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Spectrophotometric determination of fluoxetine hydrochloride in bulk and in pharmaceutical formulations

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Abstract

Two new rapid, sensitive and economical spectrophotometric methods are described for the determination of fluoxetine hydrochloride in bulk and in pharmaceutical formulations. Both methods are based on the formation of a yellow ion-pair complex due to the action of methyl orange (MO) and thymol blue (TM) on fluoxetine in acidic (pH 4.0) and basic (pH 8.0) medium, respectively. Under optimised conditions they show an absorption maxima at 433 nm (MO) and 410 nm (TB), with molar absorptivities of 2.12×10^{-4} and 4.207×10^{-3} l mol^{-1} cm^{-1} and Sandell's Sensitivities of 1.64×10^{-2} and 0.082 μg cm^{-2} per 0.001 absorbance unit for MO and TB, respectively. The colour is stable for 5 min after extraction. In both cases Beer's Law is obeyed at 1–20 μg mol^{-1} with MO and 4–24 μg mol^{-1} with TB. The proposal method was successfully extended to pharmaceutical preparations—capsules. The results obtained by both the agreement and E.P. (3rd edition) were in good agreement and statistical comparison by Student's *t*-test and variance ratio *F*-test showed no significant difference in the three methods.

Keywords: Fluoxetine hydrochloride; Spectrophotometry; Methyl orange (MO); Thymol blue (TB); Pharmaceutical formulations

1. Introduction

Fluoxetine hydrochloride, (±)-*N*-methyl-3-phenyl-3-[(α, α, α-trifluoro-*p*-tolyl)] propylamine hydrochloride, is a potent antidepressant agent. It is a selective serotonin re-uptake inhibitor which is clinically effective for the treatment of depression.

Fluoxetine hydrochloride is official only in European Pharmacopoeia [1], which suggests HPLC method for the estimation of fluoxetine in bulk. The different analytical methods that are reported for its determination include Gas chromatography [2–7], Gas Liquid chromatography [8], Liquid Chromatography [9] and High Performance Liquid Chromatography [10–13]. No spectrophotometric method has been reported till date for the estimation of fluoxetine. Hence, it was thought

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worthwhile to develop spectrophotometric method for the same.

In the present study, two colorimetric methods for the determination of fluoxetine in bulk and in its Pharmaceutical formulations are described. The methods are based on the yellow coloured ion-pair formation of fluoxetine with MO at pH 4.0 and with TB at pH 8.0. The absorbance measurements were made at λ_{max} 433 nm with MO and 410 nm with TB after extraction with chloroform. These methods are simple, rapid, sensitive, easy to apply in routine usage and do not need costly instrumentation.

2. Experimental

2.1. Apparatus

Absorbance measurements were made on Hitachi U-2000 UV-Visible Spectrophotometer (Germany make) with 10 mm matched quartz cells.

2.2. Reagents and solutions

All chemicals were of analytical reagent grade and solutions were prepared with doubly distilled water of I.P. [14] grade.

Pharmaceutical grade of fluoxetine hydrochloride was kindly gifted by Sun Pharmaceutical Advanced Research Centre (SPARC), Baroda, India, and certified to contain 99.11% of Fluoxetine hydrochloride. It was used without further purification.

2.2.1. Methyl orange (MO) standard solution (3.06×10^{-3} M)

A standard solution was prepared by dissolving 0.1 g of MO (E. Merck) in 20% alcohol and diluted to 100 ml with 20% alcohol.

2.2.2. Thymol blue (TB) standard solution (8.57×10^{-4} M)

A saturated solution was prepared by warming 0.1 g of TB with 4.3 ml of 0.05 M sodium hydroxide and 5 ml of 90% alcohol, and thereafter it was diluted to 250 ml with 20% alcohol.

2.2.3. Fluoxetine hydrochloride stock solution (2.9×10^{-3} M)

A stock solution was prepared by dissolving 0.1 g of fluoxetine hydrochloride in distilled water and diluting to 100 ml again with distilled water.

2.2.4. Phthalate buffer solution (pH 4.0)

Phthalate buffer solution of pH 4.0 was prepared by adding 0.1 ml of 0.2 M hydrochloric acid solution to 50 ml of 0.2 M potassium hydrogen phthalate solution in a 200 ml volumetric flask and making the volume with distilled water [14].

2.2.5. Phosphate buffer solution (pH 8.0)

Phosphate buffer solution of pH 8.0 was prepared by adding of 46.1 ml of 0.2 M sodium hydroxide solution to 50 ml of 0.2 M potassium dihydrogen phosphate in a 200 ml volumetric flask and making the volume with distilled water [14].

2.2.6. Chloroform (E. Merck)

Chloroform was purified as described by vogel [15] and used.

Table 1
Optimum conditions, Optical characteristics and statistical data of the regression equations for ion-pair complex formation with Fluoxetine hydrochloride

Parameters	MO	TB
Drug Aliquot (ml)	0.025–0.5	0.1–0.6
pH of Buffer	4.0	8.0
Amount of buffer (ml)	2.0	2.0
Amount of reagent (ml)	5.0	2.0
Absorption maxima (nm)	433	410
Beer's law limits (μg ml^{-1})	1–20	4–24
Apparent molar absorptivity (1 mol^{-1} cm^{-1})	2.12×10^{-4}	4.27×10^{-3}
Sandell's sensitivity (μg cm^{-2} per 0.001 A)	1.64×10^{-2}	0.082
Regression equation[a]		
Intercept (a)	3.91×10^{-2}	9.36×10^{-3}
Slope (b)	5.14×10^{-2}	1.121×10^{-2}
Correlation coefficient (r)	0.9994	0.9990

[a] $n = 45$ for MO; $n = 30$ for TB.

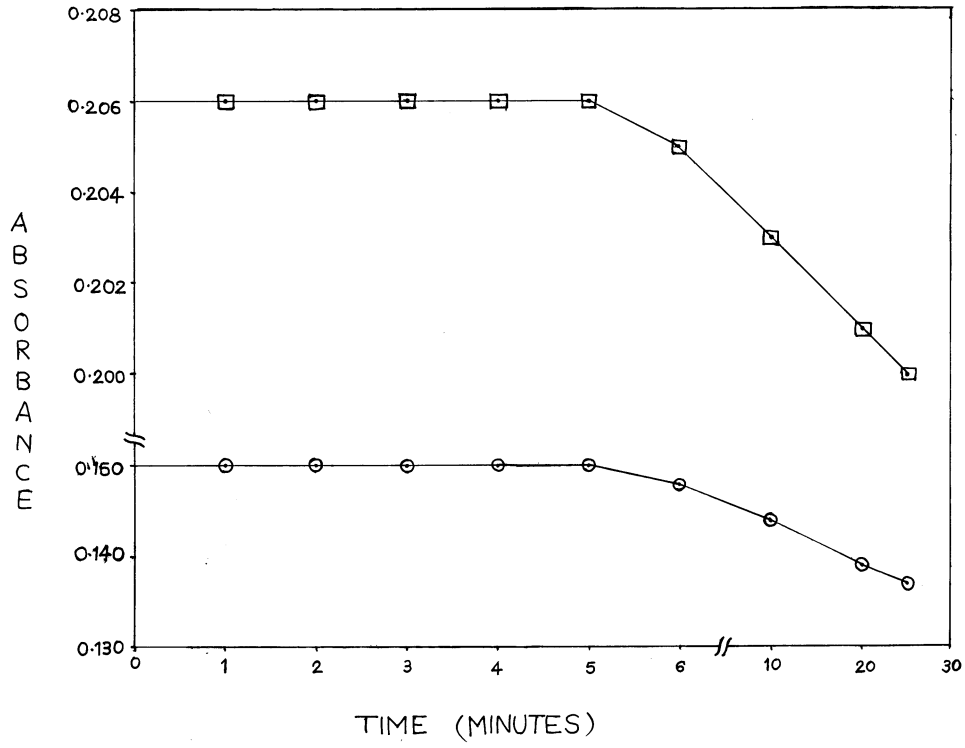

Fig. 1. Effect of time on the stability of yellow colour product after extraction. □, MO; ○, TB.

2.3. Procedure for calibration curve

Into a series of separating funnels, appropriate aliquots of the standard drug solution (Table 1) was pipetted out. To each funnel was added buffer and dye solutions as mentioned in Table 1. The solution was mixed thoroughly and successively extracted with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulphate and the volume was made to 25 ml with chloroform. The absorbance was measured within 5 min of extraction against their respective reagent blanks at the absorption maxima mentioned in Table 1. The determinations were repeated five times for each method.

2.4. Procedure for the assay of pharmaceutical capsules

Twenty capsules were emptied, weighed accurately and the contents were mixed thoroughly. A quantity of the powder equivalent to 20 mg of fluoxetine hydrochloride was dissolved in doubly distilled water with the aid of ultrasonics for 15 min. The solution was filtered through Whatman No. 42 filter paper into a 25 ml volumetric flask and diluted to the mark with distilled water. An appropriate aliquot was then taken in such a way that the final concentration in 25 ml flask lie within the range tested. The assay for fluoxetine content was completed both with MO and TB as described in Section 2.3.

3. Results and discussion

3.1. Optimization of parameters

Fluoxetine was found to yield a clear yellow product with MO in acidic medium (pH 4.0) and with TB in alkaline medium (pH 8.0), followed by extraction with chloroform having absorbance

Table 2
Evaluation of the accuracy and precision of the two proposed procedures

Compared method	Added[d]	Found ± SD[a]	RSD (%)	SAE[b]	Confidence limits[c]
MO	4.0	4.01 ± 0.039	0.973	0.017	4.01 ± 0.0484
	5.0	4.98 ± 0.042	0.843	0.019	4.98 ± 0.0521
	6.0	6.02 ± 0.064	1.063	0.029	6.02 ± 0.0795
Mean			0.960	0.022	
TB	4.0	3.98 ± 0.038	0.955	0.017	3.98 ± 0.0472
	5.0	5.01 ± 0.044	0.878	0.020	5.01 ± 0.0546
	6.0	6.00 ± 0.059	0.983	0.026	6.00 ± 0.0732
Mean			0.939	0.021	

[a] Mean ± standard deviation for five determinations.
[b] SAE, standard analytical error.
[c] Confidence limits at $P = 0.95$ and four degrees of freedom.
[d] Concentration in mg.

maxima at 433 nm and 410 nm respectively. The coloured product is due to ion-pair complex formation of the drug with the dye, MO and TB. Therefore, investigations were carried out to establish the most favourable conditions for the formation of the coloured product.

The influence of the concentration of reagent on the reaction has been studied. The effect of changing the concentration of MO (3.06×10^{-3} M) over the range of 1–10 ml and of TB (8.57×10^{-4} M) over the range of 1–10 ml was examined. It was observed that the absorbance started decreasing above 5 ml for MO and 2 ml for TB hence, 5 ml of 3.06×10^{-3} M MO and 2 ml of 8.57×10^{-4} M TB was used in further studies.

There was no effect of time on the stability of the colour (in both the methods) up to 5 min after extraction. However, a slight decrease in the absorbance was noted after this period. Therefore, it is recommended that the absorbance be measured within this time (Fig. 1).

3.2. Conformity with Beer's law

Beer's law is obeyed in the concentration (c, μg ml^{-1}) range 1–20 μg ml^{-1} of drug with MO and 4–24 μg ml^{-1} with TB. The optical characteristics such as Beer's law limits, molar absorptivities, Sandell's sensitivities [16] are recorded in Table 1. The regression analysis using the method of least square was made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations. The results are summarised in Table 1.

3.3. Selectivity

The selectivity of the method was checked by monitoring a standard solution of fluoxetine hydrochloride in the presence of other compounds of the capsules (excipients) at the same concentration levels as for the capsules. The response was not different from that obtained in the calibration. The absorbance values of solution of the excipients alone were measured too, at 433 nm with MO and at 410 nm with TB, showing no significant difference from the baseline. The excipients caused no effect upon the estimation of fluoxetine. Hence, the determination of the active compound of these pharmaceuticals, is considered to be free from interference due to excipients.

3.4. Precision and accuracy

In order to determine the precision and accuracy of the methods, solutions containing known amount of drug were prepared and analysed in five replicates. The analytical results obtained from these investigations are summarised in Table 2. The mean relative standard deviation (RSD) and the mean standard analytical error (SAE) can be considered to be very satisfactory.

Table 3
Determination of Fluoxetine hydrochloride in commercial capsules using the proposed procedures compared statistically with an official method

Formulation	Recovery ± SD (%)[b]		
	Proposed procedure		Official method[a]
	MO	TB	
Fludac[e] (Cadila, India)	20.10 ± 0.19	20.13 ± 0.25	19.99 ± 0.32
t[c]	0.661	0.771	
F[d]	2.84	1.64	
Prodep[e] (Sun, India)	20.30 ± 0.17	20.25 ± 0.35	20.37 ± 0.22
t[c]	0.563	0.649	
F[d]	1.68	2.53	
Oxedap[e] (Torrent, India)	20.19 ± 0.58	19.97 ± 0.44	20.00 ± 0.42
t[c]	0.593	0.110	
F[d]	1.91	1.10	
Prodac[e] (Searl, India)	19.96 ± 0.16	19.83 ± 0.25	19.91 ± 0.13
t[c]	0.542	0.635	
F[d]	1.52	3.70	
Nuzac[e] (Protec, India)	18.99 ± 0.65	19.02 ± 0.93	18.97 ± 0.72
t[c]	0.046	0.095	
F[d]	1.23	1.67	
Loftil[e] (S.G., India)	20.12 ± 0.28	19.78 ± 0.34	19.92 ± 0.22
t[c]	1.256	0.773	
F[d]	1.62	2.38	
Flunat[e] (Natco-fine, India)	19.98 ± 0.19	19.91 ± 0.37	20.17 ± 0.44
t[c]	0.886	1.011	
F[d]	5.36	1.41	
Trizac[e] (Unisearch, India)	20.28 ± 0.46	19.78 ± 0.59	19.99 ± 0.54
t[c]	0.914	0.587	
F[d]	1.38	1.19	

[a] E.P. [1].
[b] Mean ± standard deviation of five determinations.
[c] Tabulated t-value for $P = 0.05$ and eight degrees of freedom is 2.306.
[d] Tabulated F-value for $P = 0.05$ and $f1 = f2 = 4$ is 6.39.
[e] All the Fluoxetine capsules were labelled to contain 20 mg of Fluoxetine hydrochloride per capsule.

3.5. Application

The proposed methods for the determination of fluoxetine were applied to commercial capsules together with the official E.P. method. These determinations were carried out on the same batch of samples. The results obtained were compared statistically by Student's t-test and Variance ratio F-test (Table 3). The experimental values did not exceed the theoretical values in either test which indicates that there was no significant difference between the methods compared.

4. Conclusion

The yellow colour complex formed under the above mentioned conditions and measured

spetrophotometrically can be regarded as an ion-pair complex formation between the dye (MO, TB) and the drug. Compared with other reported methods, the proposed methods have the advantages of simplicity, sensitivity, reproducibility and it satisfies the need for a rapid procedure for the determination of fluoxetine hydrochloride in bulk and in its dosage forms. Hence, the proposed methods should be useful for routine quality control purposes.

Acknowledgements

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