ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 699–706

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Analytical methods for the quality control of Prozac® capsules

M.A. Raggi [a,*], F. Bugamelli [a], G. Casamenti [a], R. Mandrioli [a], D. De Ronchi [b], V. Volterra [b]

[a] *Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*
[b] *Psychiatric Clinic of the University of Bologna, Bologna, Italy*

Received 15 May 1998; received in revised form 18 July 1998; accepted 15 September 1998

Abstract

Some analytical methods (two spectrophotometric and two chromatographic procedures) for the determination of fluoxetine in Prozac® capsules are described. All of them are applied to the samples after extracting the drug with a methanol–water mixture. The direct and derivative spectrophotometric methods are simple and reliable; the derivative method gives better recovery and lessens interference. Both methods show linearity in the 5–30 μg ml^{-1} range of the fluoxetine concentration range. Both HPLC methods (spectrophotometric and spectrofluorimetric detection) use a tetramethylammonium perchlorate buffer–acetonitrile mixture as the mobile phase and a C8 reversed phase column. The UV detection is performed at 226 nm, while the fluorimetric detection is performed by exciting at 230 nm and revealing the emission at 290 nm. The HPLC method with UV detection is more precise, but the procedure with fluorimetric detection is more sensitive.

Keywords: Column liquid chromatography; Fluorescence detection; UV detection; Fluoxetine; Direct spectrophotometry; Derivative spectrophotometry; Capsule quality control

1. Introduction

Fluoxetine, F, (D,L-*N*-methyl-3-phenyl-3-[(α,α,α-trifluoro-*p*-tolyl)oxy]propylamine) (Fig. 1) is a strong and selective serotonin reuptake inhibitor.

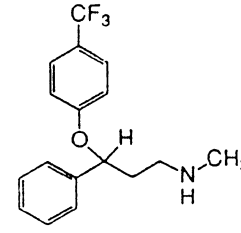

Fig. 1. Chemical structure of fluoxetine (D,L-*N*-methyl-3-phenyl-3-[(α,α,α-trifluoro-*p*-tolyl)oxy]propylamine).

* Corresponding author. Tel.: +39-51-259700; fax: +39-51-259734; e-mail: raggima@kaiser.alma.unibo.it.

0731-7085/98/$ - see front matter
PII: S0731-7085(98)00215-5

Fluoxetine efficacy is very similar to that of traditional tricyclic antidepressants, but at much lower doses [1]. It causes very few dangerous side-effects when compared to tricyclic antidepressants: the treatment with fluoxetine has a very low risk of causing overdose lethality and the anticholinergic, antihistamine and antiadrenergic adverse effects which are common during the treatment with traditional antidepressants [2]. Therefore, it is often used as the drug of choice in the treatment of severe depressive disorders, at doses ranging from 20 to 80 mg daily. Doses of 60 mg daily are used in the management of bulimia nervosa and of obsessive-compulsive disorder (in combination with behavioural and psychosocial methods) [3,4]. Fluoxetine hydrochloride is most widely marketed as Prozac® (Eli Lilly Italia S.p.A. Sesto Fiorentino, Florence, Italy) and it is commercially available in several countries as capsules and as a mint syrup formulation.

Several procedures for the determination of F in biological fluids are reported in literature [5]; they include gas chromatography [6,7] and high performance liquid chromatography (HPLC) with spectrophotometric [8–12] or spectrofluorimetric [13–16] detection; only a few works are present on the determination of F content of pharmaceutical formulations [17,18].

In previous work [19], a HPLC procedure with fluorimetric detection was developed and applied to the determination of Fluoxetine levels in plasma samples of patients subjected to therapy with Prozac® capsules.

The aim of this research is the development of analytical methods for a reliable quality control of Prozac® capsules. For this purpose, two spectrophotometric and two HPLC procedures have been implemented and compared in terms of accuracy, precision and rapidity.

2. Experimental

2.1. Chemicals

Fluoxetine hydrochloride (99% purity) was kindly provided by Eli Lilly. Methanol, acetonitrile and perchloric acid were of HPLC grade and were purchased from Carlo Erba (Milan, Italy). Tetramethylammonium perchlorate and Maprotiline hydrochloride were produced by Sigma (St. Louis, MO). Ultrapure water was obtained by means of a MilliQ apparatus by Millipore (Milford, MA).

The commercial pharmaceutical formulation analyzed was Prozac® capsules (Eli Lilly); each capsule contains 22.4 mg of fluoxetine hydrochloride, which corresponds to 20 mg of Fluoxetine base, and starch (205.64 mg) and dimethylpolysiloxane (2 mg) as excipients.

2.2. Apparatus and chromatographic conditions

The chromatographic system for HPLC analysis was composed of a Varian (Walnut Creek, CA, USA) model 9001 chromatographic pump, a Jasco-975 UV detector (Tokyo, Japan), and a Varian 9075 fluorescence detector.

Separations were obtained on a reversed phase column (Res Elut, C8 150 × 4.6 mm i.d., 5 μm, Varian) connected with a precolumn (Res Elut, C8 30 × 4.6 mm i.d., 5 μm, Varian). The injection was effected through a 20 μl loop. The mobile phase was composed of a mixture of acetonitrile–tetramethylammonium perchlorate (pH 2.6; 17 mM) (1:1, v/v). The mobile phase was filtered through a Phenomenex membrane filter (47 mm membrane, 0.2 μm, NY) and degassed by an ultrasonic apparatus. The flow rate was maintained at 1 ml min^{-1}. The fluorescence intensity was monitored at 290 nm (excitation at 230 nm), while the absorbance values were monitored at the wavelength of 226 nm. Data processing was handled by a model 745 integrator (Waters). The column was maintained at room temperature.

A Jasco UVIDEC-610 double-beam spectrophotometer, a MicropH 2000 Crison (Barcelona, Spain) pHmeter and an ALC 4225 (Milan, Italy) centrifuge were used.

2.3. Solutions

Fluoxetine stock solution (1000 μg ml^{-1}), was prepared by dissolving 22.4 mg of fluoxetine hydrochloride in 20 ml of a methanol–water (1:1, v/v) mixture; standard solutions were obtained by

diluting the stock solution with the same mixture, or with ultrapure water.

Maprotiline stock solution (1000 μg ml^{-1}) was prepared by dissolving 22.6 mg of maprotiline hydrochloride in 20 ml of methanol; maprotiline standard solutions were obtained by diluting the stock solution with ultrapure water.

Prozac® stock solution containing fluoxetine (1 mg ml^{-1}), was prepared by removing, as completely as possible, the contents of 20 capsules and mixing. An accurately weighed portion of the powder, equivalent to 20 mg of fluoxetine, was transferred into a test tube with 20 ml of methanol–water (1:l, v/v) mixture and, after agitation, stored for 5 min at 4°C. It was successively centrifuged for 15 min at 3000 rpm. Finally, the supernatant was filtered through a Whatman 540 filter paper.

The stock solutions were preserved in tight, light-resistant containers, where they were stable for at least 1 month at 4°C.

2.4. Analytical procedure

2.4.1. Spectrophotometric methods

The spectrophotometric assays, using the direct UV spectra, were performed at a wavelength of 226 nm. A calibration curve was set up by plotting absorbance values against fluoxetine standard solution concentrations, in the 5–30 μg ml^{-1} range.

The absorbance values of the first derivative spectra were calculated as the sum of the height of the maximum at $\lambda = 234$ nm and the minimum at $\lambda = 221$ nm. A calibration curve was set up by plotting derivative absorbance values against fluoxetine concentration in the same range as the direct assays.

2.4.2. HPLC analysis with UV detection

The analyses were performed setting the detection wavelength to 226 nm and injecting into the HPLC fluoxetine standard solutions in the 25–1000 ng ml^{-1} range.

A calibration curve was set up by plotting the values of fluoxetine/maprotiline peak area ratios against fluoxetine concentrations (ng ml^{-1}).

After diluting the Prozac® stock solution up to 250 ng ml^{-1} with ultrapure water, the samples were analyzed into the HPLC.

The recovery was calculated interpolating the fluoxetine–maprotiline peak area ratios on the calibration curve.

As an alternative procedure, the extrapolation method was used. Four different samples were prepared; to 0.5 ml of a Prozac® solution (declared concentration of 250 ng ml^{-1}), 0, 0.15, 0.3, and 0.6 ml of fluoxetine 2.5 μg ml^{-1} standard solutions were added (the maprotiline concentration was maintained constant by adding suitable amounts of maprotiline standard solution), and bringing to 3 ml of volume with water. Peak area ratios of the four samples were plotted against the additive fluoxetine concentrations. The extrapolation of the obtained plotline to zero of the ordinate gave the value of the fluoxetine concentration (which had to be multiplied by six according to the dilution) in the Prozac® sample.

In order to verify the accuracy of the method, a known amount of fluoxetine standard solution was added to a Prozac® solution (250 ng ml^{-1}). The sample was injected into the HPLC and the mean recovery was calculated on three trials.

2.4.3. HPLC analysis with fluorimetric detection

An excitation wavelength of 230 nm and an emission wavelength of 290 nm were used.

A calibration curve was constructed in the 10–200 ng ml^{-1} range, plotting fluoxetine–maprotiline peak area ratios against the fluoxetine concentrations. Fluoxetine standard solutions were prepared in ultrapure water, in the presence of a constant maprotiline concentration of 25 ng ml^{-1}.

The above described procedure was used on a fluoxetine standard solution of lower concentration (100 ng ml^{-1}) in order to calculate the drug recovery.

3. Results

3.1. Spectrophotometric methods

The only reported spectrophotometric method [17] on the F assay in pharmaceutical formula-

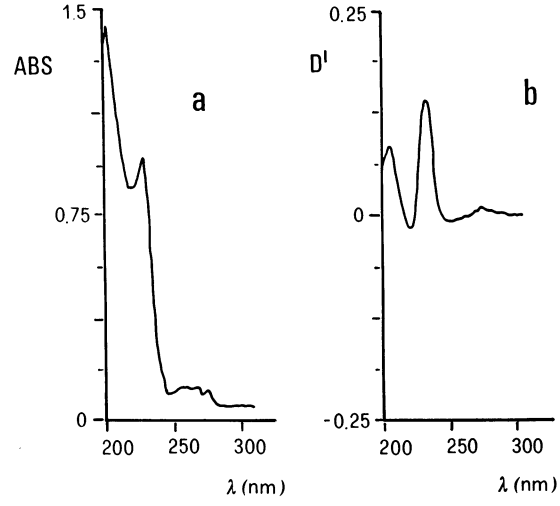

Fig. 2. Direct (a) and derivative (b) spectra of a fluoxetine standard solution in methanol (20 μg ml^{-1}).

tions is based on the complexation of the drug with bromocresol purple and the subsequent measurement of the formed complex absorbance at 405 nm. The spectrophotometric method proposed here is much simpler and faster, because it needs no complexation reaction, but uses the direct measure of the absorbance and of its first derivative.

The absorbance spectrum of a fluoxetine standard solution in water–methanol (1:1, v/v) presents a large band at $\lambda = 226$ nm (molar extinction coefficient 15900) and two small bands at 260 and 275 nm (Fig. 2(a)). The first, second, third and fourth derivative spectra were also examined. The first derivative spectrum presents very neat bands (Fig. 2(b)), and was selected because it allows for a better sensitivity than the other derivative spectra.

The first calibration curve was set up by plotting the absorbance values obtained at $\lambda = 226$ nm from the direct spectra against the fluoxetine concentration (μg ml^{-1}), in the 5–30 μg ml^{-1} range. A good linearity was found in the examined concentration range; the linearity equation, calculated by means of the least square method, was $y = 0.0456x + 0.0185$, correlation coefficient, $Rc = 0.9996$.

A second calibration curve was set up by using the difference of the values obtained at $\lambda = 234$ and 22 l nm (from the first derivative spectra), in the same concentration range as above. The obtained linearity was also good, and the equation was $y = 0.0082x + 0.0005$, $Rc = 0.9996$.

In order to verify the method precision, a 20 μg ml^{-1} fluoxetine standard solution was subjected to spectrophotometric analysis for five trials. The intraday assays (repeatability) on the direct spectra gave a relative standard solution (RSD) value of 1.32%, while the interday assays (intermediate precision) gave a RSD value of 1.80%. Using the derivative method, the RSD was 1.17% for the repeatability and 1.47% for the intermediate precision.

Application to Prozac® capsules: Having thus validated the method, it was applied to the assay of F in Prozac® capsules as described in the Section 2. The choice of the solvent used for the extraction of fluoxetine from Prozac® capsules resulted to be of great importance. Preliminary assays using methanol as the solvent led to percentage recoveries (drug found of declared) of 109%, clearly indicating the presence of interference. The interference was eliminated by using methanol–water (1:1, v/v) as the extraction solvent.

Under these conditions, the mean recovery was 97.83% (repeatability: RSD = 1.22%) intraday and 97.49% (intermediate precision: RSD = 1.43%) interday, both on five assays. The first derivative method gave better mean recoveries on five assays: 99.37% (RSD = 1.45%) intraday and 99.1 1% (RSD = 1.46%) interday.

The accuracy of both methods was verified by adding known amounts of fluoxetine standard solutions to known amounts of capsule content. From the spectrophotometric measurements, the mean percentage recovery obtained was 97.42% for the direct procedure (RSD = 1.16% interday, $n = 5$) and 98.91% for the derivative procedure (RSD = 1.05% interday, $n = 5$).

3.2. HPLC methods

UV detection: Recently, the European Pharmacopeia [20] and the United States Pharmacopeia [21] report fluoxetine determinations in pure substance and in capsules by means of HPLC proce-

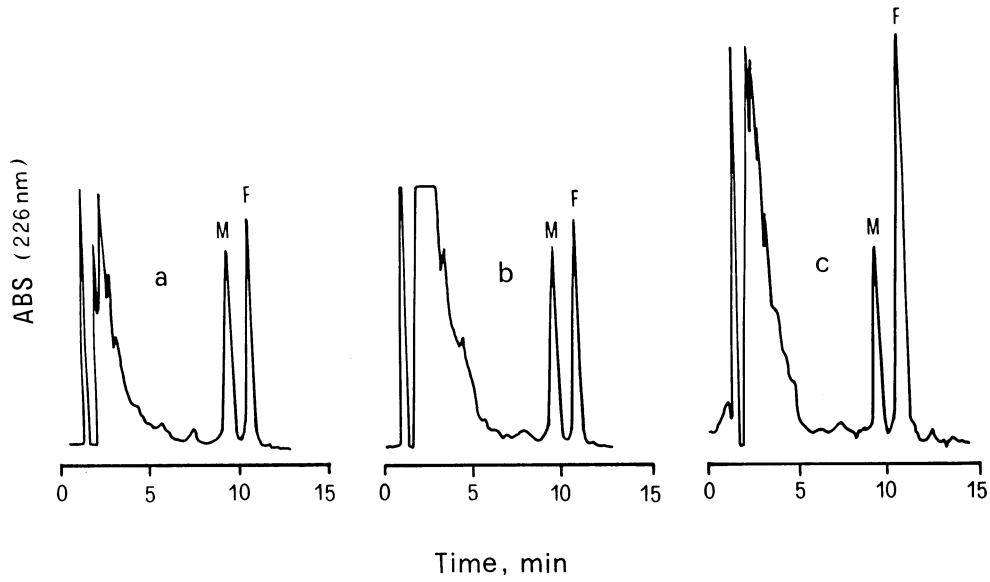

Fig. 3. HPLC chromatograms (UV detection: $\lambda = 226$ nm): (a) fluoxetine standard solution (250 ng ml^{-1}); (b) Prozac® solution (nominal concentration 250 ng ml^{-1}); (c) the same solution as in (b) spiked with a fluoxetine standard solution (500 ng ml^{-1}). Each sample contains maprotiline (500 ng ml^{-1}) as the internal standard.

dures with UV detection. The leading chromatographic conditions of this work are the same used by the authors in a recent paper [19], with the detection wavelength set to 226 nm, according to the above described spectrophotometric analysis, and are very different from those proposed by both Pharmacopeias (different column and mobile phase). The method proposed by us is simpler and faster, furthermore the mobile phase is easier to prepare and more stable.

The mobile phase was a tetramethylammonium perchlorate (pH 2.6; 17 mM)-acetonitrile (1:1, v/v) mixture (stationary phase: C8 column, 150 × 4.6 mm, 5 μm) with a flow rate of 1 ml min^{-1}.

The chromatogram of a 250 ng ml^{-1} fluoxetine standard solution, obtained at $\lambda = 226$ nm (maximum of the absorption band), is reported in Fig. 3(a). As can be seen, fluoxetine is revealed as a peak with retention time (t_r) = 10.3 min, while maprotiline, used as an internal standard, has a $t_r = 9.1$ min.

A calibration curve was set up, plotting the values of the fluoxetine/internal standard peak area ratios against the concentration of fluoxetine, expressed as ng ml^{-1}. A good linearity was found in the 25–1000 ng ml^{-1} range; the regression equation was $y = 0.00475x + 0.01546$, $Rc = 0.9994$.

The method reproducibility was verified by injecting five standard fluoxetine solutions (250 ng ml^{-1}). The resulting RSD values were 0.93% for the intraday (repeatability) assays and 1.25% for the interday (intermediate precision) assays.

Application to Prozac® capsules: After extracting fluoxetine from Prozac® capsules by means of a methanol–water (1:1, v/v) mixture and subsequent suitable dilutions with ultrapure water, the resulting solution was analyzed by means of HPLC. The chromatogram of a solution having a nominal content of 250 ng ml^{-1} of fluoxetine is reported in Fig. 3(b). No interfering peak is detected, and the chromatogram is very similar to that obtained by injecting a fluoxetine standard solution.

The fluoxetine content was determined by interpolating on the calibration curve. The mean percentage recovery ($n = 5$), expressed as fluoxetine found of declared, was 99.65% intraday (RSD = 1.51%) and 100.9% interday (RSD = 2.14%).

In order to better eliminate the possible interference from the matrix, the amount of fluoxetine

in the capsules was determined by means of the extrapolation method. Three different amounts of fluoxetine standard solutions were added to known amounts of diluted fluoxetine solution extracted from Prozac® (as described in Section 2), then injected in the HPLC.

The chromatogram of a 500 ng ml^{-1} fluoxetine standard solution added to a Prozac® solution (nominal concentration 250 ng ml^{-1}) is reported as example in Fig. 3(c). The resulting fluoxetine/internal standard peak area ratios were plotted against the added fluoxetine concentrations (ng ml^{-1}). A good linearity was obtained; the regression equation was $y = 0.0048x + 0.196$, $Rc = 0.9983$. The original concentration of fluoxetine was found by extrapolating to zero ordinate (then multiplying by six in order to account for the dilution). A value of 252 ng ml^{-1} of fluoxetine was obtained; this led to a 100.8% recovery value. This value agrees strongly with the value obtained by means of the interpolation procedure (100.9%).

The accuracy of the method was verified by adding a known quantity of fluoxetine standard solution to known amounts of the pharmaceutical formulation. The mean recovery was 99. 1%.

Fluorimetric detection: The chromatographic conditions and the internal standard are the same as those used for the UV detection method. The analysis were conducted by exciting at $\lambda = 230$ nm and monitoring the emission intensities at $\lambda = 290$ nm. Fluoxetine is detected as a neat peak at $t_r = 10.3$ min, while maprotiline (25 ng ml^{-1}) has a retention time of 9.1 min. It should be noted that the internal standard maprotiline has a molar extinction coefficient comparable to that of fluoxetine, but a much higher fluorescence emission.

A calibration curve was set up, by plotting the fluoxetine/maprotiline peak area ratio against fluoxetine concentration. A good linearity was obtained in the 10–200 ng ml^{-1}; the regression equation was $y = 0.01473x + 0.00804$, $Rc = 0.9989$.

The reproducibility of the method was evaluated by means of five assays performed on 100 ng ml^{-1} fluoxetine standard solutions. The resulting RSD values were 1.8% for repeatability (intraday) and 1.94% for intermediate precision (interday).Application to Prozac® capsules: After having optimized the method, the determination of fluoxetine in Prozac® capsules was performed. The chromatogram of a solution having a nominal content of 100 ng ml^{-1} of fluoxetine is reported in Fig. 4. No interfering peak is detected, and the chromatogram is very similar to that obtained by injecting a fluoxetine standard solution.

The fluoxetine content was determined by interpolating on the calibration curve. The mean percentage recovery ($n = 5$), expressed as amount of drug found of declared, was 98.2% intraday (RSD = 1.6%) and 100.2% interday (RSD = 1.8%).

In order to evaluate the accuracy of the procedure, the standard addition method was applied. Known amounts of fluoxetine standard solutions were added to known amounts of diluted fluoxetine solution from Prozac® (as described in the Section 2), then injected into the HPLC. A 75 ng ml^{-1} fluoxetine standard solution was added to a Prozac® solution (nominal concentration, 100 ng ml^{-1}).The percentage recovery was 100.2%.

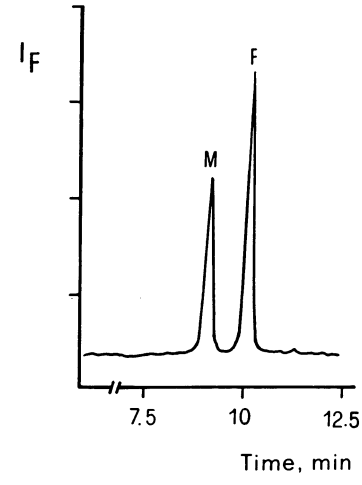

Fig. 4. HPLC chromatogram (Fluorimetric detection: $\lambda_{exc} = 230$ nm, $\lambda_{em} = 290$ nm) of a Prozac® solution (nominal concentration 100 ng ml^{-1}), containing maprotiline (25 ng ml^{-1}) as the internal standard.

Table 1
Fluoxetine assays in pharmaceutical formulations

Method	Sample concentration	Percentage of label claim[a]	RSD%		Accuracy (% recovery)[b]
			Intraday	Interday	
A. Direct spectrophotometry	20 μg ml^{-1}	97.49	1.22	1.43	97.42
B. Derivative spectrophotometry	20 μg ml^{-1}	99. 11	1.45	1.46	98.91
C. HPLC (UV)	250 ng ml^{-1}	100.9	1.51	2.14	99.1
D. HPLC (Fluorimetric)	100 ng ml^{-1}	100.2	1.6	1.8	100.2

[a] Each value is the mean of five determinations.
[b] Each value is the mean of three determinations.

4. Conclusion

We have developed four precise and accurate analytical procedures, suitable for a reliable determination of fluoxetine in Prozac® capsules. The main data are summarized in Table 1. As shown in the Table, all of the recovery values fall within the range required by the US Pharmacopeia for fluoxetine capsules (precisely within 90 and 110%) [21].

Of the two spectrophotometric procedures, the derivative one is surely more accurate than the direct one; it is thus suitable for rapid and inexpensive quality control testing of Prozac® capsules.

The results obtained using the HPLC methods (with UV and fluorescence detection) are similar, and clearly indicate that both methods are suitable, in terms of precision and accuracy, for the determination of fluoxetine in commercial capsules (Tables 1 and 2). The HPLC method with fluorimetric detection, owing to its sensitivity, seems to be very promising for the fluoxetine assay in biological fluids. Preliminary studies showed that the HPLC methods could also allow for the detection and determination of some impurities, such as norfluoxetine and others which can be toxic, often present in the pharmaceutical formulations. Moreover, galenic preparations containing fluoxetine often contain also excipients which can complex or react with the drug, thus inactivating it. More extensive studies are in progress, in order to extend the analysis to various impurities and to the monitoring of the drug stability.

Acknowledgements

This research was supported in part by a grant from 60% MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Italia). We are grateful to Eli Lilly Italia S.p.A. for their financial contributions.

Table 2
Comparison of the performance of the methods used for Fluoxetine determination

Method	Concentration range	RSD%[a]		Measure time (min)
		Intraday	Interday	
A. Direct spectrophotometry	5–30 μg ml^{-1}	1.32	1.80	5
B. Derivative spectrophotometry	5–30 μg ml^{-1}	1.17	1.47	5
C. HPLC (UV)	25–1000 ng ml^{-1}	0.93	1.25	12
D. HPLC (Fluorimetric)	10–200 ng ml^{-1}	1.80	1.94	12

[a] Each value is calculated by five determinations on Fluoxetine sβtandard solutions having concentrations of 20 μg ml^{-1} for A and B. For the HPLC methods, the standard concentrations were 250 and 100 ng ml^{-1}, respectively.

References

[1] P. Benfield, R.C. Heel, S.P. Lewis, Drugs 32 (1986) 481–508.
[2] Martindale—The Extra Pharmacopoeia, 31st ed., R. Pharm. Soc., Lond., 1996, pp. 312–314
[3] D.T. Wong, F.P. Bymaster, E.A. Engleman, Life Sci. 57 (1995) 411–441.
[4] S.H. Preskorn, Clinical Pharmacology of Serotonin Selective Reuptake Inhibitors, Professional Communications, Caddo, OK, 1996, pp. 33–39.
[5] D.S. Risley, R.J. Bopp, in: Brittany (Ed.), Analytical Profiles of Drug Substances, vol. 19, Academic Press, London, 1990, pp. 193–219
[6] J.F. Nash, R.J. Bopp, R.H. Cannichael, K.Z. Farid, L. Lemberger, Clin. Chem. 28 (1982) 2100–2102.
[7] R.J. Lantz, K.Z. Farid, J. Koons, J.B. Tenbarge, R.J. Bopp, J. Chromatogr. 614 (1993) 175–179.
[8] S.H. Wong, S.S. Dellafera, R. Fernandes, H. Kranzler, J. Chromatogr. 499 (1990) 601–608.
[9] J.H. Nichols, J.R. Charlson, G.M. Lawson, Clin. Chem. 40 (1994) 1312–1316.
[10] I.M. McIntyre, C.V. King, S. Skafidis, O.H. Drummer, J. Chromatogr. 621 (1993) 215–223.
[11] S. Joron, H. Robert, Biomed. Chromatogr. 8 (1994) 158–164.
[12] G. Misztal, H. Hopkala, Pharmazie 52 (1997) 854–856.
[13] S. Piperaki, M. Parissi-Poulou, J. Liq. Chromatogr. 19 (1996) 1405–1421.
[14] R.N. Gupta, M. Steiner, J. Liq. Chromatogr. 13 (1990) 3785–3797.
[15] R.F. Suckow, M.F. Zhang, T.B. Cooper, Clin. Chem. 38 (1992) 1756–1761.
[16] A.L. Peyton, R. Carpenter, K. Rutowski, Pharm. Res. 8 (1991) 1528–1532.
[17] G.P. Pokharjyal, K. Mamgain, A. Sahu, J. Inst. Chem. Inotia 68 (1996) 148–149.
[18] S. Atmaca, Pharmazie 50 (1995) 300–301.
[19] M.A. Raggi, R. Mandrioli, G. Casamenti, F. Bugamelli, V. Volterra, J. Pharm. Biomed. Anal. (in press).
[20] European Pharmacopoeia, 3rd ed., Council of Europe, Strasbourg, 1997, p. 868
[21] United States Pharmacopeia, 23rd Edition, 7th Supplement, United States Pharmacopeial Convention, Rockville, MD, 1997, pp. 3890–3891.