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Therapeutic monitoring of serum digoxin for patients with heart failure using a rapid LC-MS/MS method

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Abstract

Objective: Here we develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the determination of digoxin in serum.

Design and methods: The serum samples were extracted with methyl tert-butyl ether using an isotope-labeled digoxin-d3 as internal standard. The analyte was separated on a reverse phase Capcell C18 column and detected in positive electrospray ionization multiple reaction monitoring mass spectrometry.

Results: The chromatographic analysis was carried out within 3 min, but the complete analysis took longer because of the liquid–liquid extraction. The lower limit of quantification was 0.1 ng/mL for digoxin. The intra- and inter-batch precisions were less than 12%, and the bias ranged from −9.1% to 10.7%. The external quality assessment (EQA) results obtained with the LC-MS/MS method were comparable to target values. Subsequently, this method has been applied to the therapeutic monitoring of digoxin in a clinical setting.

Conclusion: In this study, we have developed a rapid and reliable LC-MS/MS method for the therapeutic monitoring of digoxin in human serum.

Keywords: Digoxin; LC-MS/MS; Liquid–liquid extraction; Therapeutic drug monitoring; Heart failure; Human serum

Introduction

Digoxin is commonly prescribed for the treatment of heart failure (HF) in clinical practice. Data from the Digitalis Investigation Group (DIG) trial, a randomized double-blinded placebo-controlled study, demonstrated that digoxin reduced hospitalizations among patients with HF and decreased the risk of death attributed to worsening HF [1–3]. The role of serum digoxin concentration (SDC) is well established, as many studies have suggested that the effectiveness of digoxin therapy in patients with HF should be optimized in the range of 0.5–0.9 ng/mL. A SDC above 1.2 ng/mL may be harmful [4–6] and the traditional range of 0.8–2.0 ng/mL for SDC is now questioned, because this new lower therapeutic window is associated with improvement of clinical outcomes [7]. Therefore, a more intensive dosage refinement is proposed [8].

The measurement and assessment of digoxin concentration are often performed inappropriately and the quality of SDC monitoring is poor [9–11]. Hence, it is necessary to introduce therapeutic drug monitoring (TDM) of digoxin, in order to optimize therapeutic efficacy and avoid the incidence of toxicity. In most cases, immunoassay techniques are the primary method used for monitoring of digoxin in clinical practice.

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Nevertheless, cross-reactivity with endogenous digoxin-like substances [12] and interference from other drugs, including a number of herbal medicines [13,14], may be the main obstacle to accurate determination of digoxin in real clinical samples. This makes LC-MS/MS, a technique with significant advantages of specificity and sensitivity, the most appropriate method for digoxin monitoring, as it is free of interferences from endogenous and exogenous compounds. Recently, many LC-MS or LC-MS/MS methods for digoxin have been reported for the purposes of drug monitoring, pharmacokinetic studies, or drug interaction investigations [15–21]. Some of these methods use gradient elution program for chromatographic separation. As a result, a long turnaround is required (14 min [15], 17 min [16], 10 min [21]) to restore the column to its original starting conditions. Some reported methods use lengthy sample preparation [15,18,20,21], or 96-well plate [16,17], which further increase both turnaround time and cost. Consequently, there is still a critical need for a method that addresses both rapid throughput and economy for the TDM of digoxin in routine clinical practice.

The aim of this paper is to build a rapid and reliable method for the TDM of serum digoxin concentration for patients with heart failure. Our protocol is based on a technique of stable isotope dilution liquid chromatography-positive electrospray ionization tandem mass spectrometry. A simple and economical liquid–liquid extraction with methyl tert-butyl ether is adopted using 0.2 mL of sample volume. The total turnaround per analysis is only 3 min and this greatly improves the assay throughput. The validated method has been subsequently applied to national EQA and clinical drug monitoring of digoxin in patients with heart failure.

Materials and methods

Chemicals and reagents

Digoxin (98.0% purity) was purchased from National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. [$^2H_3$] digoxin (digoxin-d3) was purchased from Toronto Research Chemicals Inc., North York, Ontario, Canada. HPLC-grade acetonitrile, methanol, ammonium acetate, formic acid, and methyl tert-butyl ether were purchased from Tedia Company Inc., Fairfield, USA. All other reagents were of analytical grade. Double distilled water was used throughout the study.

Liquid chromatography

HPLC analysis was performed on a Shimadzu system (Kyoto, Japan) equipped with two LC-20AD pumps, a SIL-HTC autosampler, and an online DGU-20A3 vacuum degasser. Chromatographic separation was achieved on a Capcell C18 MG III analytical column (100 mm × 2.0 mm I.D. 5 μm, Shiseido, Japan) coupled with a C18 guard column (4.0 mm × 3.0 mm I.D. 5 μm, Phenomenex, USA) at a flow rate of 0.3 mL/min. The column and autosampler were kept at room temperature. The mobile phase consisted of 10 mM NH_4Ac/0.1% formic acid in water and 0.1% formic acid in acetonitrile and was run at an isocratic elution (60:40, v:v). The sample injection volume was 20 μL and the total run time was 3 min per injection.

Mass spectrometry

A 3200 QTRAP tandem mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Canada) equipped with Turbo Ionspray source was used for quantitative analysis. The instrument was operated in positive ionization mode with an ion spray voltage at 5.5 kV and the source temperature at 400 °C. Multiple reaction monitoring (MRM) was used to detect digoxin and digoxin-d3, with precursor to product ion transitions of *m/z* 798.6/651.5 and *m/z* 801.6/654.5, respectively. The collision-activated dissociation (CAD) was set at medium. High purity nitrogen was used as the collision gas. The curtain gas, gas 1, and gas 2 were set at 20, 50, and 50, respectively. Dwell time of 200 ms was selected. Analyst 1.4.2 software was used for instrument control and data acquisition.

Standard solutions

Standard stock solutions of digoxin and digoxin-d3 (internal standard) were separately prepared at 0.1 mg/mL in methanol and stored at 4 °C. Dilutions were made to prepare calibration standards, at serial concentrations of 0.1, 0.4, 1, 4, and 10 ng/mL, by spiking appropriate amount of digoxin stock solution into blank serum. Quality control (QC) samples were prepared in the same way, at concentrations of 0.3, 1.5 and 8 ng/mL. All of the spiked standards were stored at −20 °C. A working solution of internal standard was prepared from digoxin-d3 stock at 10 ng/mL in 40% methanol and stored at 4 °C.

Liquid–liquid extraction

A 200 μL serum sample was mixed with 20 μL isotope-labeled internal standard working solution (10 ng/mL) and then extracted with 1 mL methyl tert-butyl ether by vortexing for 5 min. The mixture was subsequently centrifuged at 12 000 rpm for 5 min. The upper layer was transferred to a clean polypropylene tube and dried with a stream of nitrogen gas at 45 °C. The residue was reconstituted in 100 μL 40% methanol and 20 μL was injected onto the LC column for LC/MS/MS analysis.

Method validation

The method was evaluated by validation of the extraction recovery, matrix effect, linearity, precision and accuracy, and stability in three independent runs. The validation procedure was performed in respect to the guideline for the bioanalytical method validation recommended by U.S. Food and Drug Administration [22].

The linearity of the method was evaluated by a calibration curve prepared in duplicate over a range of 0.1–10 ng/mL digoxin in serum. A linear regression using $1/x$ weighting was