Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 58 (2003) 1209–1216
IL FARMACO
www.elsevier.com/locate/farmac

Rapid and simple methods for quantitative analysis of some antidepressant in pharmaceutical formulations by using first derivative spectrophotometry and HPLC

Nevin Erk

Department of Analytical Chemistry, Faculty of Pharmacy, University of Ankara, 06100 Ankara, Turkey

Received 4 March 2003; accepted 18 July 2003

Abstract

Two rapid, simple and accurate first derivative spectrophotometry and HPLC method for the determination of nefazodone hydrochloride and sertraline hydrochloride in pharmaceutical formulations are discussed. The first one is a derivative spectrophotometric procedure and the second one is based on a HPLC method with a UV detector. In the first method, first derivative spectrophotometry, nefazodone hydrochloride or sertraline hydrochloride by measurement of their first derivative signals at 241.8–256.7 nm (peak-to-peak amplitude), or 271.6–275.5 nm (peak-to-peak amplitude), respectively. Calibration graphs were established for 10.0–42.0 μg ml^{-1} nefazodone hydrochloride, or 8.0–46.0 μg ml^{-1} sertraline hydrochloride. In the other method, HPLC, the UV detection was carried out at 265.0 nm (nefazodone hydrochloride) and 270.0 nm (sertraline hydrochloride). The samples were chromatographed on a Supercosil RP-18 column. The mobile phases were methanol:acetonitrile:phosphate buffer at pH 5.5 (10:50:40 v/v/v) (nefazodone hydrochloride) and methanol:phosphate buffer at pH 4.5 (20:80 v/v) (sertraline hydrochloride). The results obtained from first derivative spectrophotometric method were comparable with those obtained by using HPLC. It was concluded that both the developed methods are equally accurate, sensitive, and precision could be applied directly and easily to the pharmaceutical formulations of nefazodone hydrochloride and sertraline hydrochloride, respectively.

Keywords: Nefazodone hydrochloride; Sertraline hydrochloride; First derivative spectrophotometry; High-performance liquid chromatography

1. Introduction

Both nefazodone hydrochloride and sertraline hydrochloride belong to generation of antidepressant drugs. Nefazodone hydrochloride appears to block both 5-HT transport and post synaptic 5-HT_2 receptors and, in these effects, resembles the clinically employed atypical antidepressant mianserin [1]. Sertraline is a potent selective serotonin reuptake inhibitor (SSRI) that has established efficacy in the treatment of depression, obsessive-compulsive disorder, depression relapse and social phobia [2].

Scientific literature reports HPLC methods for quantitative determination of nefazodone hydrochloride, its metabolites in human plasma and in pharmaceutical formulations [3–7].

On the other hands, various methods have been used for the determination of sertraline hydrochloride, its metabolites in human plasma and in pharmaceutical dosage forms, including HPLC method [8–12], gas-chromatography-mass spectrometry [13,14] and spectrophotometric method [15].

Nefazodone hydrochloride and sertraline hydrochloride are not included in any pharmacopoeia. With these ever increasing use and the number of formulations entering into the market, there is always a need for simple, sensitive, accurate, rapid analytical method for the estimation of nefazodone hydrochloride and sertraline hydrochloride in pure form and in pharmaceutical preparations which can be easily adapted for routine in quality testing laboratories. On extensive survey of literature, no derivative spectrophotometric method came to my notice for the determination of both drugs in pure form and in pharmaceutical formulations. In the

E-mail address: erk@pharmacy.ankara.edu.tr (N. Erk).

0014-827X/03/$ - see front matter © 2003 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.
doi:10.1016/j.farmac.2003.07.008

present study, two simple, sensitive and accurate analytical methods with better detection range for estimation of nefazodone hydrochloride and sertraline hydrochloride in pure form and in pharmaceutical formulations were developed. In both the methods, no extraction step is utilized thus reducing the time and error involved in the estimation thus not necessitating the use of any internal standard. The proposed methods were applied to the determination of both analytes in pure form and pharmaceutical preparations, with satisfactory results in both cases.

2. Experimental

2.1. Chemicals used

Two commercial preparations, Serzone® tablets (produced by Bristol–Myers Squibb Pharm. Ind., Turkey, containing 200.0 mg of nefazodone hydrochloride per tablet) and Lustral® tablets (produced by Pfizer Pharm. Ind., Turkey, containing 50.0 mg of sertraline hydrochloride per tablet) were assayed.

Methanol and acetonitrile were of HPLC grade (Merck Chem. Ind.). All other chemicals were of analytical-reagent grade.

2.2. Spectrophotometric equipment and conditions

A double beam, Shimadzu 1601 spectrophotometer model with a fixed slit width (2 nm) connected to an IBM-PC computer loaded was used. The derivative UV spectra of standard and test solutions were recorded in 1 cm quartz cells.

2.3. Chromatographic system and conditions

The high-performance liquid chromatography system consisted of a JASCO model PU-980 pump with a 7725 rheodyne value injector 20 μl fixed loop, equipped with a JASCO UV-975 UV–Vis detector. The detector was set at 265.0 and 270.0 nm (0.02 a.u.f.s.) and peak areas were integrated automatically by computer using BORWIN software programme.

3. Analytical procedures

3.1. Procedure for spectrophotometric method

3.1.1. Calibration

Stock solutions of 1 mg ml^{-1} of nefazodone hydrochloride and sertraline hydrochloride were prepared in methanol, respectively. These solutions were used in the preparation of calibration graphs and for spectra.

3.1.2. Assay procedure for dosage forms

Twenty tablets were weighed and pulverized. A weighed quantity of the powder equivalent to one tablet was transfer into a 100-ml conical flask in methanol. After 30 min of mechanical shaking, the solution was filtered in a 100-ml calibrated flask through Whatman no. 42 filter paper. The residue was washed three times with 10 ml of solvent and then the volume was completed to 100 ml with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrates and diluting them with methanol.

3.2. Procedure for high performance liquid chromatography

3.2.1. Chromatographic conditions

Solutions and mobile phases were prepared in the moment of use. The mobile phases used were methanol:acetonitrile:phosphate buffer at pH 5.5 (10:50:40 v/v/v) (nefazodone hydrochloride) and methanol:phosphate buffer at pH 4.5 (20:80 v/v) (sertraline hydrochloride). The analytical column was a Supercosil RP-18 (5 μm, 15 cm × 6.0 mm) column. All analysis were done under isocratic conditions at a flow rate of 1.0 ml min^{-1} and at room temperature.

All solvents were filtered through 0.45 μm milipore filter to use and degassed in an ultrasonic bath.

3.2.2. Calibration

An external standard method was used for quantitative determinations. Calibration graphs were prepared from stock solutions of nefazodone hydrochloride and sertraline hydrochloride in the mobile phase, respectively. Triplicate 20-μl injections were made for each solution. The final concentrations nefazodone hydrochloride and sertraline hydrochloride in the samples were calculated by comparison of sample and standard peak area obtained with the average of three injections of standard solutions.

3.2.3. Analysis of tablets for HPLC

Ten commercial tablets (Serzone® and Lustral® tablets) were weighed separately and powdered in a different mortars. A portion of the powder equivalent to about one tablet was weighed accurately, transferred to a 100 ml calibrated flask and suspended in mobile phase for HPLC method. The flasks were completed to volume with the same solvent. The samples were filtered through a 0.45-μm membrane filter, then further diluted to suit the calibration graphs.

3.2.4. Recovery studies

To keep an additional check on the accuracy of the developed assay methods and to study the interference of formulation additives, analytical recovery experiments were performed by adding known amount of

pure drugs to pre-analyzed samples of commercial dosage forms. The percent analytical recovery values calculated by comparing concentration obtained from the spiked samples with actual added concentrations are also listed in Table 3.

4. Investigations, results and discussion

4.1. Analysis of nefazodone hydrochloride and sertraline hydrochloride by first derivative spectrophotometry

To develop a sensitive first derivative spectrophotometric method various solvent system were tried, such as water, methanol, 0.1 N HCl, 0.1 N NaOH and acetonitrile a lone or in combinations of different proportions. The final decision of using methanol was based on sensitivity, interference, ease of preparation, suitability for drugs content estimation, and cost in that order.

The direct UV spectrum (Figs. 1a and 2a) or the first derivative spectrum (Figs. 1b and 2b) of nefazodone hydrochloride and sertraline hydrochloride methanolic standard solutions are given, respectively. Both spectra could be used for the determination of these drugs. In the direct UV spectrum, nefazodone hydrochloride show a single well-defined peak at 247.5 nm. Fig. 2 contains three bands with absorbance maxima at $\lambda =$ 265.1, 275.3 and 282.6 nm; but resolution of the peaks in the first derivative spectrum prompted to use this spectrum for sertraline hydrochloride analysis. In the derivative method, odd-numbered derivatives are of most use in determining the exact points of absorbance maxima of the zero-order absorption spectrum and hence, the qualitative properties of the substance under investigation; even-numbered derivatives are helpful in quantitative determinations [16]. For the determination of nefazodone hydrochloride or sertraline hydrochloride in bulk and dosage forms by measuring the peak-to-peak amplitude in the first derivative spectra at 241.8–256.7 nm (peak-to-peak amplitude), and 271.6–275.5 nm were used, respectively. By measuring the values of the first derivative, the concentration of cited drugs can be directly calculated since the first derivative measure-

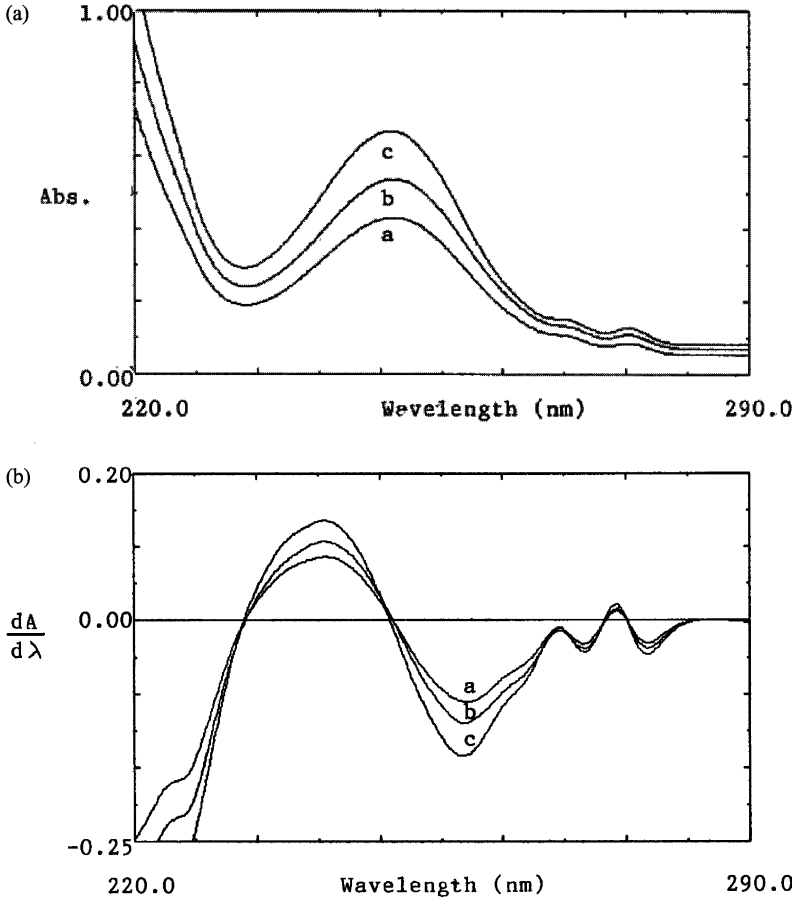

Fig. 1. Zero-order spectra of (a) and first derivative spectra (b) of (a) 10.0 μg ml^{-1}; (b) 22.0 μg ml^{-1}; and (c) 42.0 μg ml^{-1} nefazodone hydrochloride in methanol.

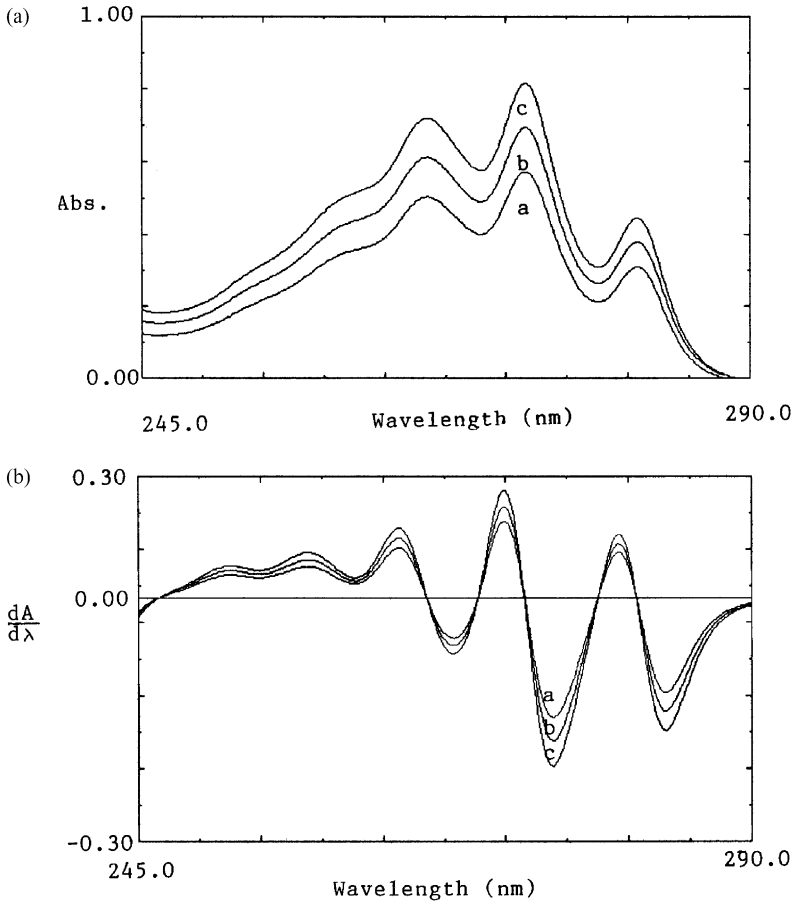

Fig. 2. Zero-order spectra of (a) and first derivative spectra (b) of (a) 8.0 μg ml^{-1}; (b) 27.0 μg ml^{-1}; and (c) 46.0 μg ml^{-1} sertraline hydrochloride in methanol.

Table 1
Results of least square regression analysis of data for the estimation of nefazodone hydrochloride and sertraline hydrochloride by the proposed methods

Parameters	First derivative spectrophotometry		HPLC	
Compound	Nefazodone hydrochloride	Sertraline hydrochloride	Nefazodone hydrochloride	Sertraline hydrochloride
Range	10.0–42.0 (μg ml^{-1})	8.0–46.0 (μg ml^{-1})	5.0–5000.0 (ngml^{-1})	40.0–1500.0 (ngml^{-1})
Detection limits	0.58 (μg ml^{-1})	0.31 (μg ml^{-1})	15.8 (ngml^{-1})	24.0 (ngml^{-1})
Qantitative limits	1.88 (μg ml^{-1})	0.96 (μg ml^{-1})	26.4 (ngml^{-1})	45.3 (ngml^{-1})
Regression equation (Y) [a]	$y = 3.56 \times 10^{-3}x + 5.91 \times 10^{-3}$	$y = 1.46 \times 10^{-4}x + 8.13 \times 10^{-5}$	$y = 0.41x + 0.18$	$y = 0.78x + 0.45$
SD on slope (S_b)	5.00×10^{-4}	1.45×10^{-5}	1.23×10^{2}	6.25×10^{2}
SD on intercept (S_a)	6.73×10^{-3}	3.68×10^{-5}	1.09×10^{2}	4.31×10^{2}
SE of estimation (S_e)	8.42×10^{-3}	2.31×10^{-5}	5.56×10^{2}	1.52×10^{2}
Correlation coefficient (r)	0.9991	0.9986	0.9980	0.9999

[a] Y = absorbance (firs.der.spec.) and peak area (HPLC); x: concentration of the drug in μg ml^{-1} (firs.der.spec.) and in ng ml^{-1} (HPLC). [b] Five replicate samples.

ment cancels the irrelevant absorbance due to the excipients in pharmaceutical dosage forms. The Beer's law range, linear regression equations, and correlation coefficient determined for each method are given in Table 1.

4.2. Analysis of nefazodone hydrochloride and sertraline hydrochloride by HPLC method

In case of HPLC, mobile phase investigated were 20–70% methanol in water, 20–70% acetonitrile in water,

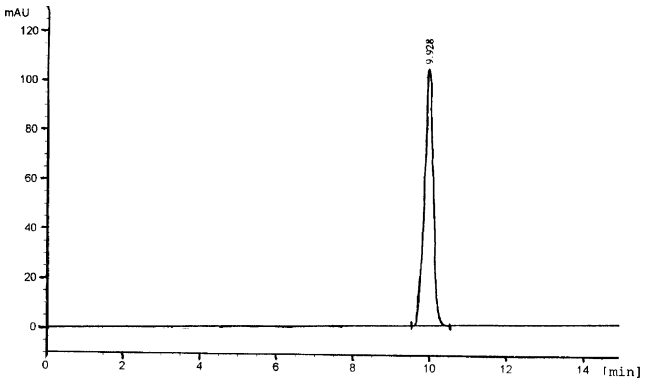

Fig. 3. A typical chromatogram of Serzone® tablet.

phosphate buffer (pH 2.8–7.8) with methanol or acetonitrile or both. Mobile phases of methanol:acetonitrile:-phosphate buffer at pH 5.5 (10:50:40 v/v/v) (for nefazodone hydrochloride) and methanol:phosphate buffer at pH 4.5 (20:80 v/v) (for sertraline hydrochloride) and flow rate selection was based on peak parameters (height, asymmetry, tailing), baseline drift, ease of preparation of the mobile phases, need for pH adjustment and cost (in that order). Internal standard was not used, as there was no extraction or separation step involved in the estimation of nefazodone hydrochloride and sertraline hydrochloride from pharmaceutical dosage forms.

The HPLC method was applied to the determination of nefazodone hydrochloride and sertraline hydrochloride in the pharmaceutical formulations. A representative chromatogram of nefazodone hydrochloride and sertraline hydrochloride obtained under the optimal conditions chosen are shown in Figs. 3 and 4. The wavelength of detection was fixed at 265.0 nm for nefazodone hydrochloride and 270.0 nm for sertraline hydrochloride, respectively. Figs. 3 and 4 show a typical HPLC chromatogram of the two pharmaceutical formulations analysed; the retention times (min) were 9.9 (nefazodone hydrochloride) and 7.5 sertraline hydrochloride. The retention times and asymmetry factors were found to be 9.9 ± 0.064 and 1.12 ± 0.14 (nefazodone hydrochloride), 7.5 ± 0.015 and 1.10 ± 0.17 (sertraline hydrochlororide), respectively. A calibration curve was obtained by plotting the area values against the concentrations of these drugs. A good linearity were obtained over the 5.0–5000.0 ng ml^{-1} range for nefazodone hydrochloride and 40.0–1500.0 ng ml^{-1} range for sertraline hydrochloride, respectively. The regression equations and statistical study are given in Table 1. The correlation coefficient values obtained were highly significant for the proposed method (Table 1). The drugs solutions were found to be stable for a period of 48 h at room temperature in the solvent system used.

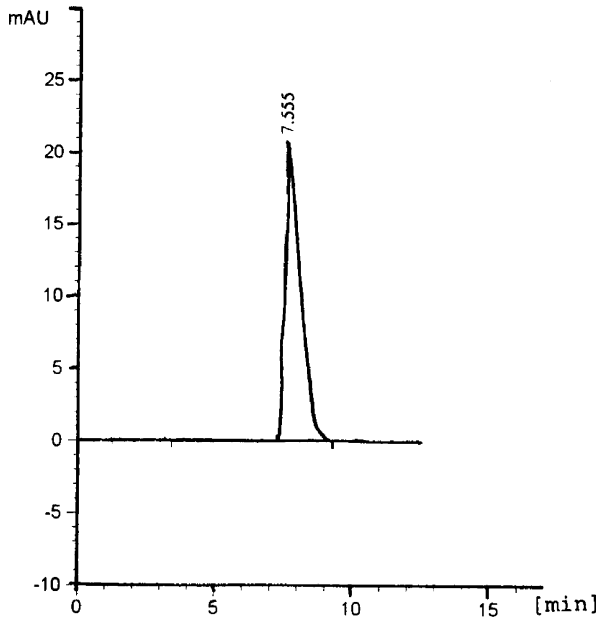

Fig. 4. A typical chromatogram of Lustral® tablet.

4.3. Validation of the developed methods

The linearity range of nefazodone hydrochloride and sertraline hydrochloride solutions in case of first derivative spectrophotometry and high performance liquid chromatography were found to be 10.0–42.0 μg ml^{-1} (nefazodone hydrochloride), 8.0–46.0 μg ml^{-1} (sertraline hydrochloride) and 5.0–5000.0 ng ml^{-1}(nefazodone hydrochloride), 40.0–1500.0 ngml^{-1} (sertraline hydrochloride), respectively.

The validation of the procedures was examined via evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability and recovery. The LOD and LOQ values were calculated from the calibration curves as kSD/b where $k = 3$ for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope of the calibration curve. The lowest LOD (0.58 μg ml^{-1} of nefazodone hydrochloride and 0.31 μg ml^{-1} of sertraline hydrochloride) for first derivative spectrophotometry and (15.8 ng ml^{-1} of nefazodone hydrochloride and 24.0 ng ml^{-1} of sertraline hydrochloride) for high performance liquid chromatography were calculated and are given in Table 1. Also, the limit of quantification (LOQ) were found to be (1.88 μg ml^{-1} of nefazodone hydrochloride and 0.96 μg ml^{-1} of sertraline hydrochloride) for first derivative spectrophotometry and (26.4 ng ml^{-1} of nefazodone hydrochloride and 45.3 ng ml^{-1} of sertraline hydrochloride) for high performance liquid chromatography.

Between-day precision and accuracy were evaluated by using three different concentrations at low, medium and high concentrations, which were prepared and analyzed on the same day (Table 2). Within-day variability was assessed using five samples of three

Table 2
Within- and between-day precision of nefazodone hydrochloride and sertraline hydrochloride standards by using first derivative spectrophotometry and HPLC method (abbreviations as in text)

Theoretical concentration (μg ml^{-1})	Within-day concentration	Measured [a]	Between-day concentration	Measured [b]	Within-day concentration	Measured	Between-day concentration	Measured
	Nefazodone	Hydrochloride	Nefazodone	Hydrochloride	Sertraline	Hydrochloride	Sertraline	Hydrochloride
	Recovery	RSD%	Recovery	RSD%	Recovery	RSD%	Recovery	RSD%
First derivative spectrophotometry								
10.0	99.0	0.48	98.0	1.04				
22.0	100.9	0.85	100.4	1.53				
42.0	100.7	0.64	98.8	1.96				
8.0					97.5	0.68	98.3	1.47
27.0					98.9	0.66	99.6	1.91
46.0					99.6	0.57	100.6	2.13
High performance liquid chromatography								
10.0	103.0	0.08	101.0	0.08				
22.0	100.4	0.05	100.0	0.13				
42.0	100.2	0.04	100.7	0.25				
8.0					98.8	0.06	98.8	1.07
27.0					100.3	0.08	99.6	1.00
46.0					100.4	0.22	100.6	1.03

[a] Mean values represent five different sample standards for each concentration.
[b] Within-day reproducibility was determined from five different runs over a 2-week period.

Table 3
Results of the assay of pure nefazodone hydrochloride and sertraline hydrochloride and its commercialformulations by the proposed methods

Sample	Recovery (mean ± SD) (%) [a]			
	Nefazodone hydrochloride		Sertraline hydrochloride	
	First der. spectr.	HPLC	First der. spectr.	HPLC
Pure drug solution	98.4 ± 0.35 $t = 0.59(2.26)$ [b]	99.1 ± 0.54	99.1 ± 0.68 $t = 0.48$	99.5 ± 0.34
Commercial tablets [c]	99.7 ± 1.43 $t = 0.97$	99.8 ± 0.58	99.7 ± 0.57 $t = 0.87$	99.7 ± 0.25

[a] Mean and relative standard deviation for ten determinations; percentage recovery from the label claim amount.
[b] Values in parentheses are the theoretical values at $p = 0.95$. Theoretical values at 95% confidence limits $t = 2.26$.
[c] Serzone® tablets were labeled to contain 200.0 mg nefazodone hydrochloride and Lustral® tablets were labeled to contain 50.0 mg sertraline hydrochloride per tablets.

different concentrations at low, medium and high concentrations analyzed on five different days over a period of two week. Table 2 represents the results obtained for between-day and within-day variability studies of nefazodone hydrochloride and sertraline hydrochloride, respectively. These results show the accuracy for quantitative determination of cited drugs. Thus, it was concluded that there was no significant difference for the assay which was tested within-day and between-day.

The two methods were further validated by estimation of nefazodone hydrochloride and sertraline hydrochloride in pharmaceutical formulations by the developed methods and analyses of pure drug solutions and the results are presented in Table 3. The estimated drugs content with low values of SD further established the precision of the proposed methods and therefore suggested the non-interferece from the formulation matrix present in the studied formulations. The method was applied to the determination of nefazodone hydrochloride in Serzone® tablets and sertraline hydrochloride in Lustral® tablets and the results are presented in Table 3.

5. Conclusion

The first derivative spectrophotometry and high performance liquid chromatographic methods were developed and validated for the assay of nefazodone hydrochloride and sertraline hydrochloride in pure forms and in pharmaceutical formulations, and works without solving equations or separation steps. The most striking feature of the derivative spectrophotometry is its simplicity and rapidity, no requiring time-consuming sample preparation such as filtration, degassing that are needed for HPLC procedure. The assay results obtained by these two methods are in fair agreement. In general, all the reported methods can be used for the routine quality control analysis of the investigated drugs in pharmaceutical preparations.

References

[1] J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, The Pharmacological Basis of Therapeutics, 9th ed., 1996, p. 454.
[2] P.V. Nickell, H.E. Ward, R.G. Booth (Eds.), Burger's Medicinal Chemistry and Drug Discovery, vol. 5, Wiley, New York, 1997, p. 183.
[3] J.E. Franc, G.F. Duncan, R.H. Farmen, K.A. Pittman, High-performance liquid chromatographic method for the determination of nefazodone and its metabolites in human plasma using laboratory robotics, J. Chromatogr. B Biomed. Sci. Appl. 570 (1991) 129–138.
[4] M. Yao, V.R. shah, W.C. Shyu, N.R. Srinivas, Sensitive liquid chromatographic-mass spectrometric assay for the simultaneous quantitation of nefazodone and its metabolites hydroxynefazodone m-chlorophenylpiperazine and triazole-dione in human plasma using single-ion monitoring, J. Chromatogr. B Biomed. Sci. Appl. 718 (1998) 77–85.
[5] M. Franklin, Determination of nefazodone and its metabolises in human plasma by high performance liquid chromatography with coulometric detection, J. Pharm. Biomed. Anal. 11 (1993) 1109–1113.
[6] S. Dodd, A. Buist, G.D. Burrows, K.P. Maguire, T.R. Norman, Determination of nefazodone and its pharmacologically active metabolites in human blood plasma and breast milk by high-performance liquid chromatography, J. Chromatogr. B Biomed. Sci. Appl. 730 (1999) 249–255.
[7] D.S. Rao, S. Geetha, M.K. Srinivasu, G.O. Reddy, LC determination and purity evaluation of nefazodone HCl in bulk drug and pharmaceutical formulations, J. Pharm. Biomed. Anal. 26 (2001) 629.
[8] G. Tournel, N. Houdret, V. Hedouin, M. Deveaux, D. Gosset, M. Lhermitte, High- performance liquid chromatographic method to screen and quantitate seven selective serotonin reuptake inhibitors in human serum, J. Chromatogr. B Biomed. Sci. Appl. 761 (2001) 147–158.
[9] A. Lucca, G. Gentilini, S. Lopez-Silve, A. Soldarini, Simultaneous determination of human plasma levels of four selective serotonin reuptake inhibitors by high-performance liquid chromatography, Ther. Drug Monit. 22 (2000) 271–276.
[10] H. Björk, F. Bengtsson, A simple method for routine TDM of the SSRI sertraline and desmethylsertraline in human plasma by HPLC, Eur. Neuropsychopharmacol. 7 (1997) S138.

[11] J. Patel, E.P. Spencer, R.J. Flanagan, HPLC of sertraline and norsertraline in plasma or serum, Biomed. Chromatogr. 10 (1996) 351–354.
[12] A.I.H. Adams, A.M. Bergold, Assay of sertraline in tablets and drug substance by liquid chromatography, J. Pharm. Biomed. Anal. 26 (2001) 505–508.
[13] K.M. Kim, B.H. Jung, M.H. Choi, J.S. Woo, K. Paeng, B.C. Chung, Rapid and sensitive determination of sertraline in human plasma using gas chromatography-mass spectrometry, J. Chromatogr. B Biomed. Sci. Appl. 769 (2002) 333–339.
[14] D. Rogowsky, M. Marr, G. Long, C. Moore, Determination of sertraline and desmethyl-sertraline in human serum using copolymeric bonded-phase extraction liquid chromatography and gas chromatography-mass spectrometry, J. Chromatogr. B Biomed. Appl. 655 (1994) 138–141.
[15] L.I. Bebawy, N. El-Kousy, J.K. Suddik, M. Shokry, Spectrophotometric determination of fluoxetine and sertraline using chloranil,2,3 dichloro-5,6 dicyano benzoquinone and iodine, J. Pharm. Biomed. Anal. 21 (1999) 133–142.
[16] A.F. Fell, G. Smith, Anal. Proc. 19 (1982) 28.