Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 320–324
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

High-performance liquid chromatography assay for moxifloxacin: Pharmacokinetics in human plasma

Sevgi Tatar Ulu *

Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul University, Istanbul 34452, Turkey

Received 16 January 2006; received in revised form 5 June 2006; accepted 7 June 2006
Available online 26 July 2006

Abstract

A sensitive high-performance liquid chromatographic (HPLC) method for the determination of moxifloxacin in human plasma using fluorescence detection was developed. The drug and an internal standard (norfloxacin) were subjected to precolumn derivatization with 4-chloro-7-nitrobenzodioxazole (NBD-CI). The chromatographic separation was achieved by HPLC using a mixture of acetonitrile–10 mM orthophosphoric acid (pH 2.5) (80:20, v/v) as the mobile phase with isocratically system, a C_{18} column. The derivative is highly fluorescent at 537 nm, being excited at 464 nm. The linear and reproducible calibration curve over the range was 15–2700 ng/mL of moxifloxacin in human plasma. The limits of detection and quantitation were 6 and 15 ng/mL, respectively. This method was applied in pharmacokinetic studies moxifloxacin preparations in healthy volunteers.

Keywords: HPLC; Moxifloxacin; Plasma; Pharmacokinetic study

1. Introduction

Moxifloxacin (mox), 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4*aS*,7*aS*)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl] 4-oxo-3 quinoline carboxylic acid [1] is a new fluoroquinolone antibacterial agent with a broad spectrum of activity, encompassing Gram-negative and Gram-positive bacteria [2]. An azabicyclo-substitution at C-7 is associated with substantially improved Gram-positive activity. Of additional importance, in studies of *S. aureus*, the presence of a methoxy group at the C-8 position was associated with a decreased propensity for development of resistance [3,4]. In particular, the potential ability of mox to kill first-step resistant mutants may greatly reduce the ability of wild-type populations to acquire resistance. Fluoroquinolones with C-8 halogenation are associated with moderate to severe phototoxicity (e.g., sparfloxacin). With mox, this was circumvented by substituting a C-8 methoxyl group [5].

Different methods for the quantification of mox in human urine have been reported which include spectrofluorimetry [6], square-wave adsorptive voltammetry [7], capillary electrophoresis [8]. Using HPLC and fluorescence detection, mox, an antimicrobial quinolones were assayed in serum [9]. Fluoroquinolones, mox, were separeted and determined in plasma by HPLC [10] with ultraviolet and fluorescence detection. Mox has been analysed using HPLC [11,12] fluorescence and ultraviolet detection, respectively. Vishwanathan et al. [13] have reported a liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) for the determination in human plasma (Fig. 1).

In this study, a new fluorimetric HPLC method has been developed. The method is based on the derivatization of mox with NBD-CI. In literature research, mox, for the first time has been derivatized by a reagent (NBD-CI) and has been determined. This paper describes a modified HPLC method that is simple, sensitive and specific for the determination of mox in plasma as the NBD-CI derivative.

2. Experimental

2.1. Chemicals and reagents

Mox and the internal standard (Norfloxacin) were obtained from Bayer (Istanbul, Turkey) and Merck Sharp Dohme (Istan-

* Fax: +90 2124400252.
E-mail address: sevgitatar@yahoo.com.

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.06.026

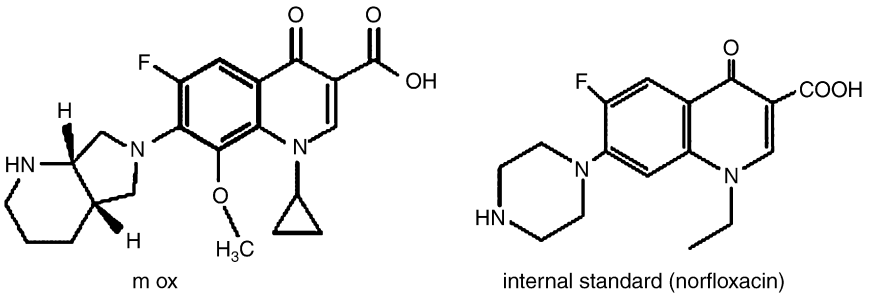

Fig. 1. Chemical structures of mox and internal standard.

bul, Turkey), respectively. All solvents used were HPLC grade (Merck Darmstadt, Germany). NBD-CI and all other reagents employed were of analytical grade Merck (Darmstadt, Germany). Water was purified by aquaMAX^{TM} ultra, Young instrument (Korea) ultrawater purification system. Venous blood samples were collected into ethylenediaminetetraacetic acid and centrifuged (4500 rpm for 15 min) and the plasma removed and frozen at −20 °C until analysis.

2.2. Solutions

Stock solutions were of mox and of the internal standard (norfloxacin) in methanol (100 μg/mL): working standard solutions and working internal standard solutions were at 10 μg/mL concentrations.

NBD-CI reagent: solution was freshly prepared at 3 mg/mL in methanol.

Borate buffer (0.1 M) was prepared from boric acid: the pH was adjusted to 9 with 0.1 M NaOH.

2.3. Instrumentation

The liquid chromatographic system was (Thermo Separation Products Liquid Chromatograph, TX, USA) consisted of a Model P 4000 solvent delivery system, a Rheodyne injection valve with a 20 μL loop, a FU 3000 detector, operating at excitation wavelength of 464 nm and emission wavelength of 537 nm and a SN 4000 automation system software. The analytical column was a Phenomenex C_{18} column (250 mm × 4.6 mm i.d., 5 μm; Thermo Separation, TX, USA), with a guard column (4 mm × 3 mm i.d., Phenomenex) packed with the same material. The mobile phase was acetonitrile–10 mM orthophosphoric acid mixture (pH 2.5) (80:20, v/v) at a flow rate 1.2 mL/min. The mobile phases were degassed by ultrasonic agitation. Under these conditions retention times of internal standard and mox were 3.834 and 4.795 min, respectively.

2.4. Sample preparation and derivatization

To 0.5 mL of plasma sample in a 12 mL glass tube 50 μL of the working internal standard solution and 0.75–135 μL working standard solutions, 1 mL methanol were added, and the sample vortex-mixed for 1–2 min. The mixture was separated by centrifugation at 4500 rpm for 15 min. An aliquot of 1 mL of the protein-free supernatant was then evaporated to dryness at 45 °C under nitrogen. To this residue, 200 μL of borate buffer and of NBD-CI solutions were added. The mixture was shaken on a vortex and heated for 10 min at 70 °C. After cooling, 200 μL of 0.1N HCl was added and the contents were extracted three times 2 mL of ethyl acetate. After extraction, 5 mL of the ethyl acetate phase was evaporated to dryness at 45 °C under nitrogen. The dry residue was dissolved in 200 μL of acetonitrile–10 mM orthophosphoric acid (pH 2.5) (80:20, v/v). 20 μL aliquots of this solution are employed for determination by HPLC.

2.5. Method validation

2.5.1. Linearity

Calibration curves were prepared by spiking different samples of blank plasma each with proper volume of one of the above-mentioned working solutions to produce the calibration curve points equivalent to 15, 30, 60, 120, 240, 480, 960,1000, 1500, 2000, 2500 and 2700 ng/mL of mox. The samples were assayed using the method described above. The standard calibration curves for mox were constructed using the mox/IS peak area ratios versus the nominal concentrations of the analytes.

2.5.2. Precision and accuracy

Mox samples (15, 1000 and 2700 ng/mL) in six replicates were analysed on the same day to determine the intra-day precision and accuracy, and on each of five separate days to determine inter-day precision and accuracy.

2.5.3. Recovery

The absolute recovery of mox from plasma was determined by comparing peak areas obtained from plasma spiked with mox at concentrations of 15, 1000 and 2700 ng/mL with the peak areas obtained from the aqueous solutions of mox of the same concentration.

2.5.4. Sensitivity

The limit of detection (LOD) was determined as the lowest concentration, which gives a signal-to-noise ratio of 3 for mox. The limit of quantitation (LOQ), defined as the lowest concentration level at which the assay was validated. The LOD and LOQ were calculated for the calibration graphs of mox as three and then times of the baseline noise level for LOD and LOQ, respectively.

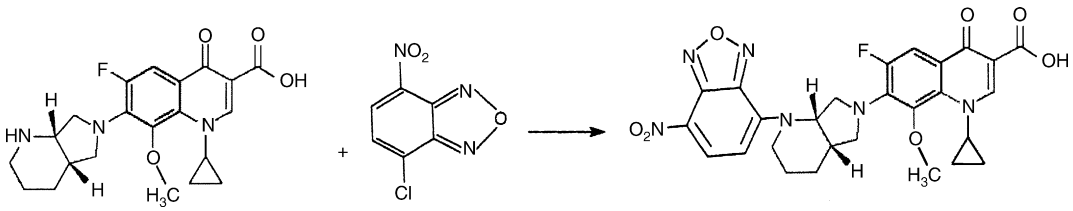

Fig. 2. The reaction between mox and NBD-CI.

2.5.5. Stability

Mox is shown to be stable under conditions of storage and processing. The stability in the ethyl acetate was determined at 4 °C and room temperature (24 h). The analyte is stable in human plasma when stored at −20 °C for 2 months and room temperature for 24 h.

The freeze-thaw stability was assessed in the mox spiked plasma samples storage at −20 °C for 2 months.

2.5.6. Specificity

Specificity was assessed by examining peak interference from endogenous substances. This was assessed by inspecting chromatograms of blank and spiked plasma samples.

2.6. Pharmacokinetic applications

Mox (400 mg) was orally administered to a 35-year-old healthy woman volunteer. Further blood samples were drawn into ethylenediaminetetraacetic acid tubes at 0.25, 0.50, 1, 1.5, 2,3, 4.5, 5.5, 6, 9, 11, 12 and 24 h after administration.

The blood was processed to plasma by centrifugation at 4500 rpm and 15 min. Plasma samples were stored −20 °C until analysis.

3. Results and discussion

The reaction between mox and NBD-CI reagent in alkaline medium (pH 9) is shown in Fig. 2.

The drug-NBD was all detected excitation at 464 nm, with emission at 537 nm with a spectrofluorimetrically monitor. Under the assay conditions described, mox was separated from the internal standard, norfloxacin, with retention times of 4.795 and 3.834 min, respectively (Fig. 3). The chromatograms obtained from the analysis of blank plasma show no interfering peaks having the same retention times as mox or internal standard derivatives.

Calibration curve obtained by plotting peak-area ratio (mox/internal standard) versus concentration were linear over the range 15–2700 ng/mL.

The calibration curve was linear for plasma and was represented by the regression equations $A = 0.018C - 3.1 \times 10^{-3}$ ($r = 0.9997$), where A is the peak–area ratio (mox/internal standard) and C is the mox concentration (ng/mL). The limit of quantification (LOQ) was 15 ng/mL; the limit of detection (LOD) was 6 ng/mL (signal-to-noise ratio of 3). The mean recovery of mox was 95.73% (Table 1).The intra-day precision was <4.74% and intra-day accuracy ranged from 2.91% to 5.73%. Inter-day pre-

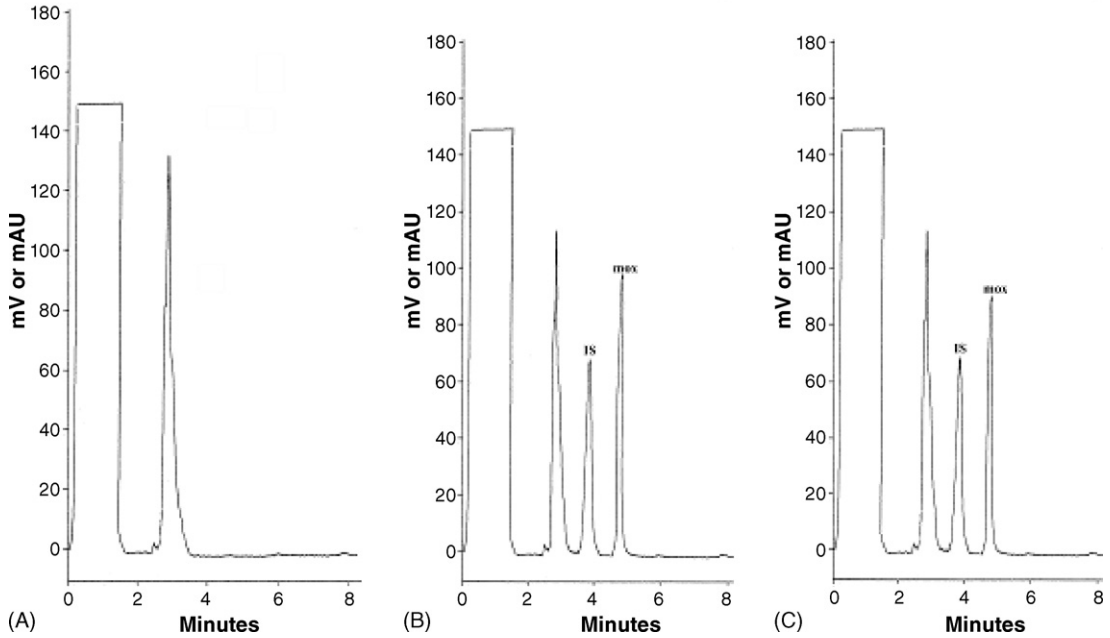

Fig. 3. HPLC chromatograms of mox and nor (IS): (A) blank human plasma, (B) human plasma spiked with 2000 ng/mL of mox and 1000 ng/mL of the (IS), (C) plasma samples obtained at 3 h after oral administration of 400 mg of mox from a healthy volunteer with 1000 ng/mL of the IS, respectively.

Table 1
Absolute recovery of mox from plasma samples ($n = 6$)

Concentration added (ng/mL)	Concentration found (mean ± standard deviation, S.D.)	Recovery (%)	Relative standard deviation, R.S.D. (%)
15	14.11 ± 0.62	94.06	4.39
1000	965.05 ± 0.40	96.51	0.041
2700	2608.97 ± 0.39	96.63	0.014

Table 2
Intra-day inter-day precision and accuracy of mox in plasma ($n = 6$)

Concentration added (ng/mL)	Concentration found mean ± standard deviation, S.D.	Precision relative standard deviation, R.S.D. (%)	Accuracy relative mean error, R.M.E. (%)	Concentration found mean ± standard deviation, S.D.	Precision relative standard deviation, R.S.D. (%)	Accuracy relative mean error, R.M.E. (%)
15	14.14 ± 0.67	4.74	5.73	14.10 ± 0.68	4.82	6.00
1000	970.89 ± 0.45	0.046	2.91	968.76 ± 0.47	0.049	3.12
2700	2611.19 ± 0.38	0.015	3.29	2611.74 ± 0.42	0.016	3.27

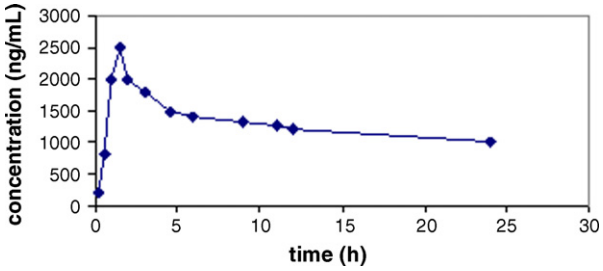

Fig. 4. Plasma concentration–time profile of mox from 35-year-old healthy volunteer following a single oral dose of 400 mg.

cision was <4.82% and inter-day accuracy ranged from 3.12% to 6% (Table 2).

In the short-term stability study, mox-NBD derivative was stable for one week at 4 °C and room temperature for 24 h. In the long-term stability study, the plasma samples spiked with mox were stored for 2 months at −20 °C and after three freeze/thaw cycles.

As mentioned above, there were no peaks of interfering with mox and IS at their retention times in the blank and spiked samples. Potential interferences by common drugs which are administered concurrently with mox, such as several drugs (enoxacin, ciprofloxacin, enrofloxacin, levofloxacin) were tested and found not to interfere with mox and IS retention time.

In the pharmacokinetic study, after a healthy volunteer was administered 400 mg of mox. Maximum plasma concentration of mox (C_{max}) and time to reach this value (t_{max}) were determined to be 2510 ng/mL and 1.5 h. Elimination half-life ($t_{1/2}$) and area under curve (AUC) of the drug were calculated as 9.5 h and 26,997 ng h/mL, respectively (Fig. 4).Pharmacokinetic parameters obtained using proposed method are in agreement with those of the studies reported previously [2] (Table 3).

Table 3
Pharmacokinetic parameters for mox in healthy volunteer

t_{max} (h)	1.5
$t_{1/2}$ (h)	9.5
C_{max} (ng/mL)	2510
AUC 0–24 (h ng/mL)	26997

4. Conslusion

In this study, a new fluorimetric HPLC method has been developed. In literature researches mox has been determined in biological fluids with HPLC method by using both UV and fluorimetric detector [9,10]. But in the method that I developed, for the first time mox has been derivatized by a reagent (NBD-CI) and has been determined in a biological fluids. The purpose of derivatization is to study in low concentration and increasing the sensitivity. Thus I have been able to study in low concentration. When this developed method is compared with the methods in literature, the concentration interval of LOD and LOQ was seen [9–12] very low. In addition the high stability of the sample of mox-NBD derivatization and plasma spiked mox is an advantage. One of the superiority of this method against the methods that are used for analysing mox in human plasma is working with the sample of 0.5 mL of plasma. In other methods a specially it has been worked with 1 mL of plasma [9]. Besides, according to the other methods the retention time is quite short [9–12]. In this study the recovery rate from the mox plasma is high, the processes of derivatization and extraction do not cost much time.

References

[1] The Merck Index (2001) 1125.
[2] J.A.B.B. Balfour, L.R. Wiseman, Drugs 57 (1999) 363–373.
[3] L.A. Mitcher, P. Devasthale, R. Zarod, Am. Soc. Microbiol. (1993) 3–51.
[4] J.M. Domagala, J Antimicrob. Chemother. 33 (1994) 685–706.
[5] X. Zhao, J.Y. Wang, C. Xu, Antimicrob. Agents Chemother. 42 (1998) 956–958.
[6] J.A. Ocana, F.J. Barragan, M. Callejon, Analyst 125 (2000) 2322–2325.
[7] M.A.G. Trindade, G.M. Silva, V.S. Ferreira, Microchem. J. 81 (2005) 209–216.

[8] J.G. Möller, H. Staß, R. Heinig, G. Blaschke, J. Chromatogr. B 716 (1998) 325–334.
[9] H.A. Nguyen, J. Grellet, B.B. Ba, C. Quentin, M.C. Saux, J. Chromatogr. B 810 (2004) 77–83.
[10] H. Liang, B. Michael, K. Sowinski, K.M. Sowinski, J. Chromatogr. B 772 (2002) 53–63.
[11] B.B. Ba, R. Etienne, D. Ducint, C. Quentin, M.C. Saux, J. Chromatogr. B 754 (2001) 107–112.
[12] T. Lemoine, D. Breilh, D. Ducint, J. Dubrez, J. Jougon, J.F. Velly, M.C. Saux, J. Chromatogr. B 742 (2000) 247–254.
[13] K. Vishwanathan, M.G. Bartlett, J.T. Stewart, J. Pharm. Biomed. Anal. 30 (2002) 961–968.