Original Article

RP-HPLC Method Development and Validation for the Estimation of Imipramine Hydrochloride in Pharmaceutical Dosage Form

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Abstract

A simple, rapid, precise and accurate RP-HPLC method has been developed and validated for estimation of Imipramine Hydrochloride (IMH) in pharmaceutical dosage form. The method is carried out on Agilent C18 (25 cm x 4.6 mm i. d., 5 µ) column with a mobile phase consisting of Methanol: Acetonitrile: Buffer (0.1M Sodium acetate adjusted to pH 4.8 using TEA) in the ratio of 50:30:20 v/v. The flow rate of mobile phase was 1.0 ml/min and the analysis was performed using UV-Visible detector at 251 nm.

The retention time of IMH was found to be 3.8 min. %RSD of the Imipramine Hydrochloride were and found to be 0.9% and 0.8% respectively. The developed assay method was validated by the guidelines of ICH Q2R1.

The method was found to be linear within the range of 20- 120 µg/ml. The % RSD for intra-day precision and inter-day precision for IMH were found to be 0.58% and 0.16% respectively. The mean recovery of the IMH is 99.83-101.04%.

The method has been successfully adopted for determination of Imipramine Hydrochloride in pharmaceutical dosage form in regular quality control analysis.

Keywords: Imipramine; Validation; Precision; Determination.

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Introduction

Imipramine Hydrochloride [1], the prototypical tricyclic antidepressant (TCA), is a dibenzazepine derivative TCA. IMH is chemically 3-(5, 6-dihydrobenzo[b] [1] benzazepin-11-yl)-N, N-dimethylpropan-1-amine Hydrochloride[2,3]. Imipramine Hydrochloride (Figure 1) works by inhibiting the neuronal reuptake of the neurotransmitters nor-epinephrine and serotonin. It binds the sodium-dependent serotonin transporter and sodium-dependent norepinephrine transporter preventing or reducing the reuptake of serotonin and norepinephrine by nerve cells. In addition to acutely inhibiting neurotransmitter re-uptake, IMH causes down-regulation of cerebral cortical beta-adrenergic receptors and sensitization of post-synaptic serotonergic receptors with chronic use. This leads to enhanced serotonergic transmission. In literature survey several analytical methods like HPLC [4-12] and UV spectrophotometric [13] methods have been reported on assay of Imipramine Hydrochloride in combination with other drugs. However few chromatographic methods were reported for estimation of Imipramine Hydrochloride individually by using HPLC. The present study was designed to develop a simple, precise and rapid analytical RP-HPLC procedure, which can be used for the analysis of assay method for estimation of IMH.

Materials and Methods

Drugs and Formulations

The reference samples of Imipramine Hydrochloride (API) were provided from Yarrow

Chemicals, Mumbai, India. The commercial formulations were procured from the local market.

Chemicals and Solvents

Methanol (HPLC grade), Acetonitrile (HPLC grade), Sodium Acetate, Glacial acetic acid, Triethylamine were purchased from E. Merck (India) Ltd., Mumbai, India. Freshly prepared triple distilled water was used throughout the experiment.

Instrumentation

Agilent Technologies 1260 Infinity Binary HPLC was used for chromatographic studies; Shimadzu UV1800 Double Beam UV-Visible Spectrophotometer was used for spectral studies. Shimadzu BL220H Digital Weighing Balance was used for weighing the materials.

Selection of Mobile Phase

The solubility of Imipramine Hydrochloride was carried out in a variety of polar and non-polar solvents as per Indian Pharmacopoeia standards. Based on the solubility of the compounds finally methanol:acetonitrile:0.1M sodium acetate in the ratio of 50:30:20 v/v/v was selected as the mobile phase for the drug due to its positive results.

Detection of Wave Length

The spectrum of diluted solutions contains 10 µg/ml of Imipramine Hydrochloride in mobile phase were recorded separately on UV spectrophotometer and the solutions were scanned between 200-400 nm by using mobile phase as blank. The overlain spectrum was observed and the wavelength was found to be 251 nm for Imipramine Hydrochloride.

Preparation of Standard Stock Solution

About 100 mg of Imipramine Hydrochloride is accurately weighed and transferred into a 100 ml (1000 µg/ml) clean dry volumetric flask containing mobile phase. The solution was sonicated for 5 min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Imipramine Hydrochloride.

Preparation of Sample Solution

10 tablets were weighed and finely powdered. An accurately weighed portion of powder sample

equivalent to 100 mg of Imipramine Hydrochloride is transferred into a 100 ml (1000 µg/ml) clean dry volumetric flask containing mobile phase. The solution was filtered and sonicated for 5 min. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Imipramine Hydrochloride. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with the mobile phase.

Chromatographic Conditions

Buffer Preparation: Dissolve 1.36 gms of Sodium acetate in 100 ml of water. Adjusted the pH to 3.5 by using glacial acetic acid and then finally adjusted the pH to 4.8 using triethyl amine and the solution is filtered and sonicated for 5 min.

Mobile Phase: Methanol:Acetonitrile:Buffer (0.1M Sodium acetate)

Ratio	: 50:30:20 v/v/v
Column	: ODS (250 × 4.6 mm, 5 µm)
Wavelength	: 251 nm
Flow rate	: 1.0 ml/min
Observation	: Peak is observed at 3.8 min.

Method Validation

System Suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters (Table 2) like theoretical plates, resolution and asymmetric factor were evaluated.

Linearity

Linearity was performed by preparing standard solution of Imipramine Hydrochloride at different concentration levels i.e., 20-120 µg/ml. The absorbance was measured at 251 nm. The correlation, linearity results were presented in Table 3.

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The concentration used for the precision studies is 100 µg/ml and was assumed as 100%. To

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study the intra-day, inter-day precision, the analysis of drugs was repeated for six times in the same day and different days. The data was represented in Table 4.

Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed drug sample solution. The standard addition method was performed at three concentration levels in triplicate at 80%, 100% and 120%. The accuracy results are presented in Table 5.

Robustness

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength (Table 6). System suitability parameters were compared with that of method precision.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. For this study six replicates of the analyte at lowest

concentration were measured and quantified. Table 7 shows the values of LOD & LOQ.

Estimation of Imipramine Hydrochloride in Tablet Dosage Forms

An accurately weighed portion of powder sample equivalent to 50 mg of Imipramine Hydrochloride was transferred to a 50 ml volumetric flask. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of mobile phase. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with the mobile phase.

The standard solutions and sample solutions were determined at 251 nm and the amount of the drugs present in the tablet dosage form was calculated. The results (Table 8) were compared with the label claim of Imipramine Hydrochloride in tablet dosage forms and yielded 99.36%.
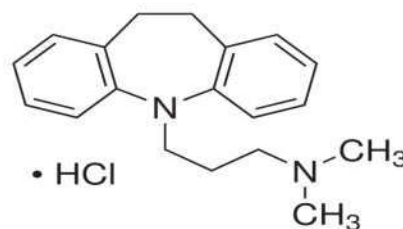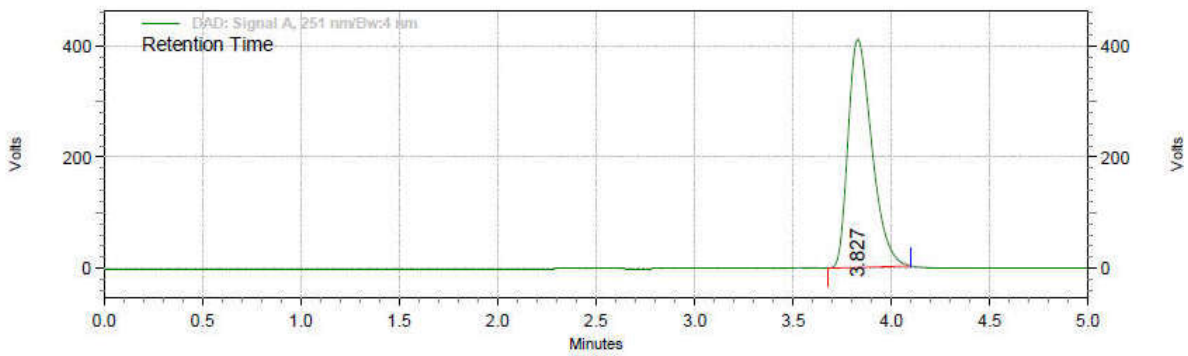

Fig. 1: Structure of Imipramine Hydrochloride

Table 1: Selection of mobile phase

S. No.	Mobile phase Compostion	RT (min)	Remarks
1	Methanol: sodium acetate buffer (pH 4.8) (70:30)	12.5	Retention time is too long
2	Acetonitrile: sodium acetate buffer (pH 4.8) (70:30)	10	Long retention time
3	Methanol:acetonitrile: sodium acetate buffer (pH 4.8) (60:20:30)	4	Slight tailing is observed
4	Methanol:acetonitrile: sodium acetate buffer (pH 4.8) (50:30:20)	3.8	Peak was eluted with good resolution

Table 2: Results for system suitability of Imipramine Hydrochloride

Injection	Retention time (min)	Peak Area	Theoretical plates (TP)	Tailing factor (TF)
1	3.833	7592163	4723	1.331
2	3.812	7615155	4791	1.329
3	3.795	7652133	5200	1.324
4	3.822	7552200	5110	1.311
5	3.799	7642259	4911	1.297
6	3.765	7582990	4822	1.349
7	3.782	7653256	4895	1.299
8	3.816	7701256	4698	1.312
9	3.832	7684352	5342	1.345
10	3.785	7712568	5123	1.362
Mean	3.8041	7638833.2	-	-
SD	0.02267867	52877.8097	-	-
%RSD	0.59616396	0.69222365	-	-

**DAD: Signal A,
251 nm/Bw:4 nm
Results**

Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry
	3.827	7237903	4758	1.45500

Fig. 2: Standard Chromatogram of Imipramine Hydrochloride
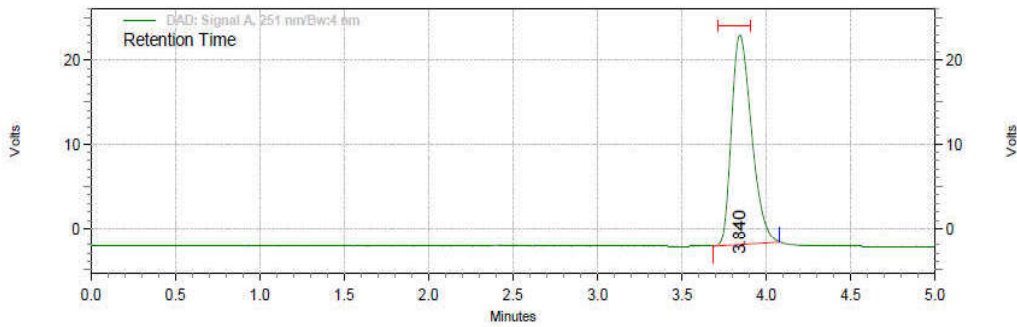
**DAD: Signal A,
251 nm/Bw:4 nm
Results**

Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry
Imipramine	3.840	434205	4866	1.40550

Fig. 3: Sample chromatogram of Imipramine Hydrochloride

S. No.	Concentration (µg/ml)	Peak area
1	0	0
2	20	1462168
3	40	2635906
4	60	4142460
5	80	5494147
6	100	7612163
7	120	8575999
	Slope	72959
	Intercept	10445
	Regression Equation(y)	y = 73011x – 105975
	Correlation Coefficient	0.994

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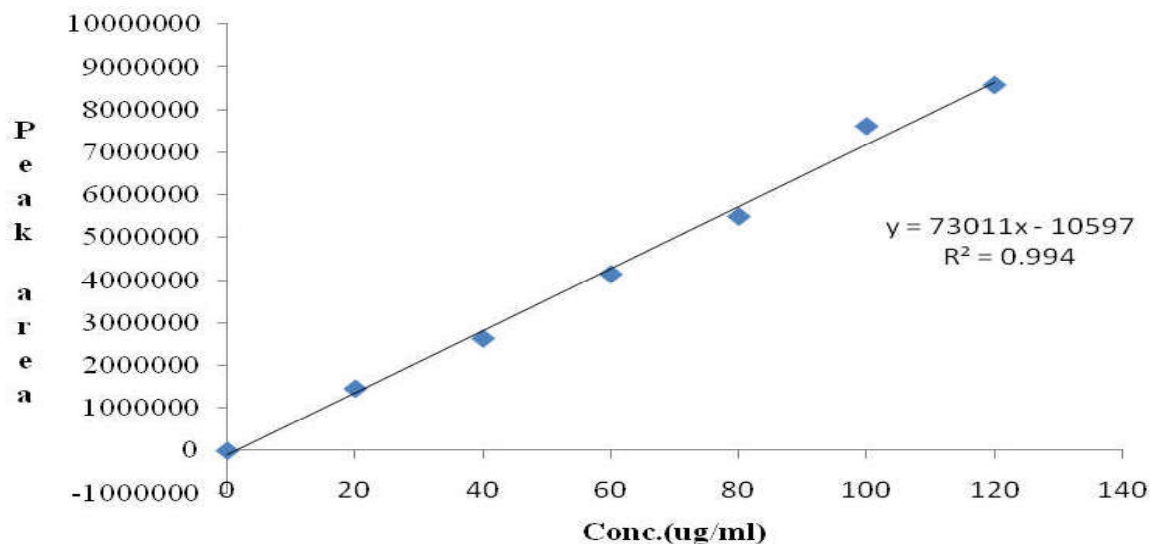
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y = 73011x - 10597
R² = 0.994

Fig. 4: Calibration graph of Imipramine Hydrochloride

S. No.	Intra-day Time (Hours)	Peak area	Inter-day (Days)	Peak area
1	0	7267889	1	7317870
2	3	7256895	2	7137889
3	6	7237903	3	7339990
4	9	7214345	4	7484339
5	12	7307160	5	7403165
6	15	7328562	6	7278567
	Mean	7268792	Mean	7326970
	SD	42674.387	SD	117403.1
	%RSD	0.58	%RSD	0.16

Table 5: Recovery studies for Imipramine Hydrochloride

Level	Standard conc. (µg/ml)	Conc. added (µg/ml)	Conc. found (µg/ml)	% Recovery	% Mean recovery
80%	10	80	79.56	99.45	99.83
80%	10	80	80.25	100.31	
80%	10	80	79.77	99.71	
100%	10	100	99.16	99.16	99.65
100%	10	100	99.56	99.56	
100%	10	100	100.23	100.23	
120%	10	120	121.22	101.02	101.04
120%	10	120	120.98	100.82	
120%	10	120	121.55	101.29	

Table 6: Robustness results for Imipramine Hydrochloride

S. No.	Parameter	Optimised	Used	Rt (min)	Peak area	%RSD
1	Flow rate	1 ml/min	0.8 ml/min	4.11	7265421	0.52
			1.0 ml/min	3.77	7237903	0.48
			1.2 ml/min	3.6	7311861	0.55
2	Wavelength	251 nm	248	3.78	7195365	0.65
			251	3.81	7248596	0.25
			254	3.85	7258463	0.36

Table 7: LOD and LOQ of Imipramine Hydrochloride

Parameter	Measured value (µg/ml)
Limit of detection	0.039
Limit of quantification	0.118

Table 8: Assay results of Imipramine Hydrochloride in marketed formulation

Formulation	Label claim	Amount found	% Assay
IMPRAMINE (Imipramine Hydrochloride)	25 mg	24.84 mg	99.36%

Results and Discussion

The present study was aimed at developing a simple, sensitive, precise and accurate HPLC method for the estimation of Imipramine Hydrochloride from bulk samples and their tablet dosage forms. Imipramine Hydrochloride, attempts were made by using mobile phases containing solvents of varying polarity, at different concentration level with implicating ODS C18 column (250 mm × 4.6 mm, 5 µm) as a stationary phase. Among the different mobile phase combinations employed, best resolution with sharp well defined peaks obtained with mobile phase composed of methanol:acetonitrile:buffer (0.1M sodium acetate) in the ratio of 50:30:20 v/v/v .The wavelengths for estimation of Imipramine Hydrochloride were found to be 251 nm. The absence of additional peaks in the absorption spectrum indicates non-interference of the commonly used excipients in the tablets and hence the method is specific. The linearity was found satisfactory in the concentration range of 20-120 µg/ml for Imipramine Hydrochloride. The regression equation of the linearity curve (Figure 4) between concentrations of Imipramine Hydrochloride over its absorbance was found to be $y = 73011x-105975$ (where y is the Peak area and x is the concentration of Imipramine Hydrochloride in µg/ml). The correlation coefficient (R^2) was found to be 0.994 for Imipramine Hydrochloride. The results show that an excellent correlation exists between absorbance and concentration the drug within the concentration range indicated. Precision of the method was studied by repeated measurements of drug solution and results showed lower % RSD values. The % RSD for intra-day precision and inter-day precision for Imipramine Hydrochloride were found to be 0.58% and 0.16% respectively (limit % RSD<2.0%). This reveals that the method is quite precise. The mean recovery of the drug is 99.83-101.04%. The high percentage of recovery indicates that the proposed method is highly accurate. The limit of detection (LOD) and limit of quantification (LOQ) of Imipramine Hydrochloride was found to be 0.039 µg/ml and 0.118 µg/ml respectively. The % assay of Imipramine Hydrochloride was found to be 99.36%. The assay results showed that the drug contents of this product to be in accordance with the labeled claims.

Conclusion

A new RP-HPLC method was developed for the determination of Imipramine Hydrochloride in bulk sample and pharmaceutical formulations. The satisfying recoveries, low correlation coefficient and accuracy results confirmed the suitability of proposed method for the routine quality control analysis for determination of Imipramine Hydrochloride in pharmaceutical formulations. Different marketed formulations were used while performing assay, hence this method can be applicable to all generic versions of dosageforms. The method was validated as per International Conference on Harmonization Guidelines and the results are within the limits. To conclude, retention time was and run time was decreased so, this HPLC method is rapid and economical for analysis of bulk drugs and pharmaceutical formulations.

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