The Analysis of Tianeptine by Reverse-Phase SPE and LC-MS/MS

UCT Part Numbers

SSHLB063
Styre Screen® HLB
60 mg, 3 mL

SPHPHO6001-10
Select pH Buffer Pouch
100 mM Phosphate Buffer pH 6.0

SCS27-C18521
SelectraCore® C18 Column
50 x 2.1 mm, 2.7 µm

SCS27-C18GDC21
SelectraCore® C18 Guard Column
5 x 2.1 mm, 2.7 µm

SLGRDHLDR-HPOPT
Selectra® Direct Connect
Guard Holder

Introduction

Tianeptine is an atypical antidepressant prescribed in some countries for depression and anxiety; however, it is not approved by the U.S. Food and Drug Administration (FDA) for medical use [1]. It is an emerging drug of abuse in the U.S., being falsely marketed in gas stations and online as a dietary supplement under names such as "gas station heroin", "Zaza", and "Neptune's Fix" [2]. At high doses, tianeptine is a full agonist at the mu-opioid receptor and a weak agonist at the delta-opioid receptor [3]. Tianeptine is not scheduled under the Controlled Substances Act, but a few states such as Florida, Alabama, Georgia, and Mississippi, have already banned it [1].

A stand-alone method for tianeptine was developed due to its unique amphoteric characteristics and abuse at high concentrations. In a previous study, low tianeptine recovery was obtained using a liquid-liquid extraction (LLE) method. This low recovery was hypothesized to result from the compound's state of being always ionized, which reduces its likelihood of partitioning into the organic phase [4]. During the development of this application note the best mode of solid phase extraction was determined by comparing extraction efficiencies. A higher calibration range is needed to quantify samples and prevent samples from needing to be re-analyzed after dilution. This application note outlines the extraction of tianeptine from blood and urine using Styre Screen® HLB and analysis via LC-MS/MS using SelectraCore® C18 LC column.

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Sample Pretreatment

In a test tube add 200 µL sample + 2 mL 100 mM phosphate buffer pH 6 + ISTDs. Mix and centrifuge.

SPE Procedure

1. Condition Column

a) 1 x 3 mL MeOH
b) 1 x 3 mL 100 mM phosphate buffer pH 6

2. Load sample

a) Load at 1 to 2 mL/minute

3. Wash Column

a) 1 x 3 mL 100 mM phosphate buffer pH 6
b) 1 x 3 mL 10% MeOH in DI H_2O

4. Dry Column

a) Dry for at least 10 minutes under full pressure or vacuum

5. Elute

a) 1 x 3 mL EtOAc:IPA:NH_4OH (78:20:2)
Note: Make elution solvent fresh daily

6. Evaporate

a) Evaporate eluate at 40°C, starting at 5 psi and increasing pressure slowly over 30 minutes

7. Reconstitute

a) 1 mL MeOH:H_2O (50:50) or other appropriate solvent and volume

LC-MS/MS Parameters	
LC-MS/MS	Shimadzu Nexera LC-30AD with MS-8050
UHPLC Column	SelectraCore® C18 Column 50 x 2.1 mm, 2.7 µm (PN: **SCS27-C18521**)
Guard Column	SelectraCore® C18 Guard Column 5 x 2.1 mm, 2.7 µm (PN: **SCS27-C18GDC21**)
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection Volume	5 µL
Mobile Phase A	5 mM ammonium formate + 0.1% formic acid in water
Mobile Phase B	5 mM ammonium formate + 0.1% formic acid in methanol

Gradient Program		
Time (min)	**Mobile Phase A (%)**	**Mobile Phase B (%)**
0	95	5
6-7	0	100
7.10-10	95	5

MRM					
Parent Ion (m/z)	**RT (min)**	**Product Ion 1 (m/z)**	**CE (V)**	**Product Ion 2 (m/z)**	**CE (V)**
436.5	3.69	292.3	-25.0	27.9	-38.0

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Chromatogram

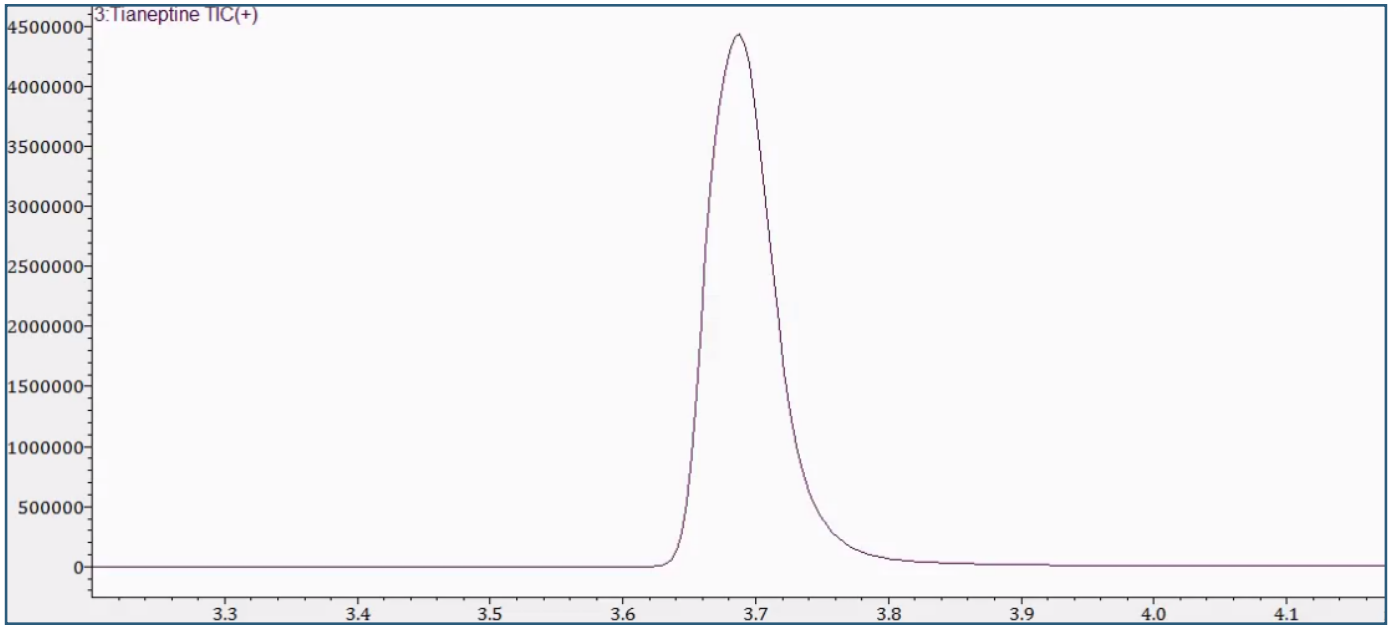

Figure 1: Chromatogram of an extracted standard prepared at 75 ng/mL

Calibration Curve

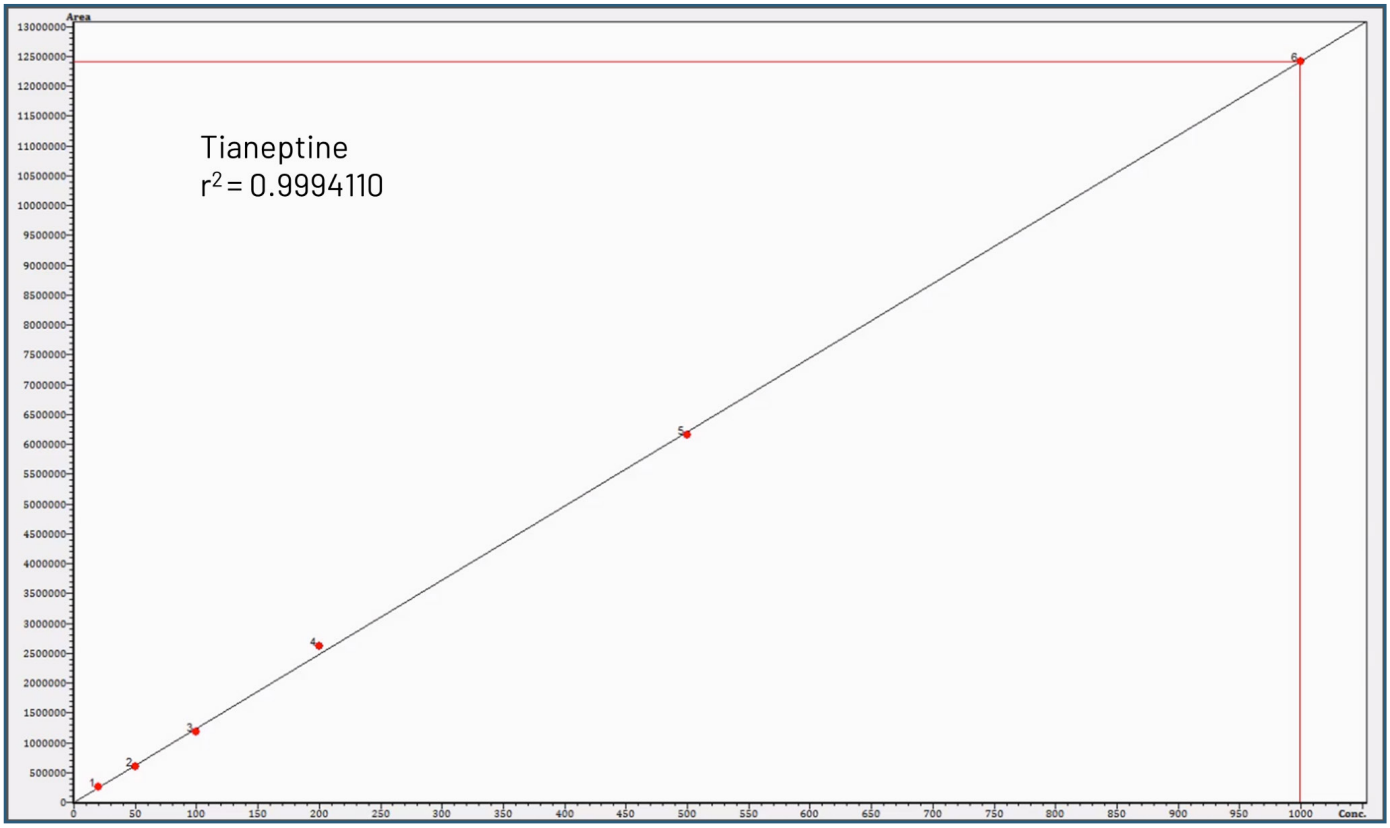

Figure 2: 6-point solvent calibration curve for Tianeptine weighted 1/c with linear equation and r^2 value (20, 50, 100, 200, 500, 1000 ng/mL).

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Results

Urine						
n=5	25 ng/mL			750 ng/mL		
Analyte	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD
Tianeptine	96%	-15%	7%	93%	-19%	5%

Blood						
n=5	25 ng/mL			750 ng/mL		
Analyte	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD
Tianeptine	89%	24%	4%	87%	-12%	5%

*Recoveries were calculated using pre and post-spiked samples. Matrix effects were calculated by comparing post-spiked and evaporated solvent standards.

Conclusion/Discussion

Tianeptine proved to be a difficult analyte to extract due to its amphoteric properties. Utilizing the ion exchange-function on UCT's flagship SPE column, Clean Screen® DAU yielded extraction efficiencies ~60%. Lower recoveries are most likely due to the competing charges; higher recoveries were achieved by using reverse-phase on HLB SPE column. Recoveries for tianeptine ranged from 87-96% across two matrices and two concentrations. Matrix effects ranged between (-19)% and 24% with relative standard deviations less than 10%. To prevent the LC-MS/MS from being overloaded, a lower sample volume and higher final reconstitution volume were utilized. This application note outlines a standalone method for the analysis of the emerging drug tianeptine from blood and urine.

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References

[1] FDA Consumer Updates (February 2022) Tianeptine Products Linked to Serious Harm, Overdoses, Death. https://www.fda.gov/

[2] The Center for Forensic Science Research and Education (February 2024) Emerging Drug Alert: Tianeptine. https://www.cfsre.org

[3] Edinoff AN, Sall S, Beckman SP, Koepnick AD, Gold LC, Jackson ED, Wenger DM, Cornett EM, Murnane KS, Kaye AM, Kaye AD. Tianeptine, an Antidepressant with Opioid Agonist Effects: Pharmacology and Abuse Potential, a Narrative Review. Pain Ther. 2023 Oct;12(5):1121-1134. doi: 10.1007/s40122-023-00539-5. Epub 2023 Jul 15. PMID: 37453966; PMCID: PMC10444703.

[4] Bakota, E. L., Samms, W. C., Gray, T. R., Oleske, D. A., & Hines, M. O. (2018). Case Reports of Fatalities Involving Tianeptine in the United States. Journal of analytical toxicology, 42(7), 503–509. https://doi.org/10.1093/jat/bky023

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