The Analysis of Tianeptine in Whole Blood and Urine by Reverse-Phase SPE and LC-MS/MS

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INTRODUCTION

Tianeptine is an atypical tricyclic antidepressant not approved by the U.S. Food and Drug Administration (FDA) for medical use. It is being falsely marketed in gas stations and online as a dietary supplement under names such as "gas station heroin", "Zaza" and "Tianna Red". At low doses, tianeptine helps regulate glutamatergic signaling making it an effective drug to treat depression and anxiety. At higher doses, tianeptine has an opioid-like effect because it is a full agonist at the mu-opioid receptor and a weak agonist at the delta-opioid receptor [1]. There is a concern in the U.S. for misuse and there has been an increase in the number of tianeptine toxicity cases over the last few years. Due to the high doses of tianeptine being consumed, forensic laboratories need a procedure with a high upper limit of quantitation. Additionally, tianeptine can be difficult to extract because it contains active acidic and basic groups. A previous study obtained low tianeptine recovery using a liquid-liquid extraction (LLE) method, which was hypothesized to result from the compound's state of being always ionized, reducing its likelihood of partitioning into the organic phase. [2]. This poster outlines an optimized solid phase extraction (SPE) procedure utilizing UCT's Styre Screen® HLB for the extraction of tianeptine from blood and urine with high recoveries.

[1] Edinoff, A. N., Sall, S., Beckman, S. P., Koepnick, A. D., Gold, L. C., Jackson, E. D., Wenger, D. M., Cornett, E. M., Murnane, K. S., Kaye, A. M., & Kaye, A. D. (2023). Tianeptine, an Antidepressant with Opioid Agonist Effects: Pharmacology and Abuse Potential, a Narrative Review. Pain and therapy, 12(5), 1121–1134. https://doi.org/10.1007/s40122-023-00539-5

[2] 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

INSTRUMENT PARAMETERS

LC-MS/MS System	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 Column 5 x 2.1 mm, 2.7 µm (**PN: SCS27-C18GDC21**)
Column Temp.	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	Conc. B: 5% (0 min) - 100% (6 to 7 min) - 5% (7.10 to 10 min)

CHROMATOGRAM

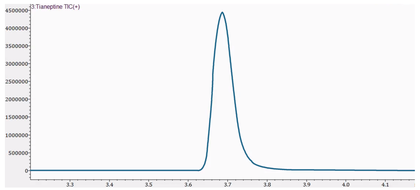

Figure 1: Chromatogram of an extracted standard prepared at 75 ng/mL

SPE PROCEDURE

Styre Screen® HLB 60 mg, 3 mL (PN: SSHLB063)

SAMPLE PRETREATMENT

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

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

STEP 2: Load
a) Load at 1 to 2 mL/minute

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

STEP 4: Dry
a) Dry for at least 10 minutes under full pressure or vacuum

STEP 5: Elute
a) 1 x 3 mL EtOAc: IPA:NH_4OH (78:20:2)
Note: Make elution solvent fresh daily

STEP 6: Evaporate
a) Evaporate eluate at 40°C, starting at 5 psi and increasing pressure slowly over 30 minutes

STEP 7: Reconstitute
a) 1 mL MeOH:H_2O (50:50) or other appropriate solvent and volume

CALIBRATION CURVE

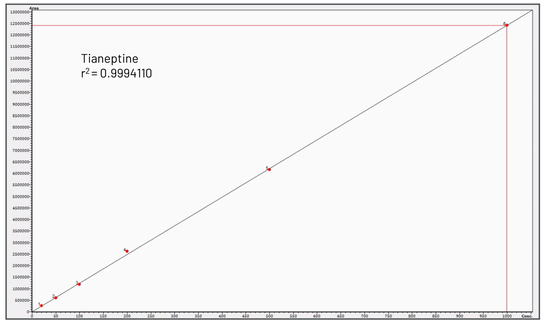

Figure 2. Calibration Curve of Tianeptine with r² value [20, 50, 100, 200, 500, 1000 ng/mL]

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%

CONCLUSION

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 SPE. 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.

Disclosure: The speaker, author, moderator, planning member and/or presenter/s do have financial relationships with UCT Inc., as defined in the AACC policy on potential bias or conflict of interest. The specific product/s: Clean Screen® DAU and the SelectraCore® column will be mentioned and/or discussed.

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