Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 857 (2007) 322–326
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Rapid and sensitive bioanalytical method for measurement of fluvoxamine in human serum using 4-chloro-7-nitrobenzofurazan as pre-column derivatization agent: Application to a human pharmacokinetic study

Gholamreza Bahrami [a,b,*], Bahareh Mohammadi [a]

[a] *Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran*
[b] *School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran*

Received 21 May 2007; accepted 26 July 2007
Available online 3 August 2007

Abstract

A sensitive and rapid high-performance liquid chromatographic method for the analysis of fluvoxamine, a selective serotonin reuptake inhibitor in human serum, is described using 4-chloro-7-nitrobenzofurazan as pre-column derivatization agent. The drug and an internal standard (fluoxetine) were extracted from 0.25 mL of serum using ethyl acetate as extracting solvent and subjected to pre-column derivatization by the reagent. A mobile phase consisting of methanol and sodium phosphate buffer (0.05 M; pH 2.8) containing 1 mL/L triethylamine (72:28 v/v) was used and chromatographic separation was performed on a Shimpack CLC-C18 (150 mm × 4.6 mm) column. The fluorescence derivatives of the drugs were monitored at excitation and emission wavelengths of 470 and 537 nm, respectively. The calibration curve was linear over the concentration range of 0.5–240 ng/mL with a limit of quantification (LOQ) of 0.5 ng/mL using 0.25 mL serum sample. The method validation was performed for its selectivity, specificity, sensitivity, precision and accuracy. In this method, which was applied in a randomized cross-over bioequivalence study of two different fluvoxamine preparations in 24 healthy volunteers, the sensitivity and run time of analysis were significantly improved.

Keywords: HPLC; Fluvoxamine; Serum; 4-Chloro-7-nitrobenzofurazan; NBD-Cl; Derivatization

1. Introduction

Fluvoxamine (FL) 5-methoxy-4(trifluoromethyl) valerophenone (E)-*O*-(2-aminoethyl) oxime maleate is a selective serotonin reuptake inhibitor which has been used for the treatment of depression with fewer adverse effects compared to the tricyclics [1]. The drug is well absorbed with time to peak plasma concentration of about 2–8 h, bioavailability of more than 90% and elimination half-life of about 18 h. FL undergoes extensive metabolism in the liver to produce a number of inactive metabolites [2]. In common with other antidepressants the role of therapeutic drug monitoring of FL in management of depressive patients is unknown [1], however, quantification methods of the drug in pharmacokinetic studies need to be sensitive and specific. There are several analytical techniques in the literature for quantification of the drug in pharmaceutical preparations and biological fluids. FL has been measured in the pharmaceutical dosage forms using capillary gas chromatography (GC) [3,4], capillary electrophoresis (CE) [5] and spectrophotometeric [6] methods. In the biological fluids, however, analysis of the drug has been reported using high-performance liquid chromatography (HPLC) coupled with UV [7–12], fluorescence [13–15] or visible [16] detectors. Determination of the drug in serum samples, using HPLC with UV detection with different sensitivities (10 ng/mL [7,8] and 25 ng/mL [9–11]) have been reported. Low-blood concentrations are achieved following single dose administration of the drug, thus, more sensitivity is needed in single dose human pharmacokinetic studies. A column switching HPLC-UV method (LOQ 0.8 ng/mL), using 1.5 mL serum sample and volume injection of 500 μL, has been reported [12]. In this method, however, toxic solvents (toluene–chloroform) has

* Corresponding author at: Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran. Tel.: +98 831 8350197; fax: +98 831 8368410.
E-mail address: gbahrami@kums.ac.ir (G. Bahrami).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.07.044

been used for drug extraction; sample preparation is tedious and time-consuming and long run time of analysis (25 min) has been reported. Different fluorogenic reagents including fluorescamine [13], dansyl chloride [14], 4-fluoro-7-nitrobenzofurazan (NBD-F) [15] and 1, 2-naphthoquinone-4-sulphonic acid [16] have been used to improve sensitivity of the assay. LOQ of 10 ng/mL has been reported using derivatization of 1 mL serum samples with either fluorescamine [13] or dansyl chloride [14], however, using of fluorescamine and dansyl derivatives are limited by their poor photo stability and band broadening of the resulted peaks. In pre-column derivatization of the drug with 2-naphthoquinone-4-sulphonic acid [16] sensitivity of 5 ng/mL and analytical run time of about 6 min have been reported. In this method, however, 30 min incubation of the reaction mixture and time-consuming multi steps extraction of the resulted derivative are needed. Pre-column derivatization of the drug using NBD-F as labeling agent has been reported by Higashi et al. [15]. Although derivatization of the drug has been achieved during 10 min yet in their method, which has not been tested in human studies, poor sensitivity (15 ng/mL using 100 μL serum sample) and long run time of analysis (18 min) have been reported. Present study describes a new, fast and sensitive method using NBD-Cl as fluorogenic agent. The procedure presented here, which has been approved in a bioequivalence study of two different FL preparations, is very sensitive with LOQ of 0.5 ng/mL using 250 μL serum sample and 20 μL injection, and fast with analytical run time of 6 min.

2. Experimental

2.1. Chemicals

FL (purity 99.7%) was from Neolux (England, UK) and was kindly provided by Bakhtar Bioshimi pharmaceutical company (Kermanshah, Iran). Fluoxetine (I.S.) was from Sigma (St. Louis, MO, USA). All reagents used were of analytical-grade except methanol which was HPLC grade and purchased from Merck (Darmstadt, Germany). Water was glass-double distilled and further purified for HPLC with a Maxima purification system (USF ELGA, England).

2.2. Standard solutions

A stock solution of FL (1000 μg/mL) was prepared in methanol. Working standards of the drug (1.25–600 ng/mL) were prepared by serial dilution of the stock solution in methanol. Working standard solutions of the I.S. (1.2 μg/mL) and NBD-Cl (5 mg/mL) were prepared in methanol. A borate buffer (0.05 M) was prepared in water and adjusted to pH 8.0 with 0.05 M potassium hydroxide solution. All solutions were stored at 4 °C and were stable for at least 3 weeks.

2.3. Calibration curve, sample preparation and derivatization

Pooled blank human serum was used for construction of the calibration curve. After evaporation of 100 μL from each working solution of the drug, under a gentle stream of nitrogen at 50 °C, the residues were reconstituted in 250 μL of drug-free human serum. In an Eppendorf tube 250 μL serum samples (blank, calibration or unknown), 100 μL of the I.S. and 1 mL ethyl acetate were added. After briefly mixing for 20 s on a vortex mixer and centrifugation for 3 min at 12000 × *g*, the organic phase was removed, transferred into a 100 × 16 mm disposable glass tube and evaporated to dryness under a stream of nitrogen at 50 °C. To the residue 125 μL of the NBD-Cl solution, 100 μL dichloromethane and 25 μL of the borate buffer were added and after a brief mixing for 10 s on a vortex mixer, the samples were kept at 60 °C for 5 min. The NBD-Cl derivatives were then analyzed by injection of a 20 μL volume of the reaction mixture onto the chromatographic column.

2.4. Equipment

The HPLC system used consisted of two pumps of Shimadzu LC-10A solvent delivery system, a system controller (SCL 10AD), a spectroflurometric detector (RF-551) operated at excitation and emission wavelengths of 470 and 537 nm, respectively, a column oven (CTO-10A), a degasser (DGU-3A) and a data processor (C-R4A) all from Shimadzu, Kyoto, Japan. The analytical column was a Shimpack CLC-ODS (Shimadzu, Kyoto, Japan), 150 mm × 4.6 mm ID., 5 μm particle size, which was protected by a Shim-pack G-ODS guard column (1 cm × 4.0 mm I.D., 5 μm particle size). A mixture of methanol and sodium phosphate buffer (0.05 M; pH 2.8) containing 1 mL/L triethylamine (72:28 v/v) was used as the mobile phase. The column oven temperature was set at 58 °C and the mobile phase was filtered, degassed and pumped at a flow rate of 2.0 mL/min.

2.5. Optimization of the derivatization conditions

Solutions of 1, 50 and 200 μg/mL of the drug were used to optimize derivatization of FL with NBD-Cl while the I.S. was reacted with the reagent at the concentration of 1.2 μg/mL. Concentrations of the NBD-Cl solutions ranging from 0.5 to 10 mg/mL, pH of the buffer solutions ranging from 6 to 12 and pH of the mobile phase ranging from 2.2 to 7 were tested to obtain optimal conditions for analysis. The polarity of the reaction solution was optimized using various organic solvent–water proportions, ranging from 1:1 to 10:1, and the reaction was allowed to proceed in a water bath at temperature ranging from 40 to 80 °C. Different organic solvents including acetone, ethyl acetate, dichloromethane, acetonitrile and chloroform were used to increase the yield of the reaction.

2.6. Method validation

Specificity of the method was tested by the analysis of 24 human blank serum samples from different volunteers. These samples were pretreated according to the sample preparation procedure without the addition of the I.S. to ensure the absence of endogenous substances with the same retention times as the analytes of interest. Average recoveries of the extraction procedure for both FL and the I.S. were estimated by comparing the