Journal of Pharmaceutical Analysis 2014;4(1):63–79

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

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ORIGINAL ARTICLE

Development of a sensitive and rapid method for quantitation of (*S*)-(−)- and (*R*)-(+)-metoprolol in human plasma by chiral LC–ESI–MS/MS

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Received 1 November 2012; accepted 18 February 2013
Available online 5 March 2013

KEYWORDS

S-(−)-metoprolol;
R-(+)-metoprolol;
Chiral column;
Chromatographic separation;
LC–ESI–MS/MS;
Human plasma

Abstract A selective, sensitive and high throughput liquid chromatography-tandem mass spectrometry (LC–ESI–MS/MS) method has been developed for separation and quantification of metoprolol enantiomers on a chiral Lux Amylose-2 (250 mm × 4.6 mm, 5 μm) column. Solid phase extraction of (*S*)-(−)- and (*R*)-(+)-metoprolol and *rac*-metoprolol-d6 as an internal standard (IS) was achieved on Lichrosep DVB HL cartridges employing 200 μL human plasma. Both the analytes were chromatographically separated with a resolution factor of 2.24 using 15 mM ammonium acetate in water, pH 5.0 and 0.1% (v/v) diethyl amine in acetonitrile (50:50, v/v) as the mobile phase within 7.0 min. The precursor→product ion transitions for the enantiomers and IS were monitored in the multiple reaction monitoring and positive ionization mode. The method was validated over the concentration range of 0.500–500 ng/mL for both the enantiomers. Matrix effect was assessed by post-column analyte infusion experiment and the mean extraction recovery was greater than 94.0% for both the enantiomers at all quality control levels. The stability of analytes was evaluated in plasma and whole blood under different storage conditions. The method was successfully applied to a clinical study in 14 healthy volunteers after oral administration of 200 mg metoprolol tablet under fasting conditions. The assay reproducibility is shown by reanalysis of 68 incurred samples. The suitability of the developed method was assessed in comparison with

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Peer review under responsibility of Xi'an Jiaotong University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jpha.2013.02.008

different chromatographic methods developed for stereoselective analysis of metoprolol in biological matrices.

1. Introduction

Enantiomeric study of drugs and/or its metabolites is of growing interest in the field of pharmaceutical and biomedical analysis. β-blockers or β-adrenergic blocking agents are one of the most explored pharmaceuticals for their stereochemical impact on pharmacodynamics and pharmacokinetics. Majority of β-blockers are marketed as racemic mixtures and hence enantiomeric analysis in biological fluids is essential to understand their stereoselective implications, therapeutic use and also in toxicological studies [1,2].

Metoprolol, a selective β-adrenoceptor antagonist, is used in clinical practice in the racemic form for the treatment of hypertension, angina pectoris and for several other cardiovascular diseases [3–5]. The (*S*)-(−)-metoprolol has significantly higher β-adrenergic receptor affinity (about 500-fold) compared to its (*R*)-(+)-antipode. In humans, the absorption of metoprolol is rapid and complete. Plasma levels after oral administration are almost 50% of levels attained following intravenous administration, indicating ~50% first-pass metabolism. A small fraction of the drug (~12%) is bound to human serum albumin and has a plasma half-life of 3–7 h. It is primarily metabolized by CYP2D6 enzymes and exhibits stereoselective metabolism that is essentially dependent on oxidation phenotype. The three principal metabolic pathways of metoprolol include (a) *O*-dealkylation to give *O*-desmethyl metoprolol, which undergoes rapid oxidation to form an acid metabolite, (b) α-hydroxylation to form α-hydroxy metoprolol and (c) oxidative deamination to give *N*-dealkyl metoprolol. About 85% of the administered drug is excreted in the urine as metabolites, along with small amounts of unchanged parent drug (less than 5%). The stereoselectivity in metoprolol pharmacokinetics is observed with higher plasma concentration of (*S*)-(−)-metoprolol (S/R-metoprolol ratio >1) and higher renal excretion of (*R*)-(+)-metoprolol in healthy volunteers and hypertensive patients after oral dose of *rac*-metoprolol [6,7].

Numerous methods have been developed for enantioselective determination of metoprolol in biological samples using different analytical techniques, such as capillary electrophoresis [8], GC–MS [9], HPLC with UV [10] and fluorescence detection [7,11–21], and LC–MS/MS [22,23]. These methods can be characterized based on two different approaches (i) direct methods, which involve use of chiral stationary phase [7,10,11–14,17–23] and (ii) indirect methods, employing derivatization with chiral reagents [9,15,16,19]. The choice of a particular approach in bioanalysis is dictated by several factors which include (a) the required assay sensitivity, (b) ready availability, purity and stability of chiral derivatizing agent, (c) efficiency and ease of derivatization, (d) suitable chiral stationary phase, (e) simple and easy optimization of chromatographic conditions and (f) overall analysis time (extraction and chromatography). This is specifically intended to facilitate application of the method in routine analysis of real samples. Lanchote et al. [19] have compared direct enantioselective separation of metoprolol enantiomers on chiral stationary phase (Chiralpak AD and Chiralcel OD-H columns) and indirect separation based on the formation of diastereomeric derivatives with *S*-(−)-menthyl chloroformate by HPLC. They concluded that the direct method with Chiralpak AD was more sensitive compared to the indirect approach, although both the methods demonstrated interchangeable use in the pharmacokinetic investigation. Mistry et al. [20] carried out a stereoselective HPLC-fluorescence assay for the enantiomers of metoprolol and diastereoisomers of its hydroxyl metabolite on Chirobiotic T bonded phase column. The analytes were extracted from 1.0 mL plasma sample by solid phase extraction (SPE) and the calibration curve was established from 0.5 to 50 ng/mL for metoprolol enantiomers. So far very few LC–MS/MS based methods are available in literature for the analysis of metoprolol enantiomers in biological matrices. Jensen et al. [22] developed and validated a stereoselective LC–MS/MS assay using Chirobiotic T column for quantification of *S*- and *R*-metoprolol in human plasma. The linear dynamic range was established from 0.5 to 50 ng/mL and the run time for the method was 11.0 min. A human dried blood spot (DBS) sampling with LC–MS/MS for enantioselective determination of metoprolol and its metabolite has also been described [23]. This is a highly rapid method (3.0 min); however, the sensitivity of the method was 2.5 ng/mL. A detailed comparative summary of different chromatographic methods developed for metoprolol enantiomers in biological samples is presented in Table 1.

The objective of the present study was to separate both the isomers chromatographically and to develop a simple and reliable LC–ESI–MS/MS method based on direct approach for their quantitation in human plasma. The proposed method exhibited superior performance in terms of sensitivity, dynamic concentration range, selectivity, ruggedness and efficiency (7.0 min per sample) due to cleaner extracts with a simple and straight forward sample extraction protocol. It ensured the estimation of both the isomers up to 24 h with desired accuracy and precision to support a bioequivalence study in healthy Indian volunteers. Additionally, this is the first report on successful demonstration of assay reproducibility through incurred sample reanalysis for metoprolol enantiomers.

2. Experimental

2.1. Chemicals and materials

Reference standards of *S*-(−)-metoprolol (99.0%) and *R*-(+)-metoprolol (99.0%) were purchased from Toronto Research Chemicals Inc. (Ontario, Canada), while *rac*-metoprolol-d6 (99.6%) used as an internal standard (IS) was from TLC Pharmachem Inc. (Ontario, Canada). HPLC grade methanol and acetonitrile were obtained from Mallinckrodt Baker S.A. de C.V. (Ecatepec, Mexico). Analytical reagent grade glacial acetic acid, formic acid, sodium hydroxide, ammonium