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SIMULTANEOUS EQUATION AND ABSORBANCE RATIO METHODS FOR ESTIMATION OF FLUOXETINE HYDROCHLORIDE AND OLANZAPINE IN TABLET DOSAGE FORM

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ABSTRACT

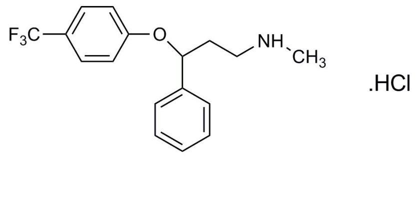
Fluoxetine hydrochloride (FH) and Olanzapine (OZ) combined tablet dosage form is used for the treatment of depressive episodes associated with bipolar disorder, called as manic depressive illness. Two spectrophotometric procedures for simultaneous estimation of FH and OZ from two component tablet dosage form have been developed. In the present investigation an attempt has been made to develop rapid and cost-effective procedures for simultaneous estimation of FH and OZ in pharmaceutical formulation. The first method is based on Vierodt's simultaneous equation method in which selected wavelengths are 225 nm and 258 nm, the wavelength maxima of FH and OZ, respectively. Additivity principle for the absorbances of FH and OZ at these selected wavelengths was validated. The second method is absorbance ratio method, wherein selected wavelengths are 236 nm and 258 nm, iso-absorptive point of both drugs and wavelength maximum of OZ, respectively. The adherence to Beer's law for both the drugs was checked by validating the linearity range at the selected wavelengths. The proposed methods were successfully applied to the estimation of FH and OZ in tablet formulations. The results suggested that the proposed procedures can be used for routine quality control of tablets containing FH and OZ.

Key words: *simultaneous equation, absorbance ratio, fluoxetine hydrochloride, olanzapine, tablets*

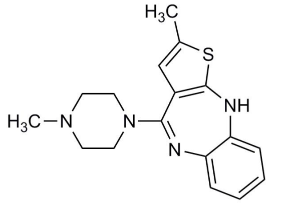
1. INTRODUCTION

Fluoxetine hydrochloride (FH) is a selective serotonin reuptake inhibitor which is one of the commonly prescribed drugs for treating depression [1]. Chemically, it is d,l-N-methyl-3-phenyl-3-[α,α,α-trifluoro-p-tolyl)oxy]propyl amine hydrochloride. A survey of literature revealed that spectrophotometric[2,3], spectrofluorimetric[4,5], electrochemical[6], gas chromatography[7] and liquid chromatography [8-10] methods are reported for determination of FH from pharmaceutical formulation. Olanzapine (OZ), 2-methyl-4-(4-methyl-1-piperazinyl)-10H[2,3-b][1,5]benzodiazepine, is an antipsychotic medicine[11]. Spectrophotometric[12-16] , linear voltametric[15], HPLC[15-17], capillary zone electrophoresis[16] methods are reported for determination of OZ from pharmaceutical dosage form. The chemical structures of FH and OZ are shown in Figure 1.

Figure 1 : Chemical structures of fluoxetine hydrochloride and olanzapine



Fluoxetine hydrochloride



Olanzapine

FH in combination with OZ is used in treatment of depressive episodes associated with bipolar disorder[18]. This combination of FH and OZ produce robust and sustained increases of extracellular levels of dopamine and norepinephrine, which were significantly greater than with either drug alone[19]. This combination drugs are available in tablet dosage form in the market. The combination of FH and OZ is not official in any pharmacopoeia. HPLC[20-23] and HPTLC[22,23] methods were reported in the literature for simultaneous determination of FH and OZ from combined dosage forms. However, the methods reported for simultaneous determination of these two drugs are found to be comparatively expensive and time consuming. No spectrophotometric method has been reported for the simultaneous determination of FH and OZ from combined dosage form. In the present investigation an attempt has been made to develop rapid and cost-effective procedures for simultaneous determination of FH and OZ in tablet dosage forms. The developed procedures are based on Vierodt's simultaneous equation and absorbance ratio methods. The proposed procedures were successfully applied for simultaneous determination of FH and OZ in tablet formulations that are available in market.

2. EXPERIMENTAL

2.1 Instrumentation

A double beam HEλIOS α UV/Visible spectrophotometer with two matched quartz cells of 1 cm path length was used for spectral measurements.

2.2 Chemicals and reagents

Fluoxetine hydrochloride (gift sample from Cadila Healthcare Pvt. Ltd., Ahmedabad, INDIA) and olanzapine (gift sample from Mangalam Organic Pvt. Ltd., Vapi, INDIA), hydrochloric acid (AR Grade, Finar Chemicals (India) Pvt. Ltd., Ahmedabad, India) and distilled water were used for the present study.

2.3 Preparation of stock solutions

FH powder (100 mg) was accurately weighed and transferred to a 100 mL volumetric flask. It was dissolved and

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*Corresponding author: vijaykparmar@gmail.com diluted to 100 mL with 0.1 N hydrochloric acid solution to obtain a stock solution of FH with final concentration of 1 mg/mL.

OZ powder (100 mg) was accurately weighed and transferred to a 100 mL volumetric flask. It was dissolved and diluted to 100 mL with 0.1 N hydrochloric acid solution to obtain a stock solution of OZ with final concentration of 1 mg/mL.

2.4 Preparation of working standard solutions

Stock solution of FH (10 mL) was transferred to a 100 mL volumetric flask and diluted to 100 mL with 0.1 N hydrochloric acid solution to obtain working standard solution of FH with final concentration of 100 µg/mL.

Stock solution of OZ (10 mL) was transferred to a 100 mL volumetric flask and diluted to 100 mL with 0.1 N hydrochloric acid solution to obtain working standard solution of OZ with final concentration of 100 µg/mL.

2.5 Procedure I – Simultaneous equation method

2.5.1 Wavelength selection

The standard solutions of FH (10 µg/mL) and OZ (2.5 µg/mL) were scanned in the range of 200 to 400 nm to record the spectra. The wavelength maxima of FH and OZ were determined from the spectra recorded.

2.5.2 Calibration curve for FH

Aliquots of working standard solution of FH (4, 8, 16, 24 and 30 mL) were transferred to series of 100 mL volumetric flask. The volume was made up to the mark with 0.1 N hydrochloric acid solution to obtain final concentration of 4, 8, 16, 24 and 30 µg/mL, respectively. The absorbance of each resulting solution was measured at 225 nm against 0.1 N hydrochloric acid solution. Calibration curve was constructed by plotting absorbance of FH against respective concentration. Regression equation for FH was calculated from calibration curve of FH.

2.5.3 Calibration curve for OZ

Aliquots of working standard solution of OZ (1, 2, 4, 8 and 12 mL) were transferred to series of 100 mL volumetric flask. The volume was made up to the mark with 0.1 N hydrochloric acid solution to obtain final concentration of 1, 2, 4, 8 and 12 µg/mL, respectively. The absorbance of each resulting solution was measured at 258 nm against 0.1 N hydrochloric acid solution. Calibration curve was constructed by plotting absorbance of OZ against respective concentration. Regression equation for OZ was calculated from calibration curve of OZ.

2.5.4 Validation of additivity principle

Working standard solutions of FH (16, 20, 24, 16, 20 and 24 mL) and OZ (4, 5, 6, 8, 10 and 12 mL) were transferred in series of 100 mL volumetric flasks, respectively. The mixture was shaken for 2 min and volume was made up to mark with 0.1 N hydrochloric acid solution. The absorbance of the resulting solutions was measured at 225 nm and 258 nm against 0.1 N hydrochloric acid solution. The observed values of absorbance were compared with values calculated from regression equations derived from calibration curves of FH and OZ at 225 and 258 nm, respectively.

2.6 Procedure II – Absorbance ratio method

2.6.1 Wavelength selection

The zero order spectra of FH and OZ were overlaid in spectrum SCAN mode. The iso-absorptive point was determined using TRACK function.

2.6.2 Calibration curve for FH

Aliquots of working standard solution of FH (8, 16, 20, 24 and 30 mL) were transferred to series of 100 mL volumetric flask. The volume was made up to the mark with 0.1 N hydrochloric acid solution to obtain final concentration of 8, 16, 20, 24 and 30 µg/mL, respectively. The absorbance of each resulting solution was measured at 236 and 258 nm against 0.1 N hydrochloric acid solution. Calibration curve was constructed by plotting absorbance of FH at selected wavelengths against respective concentration. Regression equations for FH were calculated from calibration curves of FH at 236 and 258 nm.

2.6.3 Calibration curve for OZ

Aliquots of working standard solution of OZ (2, 4, 8, 12 and 16 mL) were transferred to series of 100 mL volumetric flask. The volume was made up to the mark with 0.1 N hydrochloric acid solution to obtain final concentration of 2, 4, 8, 12 and 16 µg/mL, respectively. The absorbance of each resulting solution was measured at 236 and 258 nm against 0.1 N hydrochloric acid solution. Calibration curve was constructed by plotting absorbance of OZ at selected wavelengths against respective concentration. Regression equations for OZ were calculated from calibration curves of OZ at 236 and 258 nm.

2.7 Analysis of sample

Twenty tablets were accurately weighed and finely powdered. Tablet powder equivalent to 5 mg of OZ was accurately weighed and transferred to 100 mL volumetric flask and 20 mL of 0.1 N hydrochloric acid solution was added. The mixture was sonicated for 30 min, diluted to 100 mL with 0.1 N hydrochloric acid solution and filtered through Whatman filter paper No. 41. The absorbance of resulting solution was measured at 225 and 258 nm for simultaneous equation method and at 236 and 258 nm for absorbance ratio method. The concentration of FH and OZ were found by fitting values of absorbance in the corresponding equations of simultaneous equation method and absorbance ratio method.

3. RESULTS AND DISCUSSION

3.1 Simultaneous equation method

In this method, both the components (FH and OZ) were quantified using simultaneous equation to resolve the interference due to spectral overlapping of two components.

3.1.1 Wavelength selection

Wavelength maxima (λmax) of FH and OZ in 0.1 N hydrochloric acid solution was selected for generation of simultaneous equation. The λmax of FH and OZ were found to be 225 and 258 nm, respectively (Figure 2).

3.1.2 Linearity of FH and OZ

The linearity of FH at 225 nm was found to be in the range of 4-30 µg/mL. The co-relation co-efficient was found to be

Table 1 : Linearity data for FH at 225 nm and OZ at 258 nm (n=5)

FH			**OZ**		
Concentration (µg/mL)	**Absorbance (Mean± S.D.)**	**% C.V.**	**Concentration (µg/mL)**	**Absorbance (Mean± S.D.)**	**% C.V.**
4	0.165±0.005	2.68	1	0.081±0.006	4.82
8	0.319±0.004	1.15	2	0.153±0.008	4.00
16	0.616±0.005	0.84	4	0.299±0.006	1.88
24	0.919±0.003	0.31	8	0.593±0.007	1.11
30	1.135±0.003	0.31	12	0.849±0.003	0.32

Table 2 : Results of checking additivity principle for FH and OZ at 225 and 258 nm (n=5)

Concentration (µg/mL)		Observed absorbance		Calculated absorbance	
FH	**OZ**	**At 225 nm**	**At 258 nm**	**At 225 nm**	**At 258 nm**
16	4	0.759	0.349	0.767	0.360
20	5	0.941	0.437	0.959	0.450
24	6	1.128	0.512	1.151	0.540
16	8	0.991	0.635	0.979	0.667
20	10	1.246	0.814	1.224	0.834
24	12	1.495	0.962	1.469	1.001

Table 3. Absorption coefficients of FH and OZ

Absorption coefficient (ε)	In Lmol^{-1}cm^{-1}	In mLg^{-1}cm^{-1}
ε at 225 nm for FH	12000.77	347.00
ε at 236 nm for FH	4244.86	122.76
ε at 258 nm for FH	1152.89	33.30
ε at 225 nm for OZ	16582.50	530.60
ε at 236 nm for OZ	17585.16	562.83
ε at 258 nm for OZ	24000.00	768.00

0.9999. The linearity of OZ at 258 nm was found to be in the range of 1-12 µg/mL. The co-relation co-efficient was found to be 0.9996. The average linear regressed equations for the corresponding curves were y=0.0373x+0.0183 (FH) and y=0.0703x+0.0153 (OZ). The linearity data for FH at 225 nm and OZ at 258 nm are shown in Table 1.

3.1.3 Additivity principle

Validation of additivity principle for the absorbances of FH and OZ at 225 nm and 258 nm was performed by measuring the absorbances of mixed solution of FH and OZ in the concentration ranges of 16-24 µg/mL and 4-12 µg/mL, respectively. The observed absorbances were found to be closer to the calculated value (Table 2).

3.1.4 Simultaneous equation

Absorption coefficients were calculated for FH and OZ at 225 and 258 nm, the wavelength maxima of FH and OZ, respectively (Table 3). The simultaneous equations derived for determination of concentration of FH and OZ are (Equation-1) CFH = (εOZ1 × A225 – εOZ2 × A258) / (εFH2 × εOZ1 – εFH1 × εOZ2) and (Equation-2) COZ = (εFH2 × A225 – ε FH1 × A258) / (εFH2 × εOZ1 – εFH1 × εOZ2), respectively, where, CFH = Concentration of FH in mol/lit, COZ = Concentration of OZ in mol/lit, A225 = Absorbance of mixture at 225 nm, A258 = Absorbance of mixture at 258 nm, εFH1 = Absorption coefficient of FH at 225 nm, εFH2 = Absorption coefficient of FH at 258 nm, εOZ1 = Absorption coefficient of OZ at 225 nm, εOZ2 = Absorption coefficient of OZ at 258 nm.

Table 4 : Linearity data for FH at 236 nm and 258 nm (n=5)

Concentration (µg/mL)	FH at 236 nm		FH at 258 nm	
	Absorbance (Mean± S.D.)	**% C.V.**	**Absorbance (Mean± S.D.)**	**% C.V.**
8	0.099±0.003	3.096	0.020±0.003	4.782
16	0.195±0.004	2.065	0.040±0.002	4.330
20	0.245±0.006	2.388	0.051±0.002	4.035
24	0.296±0.004	1.472	0.065±0.004	6.153
30	0.367±0.006	1.680	0.078±0.003	3.846

Table 5 : Linearity data for OZ at 236 nm and 258 nm (n=5)

Concentration (µg/mL)	OZ at 236 nm		OZ at 258 nm	
	Absorbance (Mean± S.D.)	**% C.V.**	**Absorbance (Mean± S.D.)**	**% C.V.**
2	0.115±0.006	4.702	0.140±0.005	4.052
4	0.227±0.004	2.018	0.278±0.002	0.903
8	0.449±0.005	1.225	0.552±0.005	1.028
12	0.659±0.005	0.888	0.810±0.009	1.172
16	0.898±0.003	0.340	1.102±0.008	0.726

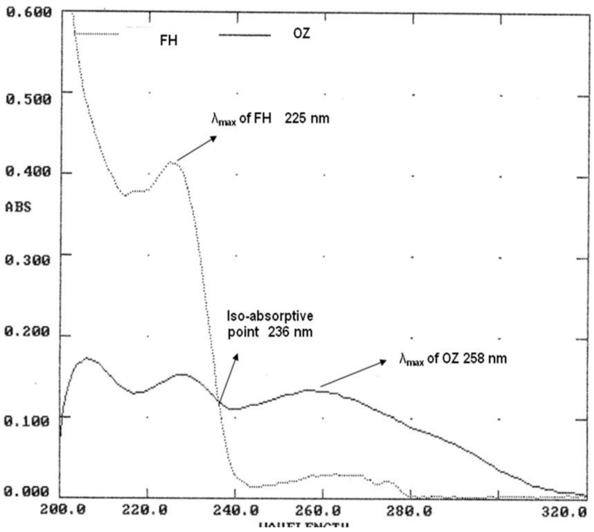
3.2 Absorbance ratio method

In Absorbance ratio method, absorbances were measured at the iso-absorptive wavelength and maximum absorption wavelength of one of the drugs.

3.2.1 Wavelength selection

The iso-absorptive point, wavelength at which FH and OZ show similar absorbance, was found to be 236 nm (Figure 2). The wavelength maximum of OZ, 258 nm, was selected as another wavelength for spectral measurement in absorbance ratio method.

Figure 2 : Overlain spectra of FH and OZ



3.2.2 Linearity of FH and OZ at selected wavelength

Regression analysis for the Absorbance ratio method was carried out and the linearity of the calibration graph and adherence of the method to Beer's law was validated for FH and OZ at selected wavelengths (236 nm and 258 nm). The linearity data for FH at 236 nm and 258 nm are shown in Table 4. The co-relation co-efficient was found to be 0.9999 and 0.9979, respectively. The linearity data for OZ at 236 nm and 258 nm are shown in Table 5. The co-relation co-efficient was found to be 0.9998 and 0.9998, respectively.

3.2.3 Absorbance ratio equation

The absorptivity coefficients of each at both wavelengths were determined (Table 3). The concentration of each drug in laboratory mixture and tablet formulation was determined by substituting the absorbance and absorptivity coefficients in following equations: Equation 3: Cx = (Qm-Qy) A / (Qx-Qy) Ax1 and Equation 4: Cy = (Qm-Qx) A / (Qy-Qx) Ay1, where, Cx = concentration of FH, Cy = concentration of OZ, Qm = ratio absorbance of sample at 236 and 258 nm wavelengths, Qx = ratio of absorptivity coefficient of FH, Qy = ratio of absorptivity coefficient of OZ, Ax1 = absorptivity coefficient of FH at 236nm, Ay1 = absorptivity coefficient of OZ at 236nm.

3.3 Analysis of Marketed Formulations

Quantitative determination of FH and OZ in tablets using proposed methods, simultaneous equation and absorbance ratio method, was performed and the results were in good agreement with the labeled amount of FH and OZ in (Table 6).

4. CONCLUSION

The proposed two procedures were developed and validated for quantitative determination of fluoxetine hydrochloride and olanzapine in tablets. The developed methods were found to be simple, rapid and economical. Results obtained for the analysis of marketed combined dosage forms were in good agreement with the labeled claim. The proposed methods can be utilized for the routine analysis of FH and OZ in tablet dosage form.

Table 6 : Assay of FH and OZ from combined tablet formulations

Tablet Formulation	Labeled value (mg)		Content of FH (% Label claim)		Content of OZ (% Label claim)	
	FH	OZ	SE$^{\#}$	AR$^{\$}$	SE	AR
OLEANZ®PLUS	20	5	103.21	97.59	100.95	98.27
OLEANZ®FORTE	20	10	101.95	99.87	101.46	97.24
OLAPADPLUS	20	5	98.64	97.64	100.33	98.64

#SE = Simultaneous equation method
$AR = Absorbance ratio method

References

[1] *Risley, D.S. and Bopp, R.J. (2005) Fluoxetine. In Analytical profiles of drug substances (Ed. Florey, K.), Academic Press, New York, 19, pp. 193-219.*

[2] Naik, M.T., Rokad, M.D. and Dhadke, P.M. (1999) Extractive spectrophotometric estimation of fluoxetine hydrochloride in pharmaceutical formulations. *Indian J Pharm Sci*, **61**: 156.

[3] Mandrioli, R., Pucci, V., Visini, D., Varani, G. and Raggi, M.A. (2002) Rapid methods for determination of fluoxetine in pharmaceutical formulations. *J Pharm Biomed Anal*, **29**: 1127.

[4] Prabhakar, A.H., Patel, V.B. and Giridhar, R. (1999) Spectrophotometric determination of fluoxetine hydrochloride in bulk and in pharmaceutical formulations. *J Pharm Biomed Anal*, **20:** 427.

[5] Berzas, J.J., Alañón, A. and Lázaro, J.A. (2002) Cyclodextrin enhanced spectrofluorimetric determination of fluoxetine in pharmaceuticals and biological fluids. *Talanta*, **58**: 301.

[6] da Silva, A.R., Lima, J.C., Oliva Teles, M.T. and Oliveira Brett, A.M. (1999) Electrochemical studies and square wave adsorptive stripping voltammetry of the antidepressant fluoxetine. *Talanta*, **49**: 611.

[7] *Berzas Nevado, J.J., Berzas Nevado, M.J., Contento Salcedo, A.M. and Aguas Nuevo, E.*J. (2000) Determination of Fluoxetine, Fluvoxamine, and Clomipramine in Pharmaceutical Formulations by Capillary Gas Chromatography. *Chromatogr Sci*, **38**: 200.

[8] El-dawy, M.A., Mokhtar, M.M. and El-dawy, F.A. (2002) Liquid chromatographic determination of fluoxetine. *J Pharm Biomed Anal*, **30**: 561.

[9] United States Pharmacopoeia 30, NF 25 (2007) United States Pharmacopeial Convention, Rockville, MD. pp. 2165-2170.

[10] British Pharmacopoeia (2007) Stationery Office, London. Vol. III, pp. 2593-2594.

[11] Howland, R.D. and Mycek, M. J. (2005) *Lippincott's illustrated reviews – Pharmacology*. B. I. Publication Pvt. Ltd., London, pp. 150-153.

[12] *Basavaiah, K., Zenita, O., Tharpa, K., Nagaraju, R., Anilkumar, U.R., Hiriyana, S.G. and Vinay, K.B. (2009)* Iodimetric assay of olanzapine in pharmaceuticals using iodate and Nile blue as reagents. *Chem Ind Chem Eng Quarterly*, **15**: 95.

[13] Krebs, A., Starczewska, B., Puzanowska-Tarasiewicz, H. and Sledz, J. (2006) Spectrophotometric determination of Olanzapine by its Oxidation with N- Bromosuccinimide and Cerium(IV)sulfate. *Anal Sci*, **22**: 829.

[14] Jasiñska, A. and Nalewajko, E. (2004) Batch and flow-injection methods for the spectrophotometric determination of olanzapine. *Anal Chim Acta*, **2**: 165.

[15] Biryol, I. and Erk, N. (2003) Voltammetric, spectrophotometric, and high performance liquid chromatographic analysis of olanzapine. *Anal Lett*, **36**: 2497.

[16] Raggi, M.A., Casamenti, G., Mandrioli, R., Izzo, G. and Kenndler, E. (2000) Quantitation of olanzapine in tablets by HPLC, CZE, derivative spectrometry and linear voltammetry. *J Pharm Biomed Anal*, **23:** 973.

[17] Prameela, R.A. and Bala, S.C. (2009) Development of HPLC method for the determination of Olanzapine in bulk and dosage forms. *Int J Pharm Tech Res*, **1**: 654.

[18] *Tohen, M., Vieta, E., Calabrese, J., Ketter, T.A., Sachs, G., Bowden, C., Mitchell, P.B., Centorrino, F., Risser, R., Baker, R.W., Evans, A.R., Beymer, K., Dube, S., Tollefson, G.D. and Breier, A. (2003)* Efficacy of Olanzapine and Olanzapine-Fluoxetine Combination in the Treatment of Bipolar I Depression. *Arch Gen Psychiatry, **60**: 1079.*

[19] Zhang, W., Perry, K.W., Wong, D.T., Potts, B.D., Bao, J., Tollefson, G.D. and Bymaster, F.P. (2000) Synergistic Effects of Olanzapine and Other Antipsychotic Agents in Combination with Fluoxetine on Norepinephrine and Dopamine Release in Rat Prefrontal Cortex. *Neuropsychopharmacology*, **23**: 250.

[20] Reddy, B.V., Reddy, S., Sreeramulu, J. and Kanumula, G.V. (2007) Simultaneous Determination of Olanzapine and Fluoxetine by HPLC. *Chromatographia*, 66: 111.

[21] Phatak, A. and Rajput, S.J. (2009) Development of a stability-indicating HPLC method for simultaneous determination of olanzapine and fluoxetine in combined dosage forms. *J Chromatogr Sci*, **47:** 605.

[22] Shah, C.R., Shah, N.J., Shuagia, B.N. and Patel, N. (2007) Simultaneous assay of olanzapine and fluoxetine in tablets by column high-performance liquid chromatography and high-performance thin-layer chromatography. *J AOAC Int*, **90**: 1573.

[23] Patel, S. and Patel, N.J. (2009) Simultaneous RP-HPLC and HPTLC estimation of fluoxetine hydrochloride and olanzapine in tablet dosage forms. *Indian J Pharm Sci*, **71**: 477.