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A Reliable Spectrophotometric Approach for Accurate Quantification of Desipramine Hydrochloride in Pharmaceuticals

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Abstract: **A short, simple, robust, and reliable UV-visible spectroscopic method has been optimized and validated for the quantification of Desipramine HCL in both active pharmaceutical ingredients (APIs) and tablet forms. This optimized technique utilizes a dual-wavelength UV-Visible spectrophotometer, set at 251nm, with a 1cm quartz cell, and is operated using Lab Solutions software. The method, validated in line with International Conference on Harmonization (ICH) guidelines, shows excellent specificity, precision, robustness, and linearity across a concentration range of 10.5µg/mL to 31.4µg/mL, with a 0.9999 correlation coefficient. Accuracy was confirmed through recovery tests, with recoveries ranging from 10.42µg/mL to 30.66µg/mL and an overall mean recovery (n=9) of 100.1%. Solution stability tests showed that both the standard and sample solutions remained stable for up to 24hours at 25°C. The method's robustness was verified by making deliberate changes to sonication time and wavelength, with all results within acceptable limits. This optimized method is fast, simple, cost-effective, and validated for quantifying Desipramine HCL, making it suitable for predicting pharmaceutical shelf life, aiding in new product formulation in research & development, and ensuring consistency in quality control.**

Index Terms: **Assay by UV, Pharmaceutical analysis, Spectrophotometric Analysis, Validation, Validated Spectrophotometric Method.**

I. INTRODUCTION

Desipramine HCL, a derivative of dibenzazepine, is a tricyclic antidepressant. Its chemical name is 5H-Dibenz [b, f] azepine-5-propanamine, 10, 11-dihydro-N-methyl-monohydrochloride (Daily Med, Desipramine hydrochloride). The chemical structure of Desipramine hydrochloride is illustrated in Figure 1. This compound is soluble in water and alcohol, with a molecular mass of 266.4 g/mol (National Library of Medicine). The typical daily adult dosage

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of Desipramine HCL ranges from 100 to 200mg, which may be increased to 300mg in more severe cases (Drugs and Supplements guide). Several analytical methods have been documented for estimating Desipramine hydrochloride. These include HPLC methods published in the USP (USP NF Desipramine HCL-2020; USP NF Desipramine Hydrochloride Tablets-2022) and other sources (Armagan et al., 2011; Torben et al., 1995; Tamara et al., 1979; Sun et al., 1995), as well as UHPLC (Jing et al., 2015) and UV spectrophotometry methods (H N Deepakumari et al., 2014; Wieslawa et al., 2000). Additionally, colorimetry (F. A. El-Yazbi et al., 1985) and gas chromatography methods (Mohammad et al., 2012) have been used. Studies on the degradation behavior of Desipramine hydrochloride (Thilak Kumar et al., 2008; Nareman D.H. et al., 2016) and its pharmacological properties (Jean D. et al., 2007) have additionally been documented. However, each of these methods has limitations, such as the high cost of chromatographic equipment (USP 621 Chromatography), which can be prohibitive for routine laboratory analysis. UV spectrophotometric (USP -857 Ultraviolet-Visible Spectroscopy) and colorimetric methods often require derivatization, increasing sample handling costs and time. According to the literature, there is a need for simple, rapid, and environmentally less toxic methods (Agnieszka Gałuszka et al; 2013) using inexpensive UV spectrophotometry for estimating Desipramine hydrochloride in APIs and pharmaceutical tablet dosage forms. Therefore, this research aims to optimize, validate a UV spectrophotometric method for the estimation of Desipramine hydrochloride in pharmaceuticals. Table 1 provides a comparison of the proposed method with existing spectrophotometric and chromatographic methods.

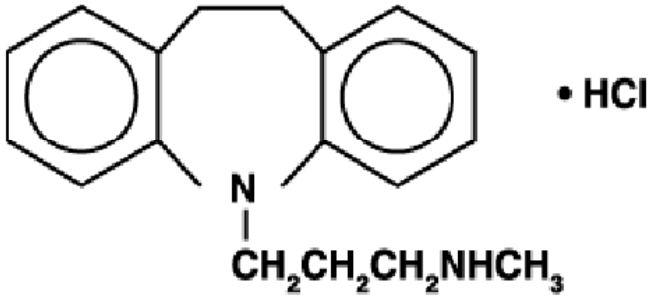


Fig. 1. Representative chemical structure of Desipramine Hydrochloride

Table I: Comparison of proposed method with existing spectrophotometric / chromatographic methods

Sr. No.	Reagents Used	Methodology	Observation	Reference
1	Sodium acetate trihydrate, glacial acetic acid, Acetonitrile, methanol and 0.1 M Hydrochloric acid, water	By HPLC	1) Requires many reagents 2) Needs costly columns and sophisticated instruments 3) Environmentally less friendly due to multiple reagents and solvent; time consuming method and solution 4) Applicable only for API 5) Commercially costly	USP NF Desipramine HCL-2020

2	Sodium acetate trihydrate, glacial acetic acid, Acetonitrile, methanol and 0.1 M Hydrochloric acid, water	By HPLC	1) Requires many reagents 2) Needs costly columns and sophisticated instruments 3) Environmentally less friendly due to multiple reagents and solvent; time consuming method and solution. 4) Applicable only for tablets 5) Commercially costly	USP NF Desipramine Hydrochloride Tablets-2022
3	Acetonitrile, KOH, Chloroform, Tetracyanoquinodimethane, water	By HPLC	1) Needs costly columns and sophisticated instruments. 2) Environmentally less friendly due to multiple reagents and solvent; time consuming method and solution 3) Commercially costly	Armagan Önal et al.,2011
4	Methanol, Acetonitrile, Sodium borohydride, Sodium di hydrogen phosphate, hexane, water	By HPLC	1) Requires derivatization. 2) Requires many reagents. 3) Needs costly columns and sophisticated instruments. 4) Environmentally less friendly due to multiple reagents and solvent; time consuming method and solution preparation. 5) Commercially costly	Torben Elm et al., 1995
5	Potassium bromide, Eriochrome blue black, Sulfuric acid, water	UV-Spectrophotometer	Bromination is needed, Exposure to bromine vapor cause severe health problems.	Jing Zhao et al., 2015
6	Ammonium metavanadate, H_2SO_4, Water	UV-Spectrophotometer	Derivatisation is needed using ammonium metavanadate and H_2SO_4. H_2SO_4 is highly dangerous and requires careful handling.	Wieslawa Misiuk et al., 2000
7	Water and Methanol	UV-Spectrophotometer	Short, simple, robust and validated, involved water and methanol reagent, less time for sample preparation and instrument set up. Lowered the solvent content, Derivatisation not needed.	Optimized method

II. MATERIALS AND METHODS

A. INSTRUMENTATION:

The analysis was conducted using a UV-1900 dual-wavelength UV-Visible spectrophotometer (Shimadzu Corporation, Japan) with a 1 cm path length quartz cell at a wavelength of 251 nm. The instrument was monitored with Lab Solution software.

B. STANDARD, SAMPLES AND CHEMICALS:

A standard Desipramine HCl with a purity of 99.60%, a commercial sample of Desipramine HCl with a labeled claim of 10 mg, and a placebo containing a mixture of microcrystalline cellulose, croscarmellose sodium, citric acid monohydrate, hydroxypropyl cellulose, hypromellose, magnesium stearate, titanium dioxide, polyethylene glycol, talc, FD&C Blue No. 1 Aluminum Lake, and iron oxide yellow were received as gift samples from Chemclues Laboratory in Mumbai. Analytical grade methanol was procured from Merck India, and Milli-Q grade water was utilized for the analysis.

1) Preparation of Diluent: Water and methanol were mixed in a 50:50 volume/volume (v/v) ratio.

2) Preparation of the standard solution:

10 mg of Desipramine HCl standard was weighed and transferred into a 25mL volumetric flask. It was dissolved using the diluent and mixed well. Then, 1mL of this solution was further diluted to 20mL in another volumetric flask using the same diluent and mixed well, resulting in 20µg/mL of Desipramine HCl.

3) Preparation of test solution:

10-tablets were weighed to determine the average weight, and then crushed into a powdered form. An amount equivalent to the average weight was transferred to a 50mL volumetric flask. Introduced 20mL of diluent, and mixed than sonicated for 15minutes with intermittent shaking, cooled to room temperature, diluted to the mark and mixed well. 2mL of this solution was then further diluted to 20mL in another volumetric flask using the same diluent and mixed well, resulting in 20µg/mL of Desipramine HCl.

C. METHOD VALIDATION:

The optimized spectroscopic method was validated inline with ICH guidelines to ensure the accuracy and reliability of the data. The validation process followed the standards outlined in ICH guidelines (ICH Q2R2), which provide a comprehensive framework for validating analytical procedures. This validation ensures the method's accuracy and reliability, making it suitable for quantitative analysis in various settings. Key validation parameters considered during this process included the following:

1) *System suitability Criteria:* System suitability tests are typically performed using standard solutions and are a prerequisite for validating the performance of the method before analyzing samples. It involves assessing various performance characteristics of the system to confirm that it meets the required standards before and during the analysis.
2) *Specificity:* The specificity parameter is evaluated to measure the degree of interference in the analysis of complex test mixtures. This parameter assesses the method's ability to accurately and specifically measure the analytes present in the test.
3) *Linearity and Range:* Linearity refers to a method's capability to yield results that are directly or indirectly proportional to the analyte concentration within a defined range of tests. Range is the interval between the upper and lower concentration levels of the analyte that have been demonstrated with suitable accuracy, precision, and linearity.
4) *Accuracy:* Accuracy refers to the closeness of test results to the true value. The method's accuracy was evaluated by performing a recovery test.
5) *Precision:* Precision assesses the consistency and reproducibility of results. These parameters help ensure the method’s validity and reliability for its intended use.
6) *Filter study:* The filter study is a critical component of the analytical method validation process. It ensures that filters used in sample preparation do not

compromise the method's accuracy, precision, or overall reliability, thereby supporting the integrity of the analytical results.

7) *Solution stability:* Solution stability is a crucial part of method validation, ensuring that the analyte remains stable over the time period between sample preparation and analysis.
8) *Robustness:* Robustness is a key parameter in analytical method validation that evaluates the method's reliability under small, deliberate variations in analytical conditions. This assessment helps ensure that the method remains accurate and precise even when there are minor changes in experimental conditions.

III. RESULTS AND DISCUSSION

A. METHOD DEVELOPMENT:

To develop a quantitative spectrophotometric method for estimating Desipramine HCl in pharmaceutical dosage forms, various diluents, including water, methanol, and a mixture of water and methanol, was tested based on the solubility of Desipramine HCl. A final diluent mixture consisting of methanol and water in a 50:50 (v/v) ratio was chosen based on the optimal solubility and extraction efficiency of the analyte from the sample. During the development process, a standard solution of Desipramine was scanned from 800nm to 200nm using a 1cm quartz cuvette. The results indicated that the active analyte exhibited maximum absorption at 251nm, which is outside the visible range. Subsequently, the specificity of Desipramine HCL was assessed by analyzing a blank solution (diluent), a placebo solution (containing excipients and color), a standard solution, and a test solution. The observations revealed that neither the blank nor the placebo solution showed any absorbance at 251nm, confirming that diluent and all excipients would not interfere with the quantitative determination of Desipramine HCL in pharmaceuticals. Consequently, 251nm was selected as the wavelength for further analyses. The extraction of the analyte in the test solution was optimized by testing different methods, including mechanical shaking, vortexing, and sonication. It was found that sonication for 15 minutes using the methanol and water (50:50 v/v) diluent yielded satisfactory results. For the observed spectra from the blank, standard, placebo, and test solutions, refers to Fig. 2. The optical characteristics of Desipramine HCl are detailed in Table II.

Table II: Optical Characteristics of Desipramine HCL

Parameter	Observed value
λ max	251nm
Beers law limit	10.5-31.4µg/mL
Molar absorptivity	7.85 x 10-5
Sandells sensitivity (µgcm-2/0.001absorbance unit)	0.038
Regression equation (Y=Mx+C)	y = 0.0264x + 0.011

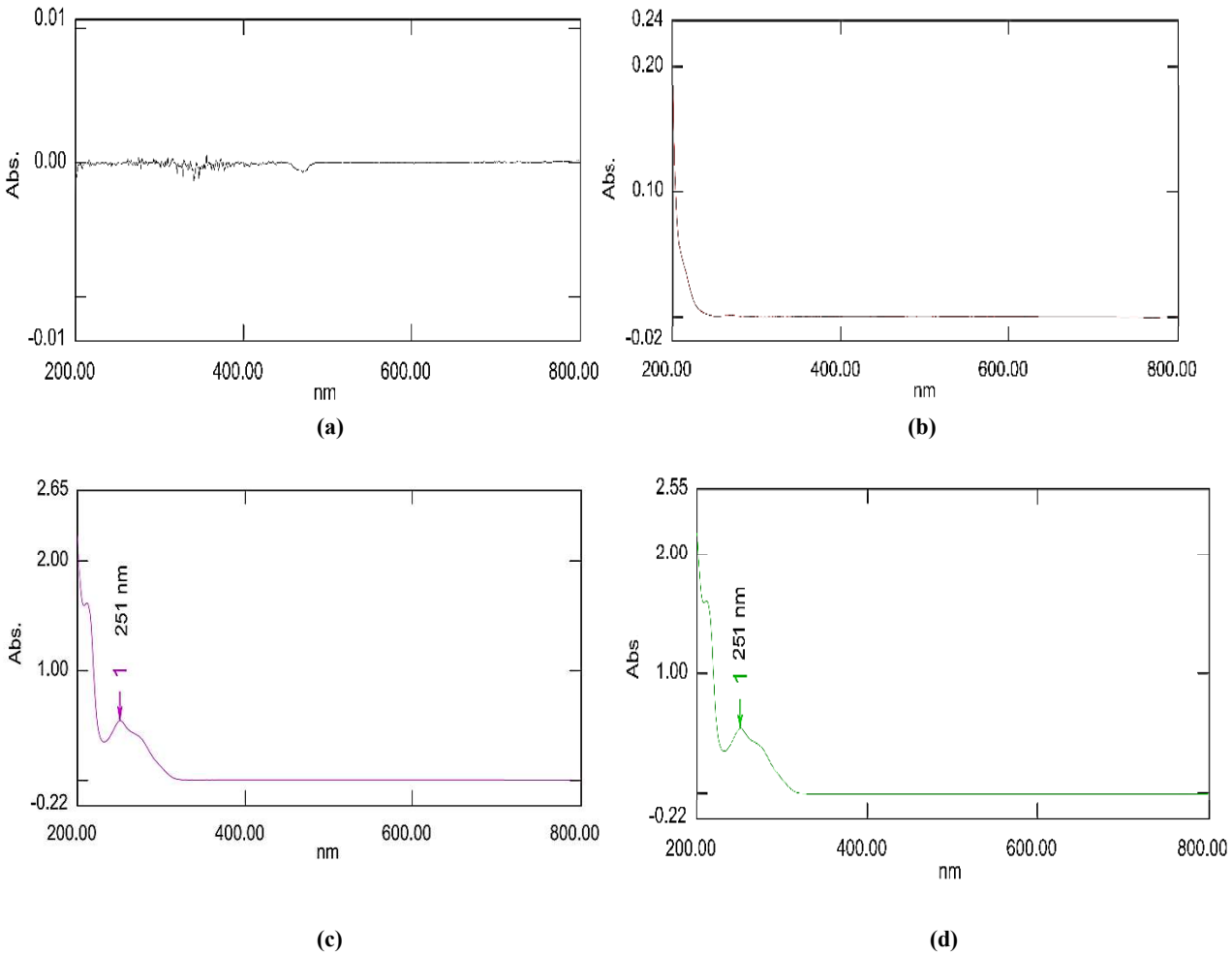


Fig. 2. Representative UV-Vis Spectra obtained from (a.) blank solution (b.) placebo solution (c.) standard solution (d.) test solution

B. METHOD VALIDATION:

The optimized spectroscopic method was validated in accordance with ICH guidelines to ensure the accuracy and reliability of the results. The validation process adhered to the standards outlined in ICH guidelines (ICH Q2R2) which provide a comprehensive framework for validating analytical procedures. These parameters ensure the method's trueness, providing confidence in its application for quantitative analysis in various settings. Key validation parameters considered during this process included following parameter.

1) System Suitability Criteria (SST):

In the system suitability criteria, the %RSD (relative standard deviation) of the absorbance from six replicates of the standard solution was monitored. The results obtained were within the acceptance criteria, indicating that the analytical system is functioning properly and that the results are accurate and reproducible. Detailed results of SST parameter represented in Table III.

Table III: Results of System suitability Parameter

Sr. No.	Absorbance of Desipramine HCL
1	0.543

2	0.543
3	0.544
4	0.543
5	0.543
6	0.543
Average	0.543
%RSD	0.08
Limit	NMT 2.0%

2) Specificity:

To ensure the specificity of the method, the method blank, placebo, standard, and test solutions were analyzed under specified conditions. It was observed that there was no interference with respect to the active analyte, indicating that the developed method can accurately measure the analytes in the presence of other substances. For the spectra obtained, refer to Fig. 2, and for the absorbance values of the different solutions at 251 nm, see Table IV.

Table IV: Results of Specificity Parameter

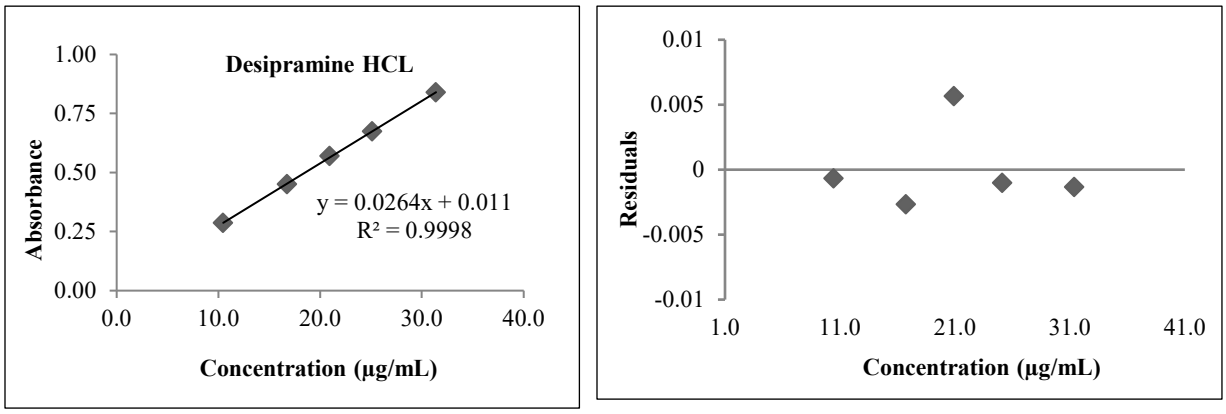
Sr. No.	Sample name	Absorbance
1	Blank solution	0.000
2	Placebo solution	0.000
3	Standard solution	0.543
4	Test solution	0.540

3) Linearity and range:

From the linearity analysis, the observed coefficient of correlation for Desipramine HCL is 0.9999, indicating that the method is linear within the concentration range of 10.5–31.4 µg/mL. For the linearity plot of Desipramine HCL, see Fig. (3a). The residuals plot, shown in Fig (3b) demonstrates that the linear model is appropriate and confirms that the assumption of linearity is valid. For detailed results of the linearity analysis, refer to Table V.

Table V: Results of Linearity Parameter

Parameter	Observed value	Acceptance Criteria
Range (µg/mL)	10.5-31.4	NA
Coefficient of correlation	0.9999	≥ 0.999
Slope of regression line	0.0264	To be reported
%Y- intercept	0.011	≤ 5%
Residual sum of square	0.00004	To be reported
Standard Deviation of Residuals	0.003	To be reported



(a) **(b)**

Fig. 3. A figure showing (a.) Linearity plot of Desipramine HCL (b.) Plot of residuals v/s concentration

4) Accuracy:

In accuracy test known concentration of the active analyte was added to a placebo solution. The recovery of the spiked analyte was then measured and calculated. The results obtained were within the acceptable range of 10.42 to 30.66µg/mL. This indicates that the method is accurate and suitable for routine analysis. For a comprehensive summary of the results, please refer to Table VI.

Table VI: Results of Accuracy Parameter

Parameter	Level-1 (50%)			Level-2 (100%)			Level-3 (150%)		
	1	2	3	1	2	3	1	2	3
Amount added (µg/mL)	10.33	10.31	10.41	19.90	19.90	19.92	30.31	30.32	30.34
Amount Recovered (µg/mL)	10.45	10.41	10.41	19.78	19.78	19.70	30.55	30.55	30.88
% Recovery	101.2	101.0	100.1	99.4	99.4	98.9	100.8	100.8	101.8
% Mean Recovery of each level (n=3)	100.8			99.2			101.1		
% RSD of each level (n=3)	0.60			0.26			0.57		
Overall % Mean Recovery (n=9)	100.1								
% RSD of each level (n=9)	0.91								

5) Precision:

The precision test was performed with six different sample preparations on different days by different analyst, evaluating the method's reproducibility by assessing intra-day (MP), inter-day (IP), and inter-analyst variations (MP and IP). The results from these precision tests were within the acceptance criteria, confirming that the method is both reliable and reproducible. For detailed results, refer to Table VII.

Table VII: Results of precision parameter (MP-Method Precision and IP-Intermediate precision)

Sr. No.	Absorbance	% Assay	Mean % assay of MP and IP(n=6)	The difference between mean % assay of MP and IP set, Limit-%difference ≤ 2	%RSD (n=6), Limit-%RSD ≤ 2	%RSD (n=12), Limit-%RSD ≤ 2
MP-1	0.540	99.0	99.6	0.4	0.40	0.49
MP-2	0.545	99.7				
MP-3	0.546	99.7				
MP-4	0.545	99.4				
MP-5	0.547	100.2				
MP-6	0.545	99.7				
IP-1	0.545	98.5	99.2		0.51	
IP-2	0.545	98.8				
IP-3	0.547	99.0				
IP-4	0.551	99.7				
IP-5	0.552	99.8				
IP-6	0.548	99.4				

6) Filter study:

The analysis was conducted by filtering the sample solution through different filters and comparing the results with those obtained from centrifuged samples. The results met the acceptance criteria, indicating that the filters did not introduce any contaminants or artifacts, nor did they adsorb the analyte. This filter study confirms that the filters used do not negatively impact the accuracy and precision of the analysis. For detailed results, please refer to Table VIII.

Table VIII: Results of Filter study Parameter

Parameter	Absorbance	% Assay	Difference of %Assay between Centrifuged and filtered solution (Limit-NMT 2.0%)
Test Centrifuged	0.545	99.8	NA
Test 0.45 Nylon	0.542	99.2	0.6
Test 0.45 PVDF	0.543	99.4	0.4
Test 0.45 PTFE	0.545	99.8	0.0

7) Solution Stability:

The analysis was performed at various time intervals after sample preparation, and the absolute difference in the percentage assay from the initial value was calculated. The results were within the acceptance limit, ensuring the accuracy and reliability of the analytical results. For detailed results, please refer to Table IX.

Table IX: Results of Solution stability Parameter

Condition	Absorbance of standard solution	Absorbance of Test solution	% Assay	Absolute %Assay difference from Initial (Limit- ≤ 2.0%)

Initial	0.543	0.540	99.0	NA
18-Hrs	0.548	0.549	99.6	0.6
24-Hrs	0.544	0.544	99.3	0.3
Average	0.545	0.544	NA	NA
%RSD (Limit- ≤ 2%)	0.49	0.83	NA	NA

8) Robustness

The robustness study was conducted by varying the wavelength by ±2 nm and altering the sonication time. System suitability and percentage assay were calculated and compared with the method's performance (MP) set. The results were within the acceptance criteria, indicating that the method is robust. This means that small changes in experimental conditions do not significantly affect the analytical results. For detailed results, please refer to Table X.

Table X: Results of Robustness Parameter

Sr. No.	Parameter	System suitability	%Assay
		%RSD	
1.	As per method (MP-Set, n=6)	0.08	99.6
2.	Sonication time (Actual 15-Min.)		
2a	13-Min.	0.00	99.2
2b	17-Min.	0.00	99.7
3.	Wavelength (Actual 251nm)		
3a	249nm	0.09	100.4
3b	253nm	0.29	100.1

CONCLUSION

The newly developed and validated UV-Visible spectrophotometric method for the analysis of Desipramine HCL demonstrated excellent linearity, precision, and accuracy across the tested concentration ranges. Robustness studies confirmed that minor variations in wavelength and sonication time do not significantly impact the results, ensuring the method's suitability for routine analysis. Additionally, filter and stability studies validated the integrity of sample preparation and the method's ability to produce consistent results over time. This method is straightforward, requiring minimal time for analysis and avoiding complex steps or derivatization processes. The instrument used is cost-effective compared to chromatographic methods, and the use of a methanol-water diluent (50:50 v/v) aligns with the principles of green

analytical chemistry (GAC) by minimizing the application of organic solvents. The method's efficiency is further enhanced by a short sample extraction time. This newly developed method is validated according to ICH guidelines, offers a cost-effective and reliable alternative for the estimation of Desipramine HCL, suitable for both research and development during formulation and for quality control in manufacturing processes. Overall, this UV-Visible spectrophotometric method is a robust and dependable tool for the quantitative analysis of Desipramine HCL in pharmaceuticals .

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