.

- LLE**
- Fortify 250 μ L blood or urine
 - 25 μ L calibrator or QC mix
 - 10 μ L internal standard
 - Add 1 mL 0.4M sodium acetate buffer (pH 3.6)
 - Add 2.5 mL N-butyl chloride
 - Rock for 5 minutes
 - Centrifuge at 3500 rpm, 5 minutes
 - Transfer organic layer to glass conical vial
 - Evaporate under nitrogen (50°C), ~10 minutes
 - Reconstitute with 30 μ L 50:50 mobile phases A:B

Figure 2: Liquid-liquid extraction protocol

RESULTS & DISCUSSION

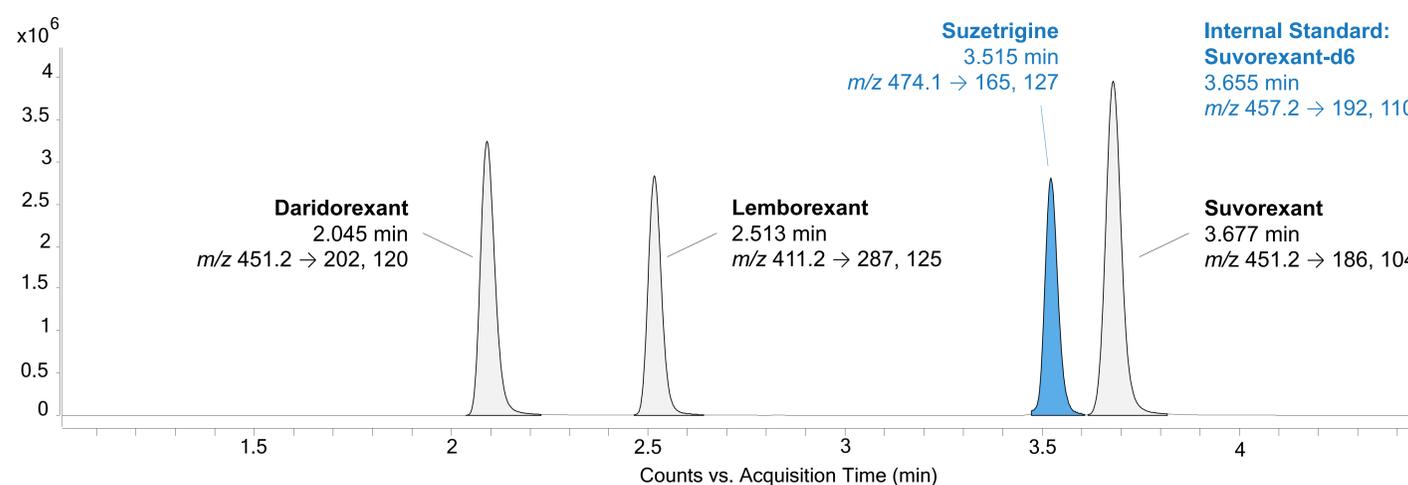


Figure 3: Example chromatogram of an extracted blood sample containing suzetrigine (1000 ng/mL) and previously validated dual orexin receptor antagonists (DORAs)². Retention time (in minutes) is plotted versus response.

Table 2: Suzetrigine method validation results summary

Parameter	Suzetrigine Validation Results
Calibration Model	Quadratic, 1/x weighted; 1.0-1000 ng/mL range
Grand Bias	Within $\pm 5.6\%$ (all QC concentrations: 1.5, 80, 400, 800 ng/mL)
Precision	Within $\pm 14.1\%$ for within- and between-run for all QC concentrations
Matrix Effects	Within $\pm 30\%$ for blood and urine (with no effect on LOD and LOQ)
Interferences	None observed from internal standard, matrices, common drugs of abuse or prescription medications
Limit of Detection (LOD)	0.25 ng/mL (blood and urine)
Lower Limit of Quantitation (LoQ)	1.0 ng/mL (blood)
Carryover (CO)	Acceptable, % CO of LoQ < 10.4%
Dilution Integrity	Values at 5X dilution within $\pm 5.5\%$ of expected concentration
Processed Sample Stability	Acceptable up to 72 hours, % difference from t ₀ within $\pm 7.56\%$ (relative peak area)

REFERENCES

- United States Food and Drug Administration (2025) FDA approves novel non-opioid treatment for moderate to severe acute pain, *FDA News Release* [30-Jan-2025]; <https://www.fda.gov/news-events/press-announcements/fda-approves-novel-non-opioid-treatment-moderate-severe-acute-pain>. Accessed July 25, 2025.
- Gomonit, M. and Skillman, B. (2025) Quantification of daridorexant, lemborexant and suvorexant in whole blood using liquid chromatography-tandem mass spectrometry, *Journal of Analytical Toxicology*; bkaf023, <https://doi.org/10.1093/jat/bkaf032>. Accessed July 25, 2025.

MATERIALS & METHODS

Chromatographic separation and detection was achieved on an Agilent 1290 Infinity II Liquid Chromatograph paired to an Agilent 6475A Triple Quadrupole Mass Spectrometer. Positive electrospray ionization (ESI+) was used with optimized parameters (Table 2).

Table 1: Optimized instrumental parameters

Parameter	Value
Column	Agilent Infinity Poroshell 120 EC-C18 (100 x 2.1 mm, 2.7 μ m) with matching guard
Column Temperature	35°C
Autosampler Temperature	6°C
Mobile Phase	A: 0.1% formic acid in diH ₂ O B: 0.1% formic acid in ACN
Flow Rate	0.4 mL/min
Gradient (7.5 min)	Start at 35% B, increase to 40% B (0.5 - 3.5 min), increase to 80% B (3.5 - 4.5 min), hold at 80% for one minute. Post-equilibration for 2 minutes.
Drying Gas	300°C, 13 L/min
Sheath Gas	350°C, 12 L/min
Nebulizer Pressure	45 psi
Capillary Voltage	3000V
Nozzle Voltage	2000V

CONCLUSIONS

This analytical workflow was developed and validated according to ANSI/ASB Standard 036 for quantitative and qualitative analysis of suzetrigine in common toxicology matrices using a simple LLE with LC-MS/MS analysis. This method will be an important tool for forensic laboratories as suzetrigine is more commonly prescribed for acute pain management.

ACKNOWLEDGEMENTS

Thank you to Sam Houston State University and the Department of Forensic Science for funding and support.