Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jopr

Original Article

# Development and validation of a liquid chromatography mass spectrometry method for the determination of donepezil in human plasma

*Prakash Katakam* [a,c], *Rama Rao Kalakuntla* [b,*], *Shanta Kumari Adiki* [a], *Babu Rao Chandu* [c]

[a] *Department of Pharmaceutical Analysis, Nirmala College of Pharmacy, Guntur, Andhra Pradesh, India*
[b] *Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India*
[c] *Department of Pharmaceutics, Faculty of Pharmacy, University of Zawia, Az Zawiyah, Libya*

ARTICLE INFO

*Article history:*
Received 31 July 2013
Accepted 19 August 2013
Available online 8 September 2013

*Keywords:*
Donepezil
Plasma
LC–MS/MS
Validation

ABSTRACT

*Aim:* A selective, and sensitive LC–MS/MS method has been developed and validated for quantification of donepezil in human plasma using donepezil D7 as an internal standard (IS).
*Methods:* The analyte and IS were extracted by liquid–liquid extraction using dichloromethane and hexane mixture and separated by isocratic elution on C18 analytical column with 0.1% formic acid and methanol in the ratio of 70:30 (flow rate of 1 ml/min) as the mobile phase in the positive ion mode. Multiple Reaction Monitoring transitions for donepezil and internal standard are 380.2/91.2 and 387.2/98.2 respectively.
*Results:* The lower limit of quantification was 50 pg/ml with the linearity range of 50 pg/ml–25,000 pg/ml and the method was validated as per international regulatory guidelines for its selectivity, stability, accuracy, precision, and recovery.
*Conclusion:* The method can be readily applicable to pharmacokinetic and bioequivalence studies to support different regulatory submissions.

Copyright © 2013, JPR Solutions; Published by Reed Elsevier India Pvt. Ltd. All rights reserved.

## 1. Introduction

Donepezil (Fig. 1) is a piperidine-based, reversible inhibitor of the enzyme acetylcholinesterase. Donepezil is indicated for symptomatic treatment of patients with mild, moderate and severe dementia of the Alzheimer's type. Alzheimer's disease is a neurodegenerative disorder characterized by progressive loss of memory followed by complete dementia. It accounts for 50% of dementia cases.[1] A consistent pathological change in Alzheimer's disease is the degeneration of cholinergic neuronal pathways that project from the basal forebrain to the cerebral cortex and hippocampus. The resulting hypofunction of the cholinergic systems is thought to account for some of the clinical manifestations of dementia. Donepezil is postulated to exert its therapeutic effect by enhancing cholinergic function and acetylcholine levels of the brain. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetylcholinesterase.

The recommended initial dose of donepezil is 5 mg taken once daily. Donepezil is well absorbed with a relative oral

* *Corresponding author*. Tel.: +91 9642518806.
E-mail address: ramarao_2k2@rediffmail.com (R.R. Kalakuntla).
0974-6943/$ – see front matter Copyright © 2013, JPR Solutions; Published by Reed Elsevier India Pvt. Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jopr.2013.08.021

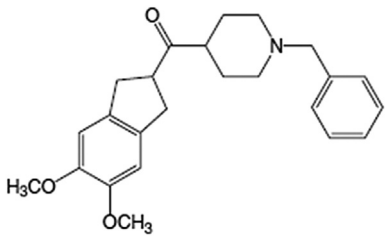


**Fig. 1 – Donepezil.**

bioavailability of 100% and reaches peak plasma concentrations ($C_{max}$) approximately 3–4 h after dose administration. In humans, donepezil is metabolized mainly by the hepatic cytochrome P-450 2D6 and 3A4 isozymes.[2] Elimination of donepezil from the blood is characterized by a dose independent elimination half-life of about 70 h.[3,4] Because plasma donepezil concentrations are related linearly to acetylcholinesterase inhibition,[5] plasma donepezil concentration is a useful tool to predict donepezil efficacy.

In the literature, methods have been reported for the quantification of donepezil in biological fluids. Methods are reported for the quantification of donepezil from biological matrix using high-performance liquid chromatography (HPLC) equipped with an ultraviolet detector,[2,3] fluorescence detector[4] and mass spectrometric[1,6,7] detector. Methods are also reported for the quantification of enantiomers of donepezil from human plasma.[8–10] Other methods are reported with estimation of donepezil in plasma by capillary electrophoresis,[11] hydrophilic interaction chromatography-tandem mass spectrometry,[12] direct measurement,[13] automated extraction.[14] The HPLC methods used to determine donepezil in human plasma are insensitive because of the lower limit of quantification (LOQ of >1.0 ng/ml). Some of the reported methods[1,4,6,10,13,14] utilized analogue internal standards like diphenhydramine, lidocaine, pindolol, loratadine, escitalopram, etc. and are validated with different calibration curve ranges for the estimation of donepezil from rat plasma, human plasma and other biological fluids. Usage of labelled internal standards is recommended during the estimation of compounds from the biological matrices to minimize the matrix effects associated with the mass spectrometric detection.

Bioequivalence and/or pharmacokinetic studies become an integral part of generic drug applications and a simple, sensitive, reproducible validated bioanalytical method should be used for the quantification of intended analyte. Bioequivalence studies for the donepezil needs to be performed with the dosage of 10 mg and 23 mg tablets to support the generic abbreviated new drug applications. For the pharmacokinetic and bioequivalence studies, quantification of donepezil was sufficient and quantification of its metabolites shall not be required. During the bioequivalence studies, appropriate lower limit of quantification needs to be used to appropriately characterize the concentration profile including the elimination phase.

The motive of the present method was to develop the method with the lower limit of quantification of 50 pg/ml and with minimal sample volume using simple and cost effective liquid–liquid extraction technique. The developed method was validated as per the current international regulatory guidelines on bioanalytical method validation. The method can be readily applicable for usage during the bioequivalence evaluation of various generic formulations for submission as part of abbreviated new drug applications.

## 2. Materials and methods

### 2.1. Chemicals

Donepezil reference standard was procured as a gift sample from a Pharma company and HPLC grade methanol, acetonitrile were commercially procured and all other chemicals were of analytical grade.

### 2.2. Preparation of solutions

0.01 N hydrochloric acid was prepared by diluting 0.1 ml of hydrochloric acid to 1000 ml in a volumetric flask with milli Q water. Mixture of dichloromethane and hexane was prepared by mixing one part of dichloromethane and four parts of hexane. 1% formic acid was prepared by adding 10 ml of formic acid to a 1000 ml volumetric flask and made up the volume with milli Q water and similarly 0.1% formic acid solution was prepared by adding 1 ml of formic acid to a 1000 ml volumetric flask and made up the volume with milli Q water. 50% methanol was prepared by mixing 500 ml of methanol and 500 ml of water in a reagent bottle. Rinsing solution which is used for auto sampler wash was prepared by mixing 0.1% formic acid and methanol in the ratio of 80:20. Mobile phase consisting of 0.1% formic acid and methanol mixture (70:30) was prepared by mixing 700 ml of 0.1% formic acid with 300 ml of methanol.

### 2.3. Preparation of standards

Donepezil and donepezil D7 stock solutions were prepared at a concentration of 0.1 mg/ml by dissolving in 0.01 N hydrochloric acid solution and the stock solutions were stored in the refrigerator. Spiking solutions of donepezil for the preparation of calibration standards and quality control samples were prepared in mobile phase and spiked in to the plasma at the ratio of 1:50. The calibration curve from 50 to 25,000 pg/ml was generated using ten calibration standards at the concentrations of 50 pg/ml (STD 1), 100 pg/ml (STD 2), 200 pg/ml (STD 3), 500 pg/ml (STD 4), 2500 pg/ml (STD 5), 5000 pg/ml (STD 6), 10,000 pg/ml (STD 7), 15,000 pg/ml (STD 8), 20,000 pg/ml (STD 9), 25,000 pg/ml (STD 10). The quality control samples were prepared at the concentrations of 50 pg/ml (LLOQQC), 150 pg/ml (LQC), 9000 pg/ml (MQC) and 18,000 pg/ml (HQC). The bulk spiked calibration standards and quality control samples were stored in the freezer. Internal standard dilution was prepared at a concentration of 3000 pg/ml using mobile phase.

### 2.4. Sample preparation and extraction

Donepezil from the plasma was extracted using liquid–liquid extraction technique. Plasma aliquot of 0.3 ml (300 μl) was added to the polypropylene tube containing 50 μl of internal standard

Download English Version:

https://daneshyari.com/en/article/8541449

Download Persian Version:

https://daneshyari.com/article/8541449

Daneshyari.com