Development and Validation of Imipramine Hydrochloride In Tablet Formulation – A New Rp-HPLC Method

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ABSTRACT

A validated HPLC method was developed for the determination of Imipramine Hydrochloride in pharmaceutical formulation. Isocratic elution at a flow rate of 1.0ml/min was employed on Phenyl Bondapak 10µm (3.9 x 300 mm) or equivalent. A mixture of 0.1 Ammonium Phosphate: Acetonitrile in the ratio of 60:40V/V was prepared and used as mobile phase. The UV detection wavelength was 254nm and 10µl sample was injected. The run time is 5min and the flow rate was found to be 1.2 ml/min. The Approximate retention time was founded as ± 8 minutes. The% R.S.D Imipramine Hydrochloride was 0.1. The LOD was found to be 15µg/ml and the LOQ was found to be 25µg/ml. The mean Percentage recovery for Imipramine Hydrochloride was found to be 81%. The method was validated as per the ICH guidelines. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of formulations. The method developed is simple and is better than the methods reported in the literature.

Key Words: *Imipramine Hydrochloride, HPLC, UV detection, Recovery.*

1. INTRODUCTION

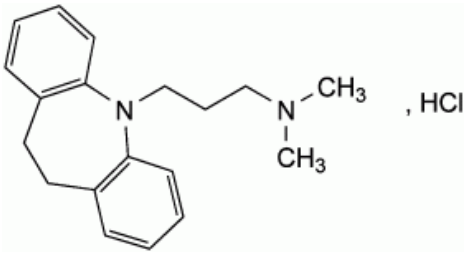

Fig: 1 Structure of Imipramine HCL

$C_{19}H_{24}N_2$.HCL is the formulae for Imipramine HCL. It is a white/slightly yellow, crystalline powder, soluble in water, alcohol and insoluble in ether. Melting point is 170 °C-174°C. Imipramine is used for treatment of depression, agitation, anxiety and to treat bedwetting. It is similar in efficacy to the antidepressant drug moclobemide.[1] It is a Tricyclic compound of dibenzazepine, in the chemical structure the side chain was attached with three rings.[2]. In British Pharmacopoeia it is official.[3-4] Literature survey concluded different methods alone and in combination with other drugs. [5-10] D. Srikantha et.al. [11] Developed a simultaneous determination of imipramine HCL and diazepam. They used the mobile phase as methanol 30V water50V and 0.1M sodium acetate 20 V. Chromosil C18 as column. Flow rate is 1.0 ml/min and wavelength 243nm. Finally satisfactory results were obtained for recovery as 100.95-101.52% for imipramine HCL, <2% is intraday and interday precision. Vishal Srivastava et.al., **[12]** proposed Diazepam and Impiramine HCL. Column as ODS C-18 (HIQ SIL 4.6mm x 25cm, 10µm). Mobile phase Methanol: Phosphate buffer (75:25 v/v). Rt for Impiramine HCL is 5.24 min. Linearity is in the range of 2-12 µg/ml. The percentage recovery is 98.81-99.96%. V.R.B. Vemula et.al. **[13]** performed chromatographic analysis on X Bridge C18 column (150×4.6 mm, 5µ), phosphate buffer with pH 3.4 & Acetonitrile (55:45) as mobile phase, at a flow rate of 1 ml/min and detected at wave length 250 nm. Results: The Rt for Imipramine 2.2 min. The regression value founded as 0.999 and linear response as 62.5-625µg/ml for Imipramine. Nagendrakumar AVD et.al.,[14] proposed stavudine by Inertsil ODS C-18, 5µm column having 250 x 4.6mm and mobile phase as Methanol 40V: 0.1 % O.P.A 50V: Acetonitrile 10V. 1.2ml/min is the flow rate and wavelength 267 nm. The Rt is 6.8 min and recovery is 97.2%. LOD and LOQ are 0.5ppm and 3.0ppm.

2. Experimental

2.1 Chemicals and Reagents: Imipramine HCL reference sample is purchased from Cipla. HPLC grade Ammonium phosphate and acetonitrile were purchased from Merck Specialties Pvt. Ltd

2.2. Instrumentation and Analytical Conditions: The analysis of drug was carried out on a PEAK HPLC system equipped with a Phenyl Bondapak 10µm (3.9 x 300 mm) or equivalent. Isocratic elution with 0.1 M Ammonium Phosphate with 60 V/V and acetonitrile with 40 V/V in the pH range 5.4 is used as a mobile phase. Before the use mobile phase was prepared freshly

and degassed by sonicating for 5min. By using Loba ultrasonic bath degassing of the mobile phase is performed. For weighing the materials a denwar analytical balance was used. The mobile composition effect on the Rt of Imipramine HCL was measured. The concentrations of the water and acetonitrile were optimized to give symmetric peak with short run time.

2.3. Chromatographic Conditions. The Stationary phase selection purely depends on sample nature, weight of the molecule and miscibility. Preferably by using column the Imipramine HCL drug in polar compounds is analyzed. For RP columns non-polar substance is highly attractive. As a mobile phase water and acetonitrile mixture was used.

2.3 Flow rate: To identify the flow rate the mobiles phase is altered in between 0.5 – 1.5mL/min to get optimum separation. From the experiment finally it is noted that flow rate of 1.0mL/min is suitable for the successful elution of the analyte.

2.4 Wave Length Detection: On UV/vis spectrophotometer 10ppm solution spectrum is recorded. Maximum absorbance wavelength peak is identified. Maximum absorbance spectra at 254nm is measured.

2.5. Stock and Working Standard Solutions.

2.5.1 Standard solution

Accurately 111 mg of Imipramine HCL reference standard is weighed and transferred into a 100ml volumetric flask. Diluted and make up to the volume with mobile phase and mixed well. Dilute 10 ml of this solution to 100 ml and dilute to volume with mobile phase. Further dilute 10 ml of this solution to 100 ml with mobile phase. Filter sample through a 0.45 µm filter. Solution must be made up immediately before use and protected from light.

2.5.2 Sample Preparation

Accurately determined 10 ml of mobile phase volume is taken. It is sonicated for 5 minutes. Squeezed the swab well. And filtered the sample through a 0.45 µm filter.

2.5.3 Blank Preparation

Place swab in 10 ml of mobile phase (volume accurately determined). Sonicate for 5 minutes. Squeezed the swab well. Filter sample through a 0.45 µm filter. Inject the blank,

standard and sample preparation according to test the system suitability to the following criteria:

2.5.4 Preparation of Standard solution (Stock Solution)

Accurately weigh 111 mg of Imipramine Hydrochloride standard into a 100 ml amber volumetric flask (11.1 mg/swab). Add 60 ml of mobile phase and sonicate for 10 minutes, cool and make up to volume with mobile phase. Dilute 10 ml of the above solution to 100 ml amber volumetric flask and dilute to volume with mobile phase 1.11 mg /swab (stock solution).

2.5.5 Preparation of Standard solutions (range)

From (1.11 mg/swab) stock solution the series of standard solutions were prepared. Nine solutions containing 0.111, 0.08325, 0.0555, 0.041625, 0.020812, 0.005203, 0.00052, 0.000052 mg/swab of Imipramine Hydrochloride, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed. The ranges of standard solutions are injected twice and the average result was used in graph.

3. Validation Procedure & Methods:

As per ICH guidelines the main objective of this proposed validation method is that this method is accurate. In this method different parameters were studies like intermediate precision, repeatability, system suitability, specificity, accuracy and stability and. Constructed different plots by using different concentrations with range of 05.0µg/mL to 30.0µg/mL prepared in triplicates to test linearity. Imipramine HCL peak area is plotted against the concentration to obtain the calibration graph. By using linear regression linearity is calculated by using least square regression method. By using five replicate injections of Imipramine HCL the repeatability is determined by using fresh Imipramine HCL solution. This method is repeated on two successive days for the intermedidate precision. Precision values are reported as percentage RSD and Imipramine HCL area is measured. By using ambient temperature in the range of 30±15°C stability values are identified in three days.

3.1 Specificity

Specificity of an analytical procedure is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The solvent and placebo solutions should not contain any components, which co-elute with the Imipramine HCL. The peak purity results from the photo diode-array analysis must show that the Imipramine HCL peak is pure – i.e. the purity angle (PA) must be less than the threshold angle (TH). The solutions listed below were injected using the conditions specified in the method of analysis. No components are seen to co-elute with the Imipramine HCL peak, and the peak Purity results indicate that Imipramine HCL peak can therefore be considered spectrally pure. For, Solvent, Detergent and placebo no significant peaks are detected. The chromatogram results were shown from the Fig:2 to 5 and peak purity are shown in Fig. 6 and Fig. 7

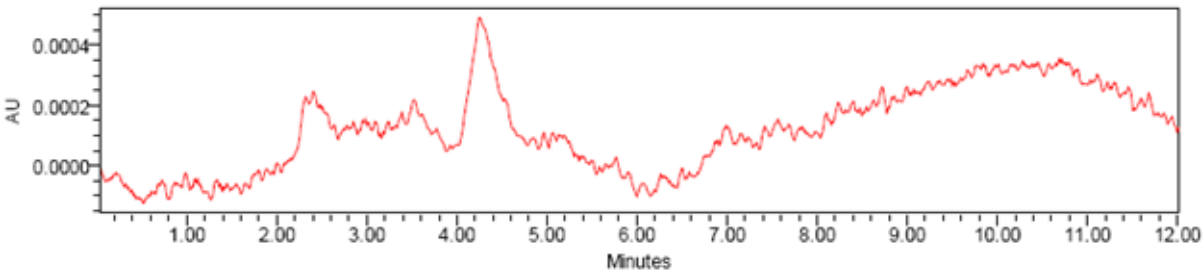

Fig: 2 Chromatogram 1 Solvent – No significant peak detected

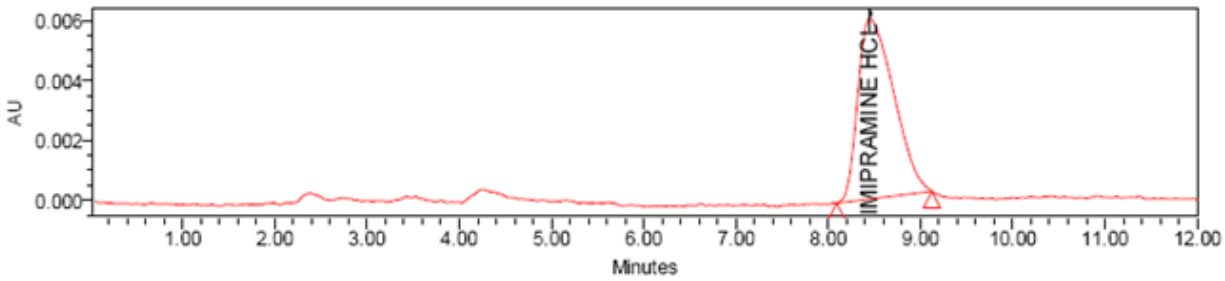

Fig: 3 Chromatogram 2 Drug active – Peak due to Imipramine HCL

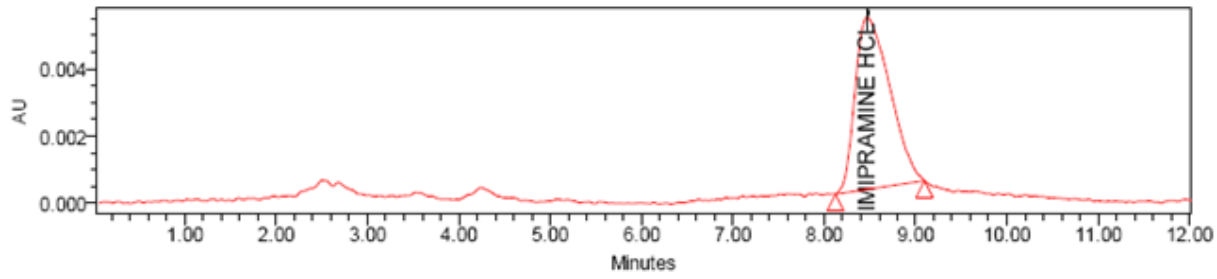

Fig: 4 Chromatogram 3 Product – Peak due to Imipramine HCL

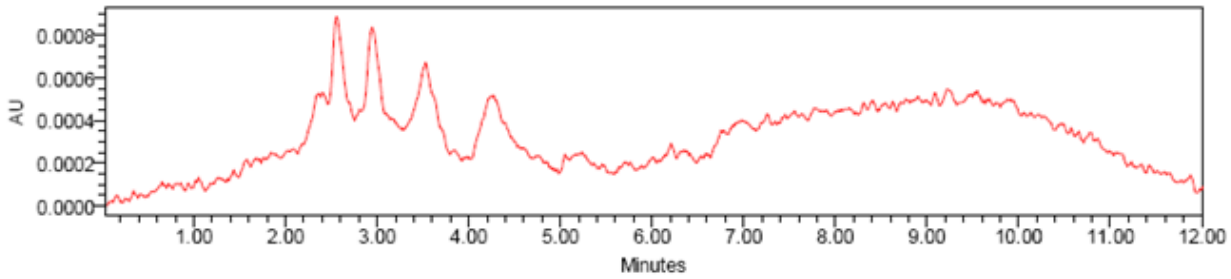

Fig: 5 Chromatogram 4 Active – UV stress: Peak due to Imipramine HCL

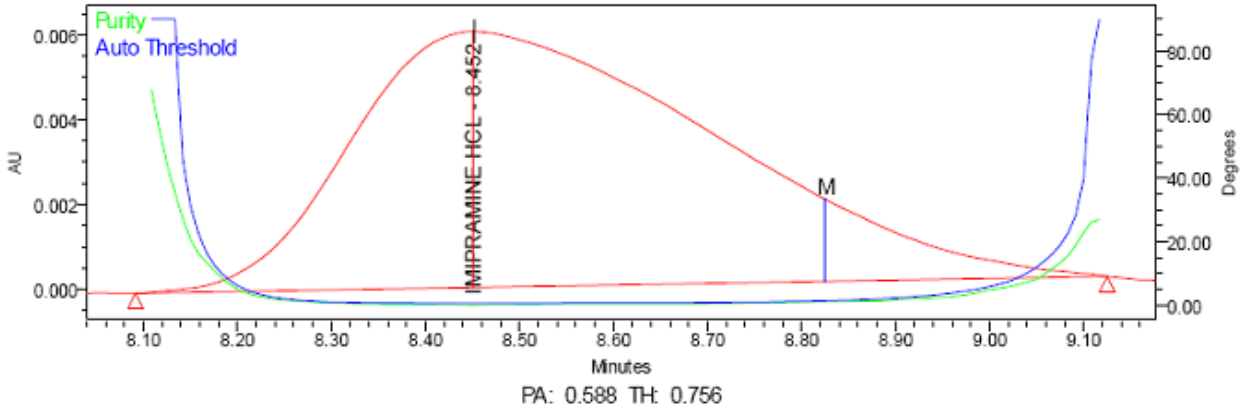

Fig: 6 Peak purity: 1 Purity angle < Threshold: 0.588 < 0.756

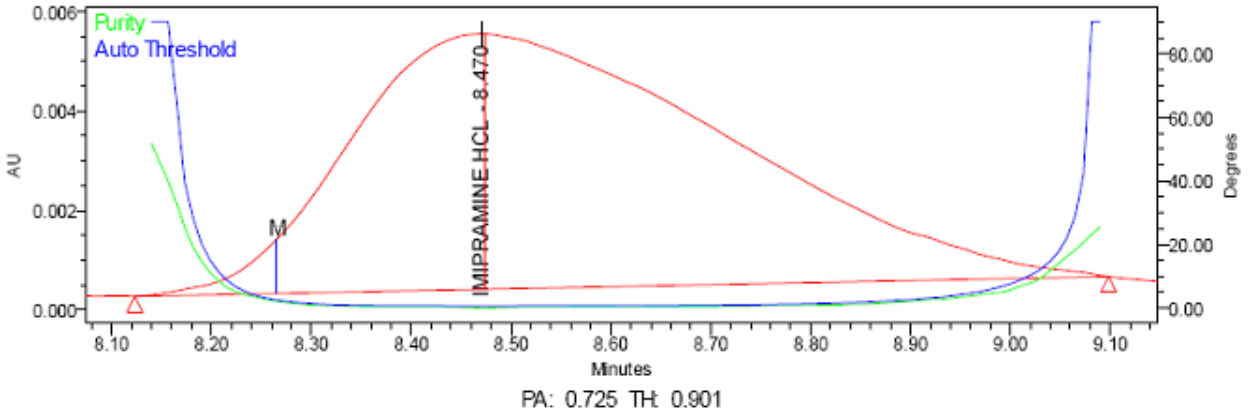

Fig: 7 Peak purity: 2 Purity angle < Threshold: 0.725 < 0.901

3.2 Detection Limit

The limit of detection by definition is a parameter of a limit test. It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions. It merely substantiates that analyte concentration is above or below a certain level. The Detection Limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliable detected. The maximum allowable carryover of Imipramine Hydrochloride is 0.110 mg/swab as determined in the Cleaning Validation Matrix.Nine solutions containing 0.111, 0.08325, 0.0555, 0.041625, 0.020812, 0.005203, 0.00052, 0.000052 mg/swab of Imipramine Hydrochloride, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed, 50% MAC is equal to 0.0555 mg/swab and the method gives linear response from 11.100 - 0.020812 mg/swab therefore the method can detect the above concentrations of API as required. The results are tabulated in the Table 1 and the calibration curve shown in the Fig.8.

Concentration mg/swab	Response 1	Response 2	Average Response
11.100	1612637	1609589	1611113
1.1100	152021	151608	1518145
0.08325	148342	149274	148808

0.04163	54822	54857	54840
0.02081	25346	25043	25195

Table : 1 Average Response

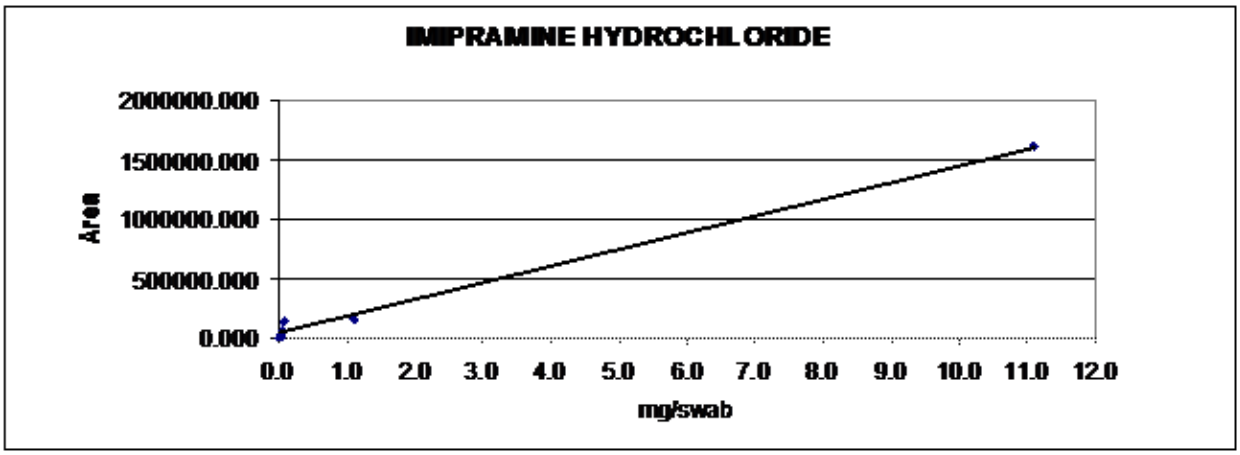

Fig: 8 Calibration curve

3.3 Linearity:

In between the range 15-140 µg/ml in 20.0 µg/ml to construct five-point graph three independent determinations are carried out. These measurements were fitted with a linear regression of the form y=ax+b and the values of regression parameters for the curves were r^2=0.989. All the linear regression parameters were statistically significant.

3.4 Precision:

The percentage of a test procedure expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The precision Percentage recovery of a known amount in the sample after swabbing. An amount of material which is to be predetermined limit is placed on a specific surface area (stainless steel) and swabbed. The precision of the analytical method is determined by assaying the swabs and calculating the % recovery of the API results. Six replicate injections of working standard solution were injected according to the method of analysis.

Sample Preparation

Placed 10 µl of solution 1 onto a specific surface area of stainless steel plate. Swab the surface area, taken the swab stick and place into a 10 ml volumetric flask. Added 10ml of solvent and sonicate for 10 minutes. Filtered the sample through a 0.45 µm filter paper. The % recovery should be greater than or equal to 65%. The results are tabulated in the table. 2.

Sample	% Recovery	% Average Recovery
1	82.59	79
	75.50	
2	91.81	90
	88.79	
3	93.08	93
	93.08	
4	84.81	88
	90.39	
5	85.88	87
	87.49	
6	96.59	95
	92.65	
Mean		89

Table: 2 Precision Values

3.5 Stability:

Stability of sample solution: Sample solution is injected after 24hr could not show any changes. Results are shown in table 3.

Drug	% Assay at 0 hr	% Assay at 24 hr
Imipramine HCL	99.21	99.81

3.6 System Suitability:

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. The % RSD of the peak responses due to Imipramine HCL for six injections must be less than or equal to 5.0 %. Six replicate injections of API MAC working standard solution were injected according to the method of analysis. The percentage relative standard deviations (% RSD) for the peak responses were determined. The analytical system complies with the requirements specified by the system suitability. The System suitability results were tabulated in the Table: 4.

Sample	Imipramine Hydrochloride Area
1	70736
2	72260
3	70575
4	71836
5	71909
6	71686
Mean	71500
% RSD	1.0

Table: 4 System Suitability Results

3.7 Robustness

Robustness Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms which demonstrated that the RPHPLC method developed is robust. The results are shown in below table 5. The data revealed that by varying the flow rate and column temperature, the acceptance criterion for system sutability is full filled for all the three parameters (ratio of peak areas). Moreover, by varying the flow rate and column temperature for sample solutions of dosage forms spiked with three known impurities, the relative retention time of all three impurities with respect to BD did not get affected. Thus, the developed method is robust. The results are tabulated in Table:5.

Parameter	Imipramine HCL	
	Retention time(min)	Tailing factor
Flow rate		
0.8ml/min	4.470	1.226
1.0 ml/min	3.493	1.186
Wavelength		
236nm	3.487	1.214
238nm	3.493	1.186

Table 5. Robustness results

4. Conclusion

A RP-HPLC method has been developed and validated for the determination of Imipramine HCL in tablet dosage form. This method is simple, rapid, accurate, precise, and specific. Its chromatographic flow rate is 1.5mins allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Imipramine HCL in pharmaceutical dosage forms.

5. Acknowledgements

The Analytical Formulation carried out for assay and content uniformity of Imipramine HCL in the drug product by using injection. The authors are thankful to management of GITAM Providing the necessary facilities.

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