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EXTRACTION SPECTROPHOTOMETRIC DETERMINATION OF ESCITALOPRAM DRUG BY ION PAIR COMPLEX METHOD WITH BROM CRESOL GREEN DYE IN TABLET DOSAGE FORM AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple Extraction Spectrophotometric method has been developed for the determination of Escitalopram in pharmaceutical formulations and in the tablet dosage form using Bromo Cresol Green (BCG). The interaction of the drug with BCG in the presence of Sodium Acetate-HCl buffer solution of pH 3.5 results in the formation of an ion- pair complex which is extracted into Chloroform and the composition of which is established as 1:1 (Drug: BCG) by Job's continuous variation method. The wavelength of maximum absorbance of the extracted Ion-pair complex is found to be 400 nm. The absorbance values of the extracted Ion-pair complex are found to increase linearly with the increase in the amount of the drug Escitalopram in the range 5 µg/mL to 35 µg/mL. This suggests the suitability of the proposed method for the determination of Escitalopram in the range 5 µg/mL to 35 µg/mL. Further it also confirms the obedience of Beer-Lambert's law in this range. The method is successfully employed to evaluate the assay of commercial tablets in pharmaceutical formulations for Escitalopram and the results agreed very well. The molar absorptivity and Sandell Sensitivity of the method are $8.887x10^3$ lit.mol^{-1}.cm^{-1} and 0.0365 µg.cm^{-2} respectively.

KEYWORDS: Extraction Spectrophotometry, Escitalopram, Ion-pair complex, Bromo Cresol Green, Pharmaceutical formulations.

INTRODUCTION

Various methods are reported in chemical literature for the determination of Escitalopram in pharmaceutical formulations which include Spectrophotometric-RP HPLC method[1], spectrophotometric method[2] Fluorimetric method[3] and Liquid chromatography-electrospray ionization mass spectrometry method.[4]

Santosh Vilashchand Gandhi et al (loc.cit) have developed simple, accurate, precise, and sensitive ultraviolet spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of Escitalopram oxalate (ESC) and Clonazepam (CLO) in combined tablet dosage form. The spectroscopic method employs an absorbance correction method using 238.6 and 308 nm as 2 wavelengths for estimation with methanol and water as solvents. Beer's law is obeyed in the concentration range of 10.0-50.0 and 0.5-3.0 µg/mL for ESC and CLO, respectively. The RP-HPLC method uses a Jasco HPLC system with HiQ SiL Cl8 column (250 x 4.6 mm id) acetonitrile-0.005 M tetrabutylammonium hydrogen sulfate (55 + 45, v/v) as the mobile phase, and satranidazole as an internal standard. The detection was carried out using an ultraviolet detector set at 287 nm. For the HPLC method, Beer's law is obeyed in the concentration range of 10.0-60.0 and 0.5-3.0 µg/mL for ESC and CLO, respectively. Both methods have been successfully applied for the analysis of the drugs in a pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

Zhangg et al(loc.cit.), have detected the absorbability of escitalopram oxalate tablets by UV spectrophotometry at a wavelength of 234.8 nm and the recovery and dissolution of which were calculated. The linear range of Escitalopram oxalate tablets was 2.5-30 µg/mL and its average recovery was 100.64%. The method is simple, accurate, reliable and suitable for the determination of Escitalopram oxalate in tablets.

D.Venkateswarlu et al[5] have evaluated the assay of Escitalopram drug in bulk dosage form and pharmaceutical formulations using Wool Fast Blue dye by Ion Pair Complex method.

Escitalopram is chemically, (lS)-l-(3-dimethylaminopropyl)-l-(4- fluorophenyl)-3H-2 benzofuran-5-carbonitrile. Chemical formula is $C_{20}H_{21}FN_2O$. Escitalopram is one of a class of antidepressants known as selective serotonin reuptake inhibitors (SSRIs). It is used to treat the depression associated with mood disorders. It is also used on occasion in the treatment of body dysmorphic disorder and anxiety. The antidepressant, antiobsessive-compulsive, and

antibulimic actions of Escitalopram are presumed to be linked to its inhibition of CNS neuronal uptake of serotonin. Chemical structure of Escitalopram is shown in the Fig. 1 below.

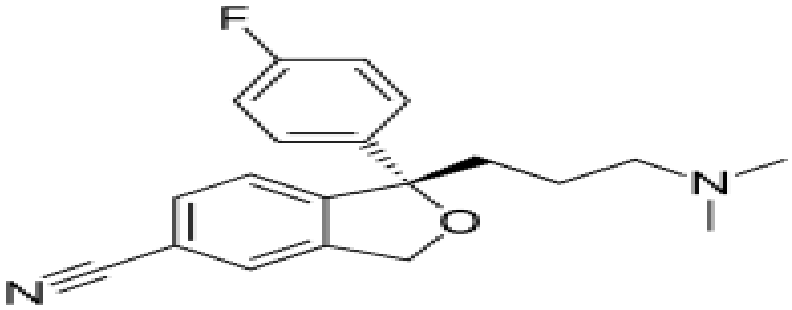


Fig. 1: Escitalopram.

MATERIALS AND METHODS

A. Apparatus: Brief description of the instruments employed

(i) Shimadzu UV-1800 Spectrophotometer

Shimadzu UV-1800 Spectrophotometer is known for better performance, ease of use with powerful functionality and it is controlled by using UV Probe software with a PC for all spectrophotometric measurements. It has the highest resolution of 1 nm.

(ii) pH meter: pH measurements are made using an Elico LI-120 digital pH meter.

B. Preparation of Reagents and Solutions

(i) Escitalopram solution: 50 mg of pure Escitalopram is dissolved in methanol and the volume of the resulting solution is adjusted to the mark in the 50 mL standard flask with methanol. This is used as the stock solution of the drug. The working solution with concentration 100 µg/mL of the drug is prepared by taking 10 mL of the stock solution and diluting it to 100 mL with methanol.

(ii) Buffer solutions: Sodium Acetate-HCl buffer solutions of pH 2-4 are prepared by mixing 0.2 M Sodium Acetate and 0.2 M HCl solutions.

(iii) Bromo Cresol Green (BCG) Solution (0.2% w/v): BCG solution is prepared by dissolving 200 mg of the dye BCG in 100 ml of distilled water.

All other chemicals, reagents and solutions used in the present investigation are of AR Grade only.

RESULTS AND DISCUSSION

Escitalopram when treated with Bromo Cresol Green (BCG) forms an Ion pair complex. This ion pair complex formation reaction is spectrophotometrically monitored to develop a method for the determination of the drug. In the process of carrying out detailed investigations, first of all, optimization of various parameters such as the wavelength of maximum absorbance (λmax), the effect of Buffer solutions (pH 2-4) and the concentration of the chosen buffer solution (pH 3.5) and Bromo Cresol Green on the absorbance of the Ion-Pair complex are established and the procedures adopted in each case are described as follows.

(a) Absorption Spectrum of Escitalopram treated with Bromo Cresol Green

The wavelength of maximum absorbance of the Escitalopram drug treated with Bromo Cresol Green solution is ascertained by the following procedure.

3 mL of Escitalopram solution (100 µg/mL) is transferred into a separating funnel. To this solution 4 mL of Bromo Cresol Green reagent and 3 mL buffer solution(pH 3.5) are added. The final volume becomes 10 mL. To this 10 mL Chloroform is added and the reaction mixture is shaken gently for 5 min and allowed to stand for 2 min so as to separate aqueous and Chloroform layer. The Escitalopram-BCG ion-pair complex gets extracted into the chloroform layer. So, the Chloroform layer is separated out and absorbance of the coloured complex is measured at various wavelengths in the range of 325 nm to 500 nm against the reagent blank.

The data obtained is shown in Table 1.

Table. 1: Absorption Spectrum.

S. No.	Wavelength in nm	Absorbance
1	325	0.456
2	350	0.728
3	375	0.932
4	400	1.072
5	425	0.768
6	450	0.456
7	475	0.324
8	500	0.304

The data shown in the above table is drawn in the form of a graph between wavelength in nm on the X-Axis and the Absorbance on the Y-Axis. The plot obtained is known as absorption spectrum which is as shown below in Fig 2.

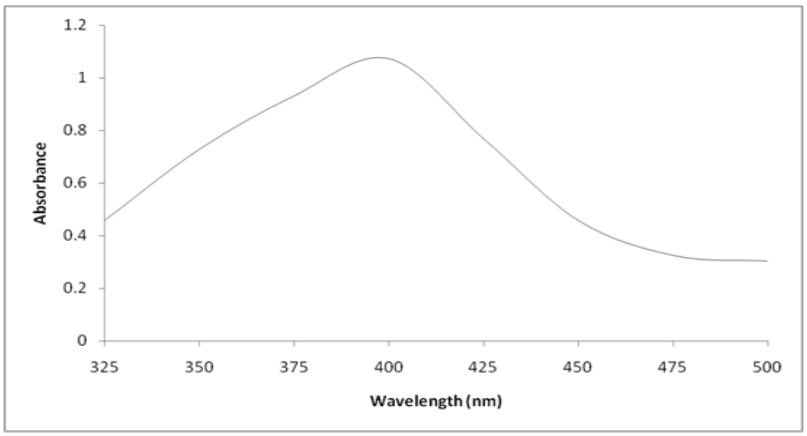


Fig. 2: Absorption Spectrum of ETP+BCG complex.

From Fig. 2, it is clear that the Escitalopram drug treated with Bromo Cresol Green solution has maximum absorbance at 400 nm. Hence, all further studies are made at 400 nm.

The optimal conditions for the determination of Escitalopram are arrived at by the following steps.

(b) Effect of pH of buffer solutions: The influence of pH on the ion-pair complex of ETP with BCG at different pH of Sodium acetate(NaOAc)-HCl buffer solution(pH 2-4) are studied adopting the following procedure and the results obtained are presented in Table 2.

3 mL of the drug ETP (100 µg/mL) + 3 mL of buffer solution of different pH of NaOAc-HCl(pH 2-4) + 4 mL BCG(0.1 % w/v), the total volume becomes 10 mL are taken in a separating funnel. To this 10 mL chloroform is added, shaken well and the extracted chloroform layer is separated and absorbances measured.

Table. 2: Effect of pH of buffer solutions.

S. No.	Volume of ETP (in mL)	Volume of Buffer Solution (in mL)	pH of buffer solution added	Volume of BCG solution (in mL)	Total Vol. (in mL)	Vol. of Chloroform added (in mL)	Absorbance of the extracted layer at 400 nm
1	3	3	2.0	4	10	10	0.472
2	3	3	2.2	4	10	10	0.506
3	3	3	2.4	4	10	10	0.748
4	3	3	2.6	4	10	10	0.802
5	3	3	2.8	4	10	10	0.854
6	3	3	3.0	4	10	10	0.942
7	3	3	3.2	4	10	10	1.020
8	3	3	3.5	4	10	10	1.075
9	3	3	4.0	4	10	10	0.732

From the data presented in Table 2, it is seen that the absorbance is almost the same in the buffer solution of pH range 3.0 to 3.5. However maximum absorbance is obtained for the buffer solution with pH 3.5. Therefore, the buffer solution with pH 3.5 is fixed for further studies.

(c) Effect of concentration or volume of buffer solution

The effect of concentration or volume of buffer solution on the absorbance of the ion-pair complex is studied by adopting the following procedure.

In a series of separating funnels containing 3.0 mL of Escitalopram (100 µg/mL), x mL of NaOAc-HCl(pH 2-4) buffer solution of pH 3.5, 4.0 mL of Bromo Cresol Green, (3-x) mL of distilled water are mixed to make the total volume 10 mL. To each separating funnel 10 mL of chloroform is added and shaken well for complete extraction of the complex into chloroform. The extracted layer is separated and the absorbance values are measured at the wavelength of maximum absorbance 400 nm. The values obtained are shown in Table 3.

Table. 3: Effect of concentration(volume) of buffer solution (pH 3.5).

S.No.	Vol. of ETP in mL (100 µg/mL)	Vol. of buffer solution of pH 3.5 in mL (x mL)	Vol. of BCG (0.1% w/v) in mL	Vol. of distilled water (3-x) mL	Total Vol. in mL	Vol. of chloroform added in mL	Absorbance of the extracted complex at 400 nm
1	3	0.5	4	2.5	10	10	0.248
2	3	1.0	4	2.0	10	10	0.487
3	3	1.5	4	1.5	10	10	0.837
4	3	2.0	4	1.0	10	10	1.016
5	3	2.5	4	0.5	10	10	1.053
6	3	3.0	4	0.0	10	10	1.073

The data in Table 3 indicates that the absorbance values are almost constant in the volume range of 2.0 mL to 3.0 mL of the buffer solution of pH 3.5. However, for all further experimental work, an optimum volume of 3.0 mL of the buffer solution of pH 3.5 is fixed since the absorbance is maximum at this volume of the buffer solution.

(d) Effect of concentration of Bromo Cresol Green

The effect of Bromo Cresol Green on the absorbance is studied by varying the volume(x mL) of Bromo Cresol Green keeping the volume of the drug ETP (100 µg/mL) fixed at 3.0 mL and the buffer solution of pH 3.5 also fixed at 3.0 mL and the total volume is adjusted to 10

mL using distilled water (4-x)mL. To each of these solutions 10 mL of chloroform is added, shaken well and the extracted chloroform layer is separated for absorbance measurement at 400 nm against the suitable reagent blank. The absorbance of the resultant solution is measured at 400 nm. The results obtained are incorporated in Table 4.

Table. 4: Effect of concentration of bromo cresol green solution on absorbance.

S. No.	Vol. of ETP in mL (100 µg/mL)	Vol. of buffer solution of pH 3.5 in mL	Vol. of BCG (0.1% w/v) in mL(x mL)	Vol. of distilled water (4-x) mL	Total Vol. in mL	Vol. of chloroform added in mL	Absorbance of the extracted complex at 400 nm
1	3	3	0.5	3.5	10	10	0.156
2	3	3	1.0	3.0	10	10	0.432
3	3	3	1.5	2.5	10	10	0.517
4	3	3	2.0	2.0	10	10	0.864
5	3	3	2.5	1.5	10	10	0.982
6	3	3	3.0	1.0	10	10	1.068
7	3	3	3.5	0.5	10	10	1.072
8	3	3	4.0	0.0	10	10	1.074

The data in Table 4 shows that the absorbance values beyond 3.0 mL of BCG(0.1% w/v) are almost constant. Therefore 3.0 mL of Bromo Cresol Green is sufficient for complete complexation and hence 3.0 mL of it is fixed for further studies.

(e) Determination of drug Escitalopram: Applicability of Beer-Lambert's Law

Calibration curve: Recommended procedure

To study the effect of drug concentration on the absorbance of the ion pair complex and to establish the suitability of the method by applying the Beer-Lambert's law to quantitatively determine the drug using the calibration curve, the following procedure is recommended.

Various aliquots(x mL) of the standard Escitalopram solution (100 µg/mL) ranging from 0.5 mL- 4.0 mL are transferred into a series of separating funnels. To each separating funnel, 3.0 mL of Bromo Cresol Green solution, 3.0 mL of buffer solution(pH 3.5) are added and the contents are made upto 10 mL using (4-x) mL distilled water. 10 mL of chloroform is added to each separating funnel and the contents are thoroughly shaken to completely extract the ion-pair complex into the chloroform layer. The extracted layer is separated and the absorbance of each is recorded at the wavelength of maximum absorbance 400 nm against the suitable reagent blank. The results obtained are tabulated and are shown in Table 5 and Fig. 3.

Table 5: Effect of drug concentration: Calibration curve

S. No.	Vol. of ETP in mL (100 µg/mL) (x mL)	Vol. of buffer solution of pH 3.5 in mL	Vol. of BCG (0.1% w/v) in mL	Vol. of distilled water (4-x) mL	Total Vol. in mL	Vol. of chloroform added in mL	Absorbance of the extracted complex at 400 nm
1	0.5	3	3	3.5	10	10	0.136
2	1.0	3	3	3.0	10	10	0.280
3	1.5	3	3	2.5	10	10	0.418
4	2.0	3	3	2.0	10	10	0.554
5	2.5	3	3	1.5	10	10	0.708
6	3.0	3	3	1.0	10	10	0.850
7	3.5	3	3	0.5	10	10	0.990
8	4.0	3	3	0.0	10	10	1.080

x mL of ETP (100 µg/mL) + 3 mL buffer solution of pH 3.5 + 3 mL of BCG (0.1% w/v) + (4-x) mL distilled water = Total volume 10 mL. To this 10 mL chloroform added, shaken well, extracted chloroform layer separated and absorbance of each solution measured at 400 nm against suitable reagent blank.

The data presented above is plotted in the form of a graph drawn between the volume of ETP (Amount of ETP) on the X-Axis and the Absorbance values obtained on the Y-Axis. The graph obtained is shown in Fig. 3 which is the calibration curve for the estimation of Escitalopram.

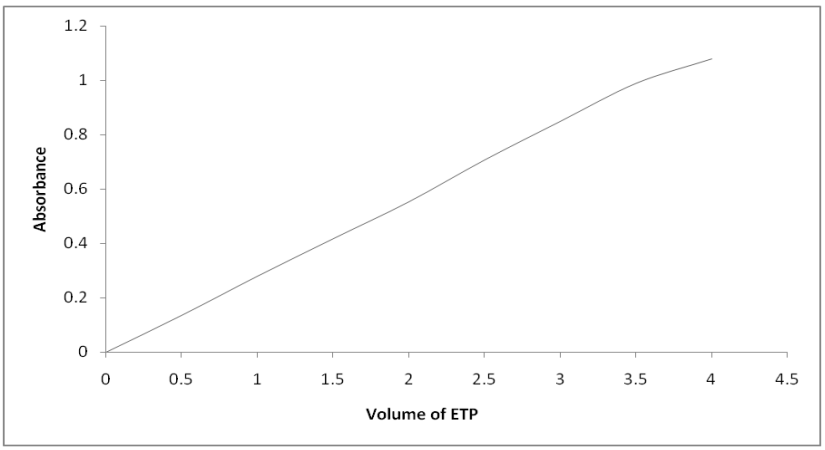


Fig. 3: Calibration curve of Escitalopram.

It is seen from the data in Table 5 and Fig. 3 that the absorbance is increasing linearly with the increase in the concentration or amount of the drug Escitalopram in the range 5 µg/mL to 35 µg/mL. This suggests the suitability of the proposed method for the determination of Escitalopram in the range 5 µg/mL to 35 µg/mL. Further it also confirms the obedience of

Beer-Lambert's law in this range. Thus this linear curve is taken as the calibration curve for the quantitative spectrophotometric estimation of Escitalopram.

(f) Stoichiometric Composition of ETP-BCG Ion-Pair Complex: Job's Continuous Variation Method: The composition of the Ion-Pair complex between the drug Escitalopram and the reagent BCG is established by the Job's continuous variation method. In this method, equimolar concentrations (1 x 10^{-3} M) of both the drug and BCG are varied continuously keeping the total volume of the mixed solution as constant at 5 mL. In each case, the absorbance is measured at 400 nm against a suitable blank. The data obtained is presented in Table 6 and Fig. 4.

Table. 6: Job's method of continuous variation λ=400 nm.

S. No.	Volume of drug(ETP) (1X10^{-3}M) V_1 in mL	Volume of Buffer Solution of pH 3.5 in mL	Volume of BCG (1 x 10^{-3} M) V_2 in mL	Volume of distilled water in mL	Total volume in mL	Volume of Chloroform added in mL	Volume fraction (X) of the extracted drug (V_1/V_1+V_2)	Absorbance at 400 nm of the extracted layer
1	0.5	3	4.5	2	10	10	0.1	0.121
2	1.0	3	4	2	10	10	0.2	0.256
3	1.5	3	3.5	2	10	10	0.3	0.367
4	2.0	3	3.0	2	10	10	0.4	0.446
5	2.5	3	2.5	2	10	10	0.5	0.508
6	3.0	3	2.0	2	10	10	0.6	0.462
7	3.5	3	1.5	2	10	10	0.7	0.376
8	4.0	3	1.0	2	10	10	0.8	0.282
9	4.5	3	0.5	2	10	10	0.9	0.161

The data in Table 6 are plotted in the form of a graph between volume fraction of the extracted drug (V_1/V_1+V_2) on the x- axis and the absorbance values on the y-axis. The graph obtained is shown in Fig. 4.

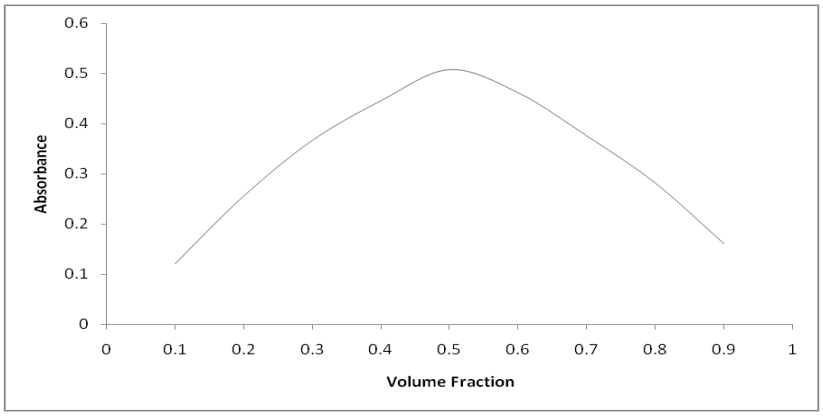
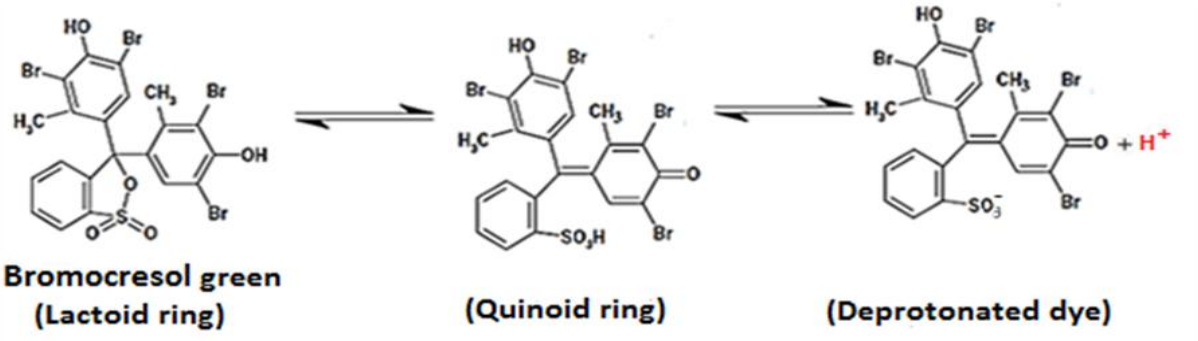
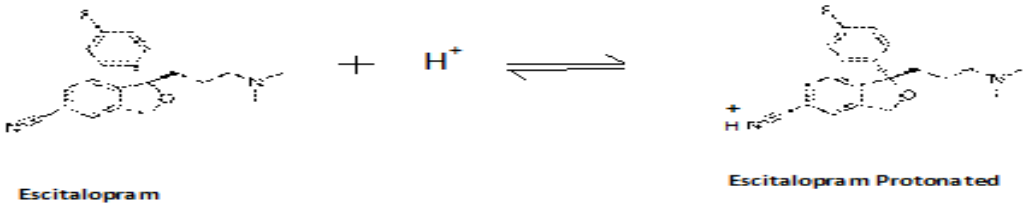
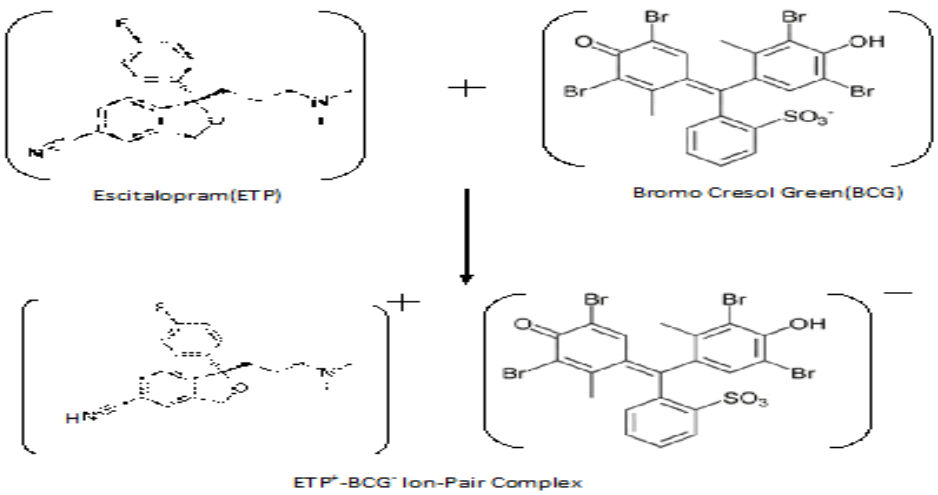


Fig. 4: Job's Continuous Variation Method.

From the Fig. 4 shown above, it is found that one mole of the drug is reacting with one mole of BCG thereby establishing the stoichiometry of the ion pair complex as 1:1 (Drug: BCG). The stability constant (K_{st}) is calculated and is found to be 9.2764 x 10^4 lit.mol^{-1}. The probable reaction sequence may be represented as shown in below.







From the above structures it can be understood that the opening of the Lactoid ring and the subsequent formation of the quinoid group is the reason for the color of the dye. It suggests that the two tautomers exist in equilibrium. It is considered that due to the strong acidic nature of the sulphonic acid group, the quinoid structure predominates. Finally the protonated drug forms an ion-pair with the dye stuff which is quantitatively extracted into Chloroform.

(g) Assay of Escitalopram in pharmaceutical formulations

The recommended procedure for the assay of Escitalopram is applied for its determination in commercial tablets.

For analysis of tablet formulation, twenty tablets of Escitalopram are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of Escitalopram was taken in a 50 mL volumetric flask containing 25 mL of chloroform, sonicated for 20 minutes. The resultant solution is filtered through Whatman filter paper No. 41 into another 50 mL volumetric flask. The filter paper was washed several times with chloroform. The washings were added to the filtrate and the final volume was made up to the mark with chloroform. 5 mL filtrate of the sample solution was diluted to 10 mL with chloroform and treated as per the procedure of the calibration curve. Amount of the drug present in sample was computed from the respective calibration curve. The results are presented in Table 7.

Table. 7: Assay of Escitalopram in tablets.

Sample	Labelled Amount (mg)	*Amount found by Proposed method ±S.D*	Percentage of Label claim	%RSD	*t_{cal}
Tablet 1	10.0	10.012±0.08	100.12	0.7990	0.3354
Tablet 2	10.0	9.97±0.078	99.7	0.7823	0.8600

*Average of five determinations based on label claim

CONCLUSION

In this method the Escitalopram dissolved in water is treated with Bromo Cresol Green dye at pH 3.5. The resultant solution is extracted with Chloroform. The ion pair complex is extractable into Chloroform layer. The absorbance of the extractable ion pair complex is measured at 400 nm against the reagent blank (prepared in a similar manner devoid of drug solution). The calibration curve is linear over the range of 5-35 µg/mL of Escitalopram. The values of standard deviation are low which indicate high accuracy and reproducibility of the method. The percent standard deviation and percent RSD calculated from the measurements of Escitalopram are shown in the above table. The molar absorptivity and Sandell's sensitivity are calculated and are found to be $8.887x10^3$ $lit.mol^{-1}.cm^{-1}$ and 0.0365 $\mu g.cm^{-2}$ respectively. The molar absorptivity and Sandell's sensitivity values show sensitivity of the method. The %RSD is less than 2, which indicates that the method has good reproducibility. The calculated 't' values are less than 't' theoretical values with 4 (n-l= 5-1= 4) degrees of

freedom at 5% level of significance. This indicates that there is no significant difference between proposed method and the standard method. There is no effect of additives and excipients such as starch, calcium lactose and glucose in the concentrations those present in general pharmaceutical preparations. The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of Escitalopram in bulk drugs samples and pharmaceutical formulations as seen from the good agreement of the amount of Escitalopram in the present method and the labeled amount of the pharmaceutical preparation.

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