Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1444–1451
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

HPLC method for the determination of fluvoxamine in human plasma and urine for application to pharmacokinetic studies

Sevgi Tatar Ulu *

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

Received 11 September 2006; received in revised form 3 November 2006; accepted 7 November 2006
Available online 6 December 2006

Abstract

A simple, specific and sensitive high-performance liquid chromatographic (HPLC) method has been developed for the assay of fluvoxamine in human plasma and urine. The method was based on reaction of fluvoxamine with 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS) forming orange colored product. The fluvoxamine–NQ derivative was separated by isocratic reversed-phase HPLC and detected at 450 nm. The chromatographic conditions were as follows: Phenomenex C_{18} (250 mm × 4.6 mm i.d., 5 μm) column, mobile phase consisting of acetonitrile/water (80:20 v/v) at a flow rate of 1 ml/min. Tryptamine was selected as an internal standard. The assay was linear over the concentration range of 5–145 and 2–100 ng/ml for plasma and urine, respectively. The limits of detection (LOD) were 1.4 and 1 ng/ml for plasma and urine estimation at a signal-to-noise (S/N) ratio of 3. The limits of quantification (LOQ) were 5 and 2 ng/ml for plasma and urine, respectively. The extraction recoveries were found to be 96.66 ± 0.69 and 96.73 ± 2.17% for plasma and urine, respectively. The intra-day and inter-day standard deviations (S.D.) were less than 1. The method indicated good performance in terms of specificity, linearity, detection and quantification limits, precision and accuracy. This assay was demonstrated to be applicable for clinical pharmacokinetic studies.

Keywords: Fluvoxamine; 1,2-Naphthoquinone-4-sulphonic acid sodium salt; Derivatization; HPLC; Pharmacokinetic study

1. Introduction

Fluvoxamine (FL) is a selective serotonin reuptake inhibitor (SSRI) belonging to a new chemical series, the 2-aminoethyl oxime ethers of aralkylketones. It is chemically designated as 5-methoxy-4′-(trifluoromethyl) valerophenone (E)-*O*-(2-aminoethyl) oxime maleate (Fig. 1) [1]. Fluvoxamine is absorbed well following oral administration to healthy volunteers, and peak plasma concentrations are achieved within approximately 2–8 h. Mean elimination half-life ($t_{1/2}$) is approximately 19 and 22 h after single and multiple doses, respectively, and is not significantly increased in the elderly [2]. No specific toxic blood level has been established, but a therapeutic level (normal steady state value that would occur after treatment with 100 mg/day) is 0.4 mg/l [3]. In common with other SSRIs, fluvoxamine is extensively metabolised in the liver, with less than 4% being excreted unchanged. The primary routes of metabolism are oxidative demethylation (accounting for approximately 42–50% of total metabolism), oxidative deamination (15–23%), =N–O bond cleavage (12%) and *N*-acetylation (8%). Fluvoxamine has no metabolites with psychotropic activity. There is therefore little possibility of metabolites causing problems if an unexpected adverse reaction occurs [4].

Several analytical methods have been published for the quantification of pharmaceutical preparations and biological fluids. A number of capillary gas chromatography [5,6] and spectrophotometric [7] methods have been described for the determination of FL pharmaceutical dosage forms, respectively. Determination of fluoxetine and FL pharmaceutical preparations by capillary electrophoresis method has also been described [8].

Several high-performance liquid chromatographic (HPLC) methods are available for determination of FL in plasma. Yasui-Furukori et al. have [9] reported a HPLC method with ultraviolet detection for the assay of FL and its metabolite. Determination of seven selective serotonin reuptake inhibitors in human serum HPLC method with ultraviolet detection has also been studied [10]. Eap and Baumann [11] have studied HPLC, gas chromatography, thin layer chromatography and detec-

* 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.2006.11.005

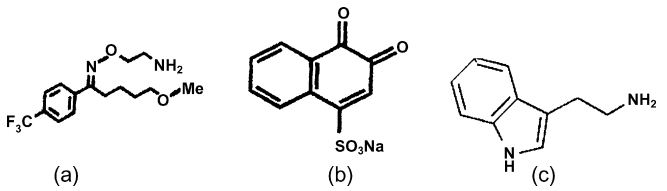

Fig. 1. Chemical structures of fluvoxamine (a), NQS (b) and tryptamine, IS (c).

tion by various detectors (UV, fluorescence, electrochemical detector, nitrogen–phosphorus detector and mass spectrometry) for the quantitative determination of selective serotonin reuptake inhibitors for therapeutic drug monitoring purposes in patients. FL has been analysed using HPLC fluorescence [12] ultraviolet [13–18] and diode array detection [19–23] for the determination in human plasma. Van Der Meersch-Mougeot and Diquet [24] have reported column liquid chromatography with ultraviolet detection for the assay of FL in human and rat plasma.

Determination of FL in rat plasma by HPLC with pre-column derivatization and fluorescence detection using 4-fluoro-7-nitro-2,1,3-benzoxadiazole has also been described [25]. Also a pre-column for the direct HPLC method has been reported for the determination of the anti-depressants clovoxamine and FL in plasma [26]. Thin-layer chromatographic or HPLC methods have been used for the determination of FL in human plasma [27]. Labad et al. [28] have studied by micellar electrokinetic capillary chromatography for the separation of new antidepressants and their metabolites. FL has been analysed using liquid chromatography–mass spectrometric [29] and gas chromatography–mass spectrometric [30,31] methods for the determination in human plasma.

This report describes a sensitive, specific HPLC procedure with ultraviolet visible detection for determining FL in human plasma and urine by means of the derivative formed with NQS. NQS usually reacts with primer and secondary amines [32–37].

In literature research, FL, for the first time has been derivatized by a NQS and has been determined by using a UV detector. This method was fully validated for its specificity, accuracy, precision and sensitivity. It was essential to establish an assay with an LOD in the low ng/ml range. Short separation times and high sensitivity, without compromising the specificity, are the main advantages of such a technique. At the same time, this method was efficient in analyzing large numbers of plasma and urine obtained for pharmacokinetic study after therapeutic doses of FL.

2. Experimental

2.1. Materials and reagents

FL was purchased from Eczacıbaşı (Istanbul, Turkey). Tryptamine (internal standard, IS) and NQS were purchased from Sigma (St. Louis, MO, USA). HPLC-grade organic solvents were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical grade.

Distilled water was prepared as required by using aquaMAX^{TM} ultra, Young instrument (Korea) ultrawater purification system. Venous blood samples were collected into ethylenediaminetetraacetic acid and centrifuged (4500 × *g* for 15 min). Plasma and urine samples were removed and frozen at −20 °C until analysis.

2.2. Instrumentation

Chromatography was preformed using a Thermo Separation Products HPLC (TX, USA) consists of a Model P 4000 solvent delivery system, a Rheodyne injection valve with a 20 μl loop, a UV 3000 detector. Separations were carried out on 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. Results were plotted and processed using SN 4000 automation system software. The mobile phase was acetonitrile/water (80:20 v/v). The flow rate was maintained at 1 ml/min. Eluted peaks were detected at 450 nm.

2.3. Solutions

Stock solutions (1 mg/ml) of FL were prepared in methanol. The initial stock solution was further diluted in methanol to produce solutions of FL (1 μg/ml). Calibration standards of FL at concentrations of 5, 10, 25, 50, 100, 125 and 145 ng/ml and 2, 5, 10, 20, 25, 75 and 100 ng/ml were prepared by spiking appropriate amount of the standard solutions in blank plasma and urine obtained from healthy volunteers. Standard solutions were stored at +4 °C.

IS stock solution was made at an initial concentration of 1 mg/ml. The IS working solution (1 μg/ml) was made from the stock solution using methanol dilution.

A reagent solution of 0.2% (w/v) NQS was prepared freshly in water. Borate buffer 0.025 M was prepared using sodiumtetraborated at pH 8.5 with 0.1N sodium hydroxide solutions.

2.4. Sample preparation

A 0.25 ml aliquot of the collected plasma and urine samples from a human volunteer was pipetted into a 12 ml centrifuge tube. The internal standard solution (20 μl, 1 μg/ml), 2 ml aliquot of acetonitrile was added. After vortexing for 3 min, the tube was centrifuged at 4500 × *g* for 20 min. A 1.5 ml aliquot of the supernatant of each sample was transferred to another 5 ml tube and evaporated to dryness under stream of nitrogen gas at 40 °C. To this residue, 300 μl of borate buffer and 200 μl of NQS solutions were added. The mixture was shaken on a vortex. The solutions were allowed to stay for 30 min in a water bath at 70 °C, cooled to room temperature and were added to 100 μl of 0.1N hydrochloric acid solutions. The mixture was extracted three times with 1.5 ml dichloromethane: *n*-butanol (4:1 v/v). The 4 ml aliquots of samples were evaporated to dryness under stream of nitrogen gas at 40 °C. The residue was reconstituted in 200 μl of the mobile phase, and a 20 μl aliquot was injected into the HPLC system.

2.5. Assay validation

2.5.1. Linearity

Calibration standards of seven concentrations of FL (5, 10, 25, 50, 100, 125 and 145 ng/ml for plasma and 2, 5, 10, 20, 25, 75 and 100 ng/ml for urine) were extracted and assayed. The linearity of the calibration curve was confirmed by plotting the peak-area ratios of FL to IS versus the FL concentrations with least-squares linear regression analysis.

2.5.2. Precision and accuracy

Intra-day and inter-day precision and accuracy were determined at low, medium and high concentrations of FL by replicate analyses of plasma and urine samples. Intra-day precision was determined by repeated analysis of each sample on 1 day ($n = 5$). Inter-day precision was determined by repeated analysis on five consecutive days ($n = 5$). The concentration of each sample was determined by using calibration standards prepared on the same day.

2.5.3. Sensitivity

Sensitivity was determined by the detection limit (LOD, three times of the value of the background noise signal) and the quantification limit (LOQ, lowest concentration of standard measurable).

2.5.4. Specificity

Control human plasma and urine, obtained from three healthy volunteers, was assessed by the procedure as described above and compared with respective plasma and urine samples to evaluate specificity of the method. Various other antidepressants and antihypertensive (milnacipran, venlafaxine, mirtazapine, amitryptiline, citalopram, norfluoxetine, amineptine, tranylcypromine, desipramine, maprotiline, aniline, cyclohexylamine, lisinopril and amlodipine) were also tested for potential interferences. The retention times for these drugs under the chromatographic conditions for the FL assay were determined.

2.5.5. Recovery

The extraction recovery for plasma and urine at three different concentrations of FL was determined. Known amounts of FL were added to drug-free plasma and the internal standard was then added. After the derivatization, chromatography processes, the peak areas were compared to the peak areas obtained from the aqueous solutions of FL at the same concentration.

2.5.6. Stability

The stability of FL and internal standard solutions were also tested at room temperature for 2, 6, 12 and 24 h and upon refrigeration (4 °C) for 24 h.

Freeze–thaw stability of the plasma samples was determined by the following three freeze–thaw cycles. The spiked plasma and urine samples at concentrations of 5 and 75 ng/ml were frozen at −20 °C for 24 h and thawed at room temperature. After completely being thawed, the samples were refrozen and this cycle was repeated three times.

For the short-term stability, three concentration levels of plasma and urine samples were kept at room temperature for a period that exceeded the routine preparation time of samples (6 and 10 h). The long-term stability was evaluated after freezing the plasma samples at −20 °C for 3 months.

2.6. Clinical study

FL was administered in a single dose of 50 mg (35–40 years old healthy woman volunteers). Further blood samples were drawn into ethylenediaminetetraacetic acid tubes at 1, 3, 4, 5, 7, 9, 10, 14, 16, 24, 36 and 48 h after administration. Plasma samples were immediately separated by centrifugation at 4500 rpm. Plasma and urine samples were stored at −20 °C until assayed.

3. Results and discussion

3.1. Optimization of the reaction conditions

The reaction between FL and NQS in borate buffer of pH 8.5 produces an orange colored product with maximum absorbance at 450 nm (Fig. 2). The different experimental parameters affecting the intensity of the color produced were studied and optimized to obtain maximum color intensity.

First, the pH was varied over the whole pH range (7–10) in borate and phosphate buffers. Because the absorbance is developed only in alkaline medium, the study of the pH was restricted to the range 7–10 using buffer. The highest absorbance readings for FL were obtained at pH 8.5 borate (Fig. 3). After studying a series of different buffer systems having the same pH value, it was found that borate buffer was superior, since the net absorbance was the highest (λ_{max}, 450 nm).

The influence of different heating temperatures and times was studied using a water bath. Effect of heating time at four different temperatures 50–80 °C for NQ derivatives. The optimization of the derivatization was performed using a standard

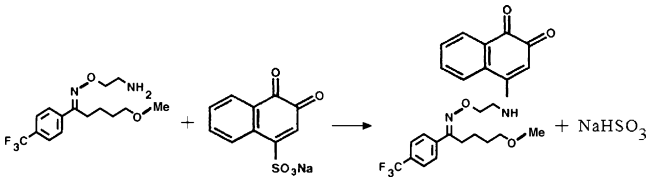

Fig. 2. The reaction between FL and NQS.

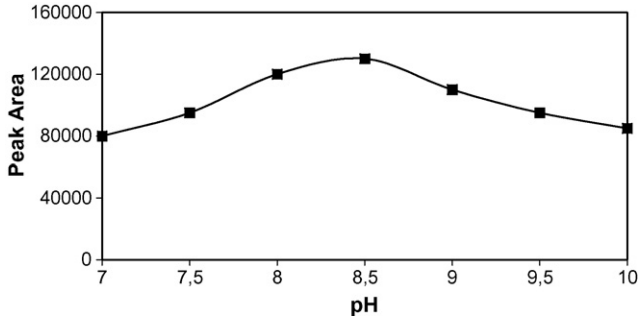

Fig. 3. Effect of pH on the reaction of FL with NQS.

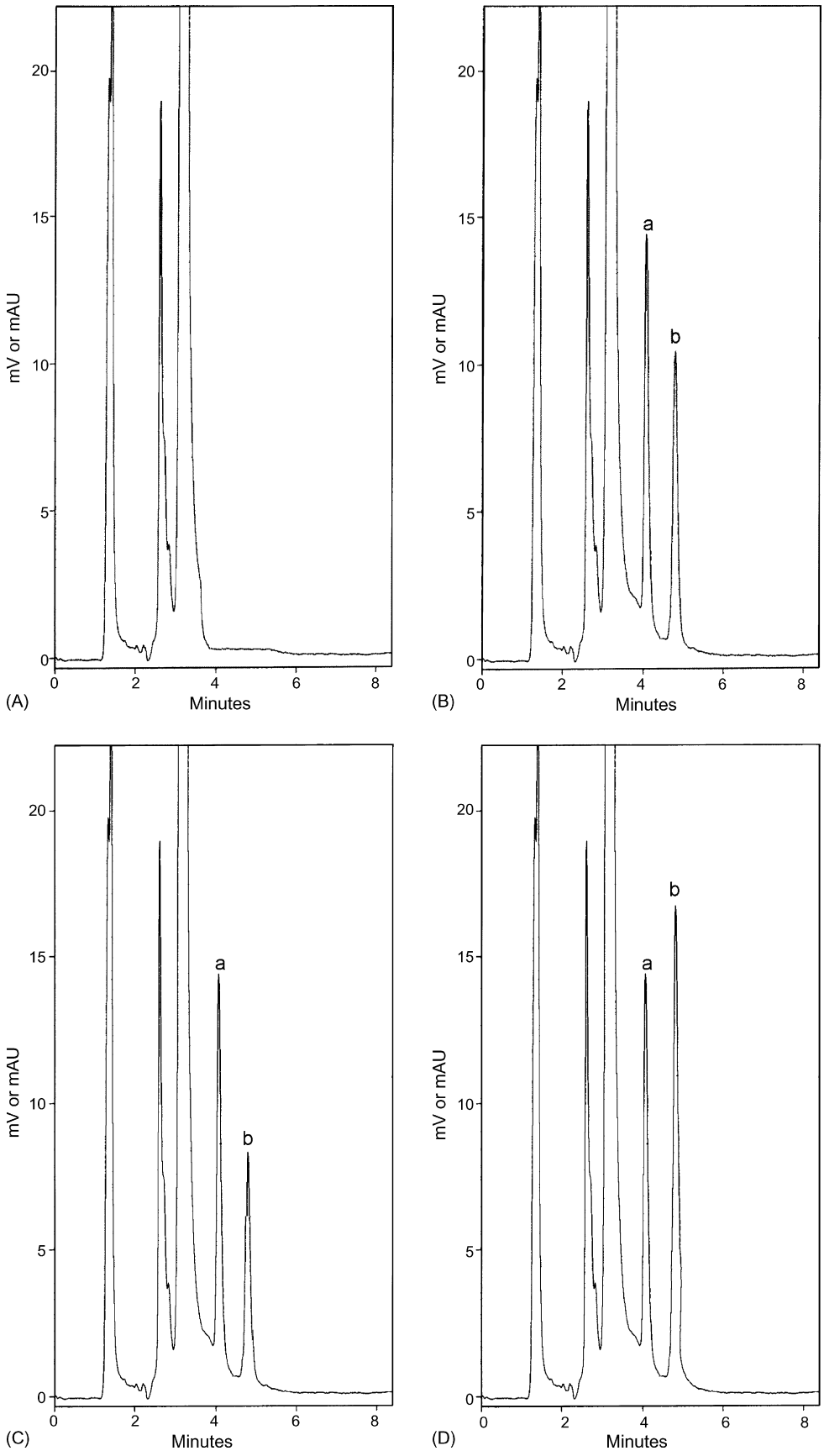

Fig. 4. Chromatogram of (A) blank human plasma, (B) plasma spiked with 100 ng/ml FL (b) and 80 ng/ml IS (a), (C) plasma sample obtained at 4 h after oral administration of 50 mg of FL from a healthy volunteer with 80 ng/ml of the IS, (D) urine spiked with 50 ng/ml FL (b) and 80 ng/ml IS (a), and (E) blank urine.

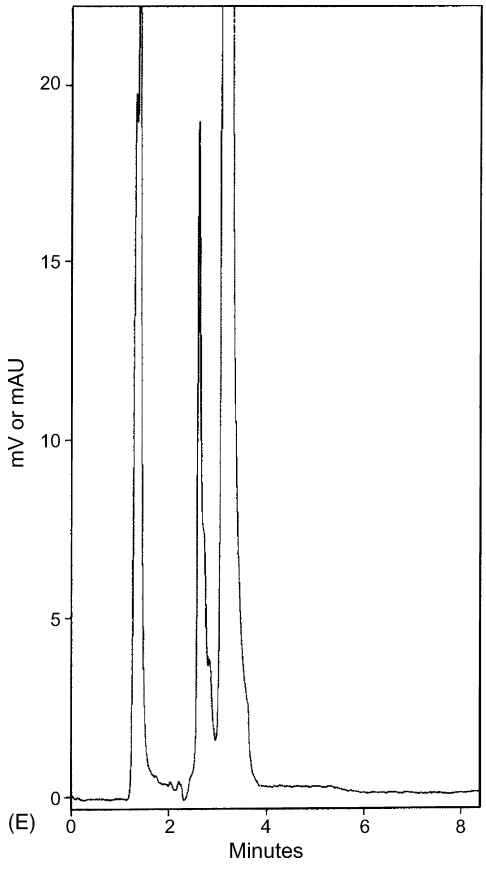

Fig. 4. (*Continued*).

solution of FL and IS. The highest and constant derivatization yield was obtained at 70 °C and a reaction time of 30 min. At room temperature, the reaction was slower. Different solvent as methanol, ethanol, acetonitrile, chloroform, dichloromethane, *n*-butanol and ethyl acetate were studied as derivatives. The maximum absorbance was obtained with dichloromethane: *n*-butanol.

3.2. Conditions of chromatography

Method development was focused on the optimization of sample preparation, chromatographic separation and column detection.

Several tests were performed for optimizing the components of mobile phase in order to achieve good chromatographic peak shape and resolution. The test results showed that the solvent system of water could improve the peak shapes of FL. Good separation of target compounds and short run time were obtained using a mobile phase system of acetonitrile water 80:20 v/v at 1 ml/min flow rate. The retention time of FL (4.807 min) was quite short than that studied by Yasui-Furukori et al. [9], Titier et al. [19], Duverneuil et al. [20] and Hartter et al. [22]. On the other hand, the mobile phase in the proposed method acetonitrile–water instead of buffered systems is used in previously reported HPLC methods [20–22]. Therefore, flushing of the column after analysis is not required.

Chromatographic separation was performed on a C_{18} reversed-phase column with a mobile phase consisting of acetonitrile and water (80:20 v/v). The λ_{max} value of FL–NQ derivative was 450 nm based on the UV–vis absorption spectra. For plasma samples, deproteinization with acetonitrile was adopted, which was simple and fast. Typical chromatograms obtained from blank human plasma and the plasma spiked with FL (100 ng/ml) and IS are shown in Fig. 4A and B, respectively. Fig. 4C represents the chromatogram of plasma obtained at 5 h after a single oral dose of FL from a healthy volunteer and urine spiked (Fig. 4D).

3.3. Assay validation

3.3.1. Linearity

Calibration curves of FL were linear over the concentration range of 5–145 ng/ml for plasma and 2–100 ng/ml for urine, which is as good as to that reported in the other papers [10–13,17,19–24].

Typical equations of calibration curves were as follows (Table 1):

$$\text{plasma}: A = 0.042C - 4.03 \times 10^{-3} \quad (r = 0.9997),$$

$$\text{urine}: A = 0.018C - 3.12 \times 10^{-3} \quad (r = 0.9998)$$

Here, A = peak-area ratio (FL/internal standard) and C = FL concentration (ng/ml).

Table 1
Results of regression analysis of the linearity data of FL

	Mean ± S.E. ($n = 6$)	
	Plasma	Urine
Slope	$4.2 \times 10^{-3} \pm 1.29 \times 10^{-2}$	$1.8 \times 10^{-2} \pm 2.22 \times 10^{-3}$
Intercept	$4.03 \times 10^{-3} \pm 7.26 \times 10^{-3}$	$3.12 \times 10^{-3} \pm 4.97 \times 10^{-3}$
Correlation coefficient (r)	$0.9997 \pm 4.57 \times 10^{-5}$	$0.9998 \pm 6.44 \times 10^{-3}$

3.3.2. Precision and accuracy

The method indicated very good precision and accuracy. Intra- and inter-day precision and accuracy for FL from plasma and urine samples data are shown in Table 2. The intra- and inter-day precisions were measured to be within 0.43 and 5.26% for plasma and 0.47 and 11.29% for urine, respectively.

3.3.3. Sensitivity

The limit of detection values defined as the lowest concentration of FL, which can be detected (signal-to-noise ratio 3) in human plasma and urine samples were 1.4 and 1 ng/ml, respectively. The quantification limits of FL in human plasma and urine samples were 5 and 2 ng/ml, respectively. The purpose of derivatization is to study in low concentration and to increase the sensitivity. The analysis has a quantification and detection limits of 1.4 and 5 ng/ml for plasma, respectively, which are as good or superior to that reported in the other papers [10–13,16,19–23,26]. Although Higashi et al. [25] use fluorescence detector, detection and quantification limits are high (0.008 and 0.015 μg/ml).

3.3.4. Extraction recovery

The mean extraction recovery of FL from human plasma and urine were 96.66 ± 0.69 and 96.73 ± 2.17%, respectively. The mean relative recovery for IS at 80 ng/ml was 95.58 ($n = 7$). Recovery data are shown in Table 3. The mean recovery is better for plasma 96.27% and for urine 96.68% than those of the studies reported by Yasui-Furukori et al. [9] and Eap and Baumann [11], in which the recoveries are 94.8 and 50–66%, respectively.

3.3.5. Specificity

Control human plasma and urine, obtained from three healthy volunteers, was assessed by the procedure as described above and compared with respective plasma and urine samples to evaluate specificity of the method. Drug-free plasma was spiked with therapeutic concentrations of such drugs which included some drugs.

Milnacipran, venlafaxine, mirtazapine, amitryptiline, citalopram, norfluoxetine, amineptine, tranylcypromine, desipramine, maprotiline, lisinopril and amlodipine. The retention times for these drugs under the chromatographic conditions for the FL assay were determined and found not to interfere with FL and IS retention time.

3.3.6. Stability

Stock solutions of FL and IS were stable for at least 15 days when stored at 4 °C. FL– and IS–NQ derivatives solutions were stable for 12 h at room temperature and 5 days at 4 °C.

The stability of FL and base at −20 °C in human plasma and urine were also assessed in FL samples after the storage at room temperature for 24 h, after three freeze processes and thaw cycles. No significant change in FL and base concentrations was found in human plasma and urine samples stored at −20 °C after three freeze–thaw cycles or at room temperature for 24 h. The short-term stability of FL in plasma and urine was stable for at least 1 month for plasma and 2 weeks for urine.

In the long-term stability study, the plasma and urine samples spiked with FL were stored for 3 and 2 months, respectively.

3.4. Application

The method was successfully used to perform the determination of plasma and urine concentrations of FL after oral administration of 50 mg dose to 35–40 years old healthy woman volunteers. Maximum plasma concentration of FL (C_{max}) and time to reach this value (t_{max}) were determined to be 58 ng/ml

Table 2
Intra-day and inter-day precision and accuracy of FL plasma and urine ($n = 5$)

Concentration added (ng/ml)	Intra-day			Inter-day		
	Concentration found (mean ± S.D., ng/ml)	Precision R.S.D. (%)	Accuracy RME (%)	Concentration found (mean ± S.D., ng/ml)	Precision R.S.D. (%)	Accuracy RME (%)
Plasma						
5	4.80 ± 0.19	3.96	−4.00	4.75 ± 0.25	5.26	−5.00
50	48.20 ± 0.35	0.73	−3.60	47.74 ± 0.46	0.96	−4.52
145	138.76 ± 0.60	0.43	−4.30	137.9 ± 0.71	0.51	−4.89
Urine						
2	1.92 ± 0.10	5.21	−4.00	1.86 ± 0.21	11.29	−7.00
20	19.31 ± 0.22	1.14	−3.44	18.98 ± 0.26	1.37	−5.10
100	96.85 ± 0.57	0.59	−3.15	95.56 ± 0.45	0.47	−3.15

Table 3
Recovery of FL from plasma and urine samples ($n = 7$)

Concentration added (ng/ml)	Concentration found (mean ± S.D., ng/ml)	Recovery (%)	R.S.D. (%)
Plasma			
5	4.72 ± 0.18	94.40	3.81
10	9.55 ± 0.23	95.50	2.41
25	23.90 ± 0.35	95.60	1.46
50	48.38 ± 0.39	96.76	0.81
75	73.45 ± 0.42	97.93	0.57
100	98.10 ± 0.59	98.10	0.60
145	142.56 ± 0.67	98.31	0.47
Urine			
2	1.88 ± 0.08	94.00	4.26
5	4.82 ± 0.12	96.40	2.49
10	9.72 ± 0.38	97.20	3.91
20	19.46 ± 0.42	97.30	2.16
25	24.35 ± 0.48	97.40	1.97
50	48.57 ± 0.55	97.14	1.13
75	72.90 ± 0.57	97.20	0.78
100	97.22 ± 0.62	97.22	0.64

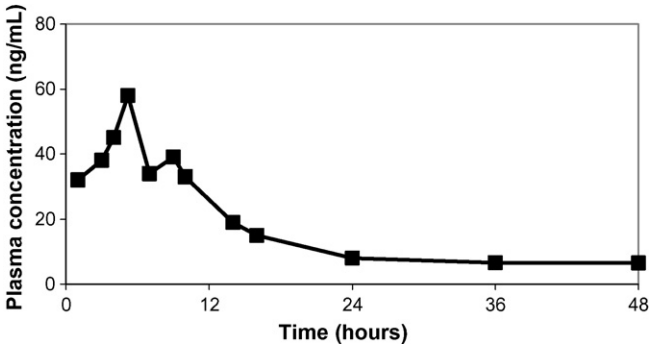

Fig. 5. Plasma concentration–time profile of FL in a healthy volunteer after a single oral administration of 50 mg.

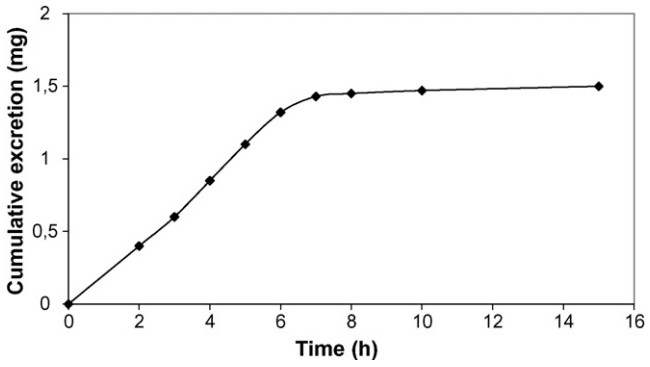

Fig. 6. Cumulative excretion of FL in urine of a healthy volunteer after a single 50 mg oral dose.

and 5.2 h. Elimination half-life ($t_{1/2}$) and area under curve (AUC) of the drug were calculated as 15.4 h and 665 ng h/ml, respectively (Fig. 5). Mean residence time (h) was calculated as 22.2 h (Table 4). Approximately 3% of the administered drug was excreted unchanged, within 15 h after oral administration (Fig. 6). Pharmacokinetic parameters obtained using proposed method is in agreement with those of the studies reported previously [2]. Furthermore, low volumes of plasma and urine (0.25 ml) are used in the proposed method, which can be advantageous in clinical pharmacokinetic studies. In the other methods especially it has been worked with 1.5 or 1 ml of plasma [9,10,12].

Table 4
Pharmacokinetic parameters for FL in healthy volunteer

t_{max} (h)	5.0
$t_{1/2}$ (h)	15.4
C_{max} (ng/ml)	58
AUC 0–48 (h ng/ml)	665
Mean residence time (MRT) (h)	22.2

4. Conclusion

As a conclusion, a new HPLC method has been developed which high reproducibility and sensitivity for the determination of FL in this study. According to the literature research, FL has been derivatized with NQS and determined using a UV–vis detector for the first time. The HPLC method is described as employing liquid–liquid extraction for sample preparation and is convenient for the quantification of FL in human plasma and urine samples. The validation data demonstrate good precision and accuracy. The assay is reproducible and accurate.

In conclusion, this paper describes a sensitive and accurate HPLC method for the quantification of FL suitable to monitor plasma concentrations during clinical pharmacokinetic studies in humans.

References

[1] The Merck Index, 2001, p. 748.
[2] M.I. Wilde, G.L. Plosker, P. Benfield, Drugs 46 (1993) 895–924.
[3] A.K. Banerjee, Br. Med. J. 296 (1988) 1774.
[4] H. Overmars, P.M. Scherpenisse, L.C. Post, Eur. J. Drug Metab. Pharmacokinet. 8 (1983) 269–280.

[5] J.J. Berzas Nevado, M.J. Villaseñor Llerena, A.M. Contento Salcedo, E. Aguas Nuevo, J. Pharm. Biomed. Anal. 38 (2005) 52–59.
[6] J.J. Berzas, C. Guiberteau, M.J. Villaseñor, V. Rodríguez, Anal. Chim. Acta 519 (2004) 219–230.
[7] S. Atmaca, S. Tatar, Acta. Pharm. Turc. 37 (1995) 33–37.
[8] J.J. Berzas Nevado, A.M. Contento Salcedo, M.J. Villaseñor Llerena, E. Aguas Nuevo, Anal. Chim. Acta 417 (2000) 169–176.
[9] N. Yasui-Furukori, Y. Inoue, S. Kaneko, K. Otani, J. Pharm. Biomed. Anal. 37 (2005) 121–125.
[10] G. Tournel, N. Houdret, V. Hédouin, M. Deveaux, D. Gosset, M. Lhermitte, J. Chromatogr. B 761 (2001) 147–158.
[11] C.B. Eap, P. Baumann, J. Chromatogr. B 686 (1996) 51–63.
[12] R.H. Pullen, A.A. Fatmi, J. Chromatogr. 574 (1992) 101–107.
[13] J.P. Foglia, L.A. Birder, J.M. Perel, J. Chromatogr.B 495 (1989) 295–302.
[14] L. Palego, D. Marazziti, L. Biondi, G. Giannaccini, N. Sarno, A. Armani, A. Lucacchini, G.B. Cassano, L. Dell'Osso, Ther. Drug Monit. 22 (2000) 190–194.
[15] A. Lucca, G. Gentilini, S. Lopez-Silva, A. Soldarini, Ther. Drug Monit. 22 (2000) 271–276.
[16] A. Belmadani, I. Combourieu, M. Bonini, E.E. Creppy, Hum. Exp. Toxicol. 14 (1995) 34–37.
[17] T. Ohkubo, R. Shimoyama, K. Otani, K. Yoshida, H. Higuchi, T. Shimizu, Anal. Sci. 19 (2003) 859–864.
[18] M.A. Saracino, L. Mercolini, G. Flotta, L.J. Albers, R. Merli, M.A. Raggi, J. Chromatogr. B 843 (2006) 227–233.
[19] K. Titier, N. Castaing, E. Scotto-Gomez, F. Pehourcq, N. Moore, M. Molimard, Ther. Drug Monit. 25 (2003) 581–587.
[20] C. Duverneuil, G.L. de la Grandmaison, P. Mazancourt, J.C. Alvarez, Ther. Drug Monit. 25 (2003) 565–573.
[21] S.H. Wong, H.R. Kranzler, S. Della Fera, R. Fernandes, Biomed. Chromatogr. 8 (1994) 278–282.
[22] S. Hartter, H. Wetzel, C. Hiemke, Clin. Chem. 38 (1992) 2082–2086.
[23] J. Pommery, M. Lhermitte, Biomed. Chromatogr. 3 (1989) 177–179.
[24] V. Van Der Meersch-Mougeot, B. Diquet, J. Chromatogr. 567 (1991) 441–449.
[25] Y. Higashi, H. Matsumura, Y. Fujii, Biomed. Chromatogr. 19 (2005) 771–776.
[26] G.J. De Jong, J. Chromatogr.B 183 (1980) 203–211.
[27] C. Schweitzer, H. Spahn, E. Mutschler, J. Chromatogr.B 38 (1986) 412–414.
[28] L. Labat, M. Deveaux, P. Dallet, J.P. Dubost, J. Chromatogr. B 773 (2002) 17–23.
[29] C.B. Eap, N. Gaillard, K. Powell, P. Baumann, J. Chromatogr. B 682 (1996) 265–272.
[30] T. Shinozuka, M. Terada, E. Tanaka, Forensic Sci. Int. 16 (2006) 108–112.
[31] C. Salgado-Petinal, J.P. Lamas, C. Garcia-Jares, M. Llompart, R. Cela, Anal. Bioanal. Chem. 382 (2005) 1351–1359.
[32] J.R.L. Smith, A.U. Smart, F.E. Hancock, M.V. Twigg, J. Chromatogr. 483 (1989) 341–348.
[33] P. Campíns-Falcó, A. Sevillano-Cabeza, C. Molíns-Legua, M. Kholmann, J. Chromatogr. B 687 (1996) 239–246.
[34] Y. Kobayashi, H. Kubo, T. Kinoshita, Anal. Biochem. 160 (1987) 392–398.
[35] J. Saurina, S. Hernández-Cassou, J. Chromatogr. A 676 (1994) 311–319.
[36] P. Edder, A. Cominoli, C. Corvi, J. Chromatogr. A 830 (1999) 345–351.
[37] Y. Nakahara, Y. Takeda, Chromatographia 26 (1988) 363–368.