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Development of New Analytical Method Validation for the Determination of Fluoxetine HCl in Bulk and Marketed Formulation by Colorimetric Method

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ABSTRACT

In the present work, simple, sensitive, rapid and accurate analytical method has been developed for the estimation of lamivudine in bulk and pharmaceutical dosage form. Method was based on reaction involving the formation of dark blue color complex between fluoxetine HCl and 0.02% crystal violet in the presence of 0.01M chloramine-T and 2M H_2SO_4, which obeyed Beer's law in the concentration range of 5 - 25 µg/ml at λmax of 603 nm. The correlation coefficient was found to be 0.9999. The methods were validated for linearity, sensitivity, accuracy, precision, LOD, LOQ, robustness.

Keywords: Fluoxetine HCl, Chloramine-T, Crystal violet, Alcohol, Colorimetric method.

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INTRODUCTION

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample. Fluoxetine hydrochloride is the first agent of the class of antidepressants known as selective serotonin-reuptake inhibitors (SSRIs). Despite distinct structural differences between compounds in this class, SSRIs possess similar pharmacological activity and formula is $C_{17}H_{18}F_3NO.HCl$ with molecular weight 309.33 g/mol. It is soluble in ethanol and dist. Water [1-4].

The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, and Limit of quantitation, Linearity and range, Ruggedness, Robustness.

Fluoxetine HCL

Fluoxetine HCl was determined spectrophotometrically in bulk and marketed formulation by using crystal violet dye and chloramine T (CT) as a strong oxidizing agent in presence of H_2SO_4.

Materials

The Chemicals and reagents used for experimental work are as follows.

FLU obtained from ELITE pharmaceuticals and YARROW pharmaceuticals

Instruments

Experiment was performed on JASCO V-630 series UV spectrophotometer and SHIMADZU 1700 with 1 cm path length matched glass cuvettes.

Preparation of standard stock solution of FLU

Standard stock solution was prepared by accurately weighing 100 mg of FLU in 100 ml calibrated volumetric flask and made up the volume with distilled water up to 100 ml.

Preparation of working standard solution of FLU

Working standard was prepared by transfer of 10 ml standard stock solution into 100 ml calibrated volumetric flask and made up the volume with distilled water to get concentration of 100µg/ml.

Preparation of Reagent

Preparation of 0.01M CT solution

Weighed accurately 0.280 gm. CT and transferred to 100 ml volumetric flask and made up the volume with distilled water.

Preparation of 2M H_2SO_4

10.8 ml of concentrated $H_2SO_4$was transferred to 100 ml volumetric flask and made up the volume with distilled water.

Preparation of crystal violet (0.02%)

Weighed accurately 20 mg crystal violet and added in 100 ml volumetric flask then diluted up to 100 ml with distilled water.

Preliminary Investigation

0.7 ml of 0.01M CT solution, 0.4ml of 2M $H_2SO_4$was transferred to 10ml volumetric flask and kept aside for 20 minutes for the completion of reaction. 1 ml of standard solution (100µg/ml) was added and kept aside for 15minutes for the completion of reaction. 0.3 ml of 0.02 % crystal violet solution was added and kept aside for 5 minutes and made up the volume with distilled alcohol. Absorbance was taken against blank at 603nm.

Parameter Fixation

Determination of absorbance maximum

An absorption maximum (or) λmax is the wavelength at which maximum absorption takes place. It is important to know the absorption maximum of the substance under study, since it helps to avoid any interfering impurities.

Procedure

0.5 ml of 0.01M CT solution, 0.7ml of 2M $H_2SO_4$was transferred to 10ml volumetric flask and kept aside for 20 minutes for the completion of reaction. 2 ml of standard solution (100µg/ml) was added and kept aside for 10 minutes for the completion of reaction. 0.2 ml of 0.02% crystal violet solution was added and kept aside for 5 minutes and made up the volume with distilled alcohol.

These solutions were scanned in U V spectrophotometer between 400-800 nm against blank.

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Figure No 1: Structure of fluoxetine HCl[3,4]

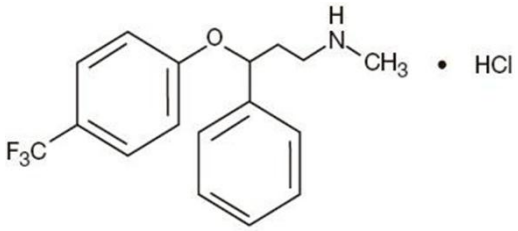
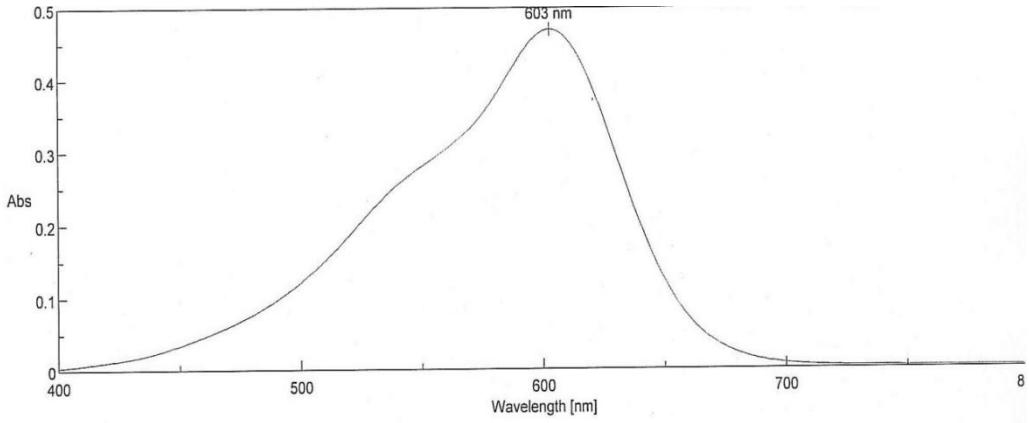


Figure No 2: λmax of FLU



Investigation

Experiments was carried out to ascertain the optimum concentrations of reagents needed for rapid and quantitative formation of dark blue colored species by measuring the absorbance of series of solutions in which one parameter was varied and others fixed.

Effect of concentration of chloramine T(CT)

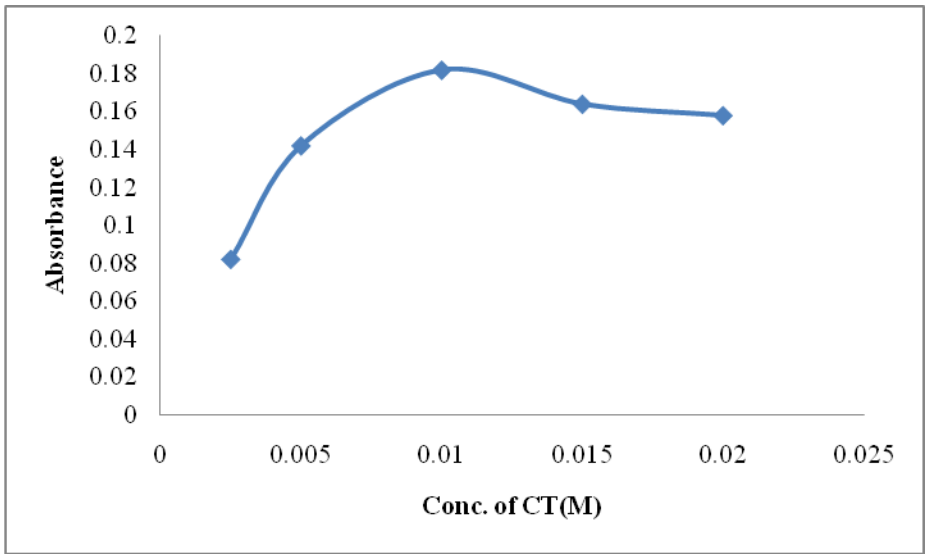
Different 5 volumetric flasks of 10ml was taken and 0.5 ml different conc. of CT solution, 0.7ml 2M $H_2SO_4$was added and kept aside for 20 minutes for the completion of reaction. 1 ml of standard solution (100µg/ml) was added into 10ml volumetric flask and kept aside for 10 minutes for the completion of reaction. 0.2 ml of 0.02% crystal violet solution was added and kept aside for 5 minutes and made up the volume with distilled alcohol. Absorbance was taken against blank at 603nm and recorded in Table no. 1 and Figure no. 3

ISSN: 0975-8585

Table No 1: Effect of Conc. of CT for FLU

SR.NO.	CONC. OF CT (M)	ABSORBANCE
1	0.0025	0.082
2	0.005	0.142
3	0.01	0.182
4	0.015	0.164
5	0.02	0.158

Figure No 3: Absorbance Vs Conc. of CT for FLU



CONCLUSION: Best absorbance found in 0.01M CT solution.

Effect of volume of chloramine T (CT)

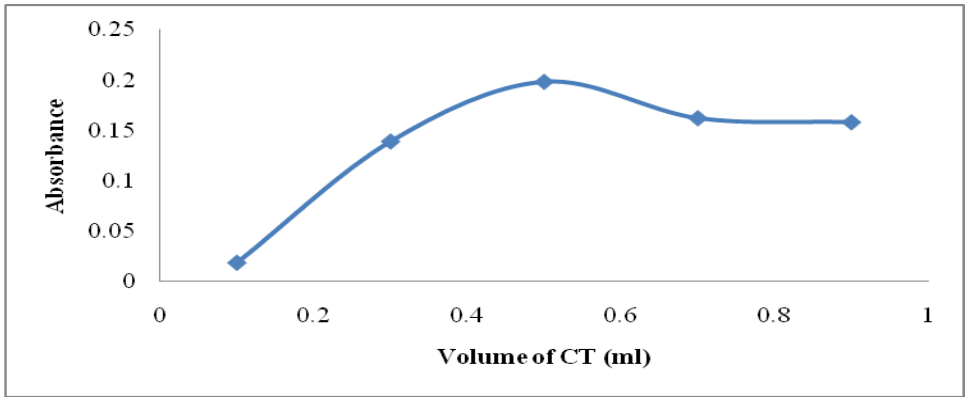
Different 5 volumetric flasks of 10ml was taken and different volume of 0.01M CT solution, 0.7ml 2M $H_2SO_4$was added and kept aside for 20 minutes for the completion of reaction. 1 ml of standard solution (100µg/ml) was added into 10ml volumetric flask and kept aside for 10 minutes for the completion of reaction. 0.2 ml of 0.02% crystal violet solution was added and kept aside for 5 minutes and made up the volume with distilled alcohol. Absorbance was taken against blank at 603nm against blank and recorded in Table no. 2 and figures no. 4.

Table No 2: Effect of Volume of 0.01M CT for FLU

SR.NO.	VOLUME OF 0.01M CT (ml)	ABSORBANCE
1	0.1	0.019
2	0.3	0.139
3	0.5	0.198
4	0.7	0.162
5	0.9	0.158

ISSN: 0975-8585

Figure No 4: Absorbance Vs Volume of CT for FLU



CONCLUSION: Best absorbance found in 0.5 ml of 0.01M CT solution.

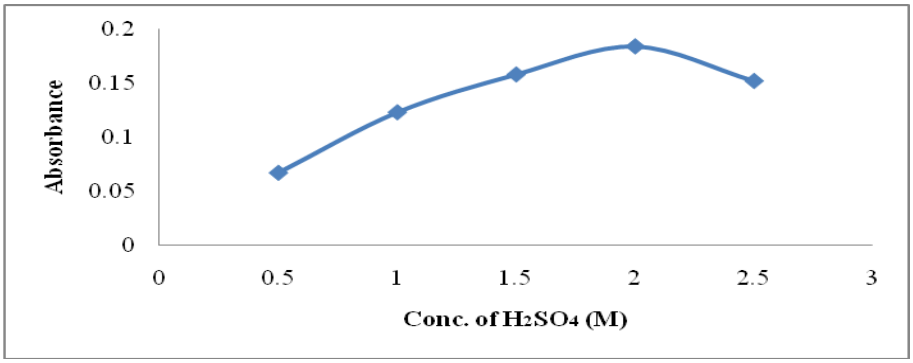
Effect of concentration of H_2SO_4

Different 5 volumetric flasks of 10ml was taken and 0.5 ml 0.01M CT solution, 0.7ml different conc. of $H_2SO_4$was added and kept aside for 20 minutes for the completion of reaction. 1 ml of standard solution (100µg/ml) was added into 10ml volumetric flask and kept aside for 10 minutes for the completion of reaction. 0.2 ml of 0.02% crystal violet solution was added and kept aside for 5 minutes and made up the volume with distilled alcohol. Absorbance was taken against blank at 603nm and recorded in Table no. 3 and Figure no. 5.

Table No 3: Effect of Conc. of H_2SO_4 for FLU

SR.NO.	CONC. OF H_2SO_4 (M)	ABSORBANCE
1	0.5	0.067
2	1	0.123
3	1.5	0.158
4	2	0.184
5	2.5	0.152

Figure No 5: Absorbance Vs Conc. of H_2SO_4 for FLU



CONCLUSION: Best absorbance found in 2M H_2SO_4.

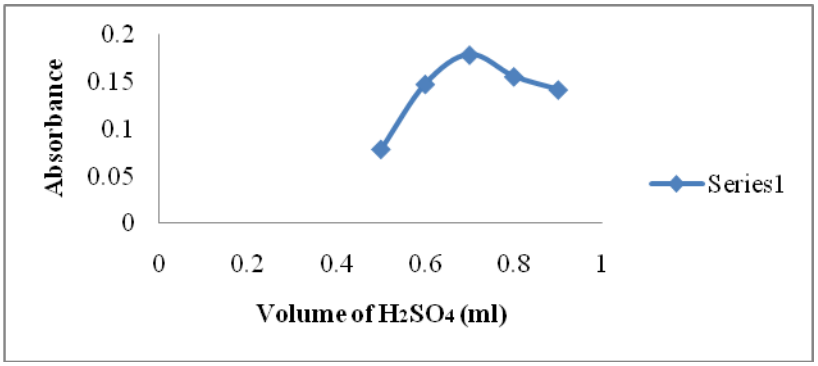
Effect of volume of 2M H_2SO_4

Different 5 volumetric flasks of 10ml was taken and 0.5 ml 0.01M CT solution, different volume of 2M $H_2SO_4$was added and kept aside for 20 minutes for the completion of reaction. 1 ml of standard solution (100µg/ml) was added into 10ml volumetric flask and kept aside for 10 minutes for the completion of reaction. 0.2 ml of 0.02% crystal violet solution was added and kept aside for 5 minutes and made up the volume with distilled alcohol. Absorbance was taken against blank at 603nm and recorded in Table No. 4 and Figure No. 6.

Table No 4: Effect of volume of 2M H_2SO_4 for FLU

SR.NO.	VOLUME OF 2M H_2SO_4(ml)	ABSORBANCE
1	0.5	0.079
2	0.6	0.148
3	0.7	0.179
4	0.8	0.156
5	0.9	0.142

Figure No 6: Absorbance Vs volume of 2M H_2SO_4 for FLU



CONCLUSION: Best absorbance found in 0.7 ml of 2M H_2SO_4.

Effect of concentration of crystal violet

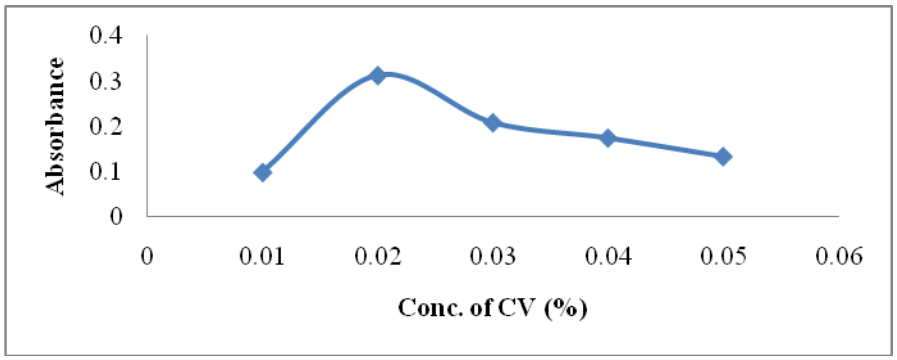
Different 5 volumetric flasks of 10ml was taken and 0.5 ml 0.01M CT solution, 0.7 ml 2M $H_2SO_4$was added and kept aside for 20 minutes for the completion of reaction. 1 ml of standard solution (100µg/ml) was added into 10ml volumetric flask and kept aside for 10 minutes for the completion of reaction. 0.2 ml of different conc. of crystal violet solution was added and kept aside for 5 minutes and made up the volume with distilled alcohol. Absorbance was taken against blank at 603nm and recorded in Table no. 5 and Figure no. 7.

ISSN: 0975-8585

Table No 5: Effect of Conc. of crystal violet for FLU

SR.NO.	CONC. OF CRYSTALVIOLET (%)	ABSORBANCE
1	0.01	0.098
2	0.02	0.312
3	0.03	0.208
4	0.04	0.174
5	0.05	0.133

Figure No 7: Absorbance Vs Conc. of crystal violetfor FLU



CONCLUSION: Best absorbance found in 0.02% conc. of crystal violet solution.

Effect of volume of crystal violet

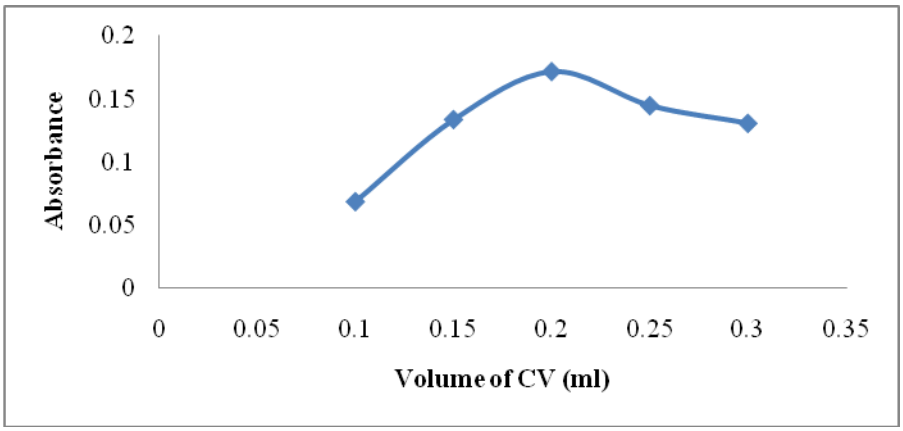
Different 5 volumetric flasks of 10ml was taken and 0.5 ml 0.01M CT solution, 0.7 ml 2M $H_2SO_4$was added and kept aside for 20 minutes for the completion of reaction. 1 ml of standard solution (100µg/ml) was added into 10ml volumetric flask and kept aside for 10 minutes for the completion of reaction. Different volume of 0.02% crystal violet solution was added and kept aside for 5 minutes and made up the volume with distilled alcohol. Absorbance was taken against blank at 603nm and recorded in table no. 6 and Figure no. 8.

Table No 6: Effect of volume of crystal violetfor FLU

SR.NO.	VOLUME OF CRYSTALVIOLET (ml)	ABSORBANCE
1	0.1	0.069
2	0.15	0.134
3	0.2	0.172
4	0.25	0.145
5	0.3	0.131

ISSN: 0975-8585

Figure No 8: Absorbance Vs Volume of crystal violet for FLU



CONCLUSION: Best absorbance found in 0.2 ml of (0.02%) crystal violet solution.

Stability of Color

0.5ml of 0.01M CT solution and 0.7 ml of 2M $H_2SO_4$was added into 10 ml volumetric flask and kept aside for 20 minutes for the completion of reaction.1 ml of standard solution (100µg/ml) was added and kept aside for 10 minutes for the completion of reaction. 0.2ml of 0.02% crystal violet was added in each volumetric flask and kept aside for 5 minutes then made up the volume with distilled alcohol. Take absorbance against blank at 603 nm. Then reading was taken for every 10 minutes intervals. The result is recorded in table no. 7 and Figure no 9 and 10.

Table No. 7: Stability study for FLU

SR.NO.	TIME IN MINUTES	ABSORBANCE (10µg/ml)	ABSORBANCE (20µg/ml)
1	10	0.2474	0.4652
2	20	0.2409	0.4609
3	30	0.2414	0.4593
4	40	0.2368	0.4629
5	50	0.2371	0.4689
6	60	0.2369	0.4589
7	70	0.2383	0.4623
8	80	0.2415	0.4655
9	90	0.2409	0.4668

ISSN: 0975-8585

Figure No. 9: Stability study for FLU

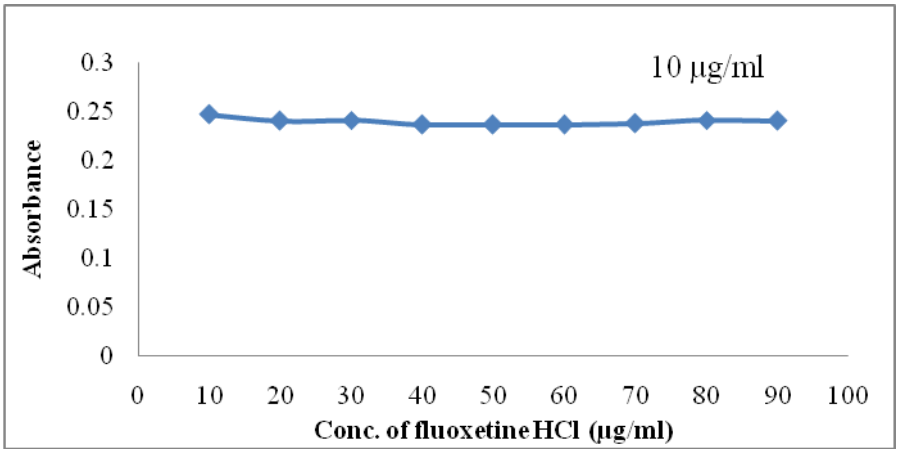
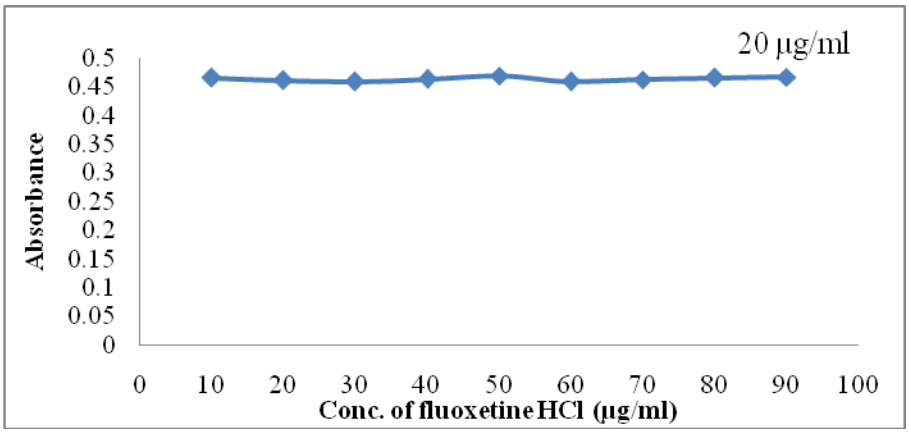


Figure No. 10: Stability study for FLU



CONCLUSION: The stability study of developed color was performed and from graph it was proved that color was stable above 1 hour.

Optical Characters

Determination of concentration range

For spectrophotometric analysis determination of the concentration range which obeys the Beer's- Lambert's law is necessary for accuracy and reproducibility.

Preparation of standard curve

Standard curve was prepared by using pure FLU in the Conc. range of 5-25 µg/ml by this method and selecting absorbance maximum at 603 nm.

ISSN: 0975-8585

Reagent and chemicals

- Working standard stock solution (100µg/ml)
- 0.01M Chloramine T
- 2M H_2SO_4
- 0.02% Crystal violet

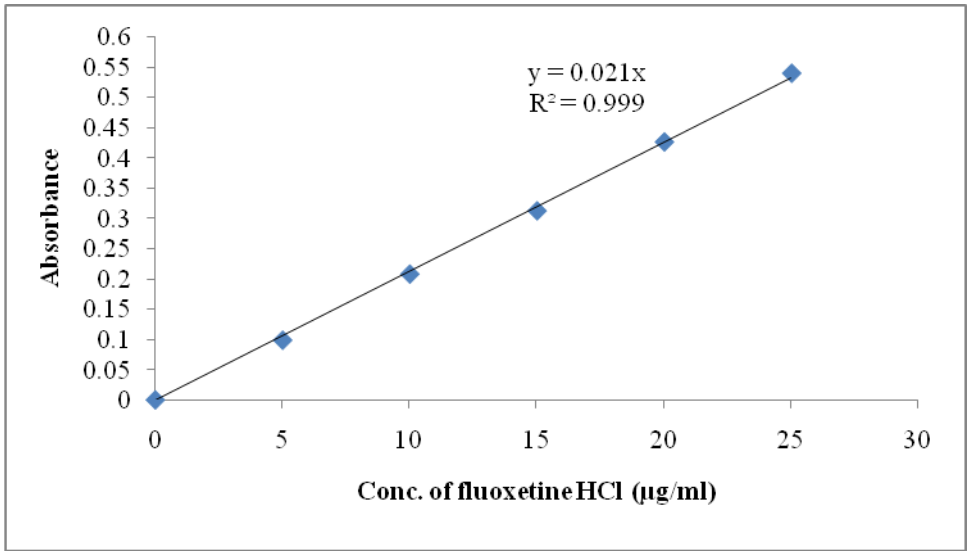
Procedure

5 volumetric flasks of 10 ml was taken and 0.5 ml of 0.01M CT and 0.7 ml of 2M H_2SO_4 was added, kept aside for 20 min. 0.5, 1, 1.5, 2 and 2.5ml of working standard of FLU were added in each volumetric flask and kept aside for 10 minutes. Then 0.2 ml 0.02% of crystal violet solution was added and kept aside for 5minutes and made up the volume with distilled alcohol. Absorbance was recorded against reagent blank at 603 nm. The result was recorded in Table no. 8 and Figure no. 11. The five such linearity was taken for regression co-efficient and eight such linearity was taken for standard deviation separately.

Table No. 8: Preparation of standard curve

SR.NO.	VOLUME OF WORKING STANDARD OF DRUG	CONCENTRATION OF DRUG (µg/ml)	ABSORBANCE
1	0.5	5	0.0986
2	1	10	0.2078
3	1.5	15	0.3124
4	2	20	0.4262
5	2.5	25	0.5397

Figure No. 11: Preparation of standard curve



ISSN: 0975-8585

Validation

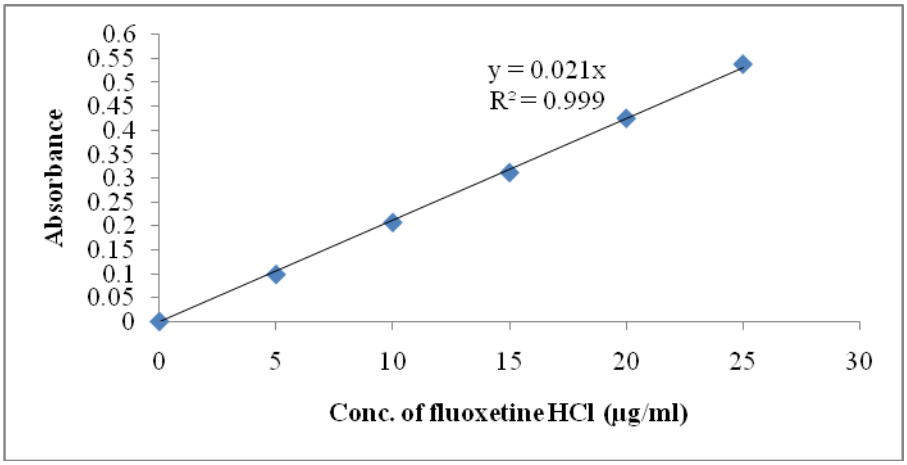
Linearity

Linearity was determined over the range of 5-25 µg/ml. 5 volumetric flasks of 10 ml was taken and 0.5 ml of 0.01M CT and 0.7 ml of 2M H_2SO_4 was added, kept aside for 20 min. 0.5, 1, 1.5, 2 and 2.5ml of working standard of FLU were added in each volumetric flask and kept aside for 10 minutes. Then 0.2 ml 0.02% of crystal violet solution was added and kept aside for 5minutes and made up the volume with distilled alcohol. Absorbance was recorded against reagent blank at 603 nm. The result was recorded in Table no 9 and Figure no 12.

Table No 9: Linearity data of FLU

SR. NO.	VOLUME OF WORKING STANDARD OF DRUG	CONC. OF DRUG (MARKETED FORMULATION) (µg/ml)	ABSORBANCE
1	0.5	5	0.0986
2	1	10	0.2078
3	1.5	15	0.3124
4	2	20	0.4262
5	2.5	25	0.5497

Figure No. 12: Linearity of FLU



Accuracy of Recovery Studies

The accuracy of the methods was determined by calculating % recovery of FLU by standard addition method. Known volumes of standard solutions of FLU were taken for recovery studies in 3 different levels 50, 100, 150% and recovery study was carried out. The three such samples were prepared and average of that readings taken for calculation of % recovery.It was mentioned in Table no. 11.

ISSN: 0975-8585

Table No 11: %Accuracy for FLU

DRUG	AMOUNT PRESENT (MARKETED FORMULATION) (µg/ml)	AMOUNT OF DRUG ADDED (BULK) (µg/ml)	AMOUNT OF DRUG RECOVERED (µg/ml)	% RECOVERY
FLU	10	-	9.96	-
		5	4.96	99.2
		10	9.95	99.5
		15	14.97	99.8

Precision

% Repeatability

System precision

The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 10 µg/ml without changing the parameters for the method. It was mentioned in Table no. 12.

Table No 12: %Repeatabilityfor FLU

CONCENTRATION (µg/ml)	ABSORBANCE	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	% RSD
10	0.206	0.2056	0.000521	0.00253	0.253 ± 0.000427
	0.206				
	0.205				
	0.206				
	0.205				
	0.206				

Method precision

The precision of the methods was checked by repeated measurement of the absorbance of marketed drug solutions (n = 6) of 10 µg/ml without changing the parameters for the method. It was mentioned in Table no. 13.

Table No 13: %Repeatability for FLU

CONCENTRATION OF DRUG (µg/ml)	ABSORBANCE	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	% RSD
10	0.205	0.2045	0.000547	0.00267	0.267 ± 0.000448
	0.205				
	0.204				
	0.204				
	0.205				
	0.204				

ISSN: 0975-8585

Intermediate precision

The intermediate precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 3) of 10 µg/ml by changing the instrument. It was mentioned in Table no. 14.

Table No 14: Intermediate precision for FLU

INTERMEDIATE PRECISION	Instrument 1	Instrument 2
CONCENTRATION (10µg/ml)	0.206	0.204
	0.206	0.205
	0.205	0.203
MEAN	0.2054	0.204
STANDARD DEVIATION	0.000583± 0.000673	0.001± 0.00115
%RSD	0.283	0.490

Reproducibility

Reproducibility expresses the precision between laboratories." The result was recorded in Table no. 15.

Table No 15: Reproducibility for FLU

REPRODUCIBILITY	SYSTEM PRESICION		METHOD PRICISION	
	Lab 1	Lab 2	Lab 1	Lab 2
CONCENTRATION OF DRUG (µg/ml)	10	10	10	10
ABSORBANCE	0.206	0.204	0.205	0.203
	0.206	0.205	0.205	0.203
	0.205	0.205	0.204	0.204
	0.206	0.204	0.204	0.203
	0.205	0.203	0.205	0.204
	0.206	0.205	0.204	0.202
MEAN	0.2056	0.2043	0.1241	0.20
STANDARD DEVIATION	0.000512	0.000602	0.000547	0.000756
COEFFICIENT VARIATION	0.00253	0.00294	0.00267	0.00372
% RSD	0.253 ± 0.000427	0.294 ± 0.000493	0.267 ± 0.000448	0.372 ± 0.000619

***At 95% confidence interval**

Stability of Solution

The intraday and interday precision of the proposed methods were performed by analysing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of FLU (5, 10,

ISSN: 0975-8585

15 µg/ml). Intraday precision was determined by analyzing drug for three times in the same day. Inter day precision was determined by analyzing the drug for three different days over a period of one week.The result was recorded in Table no. 16 and 17.

Table No 16: Intraday precision for FLU

CONCENTRATION (µg/ml)	INTRADAY (IN HOUR)	MEAN ABSORBANCE	STANDARD DEVIATION	COEFFICIENT VARIATION	% RSD
5	3	0.0953	0.000578	0.00606	0.606 ± 0.00066
	6				
	9				
10	3	0.3103	0.000578	0.00186	0.186 ± 0.00066
	6				
	9				
15	3	0.5376	0.000583	0.00108	0.108 ± 0.00067
	6				
	9				

Table No 17:Interday precision for FLU

CONCENTRATION (µg/ml)	INTERDAY (IN DAY)	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	% RSD
5	1	0.0933	0.000578	0.00620	0.620 ± 0.00066
	4				
	7				
10	1	0.309	0.001	0.00323	0.323 ± 0.00115
	4				
	7				
15	1	0.534	0.001	0.00187	0.187 ± 0.00115
	4				
	7				

LOD and LOQ

LOD and LOQ were calculated by using following formula and the result was recorded in Table no. 18

$$LOD = \frac{3.3\,\sigma}{S} \qquad LOQ = \frac{10\sigma}{S}$$

σ= standard deviation
s= slope of the calibration curve

Table No 18: LOD and LOQ for FLU

DRUG	LOD(µg/ml)	LOQ(µg/ml)
Fluoxetine HCl	0.117	0.354

Robustness

The robustness of FLU was determined with different suppliers (1) yarrow pharmaceuticals (2) Elite Pharma, Ahemdabad for the preparation of stock solution of standard drugs. The result was recorded in Table no.19.

Table No. 19: Robustness of FLU from two different suppliers

Different suppliers of bulk formulation	FLU Taken (mg)	FLU Obtained (mg)	% Recovery of FLU
Elite Pharma	10	9.93	99.37
Yarrow pharma	10	9.61	99.61

Recovery Experiments

Reagent and chemicals

- Working stock solution of marketed formulation(100µg/ml)
- 0.01M Chloramine-T solution
- 2M H2SO4
- 0.02% Crystal violet

Analysis of marketed formulation

Capsule is marketed as PRODEP capsule (100mg) manufactured by SUN PHARMA were taken for analysis.

Preparation of sample solution

Capsule powder equivalent to 100mg was weighed accurately and transferred to 100ml volumetric flask and made up the volume with distilled alcohol to get 1000µg/ml concentration. This solution was further diluted to get concentration of 100µg/ml. From this solution 2 ml working standard of LAM were added in volumetric flask and kept aside for 10 minutes. Then 0.2 ml 0.02% of crystal violet solution was added and kept aside for 5minutes and made up the volume with distilled alcohol. Absorbance was taken against blank at 603nm. The result was recorded in Table no 20.

ISSN: 0975-8585

Table No. 20: Recovery studies for FLU

TABLET	LABEL CLAIM CONC. (mg) FLU	ABSORBANCE AT 623 NM FLU (20µg/ml)	AMT OF DRUG FOUND IN CONC. FLU (mg)	%RECOVERY
PRODEP	20	0.424	19.89	99.46

RESULTS

The colorimetric methods obeyed Beer's Law in low concentration, which is an advantage in routine analysis.The results obtained by the proposed method were found to be satisfactory are mentioned in Table No. 21

Table No. 21: Result of Colorimetric analysis

METHOD	FLU
Wavelength	603 nm
Beer's range (µg/ml)	5-25µg/ml
Sandell's sensitivity(µg.cm^2/0.001AU)	0.0507
Molar absorptivity (l/mol.cm)	6.71×10^2
Correlation efficient(R^2)	0.9991
Slope	0.0213
Intercept	0.000
Regression Equation	y = 0.1065
% Recovery	99.46
LOD(µg/ml)	0.117
LOQ(µg/ml)	0.354

DISCUSSION

Two simple, sensitive, rapid and accurate colorimetric methods have been developed for the estimation of fluoxetine HCl in bulk and pharmaceutical dosage form.

Estimation of fluoxetine HCl is based on oxidation reaction, fluoxetine HCl is reacted with chloramine T a strong oxidizing agent in presence of H_2SO_4andit produced colorless complex of fluoxetine HCl. After completion of reaction known amount of crystal violet is added, and excess of chloramine T is reacted with crystal violetdye, oxidized it and produced leuco form of dye. Remaining unreacted molecules of crystal violet gives dark blue color.So the color of the final solution indicates the amount of drug present.

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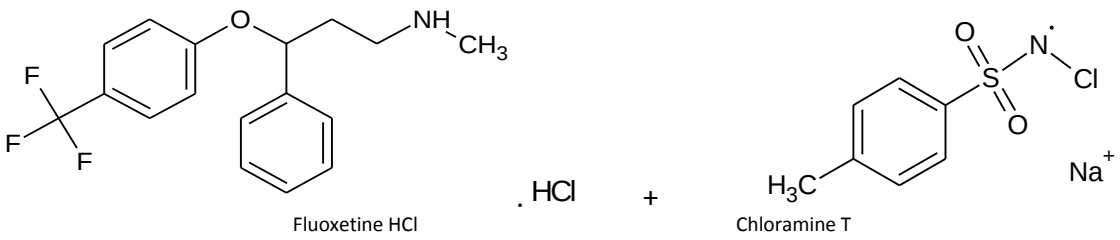
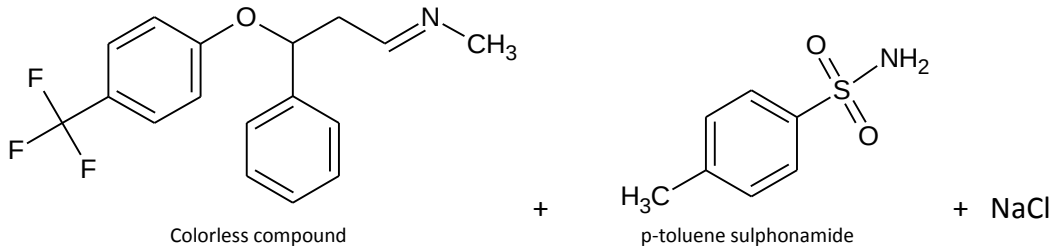
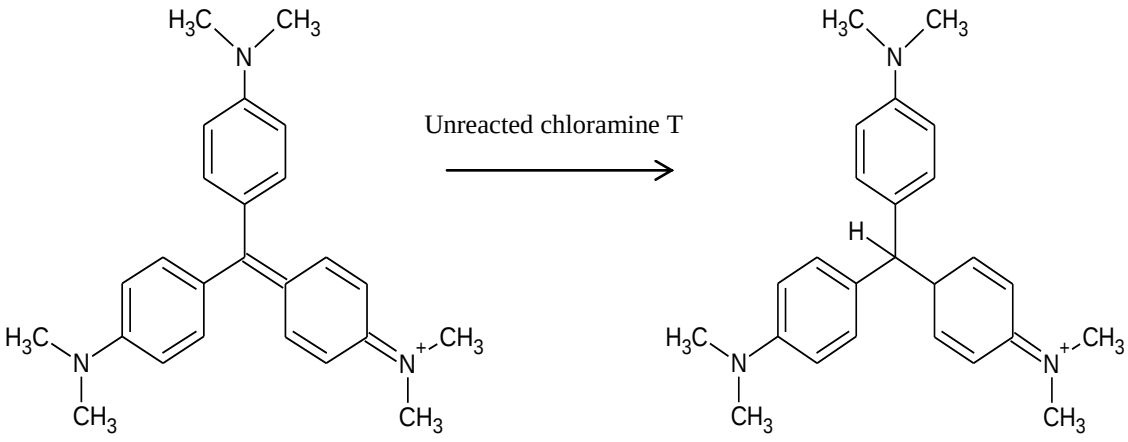


Fluoxetine HCl Chloramine T



Colorless compound p-toluene sulphonamide



Crystal violet Leuco form (CV) (Coloured)

CONCLUSION

Development of methods to achieve the final goal of ensuring the quantity of drug substances and drug products is not a trivial undertaking. It should be viewed as iterative process.

The colorimetric analysis demonstrated herein, are applicable to the estimation of FLU in pure as well as in existing dosage forms. In order to ensure that the data generated each of the above methods are both accurate and precise. The experiments have been performed on calibrated equipments using suitable reference standards.

To prove and documents the reliability of the methods, validation as per ICH guide lines have been carried out to a possible extent.

The capabilities of the methods are complementary to each other. Hence they can be regarded as simple, specific and sensitive methods for the estimation of FLU in pure and dosage forms.

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