

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

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Key Words

- TSQ Quantum
- Clinical Research
- Toxicology

Introduction

Digoxin is a cardiac glycoside that can be used at very low concentrations. Identification and quantitation of this compound 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 for research applications.

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 μ L of a 2.5 μ g/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 μ L of acetonitrile : pH 3.0, 2 mmol/L ammonium formate (30:70 by volume) and 10 μ L were injected into the chromatographic system.

Instrumentation Methods

HPLC Conditions

The chromatographic system consisted of a CTC HTS PAL Autosampler kept at 6°C and a binary high-pressure pump. A C18, 5 μ m (50 \times 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 μ L/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: Thermo Scientific TSQ Quantum
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 μ L/min a 1 μ g/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

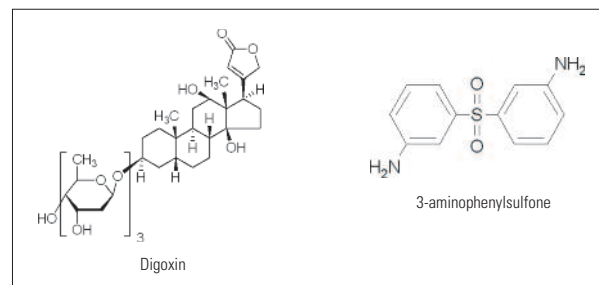


Figure 1: Structures of the investigated compounds

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.

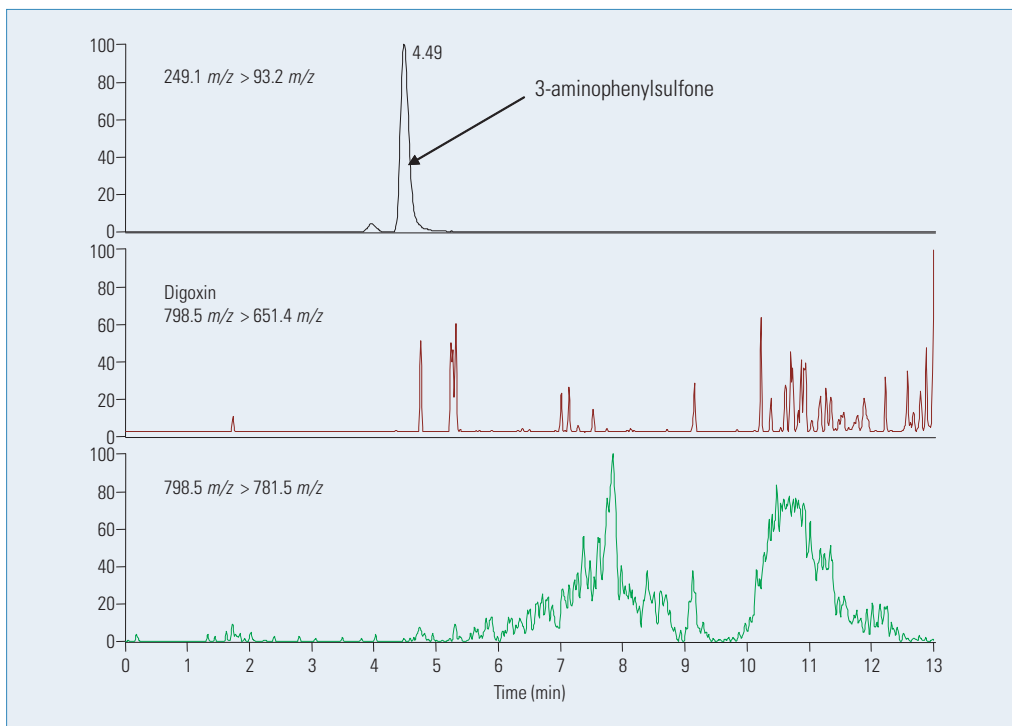


Figure 2A: Chromatogram of a blank serum

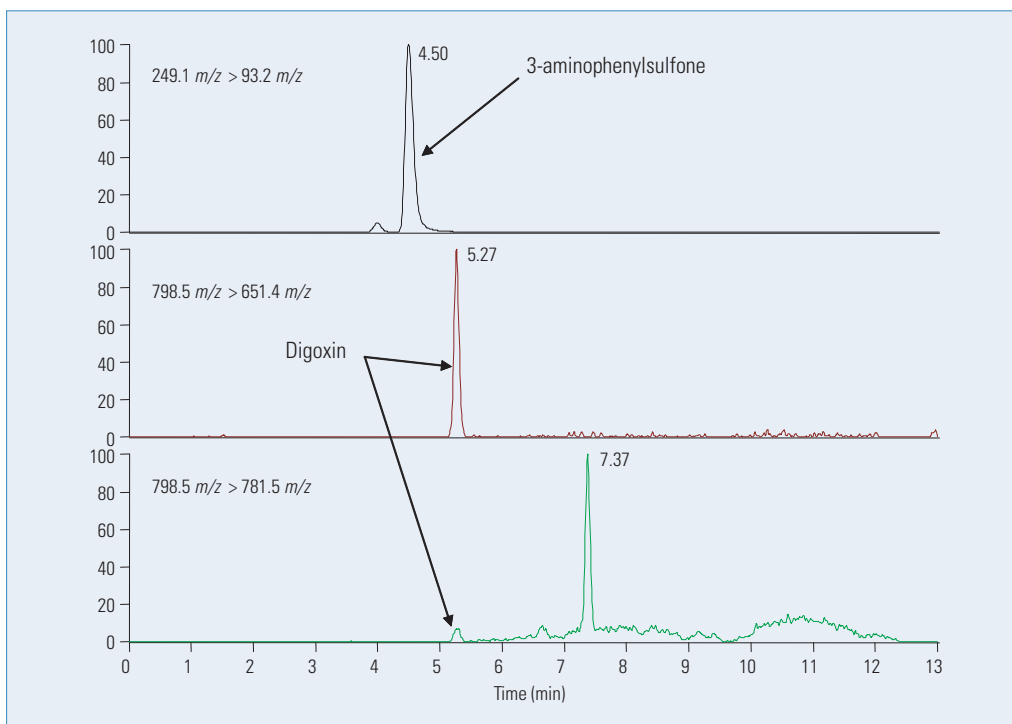


Figure 2B: Chromatogram of a blank serum spiked at 0.5 ng/mL

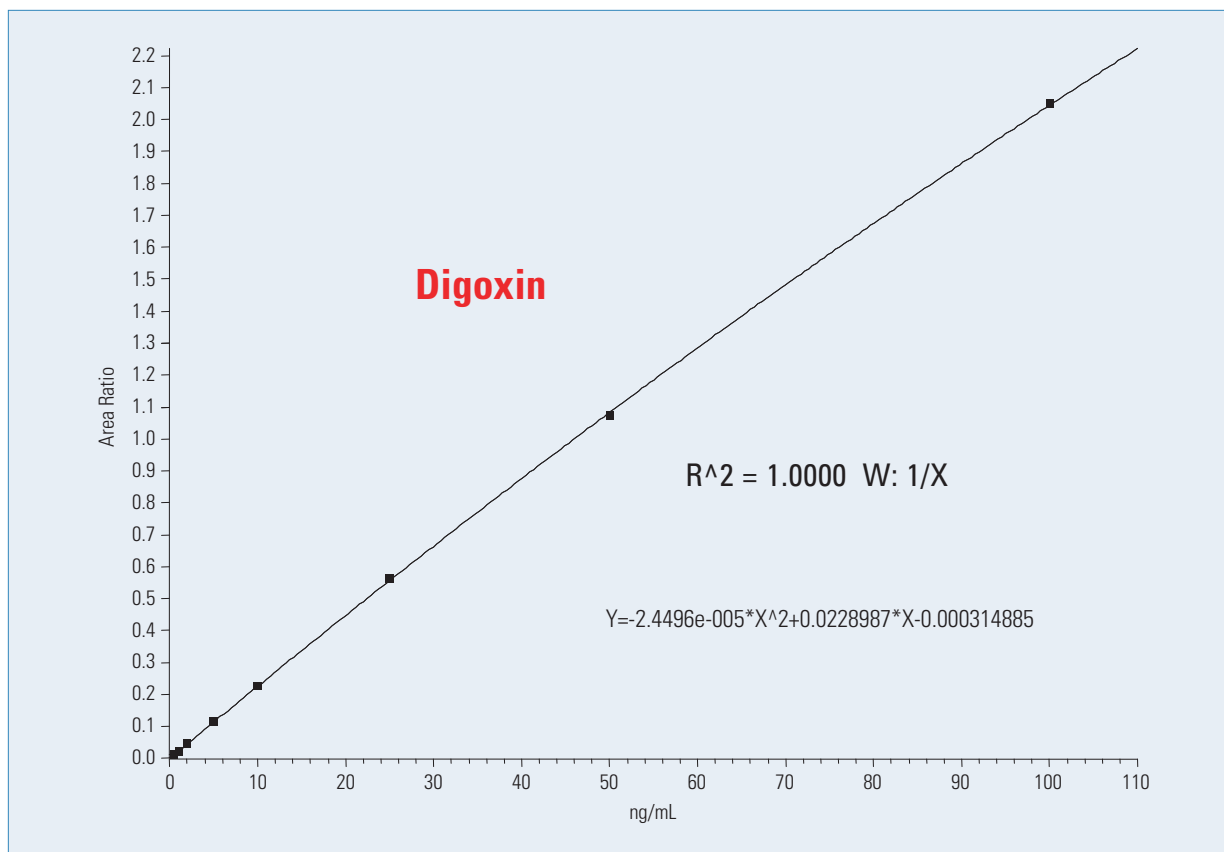


Figure 3: Representative calibration curve from standards spiked in serum

Corresponding Results of Calibration Standards

Specified Concentration (ng/mL)	Quadratic 1/x	
	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

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 for research applications.

Intra-assay Accuracy and Precision (n=6)

Specified Concentration (ng/mL)	Quadratic 1/x	
	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

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