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Research Article

LC-MS-MS METHOD FOR THE DETERMINATION OF DIGOXIN IN HUMAN PLASMA

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ABSTRACT

A rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed and validated for the quantification of Digoxin in human plasma using Triple quad 5500 *AB Sciex***.** The mass transition ion pair has been followed as *m/*z 798.500→435.200 for Digoxin. Liquid liquid extraction was carried out and spectrometric detection using an API 5500 instrument that enables detection at picogram levels. Separation was achieved on Acquity Uplc Beh C_{18} (130Å, 2.1 mm X 100 mm, 1.7 µm) column with a mobile phase consisting of Methanol : 0.1 % aqueous formic acid solution (80 : 20 V/V). Protonated ions formed by a turbo ionspray in positive mode were used to detect analyte and internal standard. Triamcinolone Acetonide was used as an internal standard. The proposed method has been validated with a linear range of 40.33-8092.75 pg/mL for Digoxin.

Keywords: Digoxin, Triamcinolone Acetonide, Triple quad 5500 LC/MS/MS

INTRODUCTION

Various methods have been reported in the literature for monitoring plasma levels of Digoxin [1-7]. Some techniques used in individual analysis of Digoxin from plasma include UV, HPLC with Mass Spectroscopy, however, these methods lacks sensitivity and also there is no specific method available in literature for the quantification of Digoxin in human plasma using LC/MS/MS system. Hence, the aim of the study is to develop a simple, sensitive, rapid and specific method for monitoring plasma levels of Digoxin. Triamcinolone Acetonide drug was used as an internal standard.

MATERIALS AND METHODS

Chemical used

Ammonium formate (sigma), Methanol (HPLC Grade), Digoxin and Triamcinolone Acetonide (LGC Promochem India Pvt. Ltd., Mumbai) ,K_2 EDTA from Sigma, Milli- Q water (Millipore, Bedford, MA, USA) were used in method development.

Instrumentation

Triple quad 5500 AB sciex LC/MS/MS was used.

MASS PARAMETERS

The mass spectrometer was operated in the positive ion mode. The developed mass and LC parameters for the estimation of Digoxin with Triamcinolone Acetonide as internal standard are given below.

The compound dependent parameters for Digoxin declustering potential was 21 eV, Entrance potential 08 eV, Collision energy 20 psi. For Triamcinolone Acetonide declustering potential was 25 eV, Entrance potential 10 eV, Collision energy 15 V. Source dependent parameters for the method are CUR- 30 psi, TEMP- 500°C, CAD- 7 psi.

Multi Reaction Monitoring (MRM)

The mass transition ion-pair has been followed as *m/*z 798.500→435.200 for Digoxin, *m/*z 435.200 →415.200 for Triamcinolone Acetonide.

Preparation of calibration curve (CC) standards and quality control (QC) samples

A stock solution of Digoxin and Triamcinolone Acetonide (1mg/mL) were prepared in methanol. Calibration standards were prepared by spiking blank plasma with Digoxin to get the concentration of 40.33, 80.67, 403.36, 746.96, 1493.92, 2872.92, 5745.85, and 8092.75 Pg/mL. Quality control samples were prepared by spiking blank plasma with 40.48, 100.71, 2869.22 and 5738.43 Pg/mL of Digoxin. The stock solutions were stored at 4-8^0 C.

Sample preparation

To 2.0 mL polypropylene centrifuge tube, 500 µL of plasma sample was spiked with 50 uL internal standard solution, 400 µL of Buffer (5mM Ammonium Formate solution), and 2.5 mL of Tertiary butyl methyl ether were added. Samples were vortexed for 10.0 minutes.

From that 2.0 mL of supernatant organic layer was taken and dried the samples under N_2 and reconstituted the samples with 300 µL of Mobile phase, vortexed for 1.0 minute and 10 µL of the sample was injected.

RESULTS AND DISCUSSION

The assay was found to be linear for Digoxin concentrations in the range 40.33 to 8092.75 pg/mL. The results are presented in Table 1. The precision and accuracy were studied satisfactorily at four Quality Control (QC) concentrations for Digoxin. The results obtained from measurement of linearity, precision and accuracy are listed in Table 2 and 3.

Table 1: Correlation coefficient (r)

Curve No.	Intercept	Slope	Correlation coefficient(r^2)
1	0.0025	0.0002	0.9934
2	0.0046	0.0002	0.9932
3	0.0010	0.0002	0.9974
4	0.0004	0.0002	0.9990
5.	0.0038	0.0002	0.9930

Table 2: Concentration of Digoxin in calibration standards prepared in human plasma.

	Nominal*	Mean	CV,%
A	40.3360	40.059	4.20
B	80.6720	81.587	8.02
C	403.3600	404.281	2.89
D	746.9620	777.390	7.90
E	1493.9220	1424.316	4.84
F	2872.9280	2851.813	1.94
G	5745.8560	6001.565	0.68
H	8092.7540	7784.714	6.39

*pg/mL; n =4

Table 3: **Data of Intra batch and Inter batch accuracy and precision**

Drug	Nominal*	Intra bach			Inter bach		
		Mean	Accuracy,%	CV, %	Mean	Accuracy,%	CV, %
LLOQ	40.486	44.503	109.92	14.94	40.243	99.40	16.75
LQC	100.710	104.933	104.19	5.24	99.681	98.98	7.91
INTQC	745.9980	811.691	108.81	1.39	776.562	104.10	4.80
MQC	2869.220	2786.028	97.10	2.88	2824.498	98.44	3.31
HQC	5738.438	5991.085	104.40	2.18	5862.066	102.15	4.48

*pg/mL n=6

CONCLUSION

The LC-MS/MS method described for Digoxin was simple, rapid, reproducible and suitable for their determination in human plasma. Most of the analytical methods reported for quantification of digoxin from human plasma was longer and lacks sensitivity. The method also has a good linearity, specificity and is also suitable for high throughput clinical sample analysis. There were no significant interferences and matrix effects by endogenous compounds throughout the analysis. The developed method can also be used as therapeutic drug monitoring technique to evaluate the pharmacokinetic parameters of drug molecules in human plasma.

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