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ABSTRACT

A simple, convenient analytical methods were developed for the estimation of Fluoxetine HCl (FLU) and Lamivudine (LAM) in bulk and pharmaceutical dosage forms. The method for Fluoxetine HCl is based on the reaction between FLU and crystal violet in presence of Chloramine T and Sulphuric Acid. The blue coloured complex obeyed the Beer-Lamberts law in the concentration range of 0-2.5µg/ml at λ-max 603nm. The correlation coefficient was found to be 0.9991. This method was validated for linearity, sensitivity, accuracy, precision, LOD, LOQ and robustness.In the case of Lamivudine (LAM) the colour reaction is based on reaction involving the formation of greenish blue complex between Lamivudine and malachite green in the presence of Chloromine T (CT) and sulphuric acid. It obeys the Beer-Lamberts law in the concentration range of 0.3-2.7 µg/ml at λ-max of 623nm. The correlation coefficient was found to be 0.9997. The method was validated for linearity, sensitivity, accuracy, precision, LOD, LOQ and robustness.

Keywords: Fluoxetine HCl, Lamivudine, Crystal violet, Malachite green, Chloramine T, LOD, LOQ.

1. INTRODUCTION :

Fluoxetine HCl [1, 2]is chemically N-methyl-3-phenyl-3-(4-(trifluoromethyl)phenoxy)propan-1-amine hydrochloride[3, 4]. It is an anti-depressant with selective serotonin – reuptake inhibitor action.

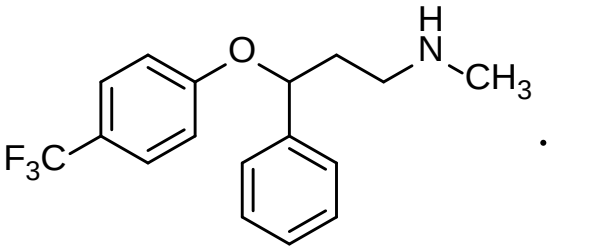


Fig.1: Structure of Fluoxetine HCl

Lamivudine[5, 6] is chemically4-amino-1-((2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl) pyrimidin-2(1H)-one[7,8]. It is a potent nucleoside analogue with reverse transcriptase inhibitor action (n RTI). It is used for the treatment of chronic hepatitis B. It is widely used and well tolerated. It is also used to inhibit both type 1 and 2 of HIV reverse transcriptase.

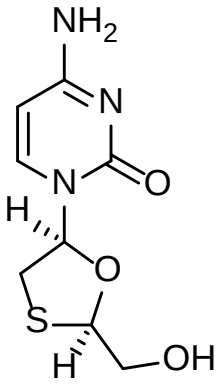


Fig 2: Structure of Lamivudine

2. MATERIALS AND METHODS :

Instrument: UV-Visible Spectrophotometer, JASCO V-360, Shimadzu 1700, with one pair of matched cells.

Reagents and chemicals: Fluoxetine HCl and Lamivudine in bulk form and marketed sample, Chloramine T (0.01M), Sulphuric Acid (2M), Crystal violet (0.02%), Malachite

green (0.02%).

Standard stock solution of FLU: 100mg of pure FLU was accurately weighed and taken in a 100ml calibrated volumetric flask, dissolved in distilled alcohol and the final volume was made upto 100ml.

Working standard solution of FLU: 10ml of standard stock solution was taken in 100ml calibrated volumetric flask, dissolved in distilled alcohol; final volume was made up to 100ml. The final concentration was100µg/ml.

Standard stock solution of LAM: 100 mg of pure LAM was weighed accurately and taken in a 100 ml calibrated volumetric flask, dissolved in distilled alcohol and final volume was made up to 100 ml.

Working standard solution of LAM: 10 ml of standard stock solution was taken in a 100 ml calibrated volumetric flask.It was dissolved in distilled alcohol and final volume was made up to 100 ml. The final concentration was 100µg/ml.

Preparation of 0.01 M Chloramine T solution: Weighed accurately 0.280 gm of CT and transferred to 100 ml volumetric flask and made up the volume with distilled water.

Preparation of 2M sulphuric acid: 10.8 ml of con. Sulphuric acid was transferred to 100 ml volumetric flask and made up the volume with distilled water.

Preparation of malachite green solution: Weighed accurately 20mg of malachite green and added in 100 ml volumetric flask. It was diluted to 100 ml with distilled water.

Determination of absorption maximum (λ-max) of FLU:1ml of working standard solution (100µg/ml) was taken in 10ml volumetric flask, to that 0.7ml of 0.01M CT solution, 0.4ml of 2M Sulphuric Acid was added, the reactants were allowed to react for 20 minutes. 0.3ml of 0.02% crystal violet solution was added after 5 minutes, the final volume was made up with distilled alcohol. The solution was used to determine the absorption maximum (λ-max). The absorption maximum was found to be 603nm.

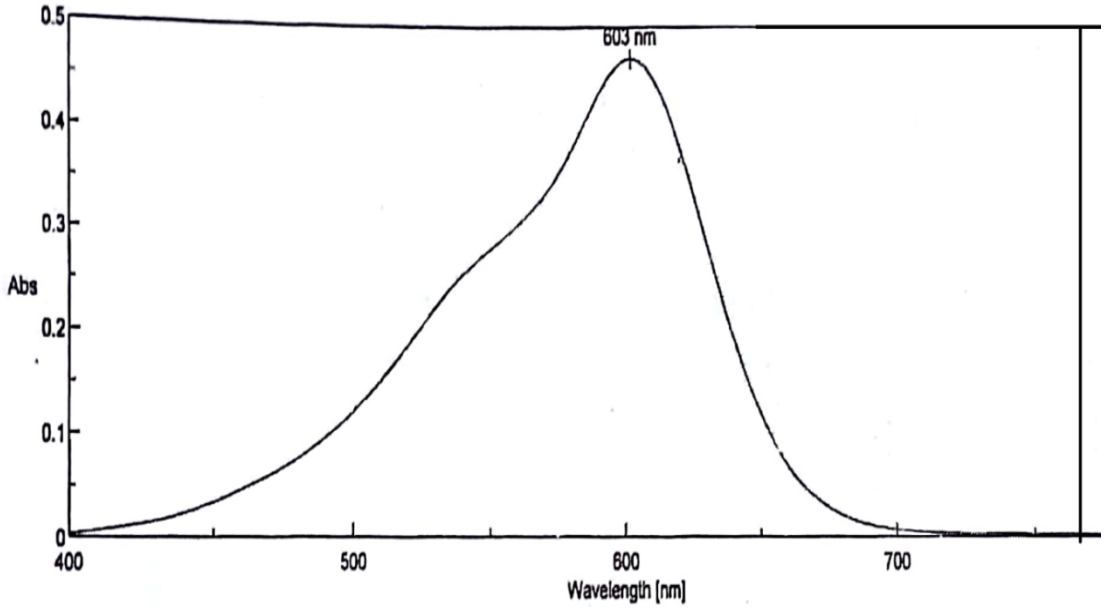


Fig. 3: λ-max of FLU

Determination of absorption maximum (λ-max) of LAM: 1ml of working standard solution (100µg/ml) was taken in a 10ml volumetric flask to that 0.7ml of 0.01M CT solution, 0.5 ml of 2 M H_2SO_4 was added and kept aside for 20 minutes to allow the reactants to react with LAM. 0.4ml of 0.02 % malachite green was added and kept aside the reaction mixture for 15 minutes. Later,it was made up to the volume with distilled alcohol. The solution was used to determine the absorption maximum (λ-max) the absorption maximum was found to be 623nm.

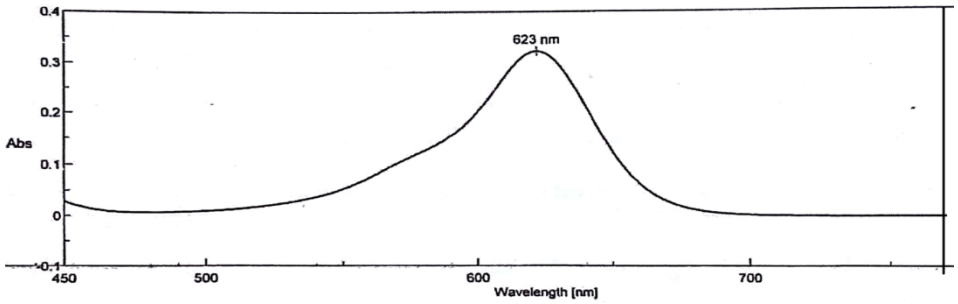


Fig. 4: λ-max of LAM

Optimum concentrations of all the reagents used in this study were determined to get the stable blue colour complex for the determination of concentration of range of FLU and LAM as per the Beer-Lamberts law [17, 18].

2.1 Stability of Colour Complex FLU:

Colour was developed using the reagents and procedure as indicated above. The absorbance readings were recorded for every 10minute interval upto 90 minutes. It was observed that colour complex was stable upto 90 minutes in the concentration of 10µg/ml and 20 µg/ml.

Table 1: Stability study of colour complex 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

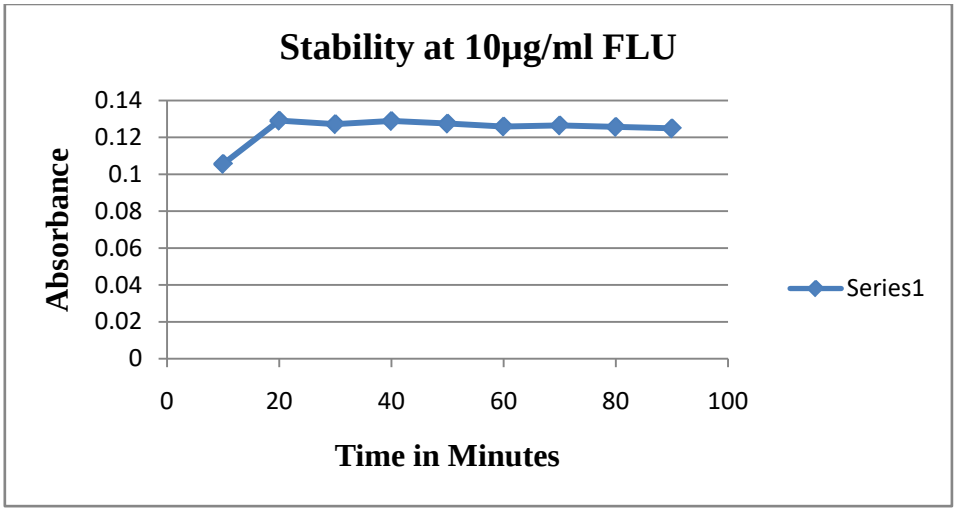


Fig. 5: Stability study for FLU (10µg/ml)

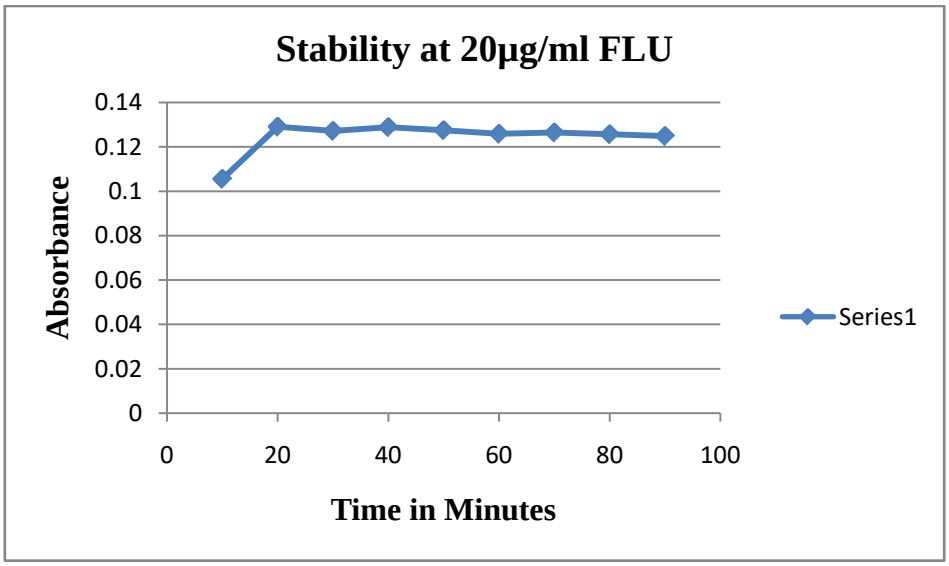


Fig. 6: Stability study for FLU (20µg/ml)

2.2 Stability of colour complex LAM:
Colour was developed using the reagents and procedure as indicated above, absorbance reading was recorded for every 10 minute intervals upto 90 minutes. It was observed that coloured complex was stable for 90 minutes in the concentration of 6µg/ml and 12µg/ml.

Table 2: Stability studies of colour complex for LAM

Sl.No.	TIMEIN Min	ABSORBANCE 6 µg/ml	ABSORBANCE 12 µg/ml
1	10	0.1056	0.2213
2	20	0.1291	0.2594
3	30	0.1272	0.2589
4	40	0.1289	0.2584
5	50	0.1275	0.2574
6	60	0.1259	0.2566
7	70	0.1265	0.2575
8	80	0.1257	0.2543
9	90	0.1249	0.2568

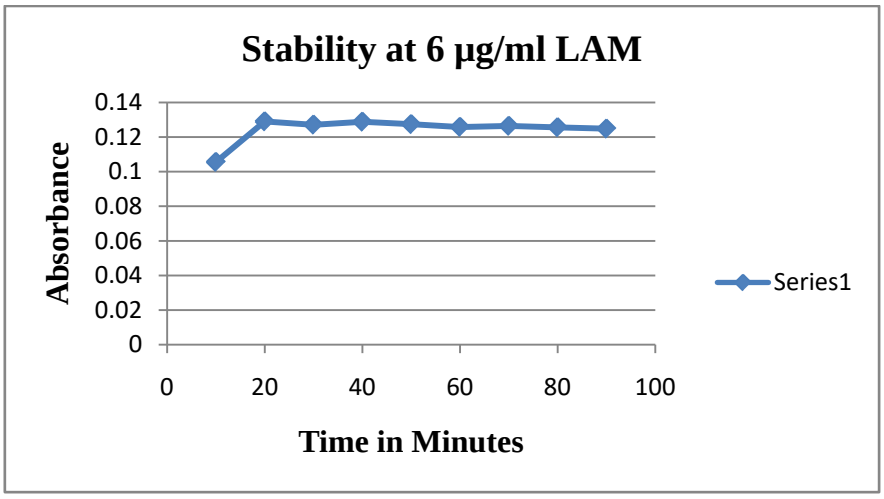


Fig. 7: Stability study for LAM (6µg/ml)

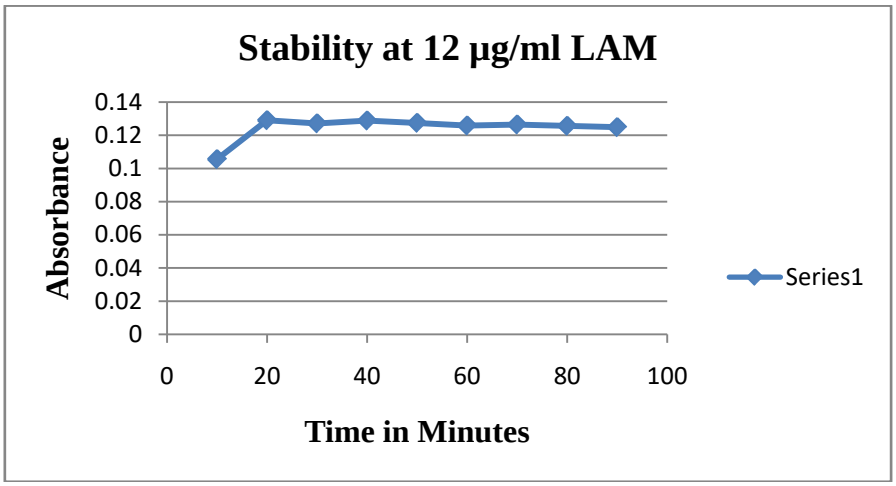


Fig. 8: Stability study for LAM (12 µg/ml)

2.3 Determination of linearity and calibration graph for FLU:

Standard graph was obtained in the concentration range of 0.5-2.5µg/ml of FLU at 603nm. 5 volumetric flasks of 10ml capacity was taken, to each flask 0.5ml of 0.1M CT, 0.7ml 2M H_2SO_4 followed by 0.5,1.0,1.5,2.0 and 2.5ml working standard solution of FLU. The flasks were kept aside for some time. Then to each flask 0.2ml of 0.02% crystal violet solution was added and made up the volume with distilled alcohol. Absorbance values were recorded against reagent blank at 603nm.

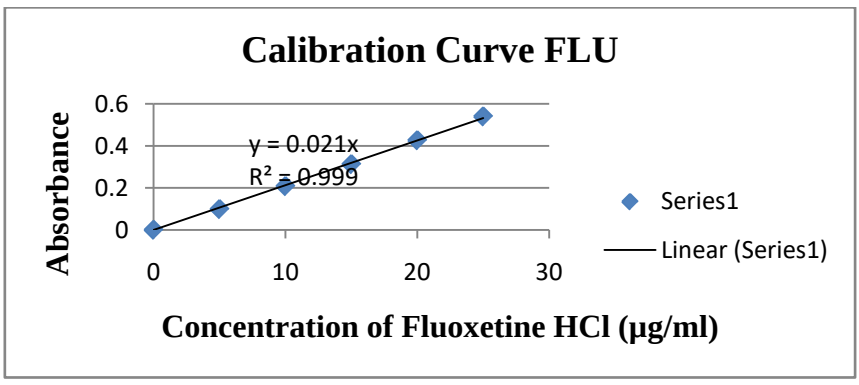


Fig. 9: Standard calibration curve of FLU at 603nm

2.4 Determination of linearity and calibration graph for LAM [15]:

Standard calibration graph [16] was obtained in the concentration range of 0.3-2.7 µg/ml of LAM at 623nm. Nine volumetric flasks of 10ml capacity was taken, to each flask 0.5 ml of 0.01M CT, 0.7 ml of 2 M H_2SO_4 was added, kept aside for 20 minutes.0.3,0.6,0.9,1.2,1.5,1.8,2.1,2.4 and 2.7 ml of working standards of LAM was added to each flask and kept aside for 10 minutes. The 0.2 ml of 0.02% of malachite green solution was added, kept aside for 10 minutes, made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 623 nm.

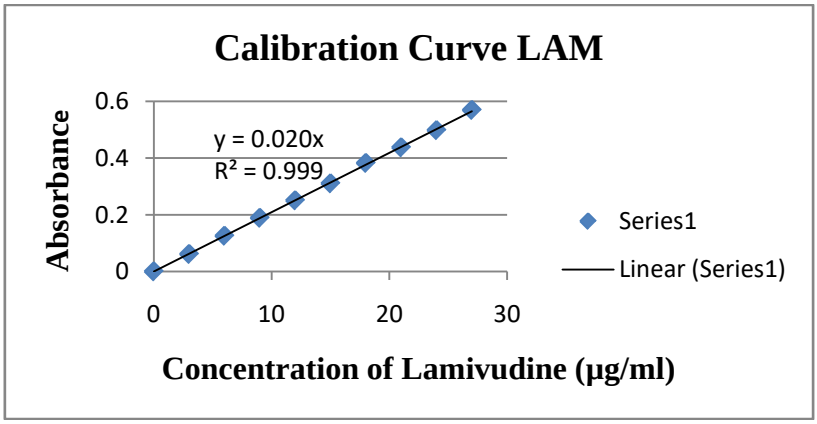


Fig 10: Standard calibration curve of LAM at 623nm

3. VALIDATION :[9,10,11]

These include study of specificity, selectivity, optimum concentration of reactants, linearity, LOD and LOQ. The parameters are summarised in table 3 and 4.

Table 3: Summary of Validation Parameters of FLU

Parameters	Fluoxetine HCL
Wavelength λ (nm)	603
Beer-Lamberts Limit (µg/ml)	0.5-2.5
Limit of Detection (µg/ml)	0.117
Limit of Quantitation (µg/ml)	0.354
Slope (b)	0.0213
Correlation Coefficient	0.9991
Relative Standard Deviation	0.372

Table 4: Summary of Validation parameters of LAM

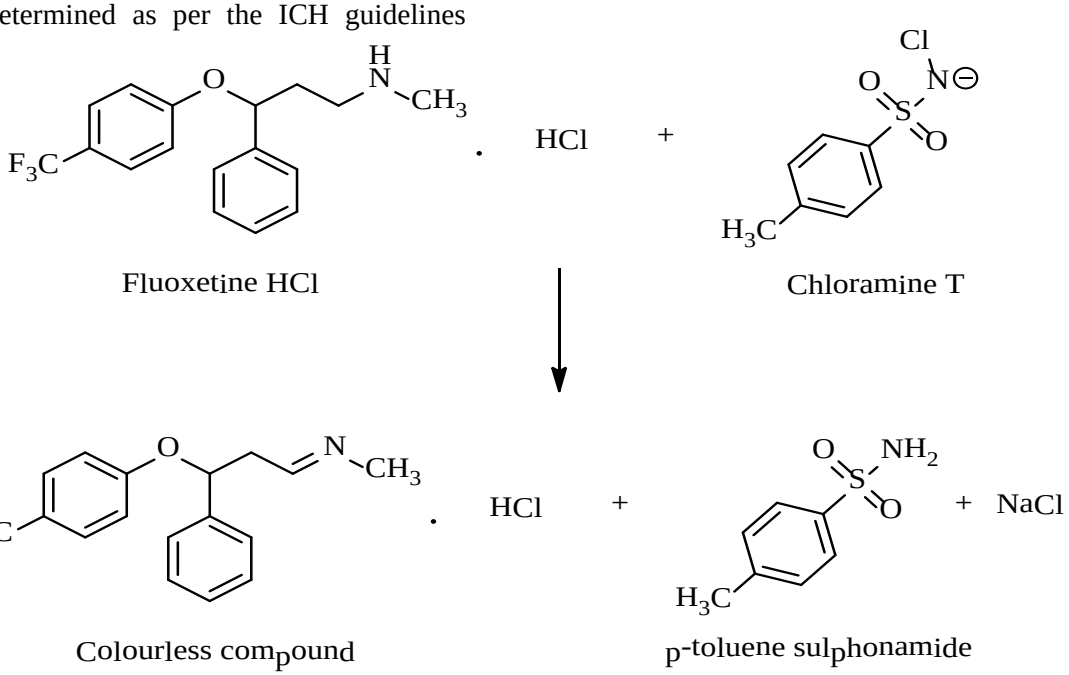
Parameters	Lamivudine
Wavelength λ (nm)	623
Beer –Lamberts limit (µg/ml)	0.3-2.7
Limit of detection (µg/ml)	0.128
Limit of quantitation (µg/ml)	0.390

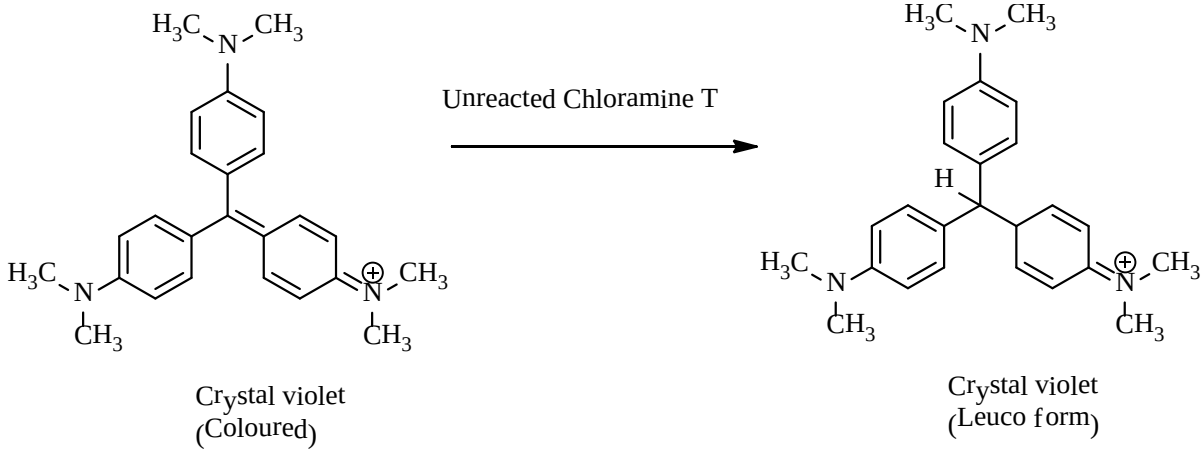
Slope (b)	0.021
Correlation coefficient	0.9993
RSD	0.550

4. RESULT AND DISCUSSION :

In the method describe only analytical and Validation parameters [13] were studied. No other procedure was investigated.Accuracy, recovery and precision studies which include intraday, interday values were also determined. Limit of detection (LOD) and Limit of quantitation (LOQ) both the values were determined as per the ICH guidelines [11, 12]. The same studies were carried out on marketed formulation, and found appreciably reliable results were observed.

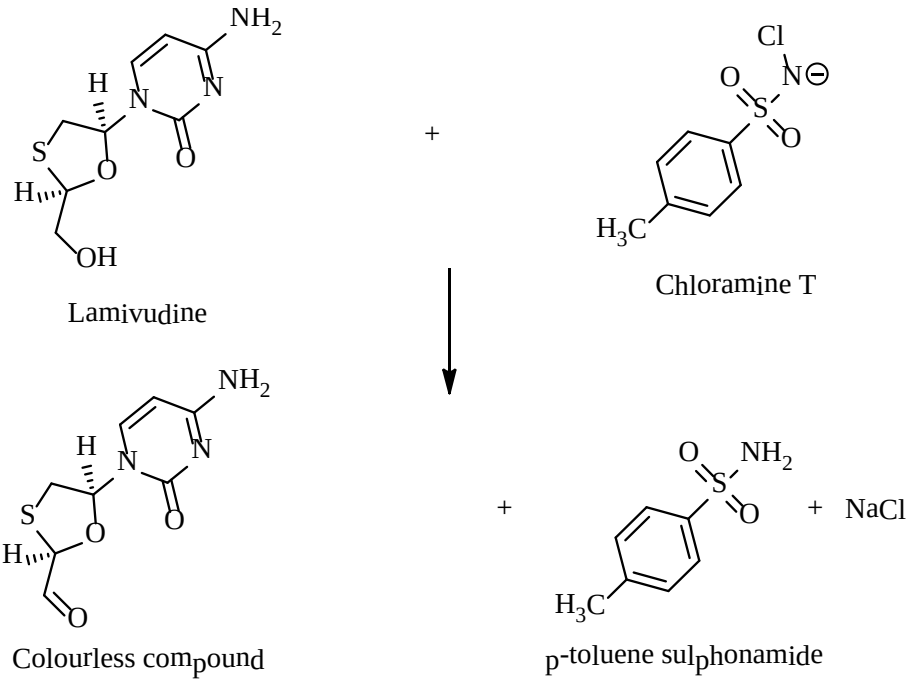
Proposed Coloured reaction of Fluoxentine-HCl is as follows:

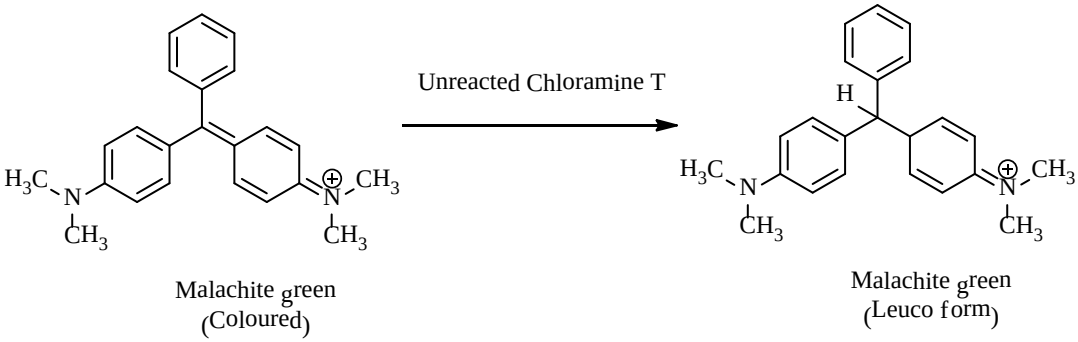




Summary of Reaction: Reaction of FLU is based on oxidation reaction [14]; FLU reacts with Chloramine T, a strong oxidizing agent in presence of sulphuric acid. It produced colourless complex of FLU. After completion of reaction known amount of crystal violet is added, and excess of Chloramine T reacts with crystal violet, oxidize it and produce Leucoform of dye. Remaining unreacted molecules of crystal violet give dark blue colour. So the intensity of the colour depends on amount of drug present.

Proposed Coloured reaction of Lamivudine is as follows:





Summary of Reaction: Estimation of lamivudine is based on oxidation reaction lamivudine reacts with chloramine T, a strong oxidizing agent in the presence of H_2SO_4. It producescolourless complex of lamivudine after the completion of reaction know amount of malachite green was added and excess chloramine T reacted with malachite green dye, oxidized it and produced leucoform of dye. The remaining unreacted molecules of malachite green gave greenish blue colour so the intensity of the final solution indicated the amount of drug present.

5. CONCLUSION :

These colorimetric methods were found to be convenient, easily reproducible and cost effective. The results obtained and validation parameters studied were noteworthy. All the reagents used were of analytical grade, glasswares were calibrated. Hence these methods could be considered as simple, specific and sensitive for the determination of Fluoxentine HCl and Lamivudine in pure and pharmaceutical formulations.

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Conflicts of interest: The authors declare no conflict of interest.

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