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RESEARCH ARTICLE

Bioanalytical Method Development and Validation for the Estimation of Metoprolol in Human K2EDTA Plasma Using Liquid Chromatography Tandem–Mass Spectrometry

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ABSTRACT

Bioanalytical methods are widely used to quantitative determination of drugs and their metabolites in biological matrix such as plasma, serum, and urine and the methods could be applied to studies in areas of human clinical pharmacology, non-human pharmacology/toxicology studies (pre-clinical studies), and bioavailability and bioequivalence studies requiring pharmacokinetic evaluation. The major bioanalytical services are method development, method validation, and sample analysis. Bioavailability is defined as "The rate and extent (amount) of absorption of unchanged drug from its dosage form." Bioavailability can be generally documented by a systematic exposure profile obtained by measuring drug/metabolite concentration in the systemic circulation over a particular time period. System suitability was performed by injecting six consecutive injections of metoprolol at higher concentration of calibration range. In conclusion, this work describes a very simple and sensitive liquid chromatography–mass spectrometry method for the determination of metoprolol suitable to monitor concentrations during clinical pharmacokinetic studies in humans.

Keywords: Bioanalytical methods, Liquid chromatography tandem–mass spectrometry, Metoprolol

INTRODUCTION

Bioanalytical methods are widely used to quantitative determination of drugs and their metabolites in biological matrix such as plasma, serum, and urine and the methods could be applied to studies in areas of human clinical pharmacology, non-human pharmacology/toxicology studies (pre-clinical studies), and bioavailability and bioequivalence studies requiring pharmacokinetic evaluation. The major bioanalytical services are method development, method validation, and sample analysis. Bioavailability is defined as "The rate and extent (amount) of absorption of unchanged drug from its dosage form." Bioavailability can be generally documented by a systematic exposure profile obtained by measuring drug/metabolite concentration in the systemic circulation over a particular time period.

Bioequivalence is a relative term which denotes that the drug substance in two or more drug products of identical dosage forms reaches the systemic circulation at the same relative rate and to the same relative extent bioequivalence is the comparative studies and mainly focuses on the release of drug substances from its dosage forms and subsequent absorption into the systemic circulation, that is, test dose plasma concentration-time will be identical with reference dose plasma concentration-

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time without showing any significant statistical differences, then test dosage form will consider as therapeutically equivalent to the reference dosage form.[1-10]

Both high-performance liquid chromatography (HPLC) and liquid chromatography tandem–mass spectrometry (LC–MS/MS) can be used for the bioanalysis of drugs in plasma. Each of the instruments has its own merits and demerits. HPLC coupled with ultraviolet (UV), photodiode array (PDA), or fluorescence detector can be used for the estimation of many compounds but it does not give the high sensitivity as required by some of the potent, low-dose drugs, and lacks selectivity. The main advantages of LC–MS/MS include low detection limits, the ability to generate structural information, the requirement of minimal sample treatment, and the possibility to cover a wide range of analytes differing in their polarities. Despite their high sensitivity and selectivity, LC–MS/MS instruments are limited to some extent due to matrix-induced differences in ionization efficiencies and ion suppression/enhancement effects due to biological matrix. HPLC coupled with UV, PDA, or fluorescence detects or offers a cost-effective bioanalytical method. Depending on the sensitivity, selectivity, and cost-effectiveness of the method, a choice needs to be made between HPLC and LC–MS/MS.[11-20]

MATERIALS AND METHODS

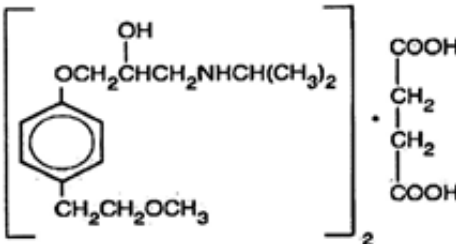
Drug profile

Metoprolol succinate

Description: Metoprolol succinate is the succinate salt form of metoprolol, a cardioselective competitive beta-1 adrenergic receptor antagonist with antihypertensive properties. Metoprolol succinate antagonizes beta-1-adrenergicreceptors in the myocardium, thereby reducing the rate and force of myocardial contraction, and consequently diminished cardiac output.

- Physical state: White crystalline powder
- Molecular formula: C34H56N2O10
- Molecular weight: 652.8 g/mol
- IUPAC name: Bis(1-[4-(2-methoxyethyl) phenoxy]-3-[(propan-2-yl)amino]propan-2-ol); but andesitic acid
- Solubility: Water and methanol

Structure:



Structure of metoprolol succinate

PK a/isoelectric point: 14.09

Drug category: Selective beta-adrenergic blocker.

Pharmacology

Pharmacokinetics and drug metabolism

Absorption

The estimated oral bioavailability of immediate-release (IR) metoprolol is about 50% because of pre-systemic metabolism which is saturable leading to non-proportionate increase in the exposure with increased dose. Extended-release (ER) metoprolol succinate is a controlled-release formulation designed to deliver metoprolol succinate at a near constant rate for approximately 20 h, independent of food intake and gastrointestinal pH. Once-daily dosing of ER metoprolol succinate 12.5–200 mg produces even plasma concentrations over a 24 h period, without the marked peaks and troughs characteristically observed with the IR formulation. This leads to consistent beta-1-blockade over 24 h, while maintaining cardioselectivity at doses up to 200 mg daily.[21-28]

Distribution

Metoprolol is extensively distributed with a reported volume of distribution of 3.2–5.6 L/kg. About 10% of metoprolol in plasma is bound to serum albumin. Metoprolol is known to cross the placenta and is found in breast milk. Metoprolol is also known to cross the blood–brain barrier following oral administration and cerebrospinal fluid concentrations close to that observed in plasma has been reported. Metoprolol is not a significant P-glycoprotein substrate.

Table 1: Standards

Name	%Purity	Supplier/Address	Storage temperature
Metoprolol succinate (Analyte)	100	Vivan Life Sciences/Mumbai	2–8°C
Metoprolol D7 Hydrochloride (ISTD)	99.86	Vivan Life Sciences/Mumbai	2–8°C
Caffeine	99.83	Vivan Life Sciences/Mumbai	2–8°C
Cetirizine HCl	99.61	Vivan Life Sciences/Mumbai	2–8°C
Paracetamol	99.98	Vivan Life Sciences/Mumbai	2–8°C
Ondansetron HCl	99.78	Vivan Life Sciences/Mumbai	2–8°C
Ibuprofen	99.98	Vivan Life Sciences/Mumbai	2–8°C
Diclofenac sodium	99.69	Vivan Life Sciences/Mumbai	2–8°C
Domperidone maleate	96.3	Clear synth/Mumbai	2–8°C

Metabolism

Metoprolol is primarily metabolized by CYP2D6. Metoprolol is a racemic mixture of R- and S-enantiomers, and when admin is treed orally, it exhibits stereoselective metabolism that is dependent on oxidation phenotype. The area under the curve for ER metoprolol succinate 100 mg once daily (3068 nmol h/l). The peak and trough concentrations of ER metoprolol succinate 100 mg were 133 ± 113 nM and 64 ± 57 nM, respectively.

Elimination

Elimination of metoprolol is mainly by biotransformation in the liver. The mean elimination half-life of metoprolol is 3–4 h in poor CYP2D6 metabolizes the half-life may be 7–9 h. Approximately 95% of the dose can be recovered in urine. In poor metabolizes, up to 30% or 40% of oral or intravenous doses, respectively, may be excreted unchanged; the rest is excreted by the kidneys as metabolites that appear to have no beta-blocking activity.

Mechanism of action

Metoprolol is a beta-1-selective (cardioselective) adrenergic receptor blocker. This preferential effect is not absolute, however, and at higher plasma concentrations, metoprolol also inhibits beta-2-adrenoreceptors, chiefly located in the bronchial and vascular musculature. Clinical pharmacology studies have demonstrated the beta-blocking activity of metoprolol, as shown by (1) reduction in heart rate and cardiac output at rest and on exercise, (2) reduction of systolic blood pressure on exercise, (3) inhibition of isoproterenol-induced tachycardia, and (4) reduction of reflex orthostatic tachycardia.

Table 2: Reagents and solvents

Name of the reagent/solvent	Manufacturer	Grade
Water	In-house	Milli-Q
Methanol	Merck	HPLC grade
Acetonitrile	Merck	HPLC grade
Ammonia	Merck	Emplura
Formic acid	Merck	Emplura
Diethyl ether	Thermo Fisher Scientific	HPLC grade
Dichloromethane	Merck	HPLC grade

HPLC: High-performance liquid chromatography

Table 3: Apparatus and equipment

Equipment	Make	Model
HPLC	Shimadzu	Prominence SIL-HTC
MS/MS	Thermo Fisher	TSQ Quantum Discovery Max
Balance	Sartorius	MF235P
Refrigerator	Videocon	VCP 314
Deep freezer (–25±5°C and –75±10°C)	Thermo	Forma 900 series/970
pH meter	Merck	Versa Star Pro
Multitube Vortex shaker	Vibromax 110 (Heidolph)	DMT-2500
Refrigerated centrifuge	Thermo	Heraeus Megafuge 40R
Nitrogen evaporator	Takahe Analytical	BioeVAP
Micropipettes and Multipette	Eppendorf	Research Plus

HPLC: High-performance liquid chromatography, MS: Mass spectrometry

Experimental work

Materials used [Tables 1-5].

Table 4: Selection of mobile phase buffer

LC condition	Method	Experiment	Conclusion
Mobile phase: acetonitrile: 10 mM ammonium formate (80:20)	1 set of CC's and 1 set of QC's (Spiking solution check)	Mobile phase buffer Selection	Reproducibility was not good
Mobilephase: acetonitrile: methanol: 0.1 % formic acid (50:50) (80:20)	1 set of CC's and 1 set of QC's	Mobile phase buffer selection	Reproducibility was good

Table 5: Selection of mobile phase

LC condition	Method	Experiment	Conclusion
Mobile phase: acetonitrile: methanol: 0.1% formic acid (50:50) (70:30) Flow rate 0.800 mL/min	1 set of CC's and 1 set of QC's (Spiking solution check)	Mobile phase buffer Selection	Reproducibility was not good
Mobile phase: acetonitrile: methanol: 0.1% formic acid (50:50) (80:20) Flow rate 0.600 mL/min	1 set of CC's and 1 set of QC's (Spiking solution check)	Mobile phase buffer selection	Reproducibility was good

Table 6: System suitability

Standard	Precision	
	Area ratio (<5%)	Retention time (NMT2%)
Drug	0.4	0.0
ISTD	NA	0.0
Drug	0.7	0.0
ISTD	NA	0.0
Drug	0.6	0.7
ISTD	NA	0.0
Drug	0.8	0.0
ISTD	NA	0.7

ISTD: Internal standard

Conclusion: 0.1% formic acid in water.
Conclusion:Mobilephase:acetonitrile:methanol:0.1% formic acid (50:50) (80:20) (v/v).

RESULTS AND DISCUSSION

System suitability was performed by injecting six consecutive injections of metoprolol at higher concentration of calibration range. The results were tabulated in the following table [Table 6].

CONCLUSION

In the present study, a rapid, sensitive, specific, precise, and accurate bioanalytical method for the estimation of metoprolol in human plasma has been developed and validated with a larger calibration curve range (0.501–349.342 ng/mL) as compared to the other reported methods which can be used for routine drug analysis and bioequivalence studies. The developed LC–MS/MS method employing liquid-liquid extraction for sample preparation is very simple and convenient for the determination of metoprolol in plasma samples. The previously reported methods for the analysis of metoprolol in biological fluids were not too satisfactory because all of them are too expensive and takes more time for estimation. LC–MS/MS operating in selected reaction monitoring mode was used to detect parent to product ion transition for analyte and internal standard. The validation data also demonstrate good linearity, precision, accuracy, sensitivity, specificity, matrix effect, recovery, system suitability, ruggedness, dilution integrity, reinjection reproducibility and stability studies, and high extraction efficiency. In conclusion, this work describes a very simple and sensitive LC–MS method for the determination of metoprolol suitable to monitor concentrations during clinical pharmacokinetic studies in humans.

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