LC/MS Application Note

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Quantitation of Digoxin in Human Plasma Using Negative Ion Electrospray Ionization LC/MS

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Introduction

A Varian 1200L LC/MS/MS system was used for quantitative measurement down to 15 picograms of the cardiac glycoside, Digoxin, in human plasma using Electrospray Ionization (ESI)-LC/MS in the negative ion detection mode. Since cardiac glycosides are very thermally labile, ESI is preferred over a hot vaporization process like APCI.

Digoxin is classified as a digitalis drug and is used to treat congestive heart failure and heart rhythm irregularities. Digoxin's mode of action is to strengthen the force of the heart contractions, and thus help to regulate the rate and rhythm of the heartbeat. The therapeutic level of Digoxin in human serum is 0.5 - 2.0 ng/mL with toxicity starting at 4.0 ng/mL. Because of Digoxin's high potency, doses are administered from 0.125 mg to a maximum of 0.5 mg, once a day. Periodic plasma level measurements are critical for establishing the proper dose administration. The onset of action from administration is from 30 minutes to two hours and when combined with the low dosing regime, plasma levels are expected to be in the low picogram to low nanogram levels. These low picogram levels are easily detected with a Varian 1200L LC/MS system in ESI negative mode.

Instrumentation

- Varian 1200L LC/MS equipped with ESI source

Materials and Reagents

- Digitoxin ordered from Sigma-Aldrich Corp., Catalog Number D5878
- Digoxin ordered from Sigma-Aldrich Corp., Catalog Number D6003
- Ammonium trifluoroacetate (ATFA) Sigma-Aldrich Corp., Catalog Number D23873-2
- All other chemicals are reagent grade or HPLC grade

Sample Preparation

A 1.0 mL of human plasma containing Digoxin is spiked with Digitoxin as the internal standard (Figure 1). The plasma is made basic and both drugs are extracted using methyl-t-butyl ether. After separation of the phases by centrifugation, the organic layer is transferred into a conical tube and evaporated to dryness. The residue is then reconstituted in 40 µL of 70:30 (v:v) water:acetonitrile. A 10 µL aliquot is analyzed by ESI-LC/MS using Selected Ion Monitoring (SIM) in the negative ion mode. The mass spectrometer is set to monitor the trifluoroacetate adduct ions $(ATFA)^-$ for Digoxin at m/z 894 and Digitoxin at m/z 878.

HPLC Conditions

Column	50 mm x 1.0 mm 3µm C18			
Solvent A	100% deionized water			
Solvent B	80:20 (v:v) acetonitrile:water with 1.4 mM ATFA			
LC Program	Time (min:sec)	%A	%B	Flow (µL/min)
	0:00	95	5	100
	6:00	55	45	100

MS Parameters

API Drying Gas	25 psi at 200 °C
API Nebulizing Gas	51 psi
Scan Time	1.0 sec
SIM Width	0.7 amu
Needle	-2800V
Shield	-800V
Capillary	-90V
Detector	1950V

Scan Parameters

Analyte	SIM Ion (m/z)
Digoxin	894
Digitoxin	878

Results and Discussion

A comparison of the LC/MS traces obtained for the Upper Limit of Quantitation (ULOQ) and Lower Limit of Quantitation (LLOQ) are shown in Figures 2 and 3. Visual examination of the chromatograms shows that no interfering peaks are present at the retention time for Digoxin. Visual examination of calibration curve for Digoxin in Figure 4 shows that excellent linearity was achieved using the weighted Linear Quadratic (1/x) model for calibration curve calculations.

During method validation, the percent recovery for Digoxin was determined to be approximately 60%. A 10 µL aliquot from a total of 40 µL of a reconstituted solution for each study sample was injected on the column. Due to limited sample recovery and using only a portion of the total recovered sample, the actual amount of Digoxin injected "on-column" for the lowest calibration point is 15 picograms. The Varian 1200L LC/MS easily detected Digoxin at the 15 picogram level.

Conclusion

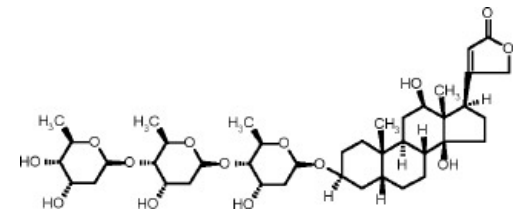
The data obtained with the Varian 1200L LC/MS demonstrates the following:

1. Excellent linearity of response is achieved for the quantitation of Digoxin from 100 pg/mL to 5 ng/mL.
2. Excellent sensitivity can be achieved using negative ion detection for Digoxin.
3. These extremely labile glycosides do not decompose at the source temperatures used in this assay (200 °C). The Varian 1200L LC/MS source assembly and metal capillary design eliminates hot spots where degradation of thermally labile compounds could take place.

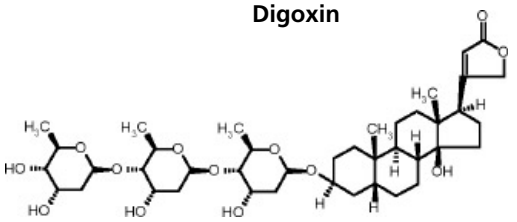
Reference

1. Guidance for Industry: Bioanalytical Method Validation, FDA (May 2001).

Compound Structures



Digoxin



Digitoxin

Figure 1. Structure of Digoxin (parent drug) and the internal standard Digitoxin.

SRM at ULOQ

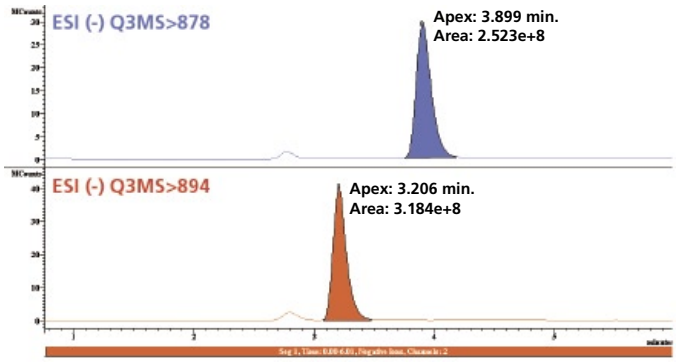


Figure 2. Excellent detector response and peak shape for ULOQ at 5 ng/mL.

SRM at LLOQ

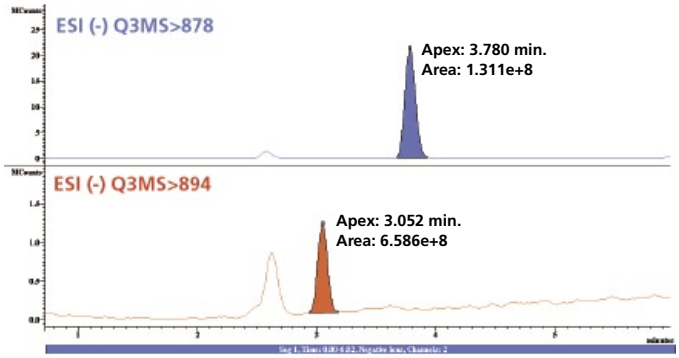


Figure 3. Excellent detector response and peak shape for LLOQ at 100 pg/mL.

Calibration Curve for Digoxin

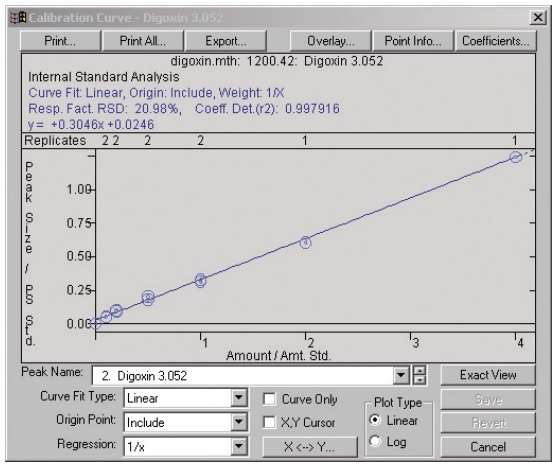


Figure 4. Excellent linearity was achieved for Digoxin using the internal standard Digitoxin.

These data represent typical results.
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