ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 561–571

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Liquid chromatographic determination of fluoxetine

Mohamed A. El-dawy *, Mokhtar M. Mabrouk, Fawzy A. El-Barbary

Faculty of Pharmacy, Tanta Univesity, Tanta, Egypt

Received 11 March 2002; received in revised form 14 April 2002; accepted 15 May 2002

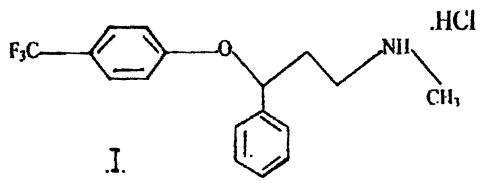
Abstract

A simple, accurate and sensitive high pressure liquid chromatographic technique is described for the determination of fluoxetine in the capsule dosage form, human plasma and in biological fluid. Analysis is performed with a reversed phase-C18 column with ultraviolet detection at 228 nm. The isocratic mobile phase (1.5 ml/min.) consists of acetonitrile and triethylamine buffer (48 + 52, V/V). A linear calibration model (correlation coefficient 0.99863) was developed using pyridoxine as internal standard. The retention times were 2.10 and 3.20 min for pyridoxine and fluoxetine, respectively. The method was applied for the quantitation of fluoxetine in spiked human plasma samples. The detection limit is 5 μg/l and the absorbance varies with fluoxetine concentrations in the range (10–300) μg/l. The mean % recovery ± S.D. was found to be 97.99% ± 2.39. The proposed method was applied successfully for monitoring of fluoxetine in human plasma after single dose administration of one prozac® capsule. © 2002 Published by Elsevier Science B.V.

Keywords: HPLC; Selective serotonin reuptake inhibitors; Fluoxetine determination; Validation; Fluoxetine monitoring

1. Introduction

Fluoxetine HCL (I), *N*-methyl-8-[4-(trifluoromethyl) phenoxy] benzene propanamine, is one of the selective serotonin re-uptake inhibitors



I.

The precise mechanism of the anti-depressant action of fluoxetine is unclear, but the drug has been shown to selectively inhibit the re-uptake of serotonin at the presynaptic neuronal membrane [1–3].

The presence of the *p*-trifluoromethyl substituent on the molecule appears to contribute to the high selectivity of the drug and its potency for inhibiting serotonin re-uptake, possibly as a result of its electron-withdrawing effect and lipophilicity [4,5].

Due to the reasonable thermal stability and volatility of fluoxetine, GC found a great application for the determination of fluoxetine, its metabolite and degradation products [6–13].

* Corresponding author. Fax: +20-40-333-4566
E-mail address: eldawymo@thewayout.net (M.A. El-dawy).

0731-7085/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0731-7085(02)00312-6

A spectrofluorimetric method has been developed by S. Atmaca, for fluoxetine determination in capsule dosage form by mixing with $Na_2B_4O_7$ buffer solution of pH (8.5) and 0.02% 7-chloro-4-nitrobenzofuran in methanol [14].

Several colorimetric methods were developed and used for the determination of fluoxetine hydrochloride in bulk form and in pharmaceutical formulations [15–19]. HPLC methods have been widely used for the determination of fluoxetine and/or its metabolite or degradation products in biological fluids or dosage form. These methods use UV detection [20–25]or fluorescence detection [26–30].

Most of the reported methods suffer from either extensive sample preparation by solid phase, or liquid–liquid extraction, or necessity of removing interferences, which can be extremely time-consuming, especially in routine work.

In the present work a new HPLC procedure for the quantitative determination of fluoxetine using pyridoxine as internal standard, was developed, validated and compared with an official chromatographic method [31].

The developed method was successfully applied for the determination of fluoxetine in bulk form, dosage form and spiked plasma sample and in biological fluids. The assay time is less than 5 min and the extraction procedure is very easy, which are of great benefit especially for the drug monitoring.

2. Experimental

2.1. Apparatus and chromatographic conditions

HPLC measurements were performed on Waters instrument equipped with a model 600 pump, U6K-injector and 486 UV/VIS variable wavelength detector. Separation was performed on μ-Bondapak C18 (300 × 3.9 mm) column. Flow rate was maintained at 1.5 ml/min and the detector wavelength was set at 228 nm. Chromatographic peaks were electronically integrated and recorded using the *Millenium* software (version 2.10) of Waters.

2.2. Materials

2.2.1. Authentic samples

Flouxetine HCl was kindly supplied by Resfar and pyridoxine HCl from Merck.

Blood samples were kindly supplied by the central blood bank of Tanta University hospital.

2.2.2. Pharmaceutical preparation

Prozac® capsule (Eli Lilly and Company), Fluoxetine® capsule (Misr Company for pharmaceutical industries, Cairo, Egypt) and Depreban® capsule (Amriya Company for pharmaceutical industries, Alexandria, Egypt).

All these preparations were purchased from the local market and claimed to contain 20 mg fluoxetine HCl.

2.2.3. Other chemicals

Methanol and acetonitrilre were of HPLC grade (BDH). Triethylamine (Aldrich) and phosphoric acid (Merck) were of analytical grade. Starch lactose and magnesium trisilicate (Prolabo).

Water was distilled and received in glass receiver and then filtered through 0.45 μm membrane filter (Millipore type).

2.3. HPLC determination of fluoxetine

2.3.1. Mobile phase

Mobile phase was prepared by mixing acetonitrile, water and triethylamine in the ratio of 480:500:20, respectively. The mixture of water and triethylamine is firstly adjusted to pH (4.7) with phosphoric acid, then filtered through a 0.45-μm membrane filter (Millipore type) and degassed by sonication for about 10 min.

2.3.2. Stock solution of standard fluoxetine

This was prepared in the mobile phase to contain 2.0 mg/ml of fluoxetine HCl.

2.3.3. Stock solution of the internal standard

This was prepared in the mobile phase to contain 1.0 mg/ml pyridoxine HCl.

2.3.4. Preparation of standard drug solutions

Aliquots of fluoxetine stock solution were diluted with the mobile phase to obtain solutions having concentrations ranging from 10 to 120 μg/l, each containing 8 μg/l pyridoxine HCl as the internal standard.

2.3.5. Assay solution of fluoxetine in a synthetic mixture

Synthetic mixture was prepared to contain in each, 10 mg fluoxetine and 50 mg of each starch, lactose and magnesium trisilicate, representing possible interfering substances, that may be present with fluoxetine in its pharmaceutical preparation. The mixture is dissolved in 25-ml methanol, then carefully transferred into a 100-ml volumetric flask, and completed to volume with the mobile phase. The solution is then filtered and the first 10 ml of the filtrate is rejected. A portion of the filtrate was diluted with the mobile phase to have a final concentration of 1 μg/ml. A total of 1.0 ml of this final solution and 1.0 ml of a 80 μg/l pyridoxine solution (prepared from its stock solution) were transferred to a 10 ml volumetric flask, mixed and diluted to volume with the mobile phase to have a solution containing 100 μg/l fluoxetine HCl and 8 μg/l pyridoxine, IS.

2.3.6. Assay solution of fluoxetine in its pharmacutical preparations

The content of about 10-capsules was accurately weighed, and a portion of the powder equivalent to about 20 mg fluoxetine HCl was transferred into a 100 ml volumetric flask, dissolved in 25 ml mobile phase, sonicated for about 10 min and the volume is completed by the mobile phase.

A portion of this solution was diluted with the mobile phase to have a solution containing 200 μg/l fluoxetine HCl.

A total of 1.0 ml of this solution was transferred into a 10 ml volumetric flask containing 1 ml of the 80 μg/l pyridoxine HCl solution and the volume was completed with the mobile phase to obtain a final solution containing 20 μg/l fluoxetine and 8 μg/l pyridoxine that was used as the assay solution.

2.3.7. Studying the effect of human plasma constituents on fluoxetine assay

2.3.7.1. Spiked drug plasma solutions. These solutions were prepared by diluting aliquots of the drug stock solutions with human blank plasma to obtain concentrations ranging from 50 to 1500 μg/l fluoxetine HCl, each contains 40 μg/l pyridoxine HCl.

2.3.7.2. Preparation of plasma samples. A total of 200 μl aliquot of each spiked plasma sample (containing 50–1500 μg/l fluoxetine HCl) was vortex mixed with 400 μl (acetonitrile:water, 20:80) mixture, for 3 min, diluted to 1.0 ml with acetonitrile (100%) in 10 ml centrifugation tubes.

The precipitated plasma protein was separated by centrifugation at 3000 r.p.m. for 15 min. The clear supernatant layer was filtered through Millipore filter (0.45 μm).

2.3.8. Application of the proposed method for monitoring fluoxetine level in human plasma after single dose administration

Two healthy volunteers (35 years old) received 20 mg single dose of fluoxetine capsule (Prozac®), then blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h after drug administration in heparinized tubes, then plasma was separated and stored at −20 °C until assayed.

A total of 1.0 ml of patient's plasma was transferred to a screw-capped sample tube containing 80 μl of the 1 mg/l internal standard stock solution. A total of 4.0 ml of 20% acetonitrile was added and the solution was vortex mixed for 3 min. A total of 4.92 ml of 100% acetonitrile was added.

The mixture was centrifuged at 3000 r.p.m. for 15 min and filtered through 0.45 m filter.

3. Results and discussion

In order to develop an HPLC method suitable for the determination of fluoxetine, mixtures of acetonitrile and water in different ratios were tried. No separation for fluoxetine was obtained except

after the addition of the basic modifier, triethylamine. Accordingly, a study has been performed to optimize the content of triethylamine and the final composition of the mobile phase. It was observed that, low concentration of the basic modifier produced tailed fluoxetine peak, so its concentration was gradually increased until obtaining the least tailed peak with the highest sample recovery. This effect may be attributed to the basic nature of triethylamine which blocks the residual silanol groups on the adsorbent surface, thus eliminates their interaction with the basic nitrogen of the drug. It was found that the pH of the mobile phase has a great influence on the shape of the peak, accordingly the effect of pH was studied using phosphoric acid. The optimum pH was found to be 4.70, which gives a highly resoluted sharp peak.

Accordingly, the optimum mobile phase composition was found to be water:acetonitrile:triethylamine in the ratio of (5:4.8:0.02). The pH of water and triethylamine mixture was adjusted to 4.70 by phosphoric acid. The flow rate was set at 1.5 ml/min and the detector wavelength was adjusted at 228 nm. The assay was performed at ambient temperature.

On searching for an optimum internal standard (IS), pyridoxine HCl was found to be a suitable one for the determination of fluoxetine by the former mobile phase.

A standard calibration curve for fluoxetine was constructed, using the ratio of peak areas of the drug at different concentrations to that of constant concentration of pyridoxine (8μg/L).

The good linearity of the method was indicated by the regression analysis in Table 1.

Other analytical parameters for the assessment of the validity of the proposed method have been performed such as follows.

3.1. Limit of detection

The limit of detection was calculated according to the recommendation for LOD for chromatographic methods, which was published in 1978 by the International Union of Pure and Applied Chemistry (IUPAC) [32] where:

$$C_L = KS_B/S$$

C_L is the lowest concentration; K is a constant for which IUPAC recommended a value 3; S is the slope of the concentration/response curve; S_B is the magnitude of noise over a representative section of the chromatographic base line.

This section is 20 times the peak width and centered at the retention time of the analyte. The value S_B is then calculated as follows:

$$S_B = Np{-}p/5$$

where $Np-p$ is the largest peak to peak fluctuation in the representative base line section.

Accordingly, the limit of detection was found to be 3 μg/l, which was proven practically.

3.2. Limit of quantitation

It was calculated, also, by the above equation, but the IUPAC recommended a value 10 for K.

Accordingly, the limit of quantitation was found to be 10 μg/l, which was proven practically.

3.3. Accuracy

To study the accuracy of the proposed method, synthetic mixtures containing various amounts of fluoxetine were prepared and analyzed by the proposed method. The mean % recovery ± S.D. was 99.88% ± 0.231 and the C.V. was calculated from the recovery experiment, and was found to be

Table 1
Quantitative parameters for the determination of fluoxetine with the proposed HPLC method in the mobile phase

Linearity range (μg/l)	S.E. intercept	S.E. slope	Correlation coefficient	LOD (μg/l)
10–120	0.004051	0.000062	0.9993998	3.0

Table 2
Recovery data of fluoxetine in mobile phase by the USP method and the proposed HPLC method*

Claimed fluoxetine concentration (μg/l)	USP method		The proposed HPLC method	
	Recovered concentration (μg/l)	% Recovery	Recovered concentration (μg/l)	% Recovery
20	19.92	99.60	20.10	99.50
40	39.86	99.64	39.95	99.87
80	80.38	100.47	79.98	99.97
100	100.77	100.77	100.10	100.10
120	120.93	100.78	120.00	100.00
Mean % recovery		100.25		99.88
S.D.		±0.590		±0.231
C.V.		0.588		0.232

* Average of three readings.

Table 3
Repeatability of different concentrations of fluoxetine using pyridoxine as IS by the proposed HPLC method

Added (μg/l)	Found (μg/l)			Mean	S.D. (±)	C.V. (%)
30	30.00	30.20	30.50	30.23	0.251	0.832
50	49.40	49.8	49.50	49.566	0.208	0.419
70	69.6	69.8	70.00	69.80	0.20	0.286
100	100.8	100.10	99.95	100.28	0.453	0.452
120	120.5	120.0	120.5	120.33	0.288	0.239

0.232%. The results were compared with those of the USP method [31] as shown in Table 2.

3.4. Precision

The precision of the method was tested by triplicate analysis of different concentrations lie in the linearity range of fluoxetine. S.D. and C.V. were calculated as illustrated in Table 3.

3.5. Specificity

For testing the specificity of the method, the percentage recovery of fluoxetine is determined in the mixture of it with possible interfering materials such as starch, lactose and magnesium trisilicate. The results obtained exhibited no interference as shown in Table 4.

3.6. Stability

Stability of fluoxetine in solution was assessed by determining the RSD of replicate injections of the same solution over a period of time under the same analysis conditions. It should be less than 1.2–2 times the value for the precision obtained by analyzing replicate injections over a relatively short time period [33].

Table 4
Recovery experiments of fluoxetine in presence of interfering substances by the proposed HPLC method, using pyridoxine as IS

	Recovered concentration (μg/l)	% Recovery
100	99.0	99.0
100	100.5	100.5
100	101.0	101.0
100	100.0	100.0
100	97.5	97.5

Mean % recovery = 99.60%; S.D. ±1.387; C.V. = 1.393%.

Table 5
Results of stability study of fluoxetine in solution over 24 h

Fluoxetine concentration (μg/l)	Recovered fluoxetine concentration (μg/l)					
	After 6 h	After 12 h	After 18 h	After 24 h	S.D.	RSD
30	30.5	30.7	31.0	31.2	0.3109	1.0078
70	69.0	69.5	68.5	70.5	0.850	1.23
100	100.0	101.5	99.5	101.0	0.913	0.9080
120	121.0	119.0	120.0	119.0	0.950	0.7990

The results obtained in Table 5 indicate adequate stability for fluoxetine in solution and during the analysis time.

Three of the pharmaceutical preparations containing fluoxetine, were assayed by the proposed HPLC method and the results are compared with those of the USP method [31] and shown in Table 6.

The high sensitivity of the proposed HPLC method allowed its application for determining fluoxetine in spiked human plasma samples. For this purpose, acetonitrile was found to be a good protein precipitant but not suitable as an extraction solvent.

By applying the Carr method [34] to measure the extraction efficiency, the optimum extraction is obtained by using a solvent mixture of acetonitrile and water (20:80).

A standard calibration curve for fluoxetine was constructed, using the ratio of peak areas of the drug at different concentrations to that of constant concentration of pyridoxine (8 μg/l).

A linear relationship existed between the peak area ratio of fluoxetine to pyridoxine and fluoxetine concentrations in the range of 10–300 μg/l.

The good linearity of the method was indicated by the regression analysis in Table 7.

The accuracy of the method was studied by carrying out the recovery experiments for different fluoxetine concentrations spiked in plasma samples, then calculating the mean % recovery ± S.D. and C.V.%, as shown in Table 8.

The proposed method was applied for the monitoring of fluoxetine in human plasma after a single dose administration of one prozac capsule in two healthy volunteers.

Fig. 1 shows the chromatogram of plasma sample supplemented only with the internal standard (pyridoxine).

Fig. 2 shows the chromatogram of a volunteer's sample after 48 h of fluoxetine administration.

Fig. 3 illustrates the concentrations observed in plasma of the volunteers (μg/l) as a function of time.

4. Conclusion

This HPLC method, with a simple extraction procedure, offers a clear advantage over GC

Table 6
Results of fluoxetine analysis in its pharmaceutical preparations by the USP method and the proposed method*

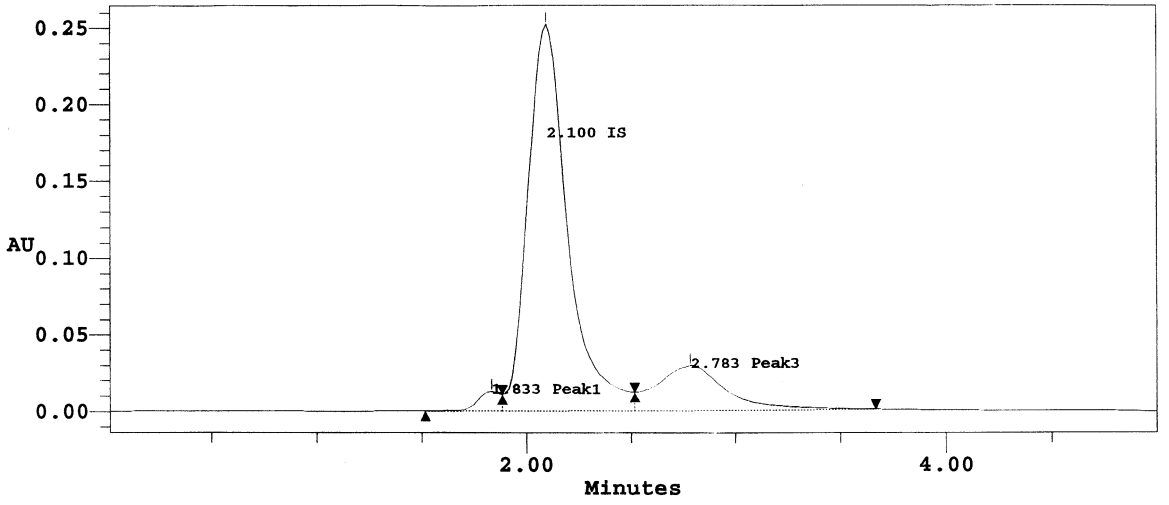
Product	Claimed concentration (mg/capsule)	USP method		The proposed method	
		Mean % recovery ± S.D.	C.V.	Mean % recovery ± S.D.	C.V.
Prozac	20	100 ± 3.889	3.889	100.45 ± 1.229	1.224
Depreban	20	98.8 ± 3.91	3.96	98.75 ± 2.46	2.49
Fluoxetine	20	100.1 ± 2.966	2.934	100 ± 0.535	0.530

* Average of five determinations.

Millennium Results Report June 9, 2002 Page: 1 of 1
Report Method: Default Version: 2.10
For Sample: theo Vial: 1 Injection: 1 Channel: 486
Proc Chan: 486 Processed: 04/06/02 02:56:37 PM
Channel Descr:

Millennium Sample Information

Project Name:	fawzy		
Sample Name:	theo		
Vial:	1	Sample Type:	Standard
Injection:	1	Volume:	10.00
Channel:	486	Run Time:	5.0 min
Date Acquired:	09/29/98 02:43:56 PM	Date Processed:	04/06/02 02:56:37 PM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	fawzy		
Processing Method:	is25		



Peak Results

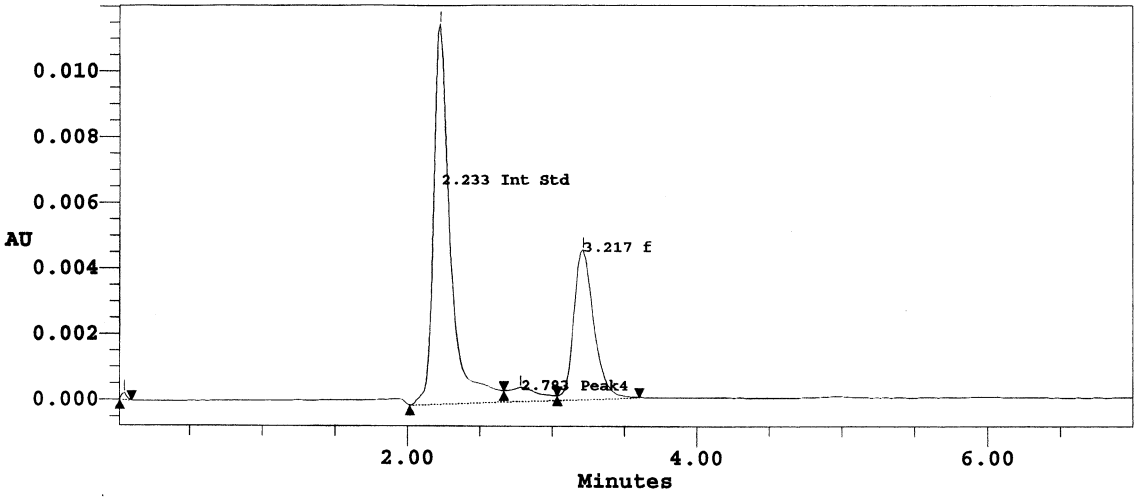
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.833	90826	12625		BV
2	IS	2.100	3341261	251843		VV
3	Peak3	2.783	671872	29193		VB

Fig. 1. HPLC chromatogram of plasma sample supplemented only with the internal standard (pyridoxine).

Millennium Results Report June 9, 2002 Page: 1 of 1
Report Method: Default Version: 2.10
For Sample: f3 Vial: 3 Injection: 1 Channel: 486
Proc Chan: 486 Processed: 10/22/97 12:55:53 PM
Channel Descr:

Millennium Sample Information

Project Name:	fawzy		
Sample Name:	f3		
Vial:	3	Sample Type:	Standard
Injection:	1	Volume:	20.00
Channel:	486	Run Time:	7.0 min
Date Acquired:	05/13/97 10:42:45 AM	Date Processed:	10/22/97 12:55:53 PM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	fawzy		
Processing Method:	fab3		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	0.050	487	213		BB
2	Peak2	1.950				Missing
3	Int Std	2.233	93798	11591	8.000	BV
4	Peak4	2.783	6325	421		VV
5	f	3.217	43803	4557	24.000	VB

Fig. 2. HPLC chromatogram of a volunteer's sample after 48 h of fluoxetine administration.

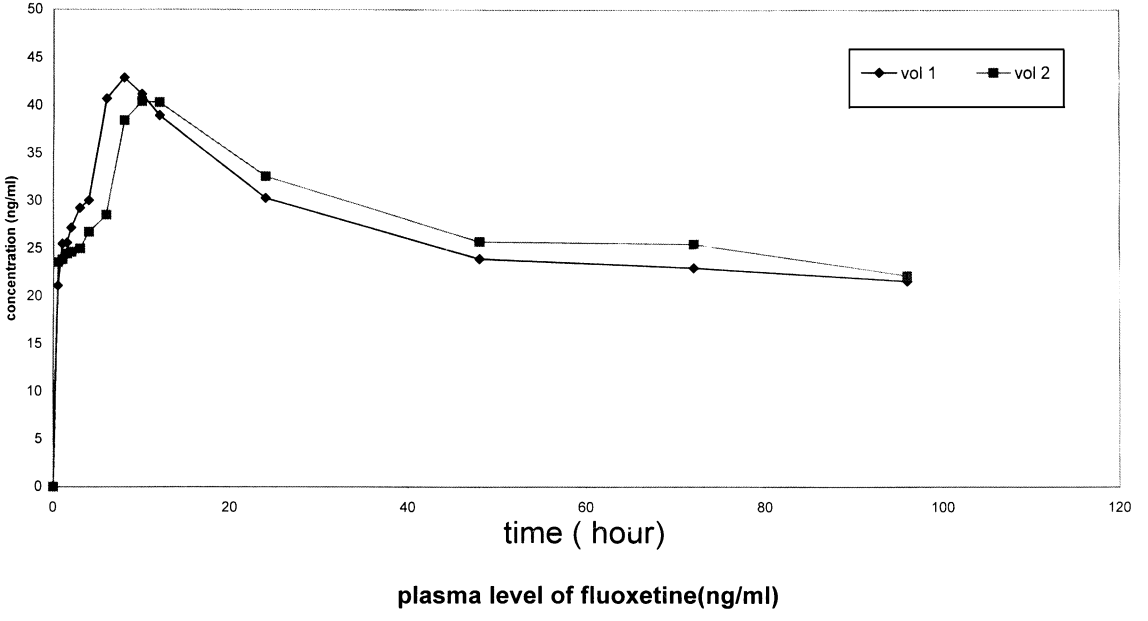


Fig. 3. Fluoxetine concentrations observed in plasma of the volunteers (μg/l) as a function of time.

Table 7
Quantitative parameters for the determination of fluoxetine with the proposed HPLC method in plasma

Linearity range (μg/l)	S.E. intercept	S.E. slope	Correlation coefficient	LOD (μg/l)
10–300	0.004511	0.000081	0.9985	3.0

Table 8
Recovery data of fluoxetine in presence of plasma by the USP method (modified) and the proposed HPLC method*

Claimed fluoxetine concentration (μg/l)	USP method		The proposed HPLC method	
	Recovered concentration (μg/l)	% Recovery	Recovered concentration (μg/l)	% Recovery
50	45.8	91.6	49.00	98.00
100	95.5	59.5	89.50	98.50
150	139.0	92.6	141.00	94.00
200	195.7	97.85	198.30	99.15
300	299.5	99.83	301.00	100.30
Mean % recovery		95.476		97.99
S.D.		±3.46		±2.39
C.V.		3.624		2.439

* Average of three readings.

technique and other HPLC methods that need solid-phase or liquid extraction procedures. With this simple elution system, the whole analysis takes only 5 min, making it more convenient for quality assurance purposes.

References

[1] Martindale, The Extra Pharmacopeia, 30th Edition, 243, Pharmaceutical Press, UK, 1993.
[2] P. Benfield, R.C. Heel, S.P. Lewis, Drugs 32 (1986) 481.
[3] R. Sommi, M. Crimson, C. Bowden, Pharmacotherapy 7 (1987) 1.
[4] R. Fuller, D. Wong, J. Clin. Psychopharmacol. 7 (supp 1) (1987) 36.
[5] D. Robertson, N. Jones, J. Swartzendruber, J. Med. Chem. 31 (1988) 185.
[6] S. Raghuveer, A. Avandhanulu, A. Pantulu, Indian Drugs 30 (2) (1993) 83.
[7] G. Torok Both, G. Baker, L. Aspeslet, J. Chromatogr. Biomed. Appl. 117 (1992) 99.
[8] L. Urichuck, L. Aspeslet, A. Holt, J. Chromatogr. Biomed. Appl. 698 (1–2) (1997) 163.
[9] R. Lantz, K. Farid, J. Konz, J. Chromatogr. Biomed. Appl. 125 (1993) 175.
[10] V. Dixit, H. Nguyen, J. Chromatogr. Biomed. Appl. 10 (1991) 379.
[11] N.H. Jourdi, P.D. Fontaneilli, G.M. Bessard, J. Chromatogr. Biomed. Appl. 692 (2) (1997) 337.
[12] R. Addison, M. Franklin, W. Hooper, J. Chromatogr. Biomed. Appl. 716 (1998) 153.
[13] J. Crifasi, C. Long, N.X. Le, J. Anal. Toxicol. 21 (6) (1997) 415.
[14] S. Atmaca, Pharmazie 50 (40) (1995) 300.
[15] B. Strazesk, K. Baranowska, J. Pharm. Biomed. Anal. 23 (2–3) (2000) 477.
[16] B. Starczewska, K. Mielech, J. Pharm. Biomed. Anal. 23 (2–3) (2000) 243.
[17] L. Bebawy, N. Elkousy, M. Shokry, J. Pharm. Biomed. Anal. 21 (1) (1999) 133.
[18] L. Bebawy, N. Elkousy, M. Shokry, Egypt. J. Pharm. Sci. 39 (4–6) (1998) 323.
[19] A. Prabhakar, V. Batel, R. Giridhar, J. Pharm. Biomed. Anal. 20 (3) (1999) 427.
[20] M. Maya, C. Domingos, J. Morais, J. Pharm. Biomed. Anal. 23 (6) (2000) 989.
[21] A. Lucca, G. Gentillini, S. Lopez, Ther. Drug Monit. 22 (3) (2000) 271.
[22] B. Olsen, D. Wirth, J. Larew, J. Pharm. Biomed. Anal. 17 (4–5) (1998) 623.
[23] G. Misztal, H. Hopkala, Pharmazie 52 (11) (1997) 854.
[24] V. Lamroussi, S. Piperaki, A. Kakoulidou, J. Planar Chromatogr. Mod. TLC 12 (2) (1999) 124.

[25] P. Zuccaro, R. Pacifici, S. Pichini, Ther. Drug Monit. 20 (1) (1998) 20.
[26] G. Battermann, K. Cabrera, D. Lubda, Labor Praxis 22 (9) (1998) 32.
[27] P. Clausing, L. Rushing, G. Newport, J. Chromatogr. Biomed. Appl. 692 (2) (1997) 419.
[28] R. Gupta, M. Steiner, J. Liq. Chromatogr. 13 (9) (1990) 3785.
[29] M. Martin, C. Perez, Anal. Lett. 30 (14) (1997) 2493.
[30] A. Ramaiya, C. March, H. Karnes, J. Pharm. Biomed. Anal. 15 (6) (1997) 729.
[31] United States Pharmacopoeia, XXIV, United States pharmacopoeia convention, Washington, DC, 2000, p. 738.
[32] J. Foley, J. Dorsey, Chromatographia 18 (9) (1984) 503.
[33] S. Zepsi, J. Chromatogr. 454 (1992) 252.
[34] G. Carr, J. Wahlich, J. Pharm. Biomed. Anal. 8 (8-12) (1990) 613.