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Zero Order and Area under Curve Spectrophotometric Methods for Determination of Fluoxetine Hydrochloride in Pharmaceutical Formulation

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ABSTRACT

Simple, fast and reliable spectrophotometric methods were developed for determination of Fluoxetine Hydrochloride in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in Distilled Water. The quantitative determination of the drug was carried out using the zero order derivative values measured at 226 nm and the area under the curve method values measured at 220-231 nm (n=2). Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Fluoxetine Hydrochloride using 5-25µg/ml (r^2=0.999 and r^2=0.997) for zero order and area under the curve spectrophotometric method. All the proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. Developed spectrophotometric methods in this study are simple, accurate, precise and sensitive to assay of Fluoxetine Hydrochloride in tablets.

Keywords: Fluoxetine Hydrochloride, UV visible spectrophotometry, AUC, Method Validation

INTRODUCTION

Fluoxetine hydrochloride, (±)- N-methyl-3-Phenyl-3-[(α,α,α-trifluoro- p -tolyl)] propylamine hydrochloride (Figure 1), is an antidepressant drug used for the handling of unipolar mental depression. Fluoxetine (FLX) is the most widely prescribed selective serotonin reuptake inhibitor antidepressant drug [1,2]. FXT has been shown to have comparable efficacy to tricyclic antidepressants but with fewer cardiovascular and anticholinergic side effects. It is also effective in treatment of the obsessive compulsive disorders. Fluoxetine is extensively metabolized by N-demethylation in liver into its active metabolite norfluoxetine. Fluoxetine hydrochloride is also used in a variety of disorders in addition to depression[3,4,5]. Beneficial responses have been reported in obsessive compulsive disorders, pain syndromes including diabetic neuropathy and fibrositis, panic disorders and nervous bulimia (American Hospital Formulary Service, Drug Information 93) [6,7].Literature survey revealed several analytical methods UV spectrophotometry [8,9] and HPLC[10] have been reported in bulk, pharmaceutical dosage form for determination of Fluoxetine Hydrochloride. To our notice, so far no UV- spectrophotometric method using Zero Order and Area under Curve (AUC) has been reported for the determination of Fluoxetine Hydrochloride in bulk and tablets. Hence an attempt has been made to develop new Zero Order and Area under Curve Spectrophotometric methods for estimation of Fluoxetine Hydrochloride in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

Molecular formula: $C_{17}H_{18}F_3NO$

Molecular weight: 309.32613 g/mol

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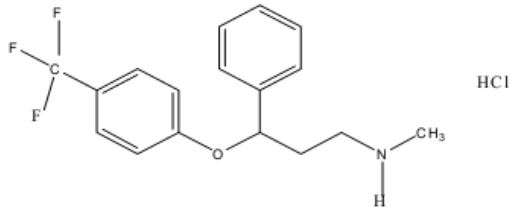

Fig. 1: Chemical Structure of Fluoxetine Hydrochloride

MATERIALS AND METHODS

Apparatus and instrumentation: A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India).Calibrated volumetric glassware (Borosil®) was used for the validation study.

Materials: Reference standard of Fluoxetine hydrochloride API was supplied as gift sample by Marksan Pharmaceutical Ltd., Verna, Goa. Tablet sample with label claim 500 mg per tablet were purchased from local market Pune.

Method development

Preparation of Standard and Sample Solutions: Stock solution of 10µg/ml of Fluoxetine hydrochloride was prepared in Distilled Water, for zero order and area under the curve spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with Distilled water in a concentration range of 5, 10, 15, 20, and 25µg/ml with Distilled water for zero order and area under the curve spectrophotometric methods. Distilled water was used as a blank solution.

Area under curve (Area calculation): Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between λ1 and λ2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 220 to 231 nm.

Area calculation: $(\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1 and λ2 are wavelength range start and end point of curve region[11].

Assay Procedure: Twenty tablets each containing 500 mg of Fluoxetine Hydrochloride were were weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Fluoxetine hydrochloride were was transferred in 100 ml of volumetric flask. A 50 ml of distilled water was added and sonicated for 15minutes. Then solution was further diluted up to the mark with distilled water. The solution was filtered using Whatmann filter paper no. 41; first 5 ml of filtrate was discarded. This solution was further diluted to obtain 15µg/mL solution with water subjected for UV analysis using Distilled water as blank. Appropriate dilutions were made with Distilled water from stock solution for both zero order and area under the curve spectrophotometric methods.

Table 1: Assay of tablet dosage form.

Sr. No	Sample Solution Concentration (µg/ml)	Amount found (%)* Zero derivative	Amount found (%)* Auc	Mean % Found zero derivative	Mean % Found Auc	%RSD zero derivative	%RSD Auc
1	15	101.39	100.71				
2	15	98.07	102.16	99.37	100.85	0.4987	0.5771
3	15	98.65	99.69				

*n=3, % RSD = % Relative Standard Deviation.

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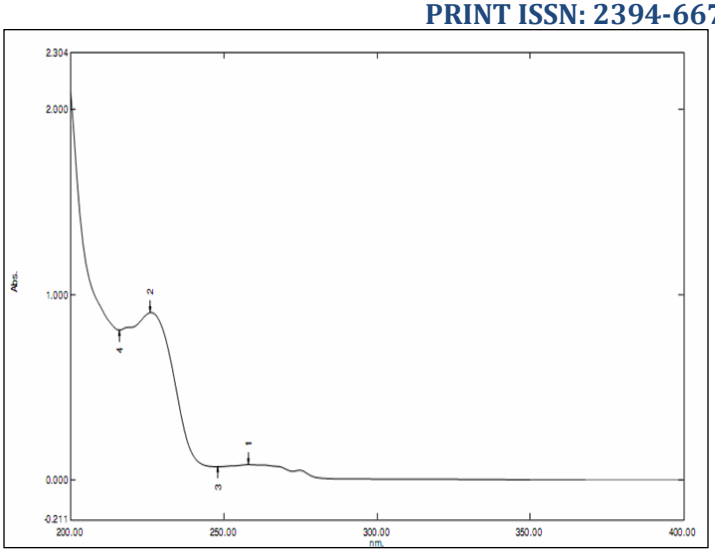

Fig. 2 Zero order derivative spectrum of Fluoxetine Hydrochloride in Distilled water (20µg/ml).

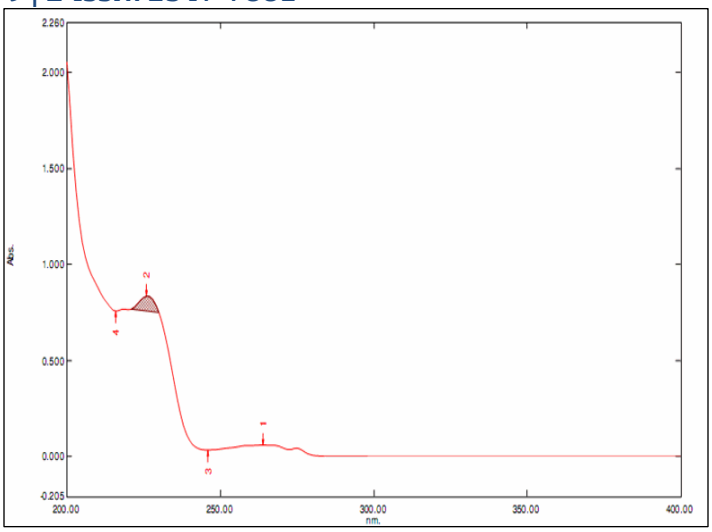

Fig. 3 UV AUC spectrum of Fluoxetine Hydrochloride Distilled water (20µg/ml).

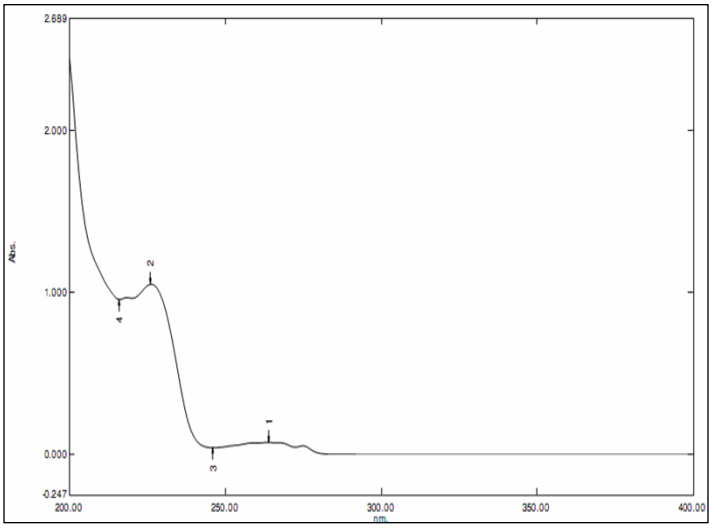

Fig. 4 Zero order derivative spectrum of Fluoxetine Hydrochloride dosage form (25µg/ml).

RESULTS AND DISCUSSION

The zero order and area under the curve spectra for Fluoxetine Hydrochloride were recorded at the wavelength of 226nm and 220-231 nm respectively [Fig. 2 and 3].

Linearity and Range:

Under the experimental conditions described, the graph obtained for zero order and area under the curve spectra showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y=0.054x+0.002 (r^2=0.999) at 226nm for zero order derivative spectrophotometry and y=0.053x+0.011 (r^2=0.9937) at 220-231nm for area under the curve spectrophotometry. The range was found to be 5-25µg/ml for both zero order and area under the curve spectrophotometric methods.

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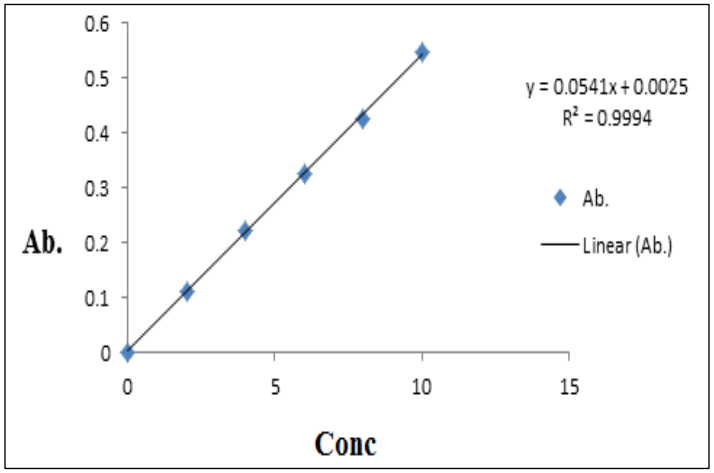

Fig.5 Linearity of Fluoxetine Hydrochloride by Absorbance.

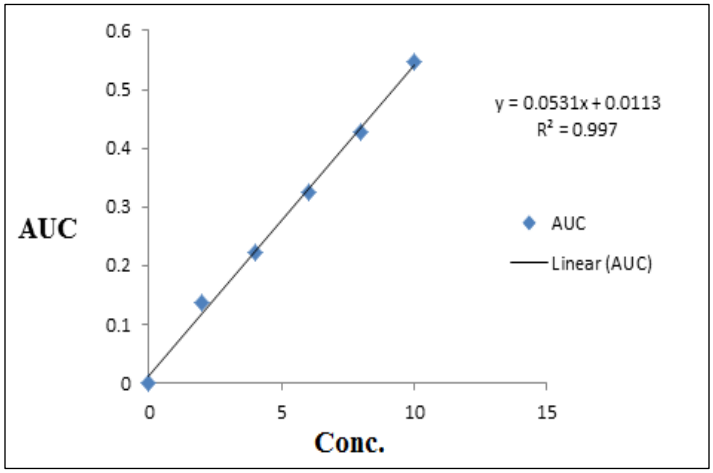

Fig.6 Linearity of Fluoxetine Hydrochloride by AUC.

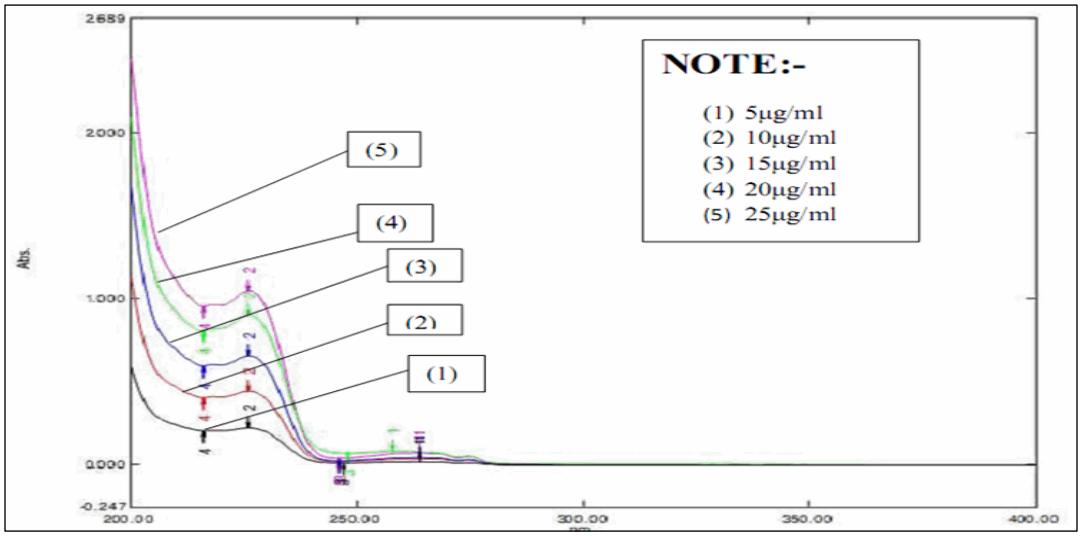

Fig. 7 Zero order derivative overlay of Fluoxetine Hydrochloride at 5,10,15,20 and 25 µg/ml Concentrations.

Table 2: Stastical data for the calibration graphs for determination of Fluoxetine Hydrochloride by Proposed methods.

Parameters	Zero order derivative	Area Under the Curve
Linearity range(µg/ml)*	5-25	5-25
r^2± S.D*	0.999	0.997

Accuracy

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 15µg/ml standard solution. For Area under curve (AUC) was measured in wavelength range 220-231 nm and For Zero order derivative at 226nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

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Table 3: Accuracy results for Fluoxetine Hydrochloride

Accuracy level	Sample conc (µg/ml)	Std. conc	Total amnt. Added (µg/ml)	%Recovery zero derivatie	% Recovery Auc*	Mean of Zero derivative*	Mean of Auc	% RSD Zero derivative	% RSD Auc
80	15	12	27	100.18	99.36				
100	15	15	30	102.92	100.29	100.57	100.16	0.716	1.149
120	15	18	33	98.63	100.83				

*n=3, % RSD = % Relative Standard Deviation.

Precision:

To determine the precision of the method, Fluoxetine Hydrochloride solutions at a concentration of 10µg/ml were analysed each three times for both zero order and area under the curve spectrophotometric methods. Solutions for the standard curves were prepared fresh everyday.

Table 4: Results of Intra and Inter Day Precision

Parameters	Intra Day Precision		Inter Day Precision	
	S.D*	% RSD*	S.D*	% RSD*
Zero derivative	0.0064	0.6617	0.0033	0.4114
Area under the curve	0.8374	0.5421	0.8612	1.6109

Sensitivity:

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3xσ/S and LOQ = 10xσ/S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.4731µg/ml and 1.3391µg/ml respectively for zero order derivative and The LOD and LOQ were found to be 0.9428µg/ml &2.8236µg/ml for area under the curve methods respectively.

Analysis of the Marketed Formulation:

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.37% and 100.85% zero order and area under the curve spectrophotometric methods respectively. It may therefore be inferred that degradation of Fluoxetine Hydrochloride had not occurred in the marketed formulations that were analysed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Fluoxetine Hydrochloride in pharmaceutical dosage form.

Table 5: Summary of validation parameters

Parameter	Zero derivative	AUC
λ range	200-400 nm	220-231nm
Regression Equation (y=mx+c)	Y=0.054x+0.002	Y=0.053x+0.011
Measured wavelength	226nm	226nm
Linearity range	5-25µg/ml	5-25µg/ml
Slope	0.054	0.053
Intercept	0.002	0.011
Correlation coefficient (R^2)	0.999	0.997

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Limit of Detection (LOD) µg/ml	0.4731	0.9428
Limit of Quantitation (LOQ) µg/ml	1.3391	2.8236
Accuracy (Mean % Recovery)	100.57	100.16
Precission (%RSD)	0.716	1.149

CONCLUSION

No UV or Area under Curve spectrophotometric methods have been described for the determination of Fluoxetine Hydrochloride. Therefore simple, fast and reliable derivative spectrophotometric methods were developed for the routine determination of Fluoxetine Hydrochloride. The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

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