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Role of Eosin Y in Spectrophotometric Determination of antipsychotic drug Clozapine via ion pair Complex Formation

Vaishali Parastekar & Ritika Makhijani[1*]

1,Department of Chemistry, V.E.S College of Arts, Science & Commerce, Mumbai – 400 071 Maharashtra, INDIA

Abstract

For the measurement of Clozapine drug in pure form and pharmaceutical formulations, a quick, sensitive, and easy spectrophotometric approach was devised and validated. The suggested procedure is based on the creation of a binary complex between these drugs and eosin Y in an aqueous acetate buffered media. The binary complex was formed under the optimum conditions which shows absorption maxima at 545 nm. Beer's law is obeyed in the concentration range of 1.0 - 10 µg/ml . Molar absorptivity and Sandell's sensitivity are found to be 1.1176 x 10^4 L $mol^{-1}cm^{-1}$ and 0.0291 µg/cm^2 respectively.

The limit of detection (LOD) was 1.1152 µg/mL & the limit of quantitation (LOQ) was 3.3796 µg/mL for the Clozapine drug. It was observed that the excipients that are commonly present in pharmaceutical formulations did not interfere. The proposed method was successfully applied to the analysis of Clozapine tablets in their dosage forms.

***Keywords*:** Ion association; Spectrophotometry; Clozapine; Eosin Y.

Introduction

IUPAC name of Clozapine is8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e] [1,4]-diazepine .It is a versatile and effective antipsychotic medication (Figure 1). It is used for the treatment resistant schizophrenia (TRS). It is observed that 30 to 40 % of patients with schizophrenia meet the criteria for TRS and clozapine plays key role in 40–70% of this subgroup. (Kane, et al.,1988), (Remington, et al., 2016). Highly potent Clozapine has reduced mortality in comparison with other antipsychotic treatments. (Taipale, et al., 2020), (Tiihonen, et al., 2009). However, due to the barrier with the use of clozapine the drug is not effectively used in spite of its many advantages. (Remington, et al., 2016), (Stroup, et al.,

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2014). Fear of severe side effects & Complex pharmacodynamic profile are among those barriers. (Cunningham, 1996)

Analytical methods for the assay clozapine include titri-metric method (The British Pharmacopoeia, 2000), liquid chromatographic (Zhou, et al., 2004), (Garcia,et al., 2003) capillary electro-phoretic (Raggi, et al., 2001), electrochemical methods (Ivon,et al., 2001), (Ozkan,et al., 1996), & few Spectrophotometric methods. (Xu, et al., 1998), (Kelani, et al., 1997)

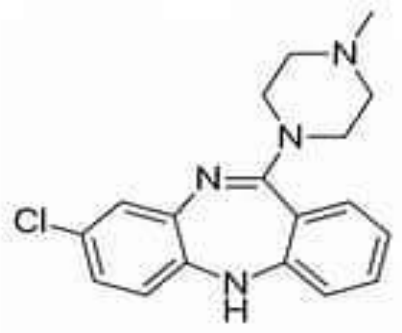

Figure 1: Structure Of Clozapine

According to a review of the literature, no spectrophotometric approach using ion pair complexation via Eosin Y has been documented for determining the presence of the medication Clozapine. Therefore, the goal of the current work was to create an easy-to-use spectrophotometric method for measuring Clozapine in both its pure form and pharmaceutical formulations through ion pair complexation utilizing eosin.

EXPERIMENTAL

Instrumentation & Chemicals

LABMAN UV-VIS Spectrophotometer, quartz cells with a 1 cm path length were employed for having the wavelength range of 320 to 1000 nm.

Chemicals used in the current study came from Loba Chemie and were of analytical quality.

Double distilled water was employed.

Preparation of standard drug solution

Crushed & accurately weighted tablets equivalent to 10 mg of Clozapine were made to dissolve in water, swirled, sonicated for 30 min, shaken well and filtered. The filtrate was

made up to 100 ml with distilled water to obtain a 100 µg ml^{-1} (100 ppm) stock solution. The working solutions 20 µg ml^{-1} were prepared by appropriate dilutions of the stock solution

Preparation of Reagents

By dissolving 3.4593 g in distilled water, thoroughly mixing it, and increasing the volume to 1000 mL in a volumetric flask, the solution of eosin Y ($5x10^{-4}$ M) was made. By combining 0.1 M sodium acetate and acetic acid solutions and adjusting the pH with a pH meter, the acetate buffer solution was made with a pH of 3.5.

Experimental procedure

To the series of 10 ml calibrated standard flasks various quantities (0.5 -- 5.0 ml, 20 ppm) of standard clozapine solution was added followed by 1.0 ml of 5 $X10^{-4}$M eosin Y & 2 ml of pH 3.5 with continuous shaking. Solution was made up to the mark with distilled water. At room temperature, the absorbance was measured at 545 nm.

RESULTS AND DISCUSSION

In an acidic solution, Clozapine and eosin Y interact through the production of an ion-pair complex, resulting in a reddish orange complex with a maximum wavelength at 545. (Fig. 2).

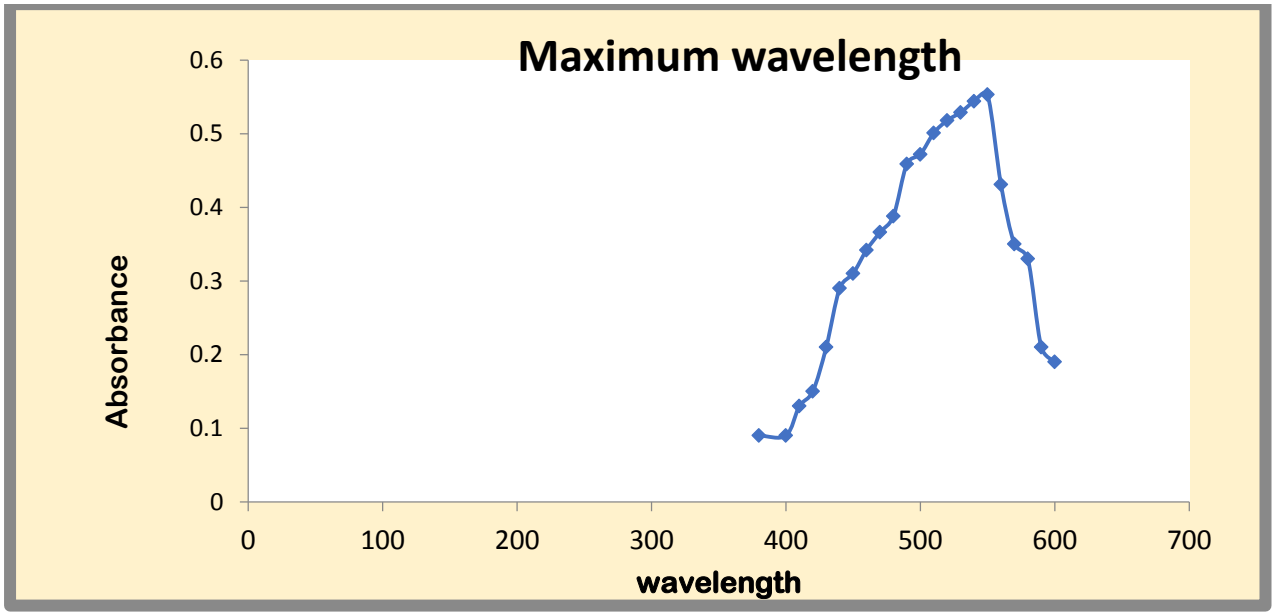

Figure 2: Absorption spectra of ion pair complex of Clozapine with eosin

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Optimization of reaction conditions

To find the ideal circumstances for the assay technique, it was researched how different parameters affected the colour development.

Effect of eosin Y concentration:

The ion-pair formation for Clozapine was optimized using 1.0 ml of 5X 10^{-4} M eosin Y, as shown in (Fig. 3), after various concentrations of eosin Y were introduced to the medications under study. Subsequent experiments made advantage of these concentrations.

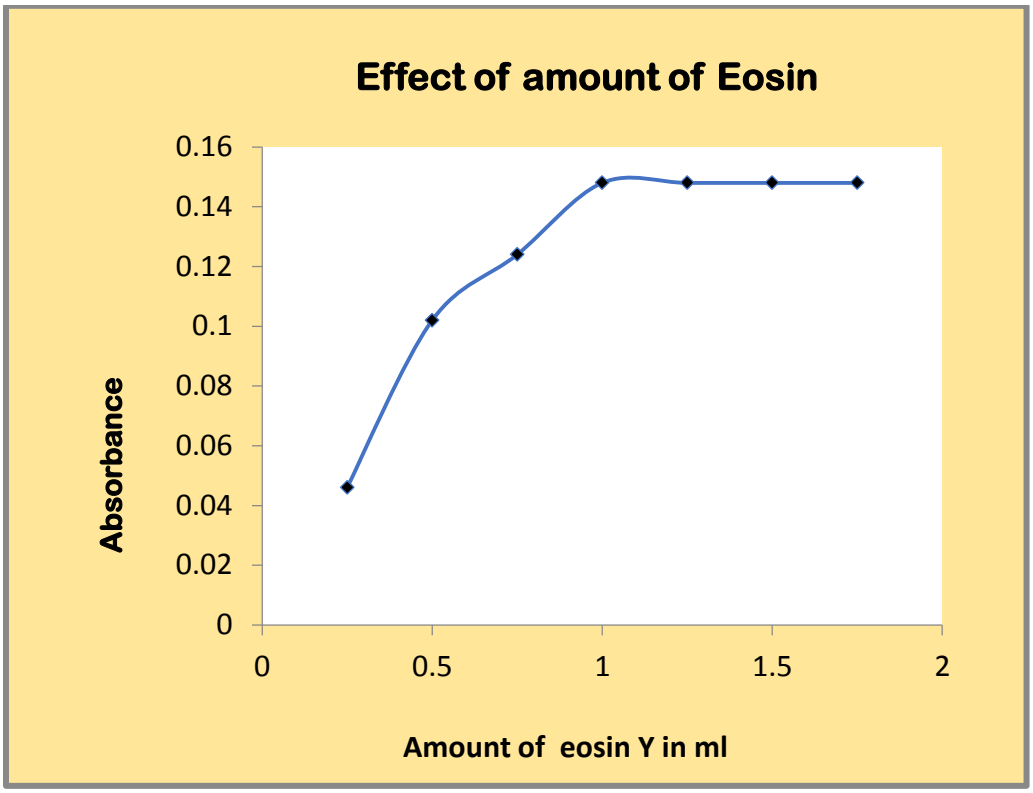

Figure 3: Effect of eosin concentration on the absorbance ion pair complex of Paracetamol with eosin

Effect of PH & buffer solution

Over the pH range of 2.6-4.5, the impact of pH on the absorbance of binary complexes was investigated. According to Figure 4, The best absorbance values for Clozapine drug was achieved at a pH of 3.5. Two milliliters of acetate buffer pH 3.5 was sufficient to reach the ideal pH level. The

buffer solution needs to be added after combining the drug-dye solution for the best colour intensity and precision. We made and tested many buffers with the above mentioned pHs. Acetate buffer produced stable complex and was thought to be ideal for the production of complex with the Clozapine (Table 1).

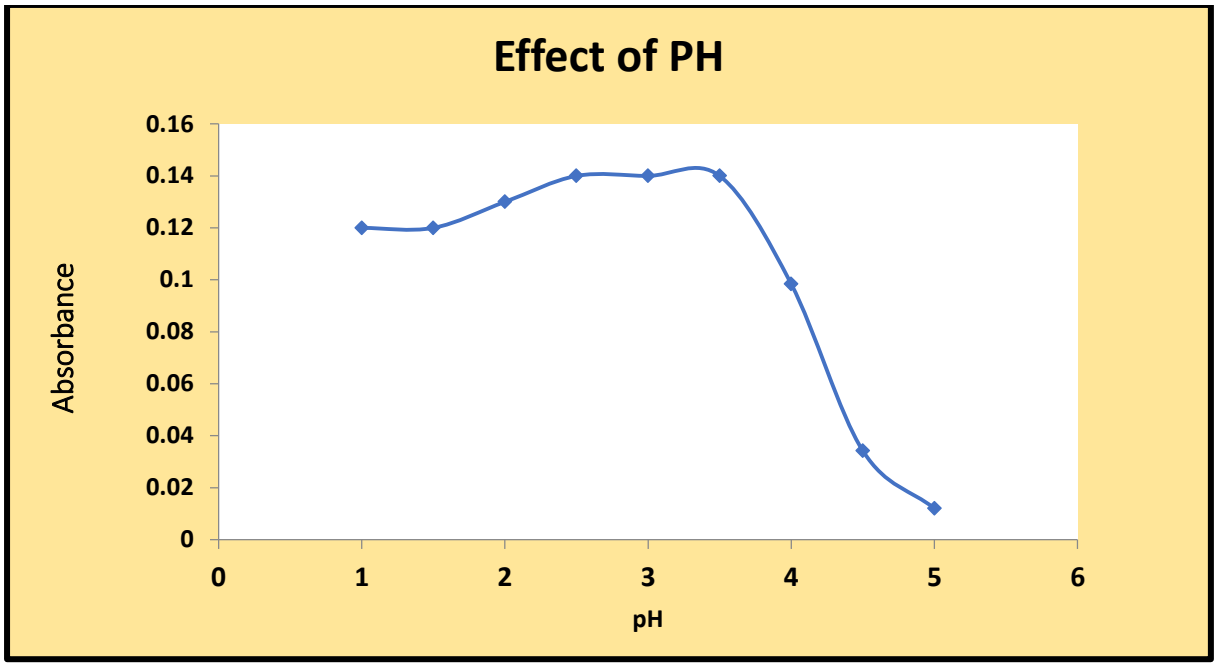

Fig 4: Effect of pH on the absorbance of ion pair complex of Clozapine with eosin

Sr. No	Buffer	Absorbance
1	Glycinate	0.98
2	Acetate buffer	0.161
3	Citrate	0.038
4	Phthalate	0.114
5	Tartrate	0.92

Table 1: Effect of buffer solutions on the absorption of ion pair complex of Clozapine with eosin

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Effect of temperature and developing time:

By observing the colour development at various temperatures, the ideal reaction time was discovered. It was discovered that the complex forms at room temperature with the greatest absorption right away and stays steady for more than 24 hours. At 40 °C, however, the complex's absorbance rapidly dropped, indicating the disintegration of ion pair complexes. Room temperature was hence proved to be ideal and applied in all ensuing studies.

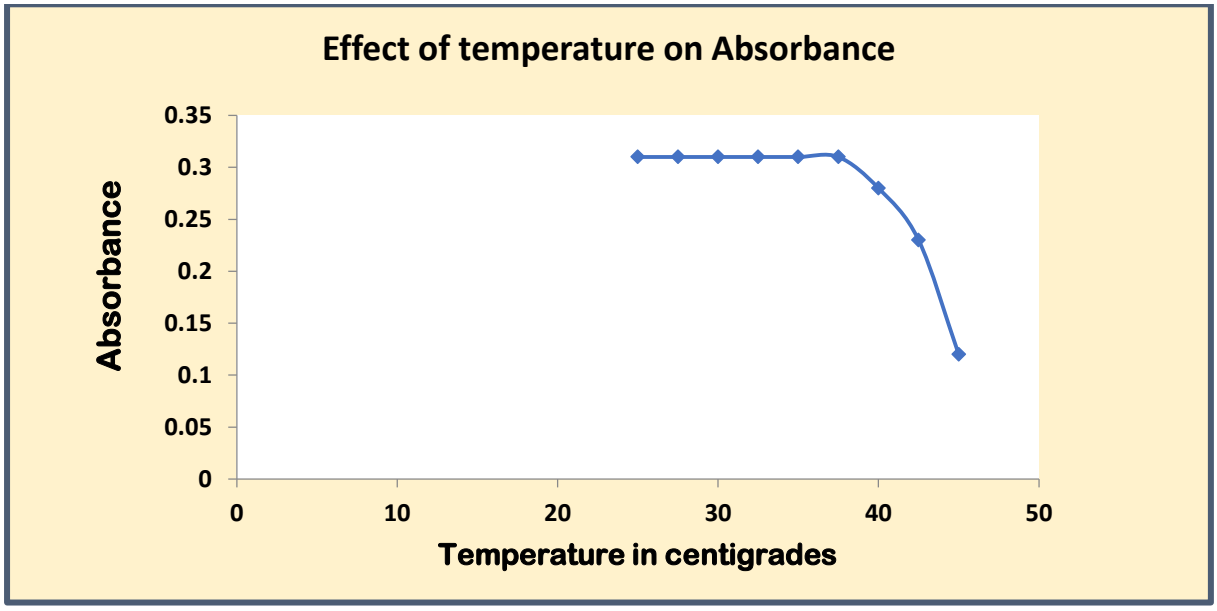

Figure 5: Effect of temperature on the absorbance of ion pair complex of Clozapine with eosin

Mechanism

In an acidic solution, Clozapine and eosin Y react through the creation of an ion-pair to produce a reddish orange complex with a maximum wavelength at 545. (Fig. 6). The most basic part of the drug molecule and the carboxylate anion of the dye engage electrostatically to produce this complex. (Walash, et al., 2011). This mostly happens in an acidic solution, enhancing the electron delocalization of eosin and causing a bathochromic shift of the dye of roughly 30 nm. The only site in Clozapine which can undergo protonation is the nitrogen bonded to an electron donating methyl group in the ring and this protonated Clozapine forms ion-pair with the eosin dye.

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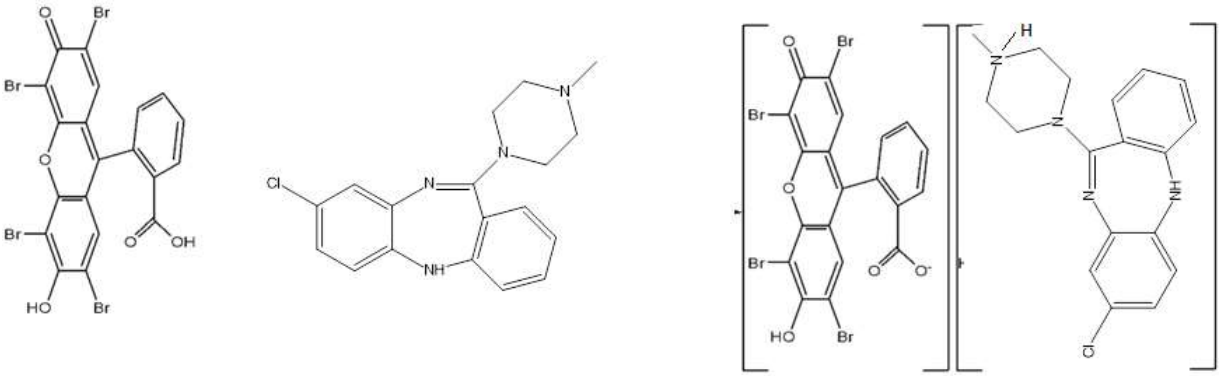

Figure 6: Mechanism showing the formation of ion pair complex of Clozapine with eosin

Composition & Stability constant of colour complex:

The composition of the ion-pair was studied by Job's continuous variation and mole ratio methods (British Pharmacopoeia, 2007) Composition was found to be 1:1 drug: eosin Y by both methods as there is only one basic center in the drug (Fig. 7 & 8)

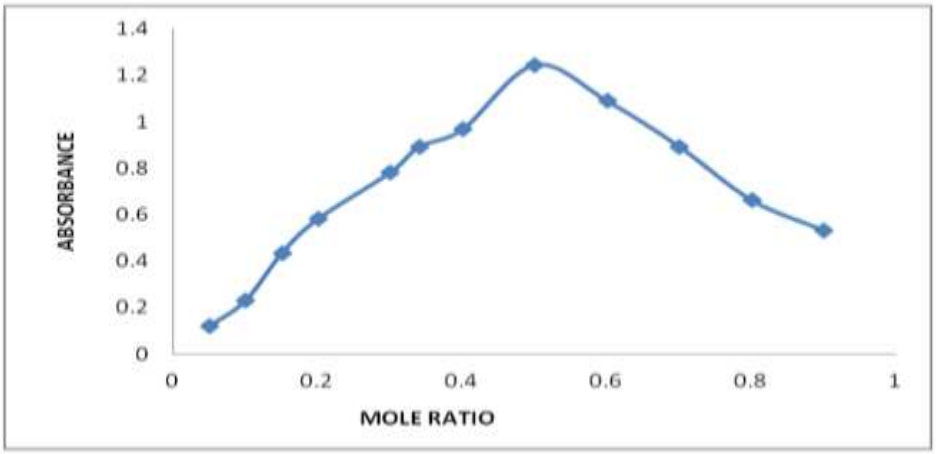

Figure 7: Job's method suggesting composition of ion pair complex 1:1 clozapine: eosin

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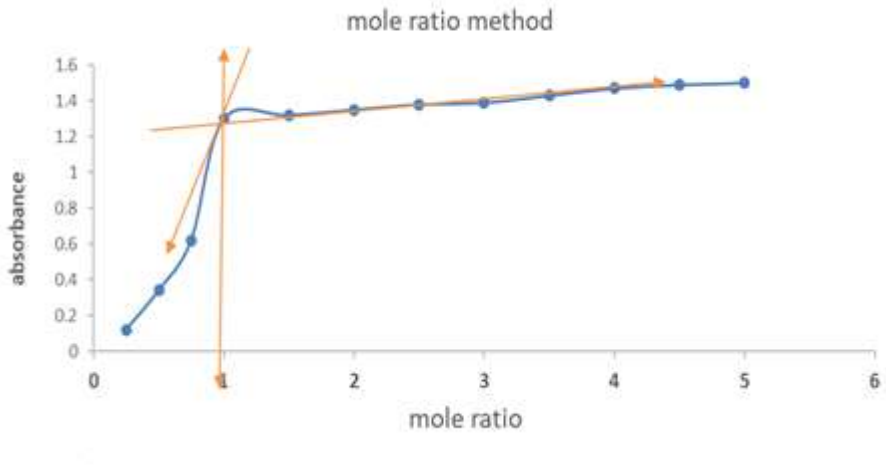

Figure 8: Mole ratio method suggesting composition of ion pair complex 1:1 clozapine: eosin

Stability Constant:

By comparing the absorbance of a solution containing the drug and eosin Y in stoichiometric proportions (As) to one having an excessive amount of the eosin Y reagent (Am), the apparent stability constant was computed. The average conditional stability constant of the ion pair complex was determined using the formula $Kc=1-\alpha/4\alpha^3C^2$ and α =Am-As/Am (where Kc is the stability constant, α is the dissociation degree, and C is the concentration of the complex, which is equal to the concentration of drug. (Dhamra, et al., 2014). This equation was calculated using the 1:1 ratio. The average stability constant for three different concentrations of clozapine was determined to be 4.728 $x10^8$ l^2 mol^{-2}.

Method Validation:

Between absorbance values and drug (concentration), the calibration curve is drawn. (Fig. 9) This is shown to be linear for Clozapine concentrations between 1.0 and 10 µg/ml. The regression equation representing linearity Sandell's sensitivity, and the molar absorptivity are discovered to be 11176.4 L mol^{-1} cm^{-1} and 0.0291 µg/cm^2 respectively.

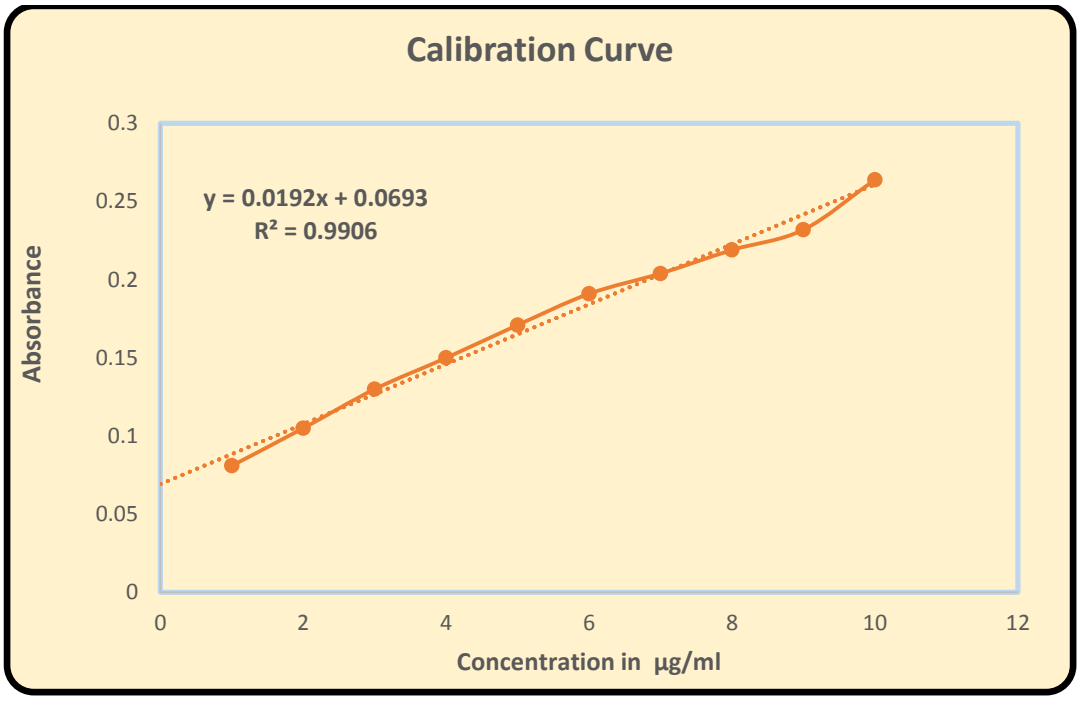

Figure 9: Calibration Curve

Limit of quantification and limit of detection:

The limit of quantification (LOQ) was determined by establishing the least concentration that can be measured according to ICH Q2(R1) recommendations (ICH Harmonized Tripartite Guideline, 2005), below which the calibration range is non- linear. The limit of detection (LOD) was determined by evaluating the lowest concentration of the analytes that can be readily detected and. The LOQ and LOD were calculated according to the following equations: LOQ = 10 Sa/b LOD = 3.3 Sa/b Where (Sa) is the standard deviation of the intercept of the regression line and (b) is the slope of the calibration curve. LOD is 1.1152 and LOQ is 3.3796

Specificity

The specificity of the method was inferred by studying any interference encountered from the common excipients by measuring the absorbances of solutions containing 2 µg/mL of Clozapine drug and various amounts of diverse species, up to 100-fold excess, in a final volume of 5 mL It was noticed that the effect of excipients was negligible. (Table 2).

Excipients	Recovery %		
	2 µg ml^{-1} of Clozapine drug per fold excess foreign added		
	5 µg ml^{-1}	10 µg ml^{-1}	20 µg ml^{-1}
Glucose	99.9048	98.3425	97.0233
Fructose	98.9245	96.5135	95.7845
Starch	98.3425	96.012	95.0102
NaCl	97.8925	95.95254	94.6587
Urea	98.3214	97.0124	95.53265
$MgCl_2$	97.8325	95.3214	94.6589

Table 2: Effect of excipients for assay of Clozapin

Parameter	Method
Maximum Wavelength λ_{max}	**545 nm**
Beer's Law Limits µg/mL	**1.0 - 10.0 µg/ml**
Sandell's Sensitivity (µg/cm^2 /0.0001 Absorbance)	**0.0291 µg/cm^2**
Molar Absorptivity Lt/mole/cm	**11176.4 L $mol^{-1}cm^{-1}$**
Slope(b)[a]	**0.0191**
Intercept(a)[a]	**0.0693**
Correlation Coefficient (r^2)	**0.9906**
Standard Deviation (S)	**0.0129**
Variation from mean at 95% level confidence limit	**±0.1984**
Limit of Detection (LOD)µg/mL	**1.1152**
Limit of Quantification (LOQ)µg/mL	**3.3796**

Table-3 Regression parameters, Optical characteristics Precision and Accuracy

Regression equation Y= a + bC, Where Y stands for absorbance and C is concentration in µg/mL b is %Relative standard deviation which is calculated for ten determinations.

Determination of pharmaceutical formulations of Clozapine

Drug	**Manufacturing company**	**Labelled amount(mg)**	**Amount found by proposed method**	***Amount found by Reference Method**
Clozaril	Aurobindo Pharma	**25.00 mg**	**24.89**	**24.99**
Clozaril	Heritage Life	**50.00mg**	**49.88**	**49.98**

Table 4: Analysis of Pharmaceutical Formulations of Clozapine.

Conclusion

For the purpose of determining the presence of paracetamol in certain of their pharmaceutical formulations, a straightforward, sensitive, quick, accurate, and precise spectrophotometric approach was devised. The recovery test data and statistical characteristics demonstrated the proposed method's good repeatability and accuracy, and the proposed method's proposed correlation coefficient for pharmaceuticals reflects outstanding linearity.

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