Application Note: 384

Determination of Digoxin in Serum by Liquid Chromatography–Tandem Mass Spectrometry

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Key Words

- TSQ Quantum™
- Forensics
- Therapeutic Drug Monitoring
- Toxicology

Introduction

Digoxin is a cardiac glycoside used in the treatment of various heart failures. Moreover, this compound is used at very low concentration due to their toxicity. Identification and quantitation of such a compound, often performed during a classical forensic expertise or for therapeutic drug monitoring, necessitate a sensitive and specific method. This study aims to describe a method using liquid chromatography/tandem mass spectrometry and permitting to quantify digoxin at low concentrations.

Goal

The goal of this study was to identify and quantify digoxin in serum. This report demonstrates the use of the TSQ Quantum for this application.

Experimental Conditions/Methods

Chemicals and Reagents

Digoxin and 3-aminophenylsulfone (internal standard) were purchased from Sigma. Ammonium formate and formic acid (>99 % pure) were also purchased from Sigma. All reagents and solvents used in the extraction procedures were of analytical grade.

Sample preparation

To 1 mL of serum were added 50 µL of a 2.5 µg/mL aqueous solution of 3-aminophenylsulfone (Internal Standard), 1 mL of a solution of pH 9.50 carbonate buffer and 8 mL of Ether-Dichloromethane-Isopropanol (30:40:30 by volume). The tubes were vortex-mixed and shaken on an oscillatory mixer. After centrifugation at 3,400 g for 5 min, the organic phase was poured in a conical glass tube and evaporated under a stream of nitrogen at 37°C. The dried extracts were reconstituted in 50 µL of acetonitrile : pH 3.0, 2 mmol/L ammonium formate (30:70 by volume) and 10 µL were injected into the chromatographic system.

Instrumentation Methods

HPLC Conditions

The chromatographic system consisted of a CTC HTS PAL Autosampler kept at 6°C, a binary high-pressure Agilent 1100 pump. A C18, 5 µm (50×2.1 mm) column, maintained at 25°C, was used with a linear gradient of mobile phase A (pH 3.0, 2 mmol/L ammonium formate) and mobile phase B (acetonitrile:pH 3.0, 2 mmol/L ammonium formate (90:10; v/v)), flow rate of 200 µL/min, programmed as follows: 0-1.2 min, 20% B; 1.2–8.2 min, 20 to 80% B; 8.2–10.2 min, 80% B; 10.2–10.7 min, decrease from 80 to 20% B; 10.7–13 min, equilibration with 20% B.

MS Conditions

Mass Spectrometer: TSQ Quantum (Thermo Scientific, San Jose, CA, USA)
Source: ESI mode
Ion Polarity: Positive
Spray Voltage: 3800 V
Sheath/Auxiliary gas: Nitrogen
Sheath gas pressure: 30 (arbitrary units)
Auxiliary gas pressure: 30 (arbitrary units)
Ion transfer tube temperature: 250°C
Scan type: SRM
Collision gas: Argon
Collision gas pressure: 1.5 mTorr

SRM Conditions

Settings were optimized by infusing at 5 µL/min a 1 µg/L solution containing the studied compound in acetonitrile: pH 3.0, 2 mmol/L ammonium formate (30:70, by volume). The structure of these compounds is shown in Figure 1.

Compounds	Quantification transition	Collision energy	Confirmation transition	Tube lens voltage
Digoxin	798.5/651.4	20	798.5/781.5	84
3-aminophenylsulfone	249.1/93.2	24		126

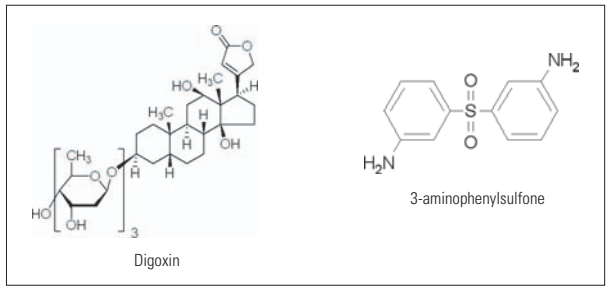

Figure 1: Structures of the investigated compounds

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Results and Discussion

The LC-ESI/SRM chromatograms for 3-aminophenylsulfone and digoxin for a blank serum sample and a blank serum sample spiked at 0.5 ng/mL are shown in Figures 2A and 2B respectively. Identification of digoxin was achieved with two characteristic SRM transitions and their relative retention time.

Linearity

Calibration curve obtained for digoxin spiked in serum samples is presented in Figure 3. Concentration range was comprised between 0.5 ng/mL and 100 ng/mL.

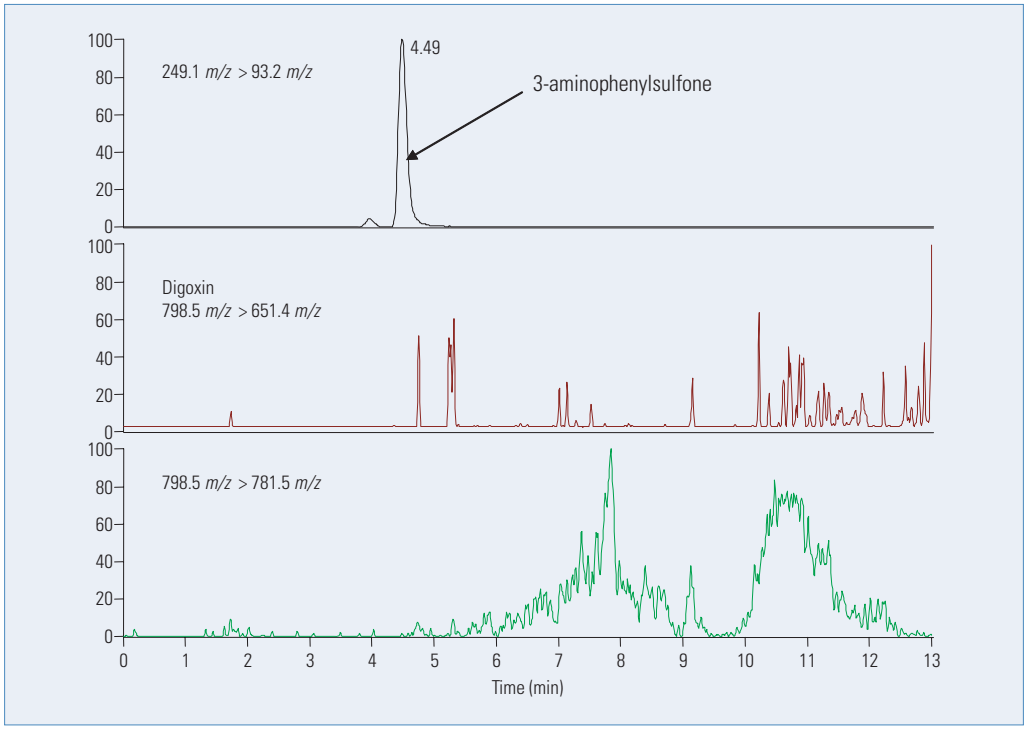

Figure 2A: Chromatogram of a blank serum

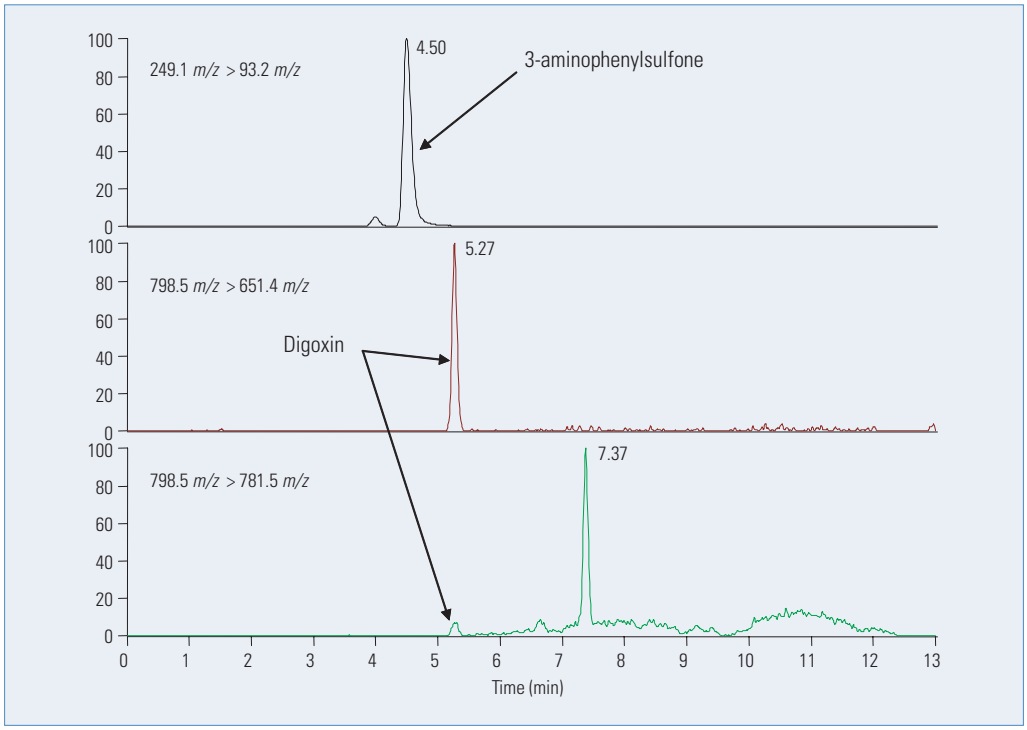

Figure 2B: Chromatogram of a blank serum spiked at 0.5 ng/mL

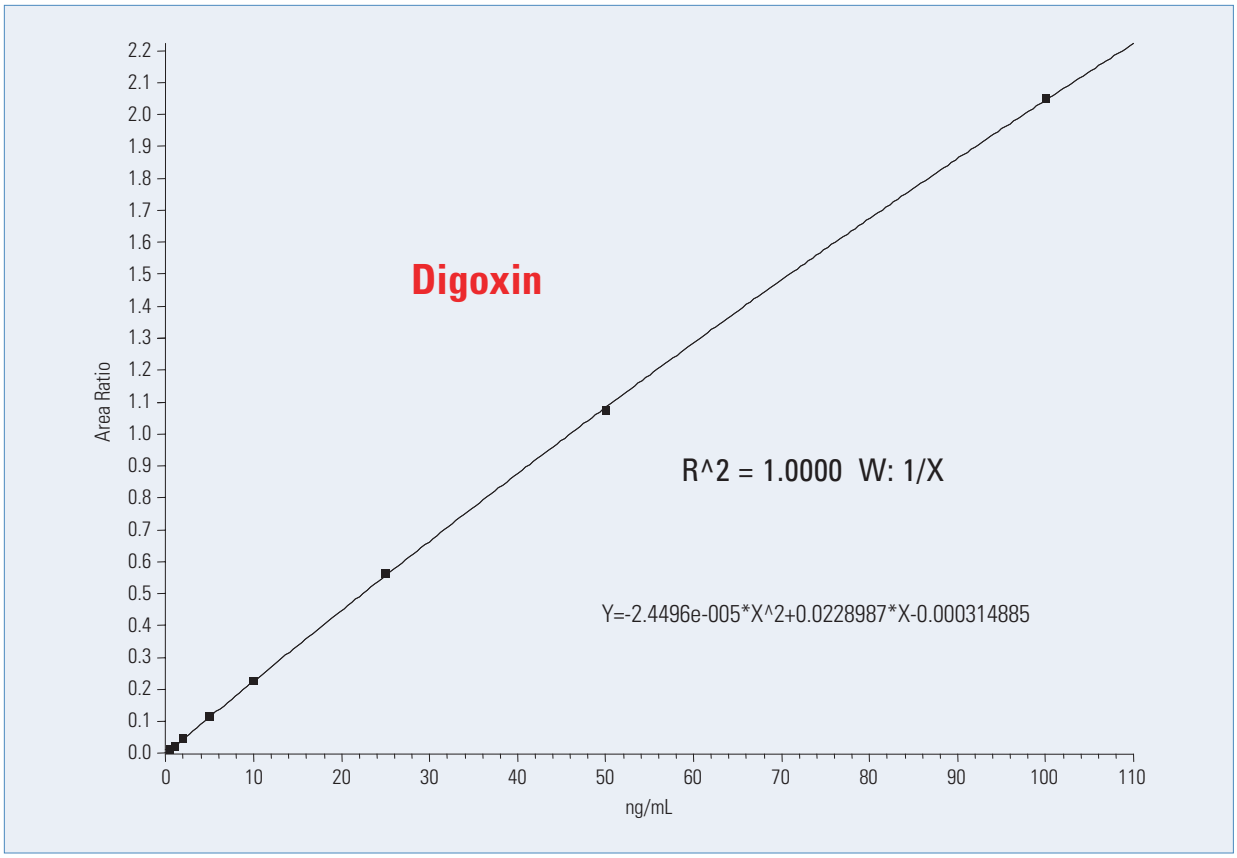

Figure 3: Representative calibration curve from standards spiked in serum

Corresponding Results of Calibration Standards

	Quadratic 1/x	
Specified Concentration (ng/mL)	Calculated Amount (ng/mL)	% Diff
0.5	0.503	0.53
1	.0972	-2.79
2	2.041	2.07
5	5.001	0.02
10	9.985	-0.15
25	25.239	0.95
50	49.614	-0.77
100	100.149	0.15

Intra-assay Accuracy and Precision (n=6)

	Quadratic 1/x	
Specified Concentration (ng/mL)	Calculated Amount (ng/mL)	% Diff
0.5	0.514	2.76
0.5	0.496	-0.75
0.5	0.546	9.10
0.5	0.558	11.62
0.5	0.510	1.97
0.5	0.563	12.62

Accuracy and precision

Intra-assay accuracy and precision (n=6) have been studied at the lowest concentration (0.5 ng/mL). Relative Standard Deviation was equal to 5.28% and Mean Relative Error to 6.23%.

Conclusion

This application note describes a sensitive and specific method developed for the quantitation of digoxin in serum.

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