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UV SPECTROPHOTOMETRIC QUANTIFICATION OF CLOMIPRAMINE IN PHARMACEUTICALS EMPLOYING MULTIVARIATE CALIBRATION TECHNIQUE AND EVALUATION OF GREENNESS PROFILE

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ABSTRACT

The current study suggests creating a unique methodology and testing it to determine the amount of clomipramine in formulations and bulk. Sensitive, precise, and quick describe this analytical method. The analytical approach was carried out with the aid of a UV spectrophotometer and a multivariate calibration methodology. This multivariate calibration approach was developed using the link between concentration and absorbance, which has been established at five distinct wavelengths, including the absorption maxima at 252 nm. The procedure was validated using ICH Q2 (R1) criteria by using the linear regression equations as well as other mathematical and statistical methods. The validation parameters for accuracy, linearity, and others mentioned in the instructions have all been fulfilled. When used in routine examination of medications and formulations, this technology is sensitive, cost-effective, and yields reliable results. The technique greenness values were calculated using the analytical Eco scale, Agree metrics, and Green Analytical Procedure Index.

Keywords: Clomipramine, Multivariate calibration technique, UV spectrophotometric, Pharmaceutical formulations, ICH guidelines, Validation

INTRODUCTION

The chemical name for clomipramine hydrochloride, which has a molecular weight of 351.31 gm/mol, is 3-chloro-5-[3-(dimethylamino)propyl]-10,11-dihydro-5H-dibenz[b,f]azepine monohydrochloride. Clomipramine is a member of the tricyclic antidepressant class of drugs **[1]**. medication that has an antidepressant effect due to the increased monoamine concentration in the synaptic cleft **[2]**.

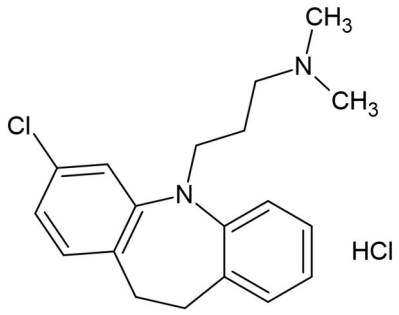

Figure 1: Structure of Clomipramine hydrochloride

Obsessive-compulsive disorder (OCD) therapy with clomipramine is only FDA-approved for those 10 years of age and over. Clomipramine was the first OCD medication to be approved by the FDA in 1989. In a meta-analysis, In comparison to sertraline, fluoxetine, and fluvoxamine, clomipramine was found to be more successful at treating OCD. Clomipramine was found to be 37% more effective in treating children and adolescents' CY-BOCS, the Children's Yale-Brown Obsessive-Compulsive Scale **[3]**. Typical TCA clomipramine, which is said to be a potent serotonin reuptake inhibitor and has a wide range of therapeutic applications, may be used as an alternative to fluoxetine due to its broad therapeutic range. Desmethyl clomipramine, which varies from clomipramine only by one methyl group, is the drug's main active metabolite and has a 50% absolute oral bioavailability **[4]**.

The pharmacokinetics of clomipramine (CMI) after oral and intravenous treatment demonstrates that the medication is swiftly absorbed from the gastrointestinal system and undergoes significant demethylation as part of first-pass metabolism in the liver, where it is converted to its primary metabolite, demethylclomipramine (DCMI) **[5]**. Because of its high permeability and high solubility, clomipramine belongs to the BCS class I substance **[6]**.

According to a survey of the literature, many methods for finding clomipramine in biological or pharmacological formulations have been documented. Few hyphenated methods for clomipramine were described, including LC-MS/MS **[7, 8]**, LC/MS **[9]** UPLC MS/MS **[10]**, few chromatographic techniques, including HPLC **[11-13]**, HPLC-UV **[14]**, GC **[15]**, GC-MS **[16]**, spectrophotometry **[17]**, and spectrofluorimetry **[1, 18]**. For this sample of

clomipramine, no multivariate calibration technique (MVC) utilizing UV spectrophotometry was described. Therefore, the development of the UV spectrophotometric MVC for the determination of clomipramine is the focus of the current technique. Applied analytical technique offers powerful, rapid, sensitivity, and low-cost quantitative analysis of investing admixtures under optimized conditions.

The following equations may be generated for each chosen wavelength when the absorbance of a sample(x) is measured at various wavelengths (λ), namely at 248, 250, 252, 254, and 256nm.

$A_{\lambda 248}$= a X C_X + k1 …… (1)

$A_{\lambda 250}$ = b X C_X + k2…… (2)

$A_{\lambda 252}$ = c X C_X + k3 …….. (3)

$A_{\lambda 254}$ = d X C_X + k4 …… (4)

$A_{\lambda 256}$ = e X C_X + k5 …… (5)

Whereas,

- A_λ= Absorbance of the sample;
- a, b, c, d, e = Slope of the straight regression functions of a sample;
- k_1, k_2, k_3, k_4, k_5 = Intercept of the straight regression;
- C_X = Concentration of the sample

The five equations mentioned before can be arranged as follows:

$A_T = a \times C_X + b \times C_X + c \times C_X + d \times C_X + e \times C_X + K_T$…… (6)

The aforementioned equation may be reduced even more to

$A_T = C_X (a+b+c+d+e) + K_T$……. (7)

Whereas,

- A_T = Sum of the absorbances acquired
- K_T = Sum of intercepts of regression equation

To determine the amount of analyte X in a solution, use the formula.

$$Cx = \frac{A_T - K_T}{(a+Ab+c+d+e)} \quad \text{……(8)}$$

Greenness evaluation techniques

According to "The Globally Harmonized System of Classification and Labeling of Chemicals (GHS)," the eco analytical scale **[19]** assigns penalty points depending on the number of signal words coupled with the pictograms. In the analytical eco scale method, each reagent is taken into account, together with its type, amount, possible occupational exposure, energy depletion, and waste. An initial score of 100 points is reduced by penalty points.

Analytical eco-scale = 100 - total penalty points …….. (9)

An additional illustration is the Green Analytical Procedure Index (GAPI) **[20]**, which is composed of five pentagons and has a unique color scheme. The colour coding of

the pictogram for each phase of an analytical procedure uses three levels of assessment. Green, yellow, and red are the three colors used by GAPI to depict the degrees of minimal, moderate, and significant environmental harm related to the analytical technique, correspondingly. In 2018, J. Potka-Wasylka provided succinct, accurate, informative reporting on GAPI **[20]**. AGREE metrics, **[21]** to quantify the greenness profile during the second evaluation procedure, specialized software is used. The software generates a circular figure with clockwise oriented numerals on the edges, ranging from 1 to 12. The 12 green analytical chemistry philosophies are depicted in these images. Depending on the data provided and their weight, the outputs of these 12 principles are rated from 0 to 1 on a scale of 1 to 12. On this overall scale, red denotes a value of zero, dark green a value of one or a number which is near one, and yellow a value in between. The twelve principles are put together with the core to generate a score that represents the degree of greenness.

MATERIALS AND METHODS:

Chemicals and solvents employed:

- Distilled water
- CLOFRANIL® TABLETS – (Label claim – 25 mg of CLP), manufactured by Sun Pharma Laboratories Pvt. Ltd., The medication formulations that were marketed were procured on a regional basis.

Solubility:

- Readily dissolving in water, methanol, and methylene chloride.

Instrumentation:

- UV-Vis double beam Spectrophotometer (Lab India UV-3092).
- Electronic balance (SHIMADZU AY-220H).
- Sonicleansonicator (model 160T, Thebarton-Australia).

METHOD DEVELOPMENT:

Selection of solvent:

Distilled water, which was used as the solvent throughout the analysis to solubilize the medication, was reported to be freely soluble.

Preparation of the standard solution

100 mg of the drug component were diluted in 100 mL of distilled water to create the clomipramine standard stock solution. We adjusted the concentration of this solution (7-13g/mL) and utilized it for further investigation.

Preparation of sample solution:

Accurately quantifying 50mg of clomipramine, it was subsequently placed into a 50ml volumetric flask. After adding 25ml of distilled water, the mixture was sonicated

for 10 minutes. The resulting volume was 50 ml (1000 µg mL^{-1}). The resulting mixture was then diluted with the solvent to generate concentrations between 7 and 13 µgmL^{-1}

λ_{max} determination and selection of wavelength for multivariate calibration:

Over the wavelength range of 200 to 400 nm, the working standard solutions for clomipramine were scanned against distilled water as the blank solution, which has a maximum absorption at 252 nm. The wavelength of the MVC method was accordingly located between these absorption peaks, at 248, 250, 252, 254, 256nm.

The proposed method's linearity, accuracy, and precision were verified in accordance with ICH recommendations **[22]**.

Linearity

The stock solution was adequately diluted with distilled water to achieve concentrations ranging from 7 to 13 µg mL^{-1}, which were then used to analyze the linearity and spectral area of clomipramine. The absorbance of linearity solutions at the appropriate wavelength was measured and analyzed for the MVC method.

Limit of Quantification and Detection

The following calculations were used to determine the Limits of Detection (LOD) and Limits of Quantification (LOQ) for clomipramine by considering the slope of the calibration curve and the standard deviation of responses for a given wavelength.

$$LOD = \frac{3.3 \text{ x standard deviation}}{\text{Slope}} \quad \text{..... (10)}$$

$$LOQ = \frac{10 \text{ x standard deviation}}{\text{Slope}} \quad \text{..... (11)}$$

Precision

The repeatability of the precision was evaluated using intraday and interday precision. A standard clomipramine solution with a concentration of 10 µgmL^{-1} was used to evaluate various degrees of accuracy. Six solutions were examined at five different wavelengths to assess repeatability. The absorbance of prepared solutions was measured three times at varying intervals on the same day for the intervariation scenario. Three more days of using the absorbance were employed to account for intravariation.

Accuracy

The clomipramine methodology's precision was assessed at 80, 100, and 120 percent of the concentrations of the previously examined sample solutions, while the recovery values percentages were estimated.

Assay

Weigh and powder 10 Tablets. Weigh accurately a quantity of the tablet powder equivalent to about 50mg of clomipramine, add 25 ml of distilled water and sonicate for 10mins. Add sufficient distilled water and make up to 50mL. The solution obtained

above is filtered and diluted with distilled water to attain 10 µg mL^{-1} concentration of clomipramine. By measuring the absorbance of the resulting solution at 252 nm, the concentration of clomipramine is identified.

RESULTS AND DISCUSSION

Clomipramine standard solution was originally scanned between 200 and 400 nm. The maximum spectrum of clomipramine has a wavelength of 252 nm. The UV spectrum of standards and samples of clomipramine was recorded using distilled water as a blank and using the nm of 252nm for MVC. **Figure 2** displays the usual spectra of clomipramine at 10 µg mL^{-1}.

Linearity

The linearity findings for the developed method for clomipramine were determined within the concentration ranging from 70 to 130 percent for 10g mL-1 (7 to 13 µgmL^{-1}), in accordance with ICH Q2 R1 requirements. In **Figure 3,** the linearity spectrum of clomipramine is depicted. By estimating the absorbance of reference solutions that had been diluted at five distinct wavelengths (248, 250, 252, 254, 256), the calibration curve was produced. **Table 1** presents the observed results in tabular form. In the chosen concentration range, it was discovered that all of the standard curve were linear. **Figure 4-8** and **Table 2** depict the calibration graphs, and regression analysis, respectively.

Limit of Detection and Limit of Quantification

The linearity slope was employed for calculating the LOD and LOQ for clomipramine, and many sample studies have supported this method. The average of all the absorbance was used to compute the LOD for clomipramine, which was found to be 1.4132 µg mL^{-1}. The average of all the absorbances was used to compute the LOQ for clomipramine, which was found to be 4.2826 µg mL^{-1}.

Precision

Figure 9 shows the system precision spectra for clomipramine. **Figure 10** depicts the clomipramine interday precision spectra. **Figure 11** for clomipramine depicts the intraday precision spectra. For clomipramine, the percentage RSD of the system's intraday and interday precision was calculated. It was discovered to be less than 2%, demonstrating the precision of the approach method. Comparing the results acquired from other accuracy approaches, the suggested method exhibits good precision.

Accuracy

Figure 12 shows the overlay spectra for clomipramine, were verified for accuracy at 80, 100, and 120%. The clomipramine

findings are displayed in **Table 3**, and it was determined that the results were within acceptable bounds.

Assay of marketed formulations:

Using the proposed spectrophotometric method, the quantity of clomipramine in the tablet's formulation was examined. The UV absorption spectra of a commercial medication was tested three times. During extraction and filtration, the pharmaceutical formulation's excellent analytical recovery values remained stable. The **Table 4** presents the findings.

Evaluation of Greenness Profile

The outcomes of the suggested approaches' greenness profile were assessed. Analytical scale findings are displayed in **Table 6**, whereas agree and GAPI metrics data are provided in **Figures 14 and 15.**

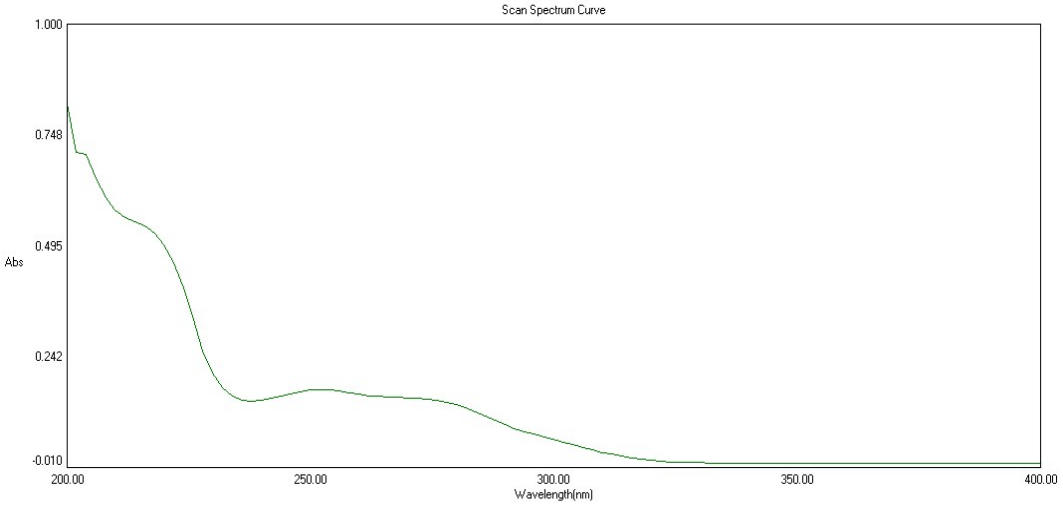

Figure 2: UV spectrum of standard clomipramine (10 µg mL^{-1}) using water as blank

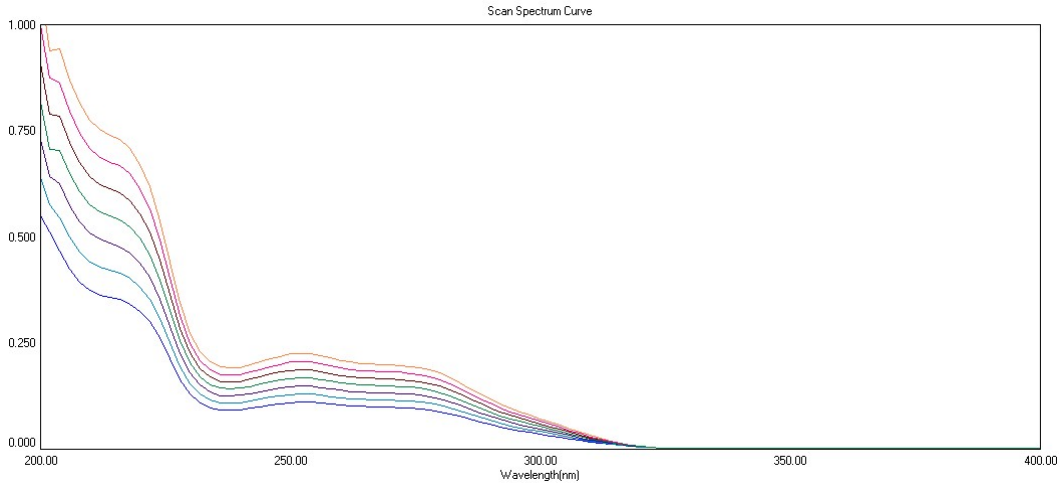

Figure 3: Linearity spectrum of clomipramine (7-13 µg mL^{-1}) using water as a blank

Table 1: Multivariate UV calibration data at five selected wavelengths

Concentration (μgmL^{-1})	248nm	250nm	252nm	254nm	256nm
7	0.106	0.109	0.110	0.109	0.107
8	0.125	0.127	0.129	0.128	0.126
9	0.144	0.147	0.148	0.147	0.145
10	0.162	0.166	0.167	0.166	0.163
11	0.181	0.185	0.184	0.185	0.182
12	0.199	0.204	0.205	0.205	0.201
13	0.218	0.223	0.224	0.224	0.219

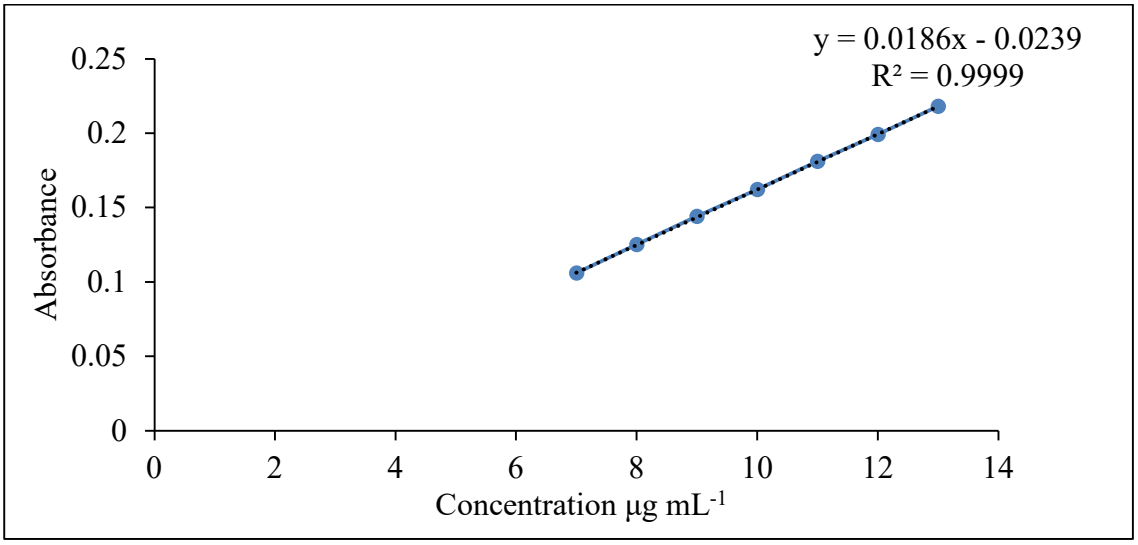

Figure 4: Calibration curve at 248nm

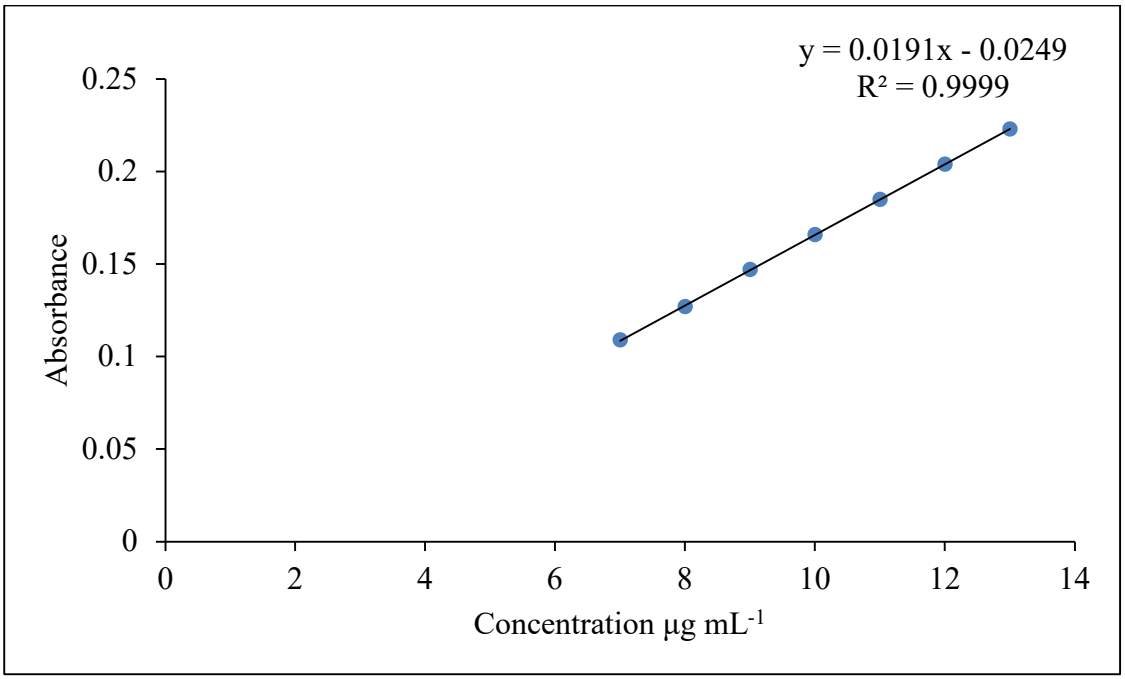

Figure 5: Calibration curve at 250nm

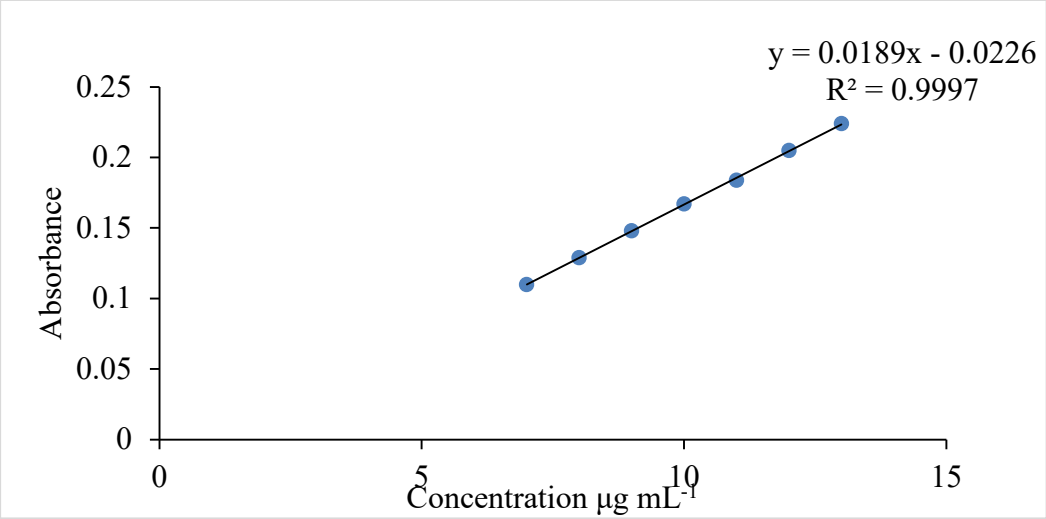

Figure 6: Calibration curve at 252nm

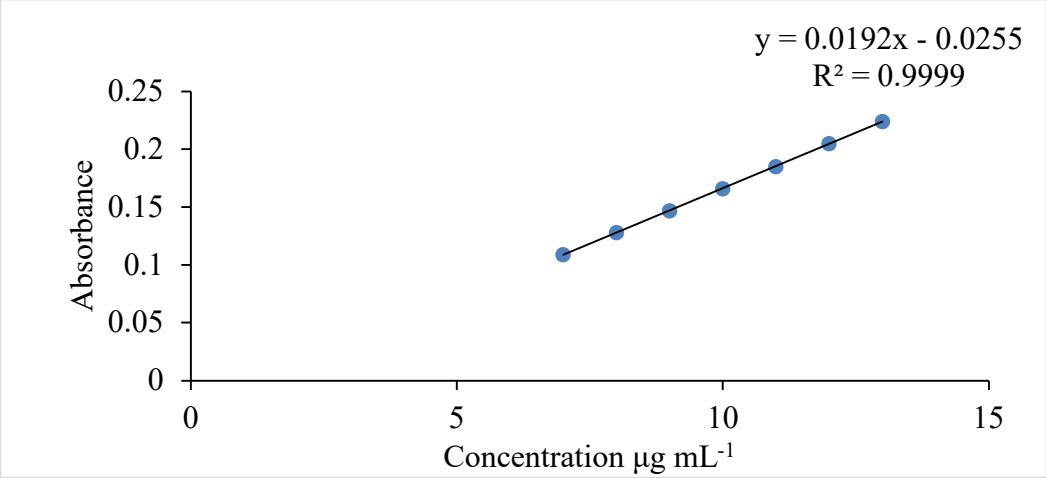

Figure 7: Calibration curve at 254nm

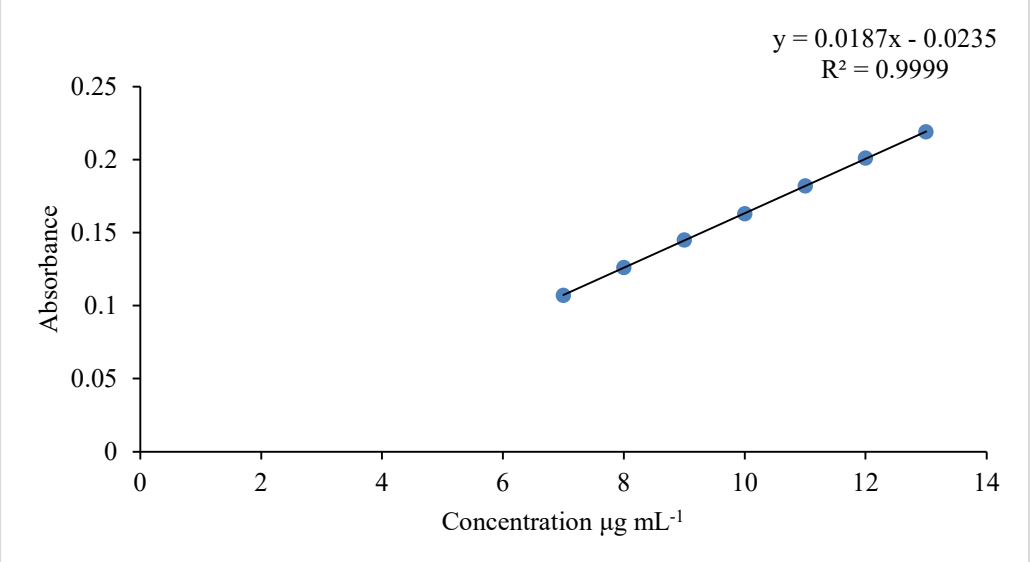

Figure 8: Calibration curve at 256nm

Table 2: Linearity data shows statistical parameters at the selected wavelengths

Wavelength(nm)	Regression equation	Slope	Intercept	R^2	LOD ($\mu g\ mL^{-1}$)	LOQ ($\mu g\ mL^{-1}$)
248	y = 0.0186x - 0.0239	0.00186	- 0.023	0.9999	0.5805	1.7591
250	y = 0.0191x - 0.0249	0.00191	-0.024	0.9999	1.4898	4.5146
252	y = 0.0189x - 0.0226	0.00189	-0.022	0.9997	1.4132	4.2826
254	y = 0.0192x - 0.0255	0.00192	- 0.025	0.9999	0.5632	1.7067
256	y = 0.0187x - 0.0235	0.00187	-0.023	0.9999	0.5782	1.7524

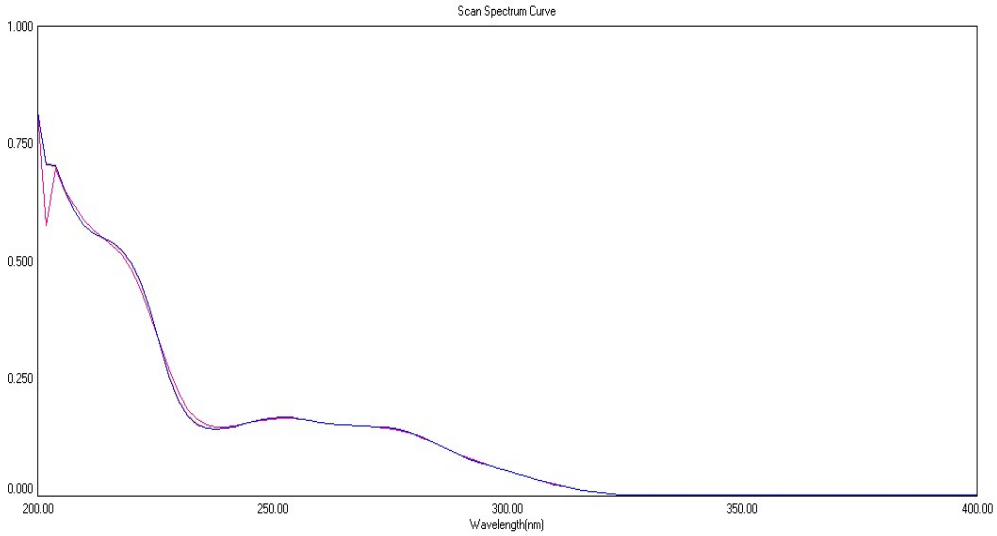

Figure 9: System precision overlay spectra of clomipramine

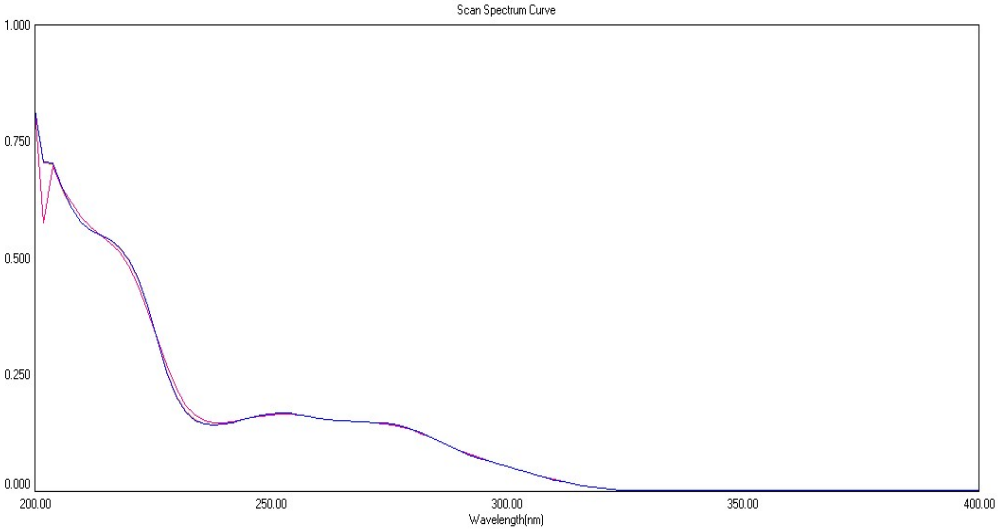

Figure 10: Interday precision overlay spectra of clomipramine

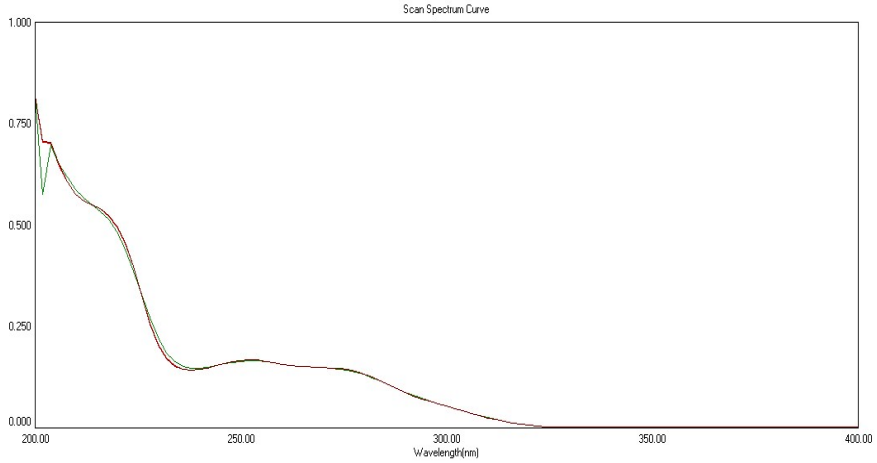

Figure 11: Intraday precision overlay spectra of clomipramine

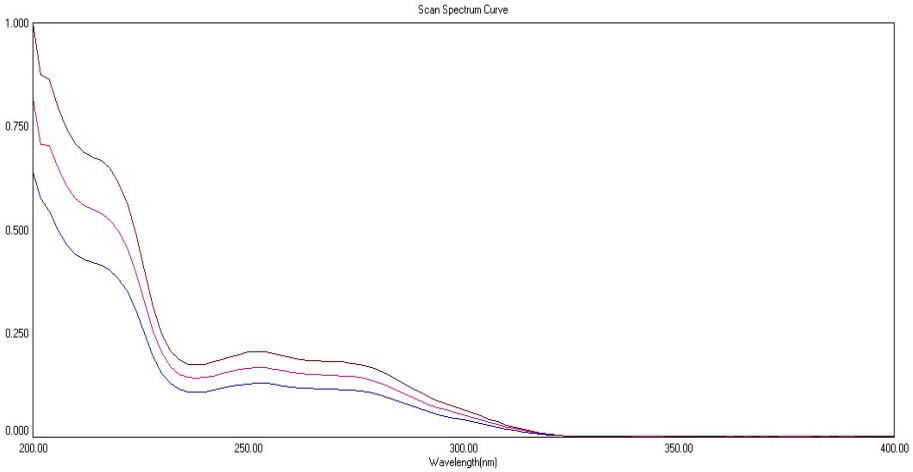

Figure 12: Overlay spectra of accuracy of clomipramine 80, 100, 120 % raising

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg mL^{-1})	Amount added (µg mL^{-1})	Absorbance	Amount recovered (µg mL^{-1})	% Recovery
248	4	4	0.125	3.97	99.25
		6	0.162	5.97	99.50
		8	0.199	8.01	100.13
250	4	4	0.128	4.02	100.50
		6	0.166	5.95	99.17
		8	0.204	7.98	99.75
252	4	4	0.129	3.98	99.50
		6	0.167	6.03	100.50
		8	0.205	7.95	99.38
254	4	4	0.128	3.99	99.75
		6	0.166	6.01	100.17
		8	0.205	7.98	99.75
256	4	4	0.126	3.97	99.25
		6	0.163	5.96	99.33
		8	0.201	7.97	99.63

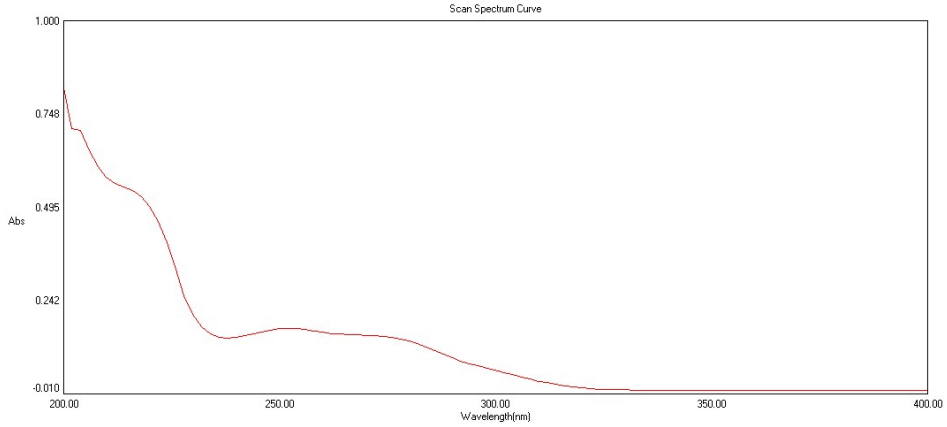

Figure 13: UV spectrum of standard clomipramine (10µg mL^{-1}) using water as a blank

Table 4: Assay of Methimazole

Label claim (mg)	Amount estimated (mg)	% Assay
25	24.98	99.92
25	25.01	100.04
25	24.95	99.80
Average	24.98	99.92
SD		0.12
% RSD		0.12

Table 6: Summary of Eco scale penalty points for the proposed method

Description	Penalty points	Total Penalty Points	Score
Water	0	3	97
Instrument	0		
Occupational hazard	0		
Waste	3		

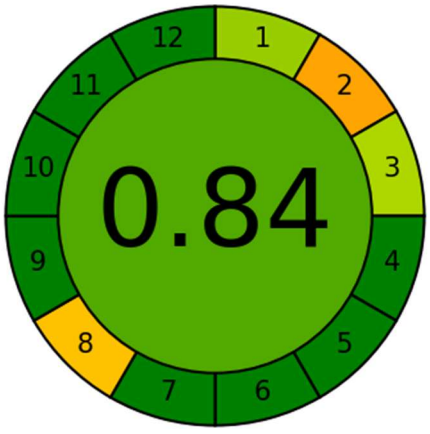

Figure 15: Agree metrics output for the proposed method

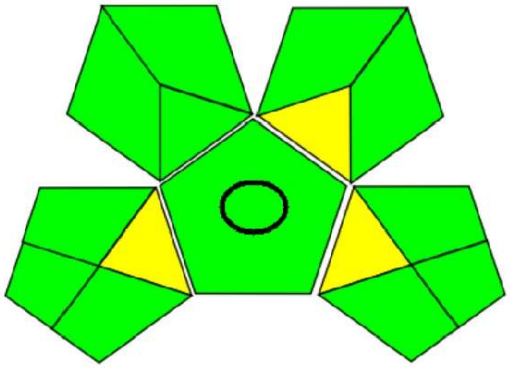

Figure 14: GAPI Pictogram for the proposed method

CONCLUSION

By assessing a variety of validation criteria, the newly developed spectrophotometric technique for the measurement of clomipramine was validated and found to be within acceptable ranges in accordance with ICH guidelines. The measurement of clomipramine in its tablet formulation was shown to be sensitive, accurate, precise, and reproducible using the described approach. We strongly advise using the proposed methodology for routine research on clomipramine in pharmaceutical formulations since it is more accurate than existing UV spectrophotometric approaches and comprises a method with straightforward mathematical components.

STATEMENT OF ETHICS

There are no animal or human participants used in this study's trials.

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DISPUTE OF INTEREST

There are no financial interests that could be at odds with this content.

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