

INTRODUCTION

Tetrodotoxin (TTX) is an extremely potent naturally occurring neurotoxin. It is primarily found in pufferfish, but has also been identified in certain marine bacteria, algae, and other marine organisms. TTX selectively blocks voltage-gated sodium ion channels on neuronal cell membranes, thereby disrupting nerve impulse transmission. Following ingestion of TTX-contaminated food, poisoning symptoms can develop rapidly, including numbness of the lips and tongue, vomiting, respiratory distress, muscle paralysis, and, in severe cases, death.

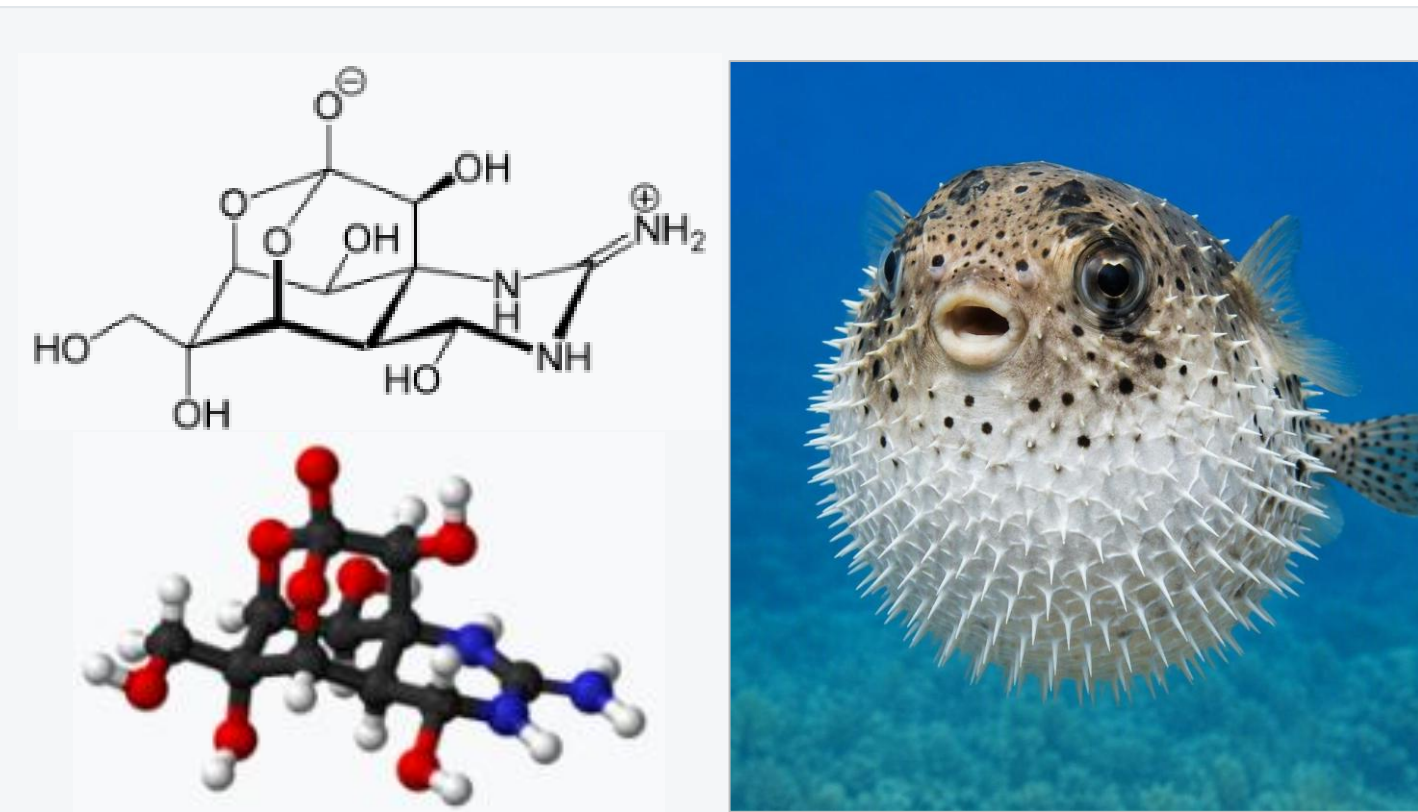


Figure 1. Chemical structure of tetrodotoxin (TTX) [left] and its principal natural source, the pufferfish [right].

Because tetrodotoxin intoxication has a rapid onset and a high fatality rate, accurate and rapid detection of TTX in biological specimens is critical. LC-MS/MS technology offers exceptional sensitivity, selectivity, and quantitative capability, enabling precise identification and quantification of tetrodotoxin in complex biological matrices such as blood and urine.

Based on the sample-preparation procedure described in SF/Z JD0107011-2011 [Determination of Tetrodotoxin in Biological Samples by Liquid Chromatography-Tandem Mass Spectrometry],¹ the method was optimized and implemented on a SCIEX mass spectrometry platform. It enables rapid identification and quantification of tetrodotoxin during the early stages of poisoning.

ADVANTAGES AND FEATURES

High sensitivity. The method achieves sub-picogram-level sensitivity, fully satisfying the requirements of the referenced standard, which specifies a detection limit of 2.0 ng/mL¹

Fast analysis. Rapid detection of tetrodotoxin is achieved with a total runtime of only 6 minutes per injection.

Method validation. Excellent linearity was obtained over the range of 0.1–5 ng/mL, with correlation coefficients greater than 0.999. Recovery in blood and urine ranged from 60.7% to 93.5%, and RSDs were below 5%.

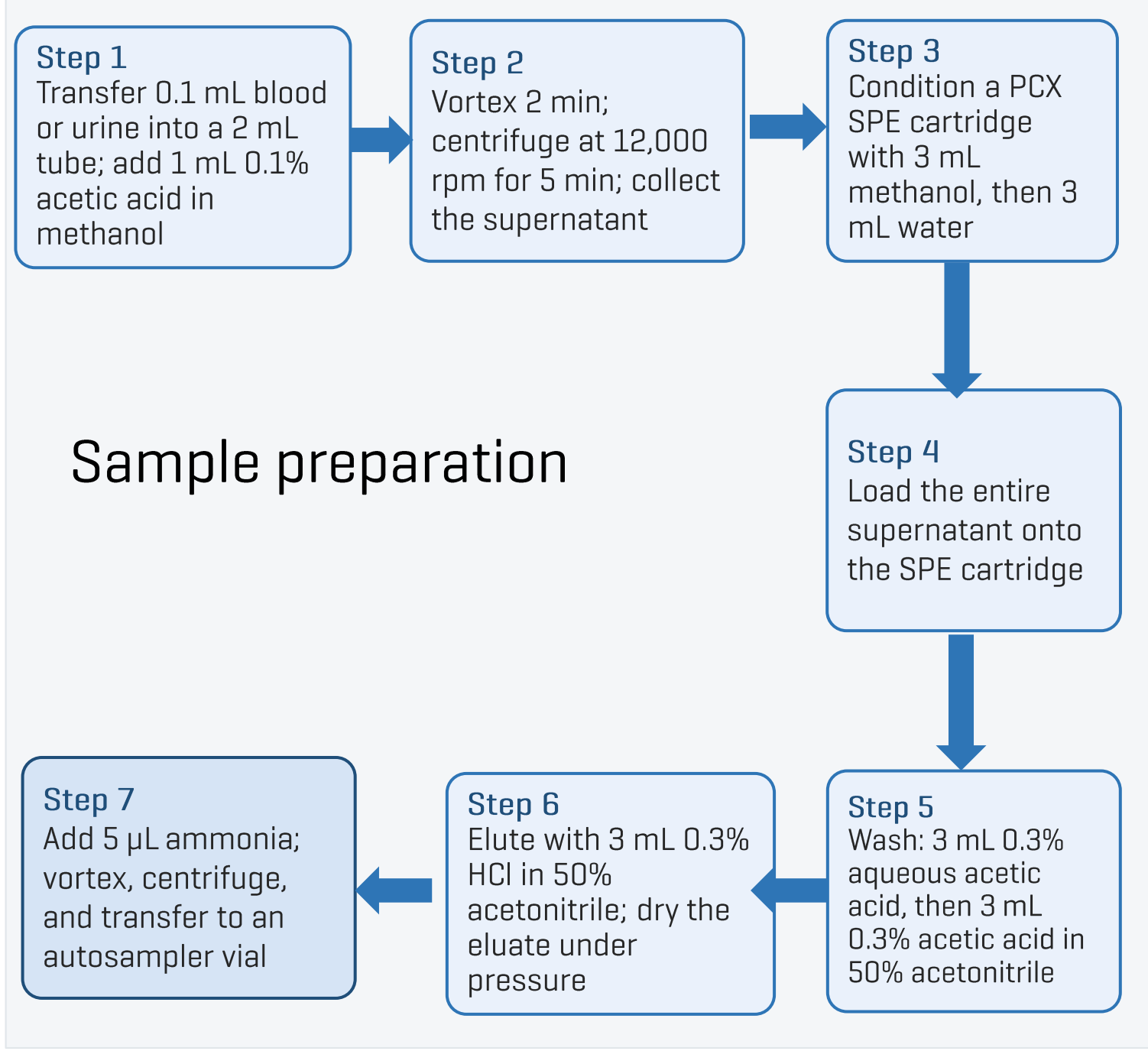
METHODS

The analysis was performed using a SCIEX Triple Quad 4500 LC-MS/MS system [triple quadrupole mass spectrometer].

Sample preparation combined a methanol/acetic-acid extraction with solid-phase extraction (SPE) cleanup on a PCX cartridge. Chromatographic separation was achieved using hydrophilic interaction liquid chromatography (HILIC) on BEH Amide bonded silica 100 × 2.1 mm, 1.7 μm column using a 6-minute gradient elution.

Analytes were detected using electrospray ionization in positive-ion mode with multiple reaction monitoring [MRM]. Matrix-matched calibration curves were prepared in blank blood and urine, with six replicates analyzed at each concentration level. Method validation included linearity, recovery, and precision.

Data were acquired and processed using SCIEX OS software.



Liquid chromatography conditions

Column: BEH Amide bonded silica [HILIC]
[100 × 2.1 mm, 1.7 μm]

Mobile phase:
A: 0.1% formic acid in water
B: Acetonitrile

Flow rate: 0.4 mL/min

Column temperature: 40° C



Time [min]	A[%]	B[%]
0.0	15	85
0.5	15	85
2.0	50	50
4.0	50	50
4.1	15	85
6.0	15	85

Table 1. Gradient elution program

Mass spectrometry conditions

Parameter	Setting
Curtain gas [CUR]	25 psi
Temperature [TEM]	650°C
Collision gas [CAD]	8 psi
Ion source gas 1 [GS1]	45 psi
Ion source gas 2 [GS2]	55 psi
IonSpray voltage [IS]	5500 V

Table 2. Source parameters



ID	Precursor ion [m/z]	Product ion [m/z]	DP [V]	CE [eV]
TTX 1	320.1	302.1	115	34
TTX 2	320.1	162.0	115	52

Table 3. MRM transition parameters

RESULTS AND DISCUSSION

Optimization of LC-MS/MS conditions

Combinations of acetonitrile, formic acid, and ammonium formate were evaluated during method development.

The combination of 0.1% formic acid and acetonitrile gave the best sensitivity and was selected as the mobile phase.

Source parameters were optimized by flow injection analysis [FIA] to maximize signal intensity and sensitivity. The extracted ion chromatogram [XIC] of tetrodotoxin at 0.05 ppb is shown in Figure 3 .

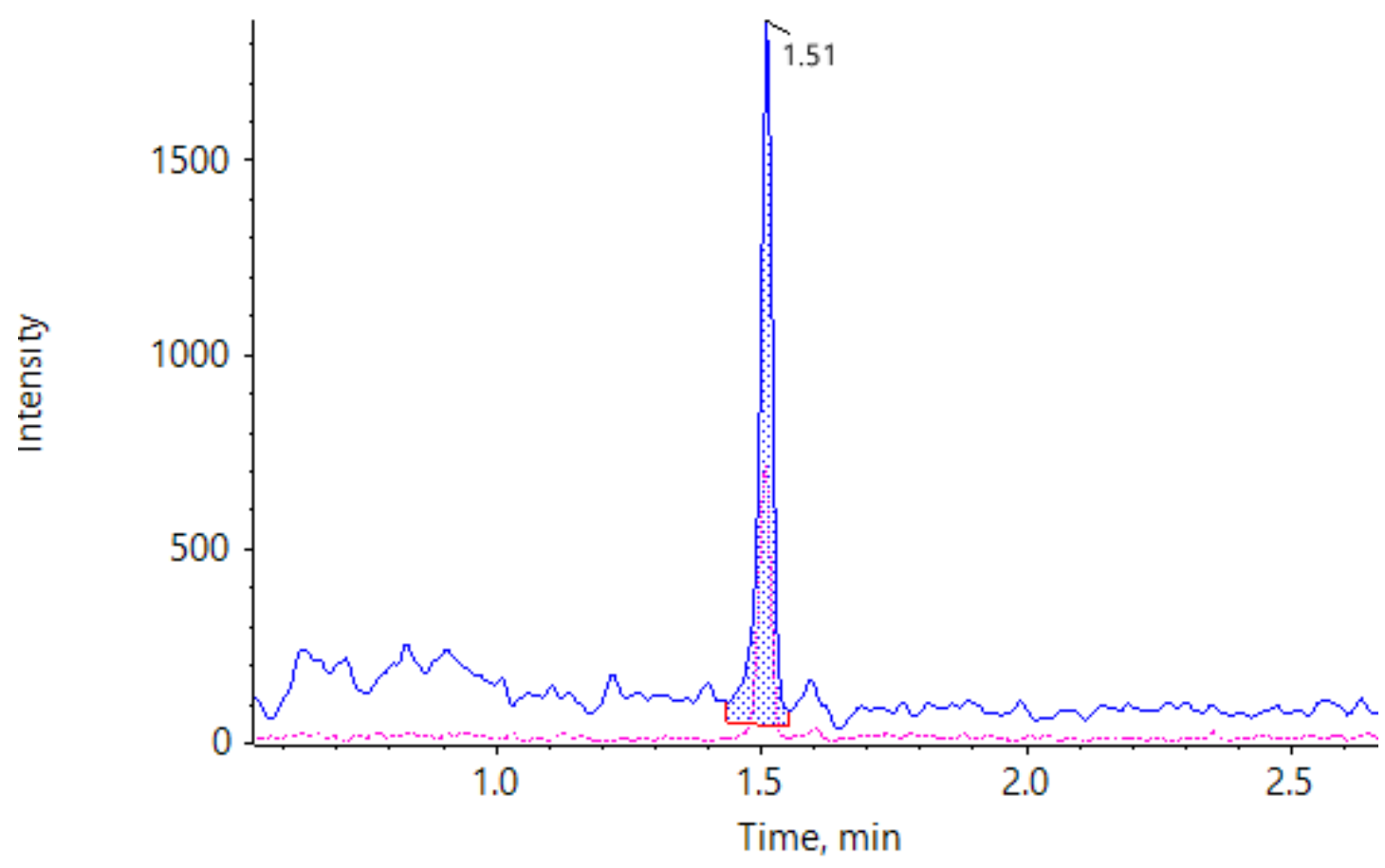


Figure 2. Extracted ion chromatogram [XIC] of tetrodotoxin [0.05 μg/L].

Linearity

Matrix-matched calibration standards were used for quantitation of tetrodotoxin in blood and urine.

Excellent linearity was achieved over the concentration range of 0.05–10 μg/L, with correlation coefficients [R] greater than 0.999 for both blood and urine matrices.

The matrix-matched calibration curve is shown in Figure 3.

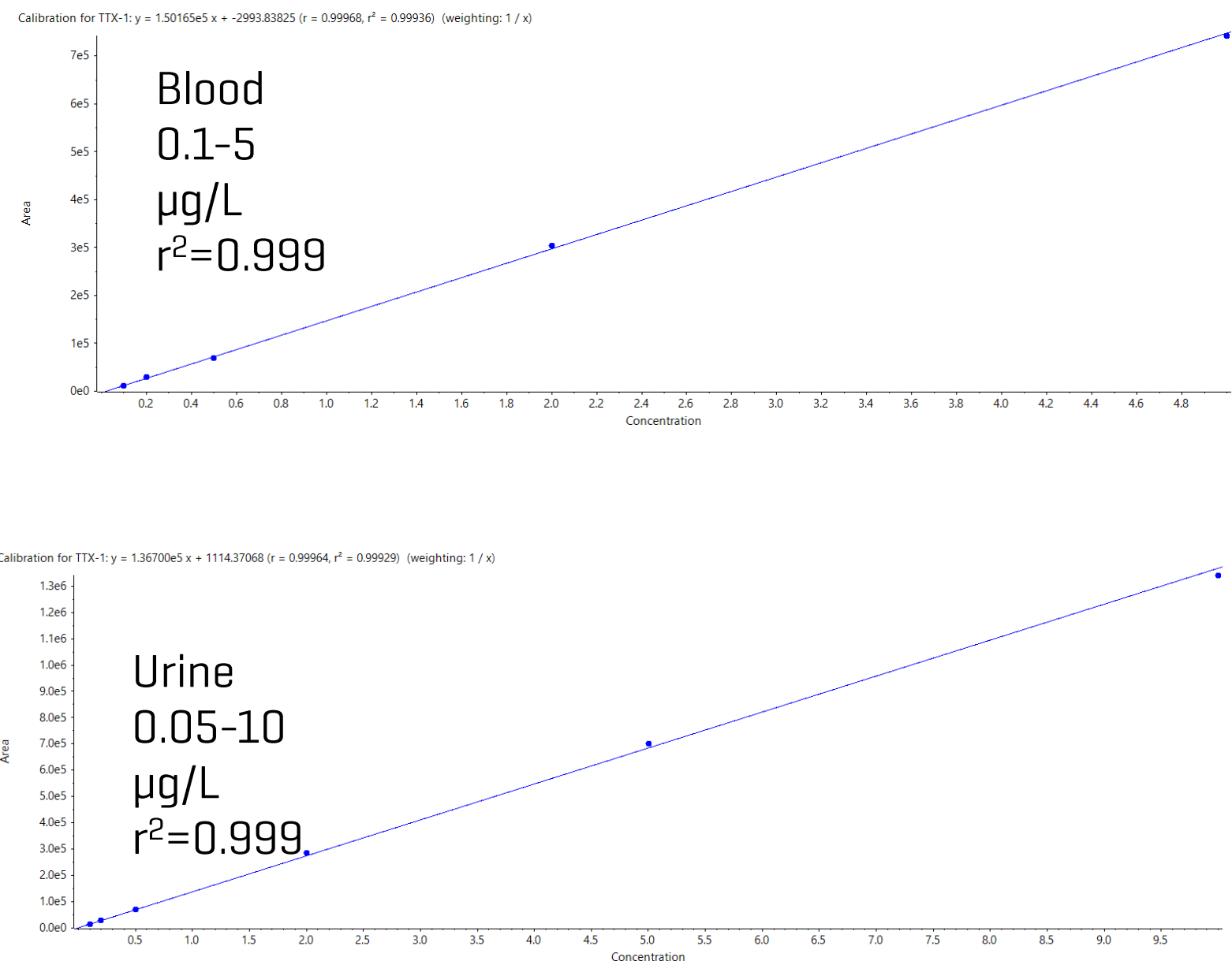


Figure 3. Matrix-matched calibration curves for tetrodotoxin in blood and urine

Recovery and precision

Tetrodotoxin standards were spiked into blank blood and urine at 1.0, 5.0, and 10.0 ng, with six replicates analyzed at each concentration level.

Recoveries ranged from 60.7% to 93.5% across blood and urine, and relative standard deviations [RSDs] remained below 5%, meeting the requirements for routine forensic toxicology testing.

Matrix / level [ng]	Recovery [%]	RSD [%]
Blood 1.0	65.7	2.7
Blood 5.0	61.4	1.6
Blood 10.0	60.7	2.0
Urine 1.0	93.5	1.9
Urine 5.0	88.2	3.6
Urine 10.0	89.4	1.7

Table 4. Recovery and precision of tetrodotoxin

PERFORMANCE HIGHLIGHTS

This validated LC-MS/MS method delivers strong analytical performance for tetrodotoxin in blood and urine.

Validated analytical performance includes:

- Linear range 0.1–5 μg/L, r² > 0.999 [blood and urine]
- Recovery 60.7–93.5%, with RSD < 5%

Total analysis time is 6 minutes per injection, with sub-picogram sensitivity that satisfies the 2.0 ng/mL detection-limit requirement of SF/Z JD0107011-2011.¹

The focus of this experiment was to optimize the sample pretreatment process, making it simple and convenient. For forensic police clients, tetrodotoxin concentrations in routine poisoning cases are generally high, and their testing priorities lie mainly in qualitative screening. Therefore, a straightforward pretreatment workflow is highly valuable, and the 60% extraction recovery can satisfy their testing demands. Examination of the corresponding chromatograms revealed negligible matrix interference even without an internal standard, so the use of an internal standard is dispensable for this method.

Critical lethal blood concentration: Blood TTX concentrations above 9 μg/L carry high risks of paralysis and respiratory arrest; fatal clinical cases show blood TTX ranging from 12.8 to 119 μg/L.

CONCLUSIONS

- Developed a robust LC-MS/MS method for the rapid identification and quantification of tetrodotoxin (TTX) in blood and urine, supporting both clinical and forensic investigations.
- Implemented a streamlined sample preparation workflow that reduces sample handling time and laboratory effort while maintaining analytical sensitivity.
- Demonstrated strong analytical performance with excellent linearity from 0.05–10 μg/L in urine and 0.1–5 ug/L in blood [r² > 0.999], spike recoveries exceeding 60%, and precision within 5% RSD.
- Enabled rapid detection of TTX during the early stages of poisoning, providing critical information to support timely diagnosis and therapeutic intervention.
- Provides a reliable, efficient, and fit-for-purpose tool for routine toxicology laboratories investigating suspected tetrodotoxin exposure.

REFERENCES

1. SF/Z JD0107011-2011 Determination of Tetrodotoxin in Biological Specimens Liquid Chromatography–Tandem Mass Spectrometry. Forensic Identification Technical Specification. Issued by Judicial Identification Administration Bureau of the Ministry of Justice of the People's Republic of China

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