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Research Article

A NOVEL RP-HPLC METHOD FOR THE QUANTIFICATION OF ICATIBANT IN FORMULATIONS

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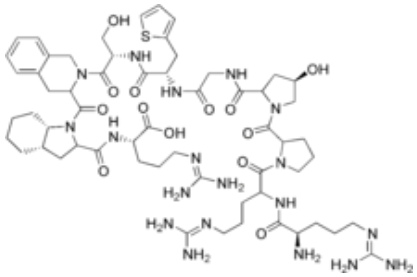
ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Icatibant tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Chromosil C18 (250mmx4.6mm I.D., 5µm particle size) at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile: water 57:30:13 v/v/v. The UV detection wavelength was 224nm and 20µLsamplewas injected. The retention time for Icatibant was 9.98mins. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Icatibantin tablet dosage form and bulk drug.

Key Words: Icatibant, RP-HPLC, UV detection, recovery, precise, 224nm.

INTRODUCTION

Icatibant (fig.1) is a peptidomimetic drug consisting of ten amino acids, which is selective and specific antagonist ofbradykinin B2 receptors. It has been approved by the European Commission for the symptomatic treatment of acute attacks,[1][2] of hereditary Angioedema (HAE) in adults (with C1-esterase-inhibitor deficiency).



EXPERIMENTAL

Materials:

Working standard of Icatibant was obtained from well reputed research laboratories. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus:

A Series HPLC [6-11] system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system.Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. (250×4.6) mm, Electronic balance-DENVER (SI-234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

Determination of wavelength of maximum absorbance:

The standard solutions of Icatibantwere scanned in the range of 200 -400 nm against mobile phase as a blank. Icatibantshowed maximum absorbance at 224nm. So, the wavelength selected for the determination of Icatibantwas 224nm.

Chromatographic equipment and conditions:

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Icatibantan isocratic PEAK LC 7000 HPLC instrument with Zodiac C18 column (250 mm x 4.6 mmi.d., 5µm particle size) was used. Theinstrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µl Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol: Acetonitrile: water 57:30:13 v/v/v, Injections were carried out using a 20 µl loop at room temperature and the flow rate was 1 ml/min. Detection was performed at 224nm

with 15mins runtime.

Standard and sample solutions:

10 mg of Icatibant standard was accurately weighed and transferred into a 10ml volumetric flask and dissolved in mobile phase and made upto the mark to get 1000 µg/ml concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 (INTELENCE) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Icatibantwas accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase was added and the solution was sonicated for 15 mins. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 10µg/ml.

Optimized chromatographic conditions:

Chromatographic conditions as optimized above are shown in Table.1. These optimized conditions were followed for the determination of Icatibant in tablet Formulations. The chromatograms of standard isshown in Figure2.

Mobile phase	: Methanol: Acetonitrile: Water (57:30:13, v/v/v)
Pump mode	: Isocratic
pH	: 5.4 (adjusted with 0.1% OPA)
Diluent	: Mobile phase
Column	: Chromosil C18 (250 mm × 4.6mm I.D., 5 µm particle size)
Column Temp	: Ambient
Wavelength	: 224nm
Injection Volume	: 20µL
Flow rate	: 1.0mL/min
Run time	: 15mins
Typical t_R of Icatibant	: 9.98mins

Table 1: Optimized chromatographic conditions for the estimation of Icatibant

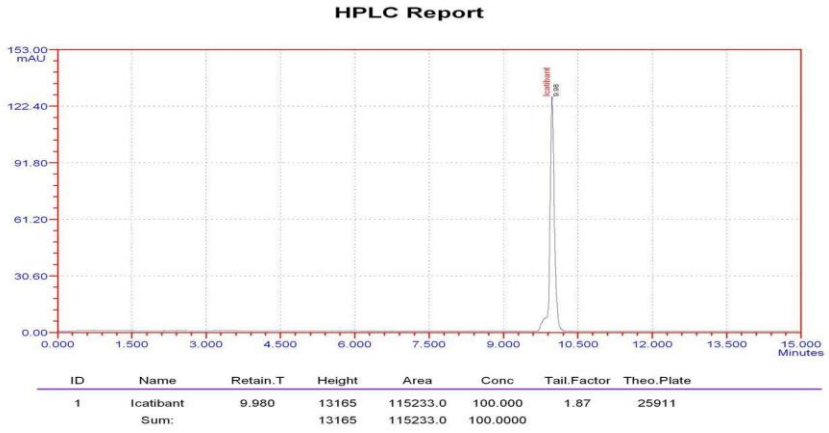


Figure 2: Standard chromatogram of Icatibant

Method validation:

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness

Specificity:

The specificity of method was performed by comparing the chromatograms of blank, standard and sample. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results are shown in table. 2.

Name of the solution	Retention Time in Min
Blank	No peaks
Icatibant	9.98

Table 2: Specificity study

Linearity:

20µL of working standard solutions of 10,20,30,40,50,60 µg/mlof Icatibant were injected into the chromatographic system. The peak area was determined for each concentration of the drug solution. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 16249x-43598 (r^2= 0.999). Linearity values are showed in Table 3.

Level	Concentration of Icatibant in (µg/ml)	Area
Level 1	10	115233
Level 2	20	300426
Level 3	30	458762
Level 4	40	621482
Level 5	50	799879
Level 6	60	953210
	Slope	16717
	Intercept	-43598
	Correlation Coefficient	0.999

Table 3: Linearity Result of Icatibant

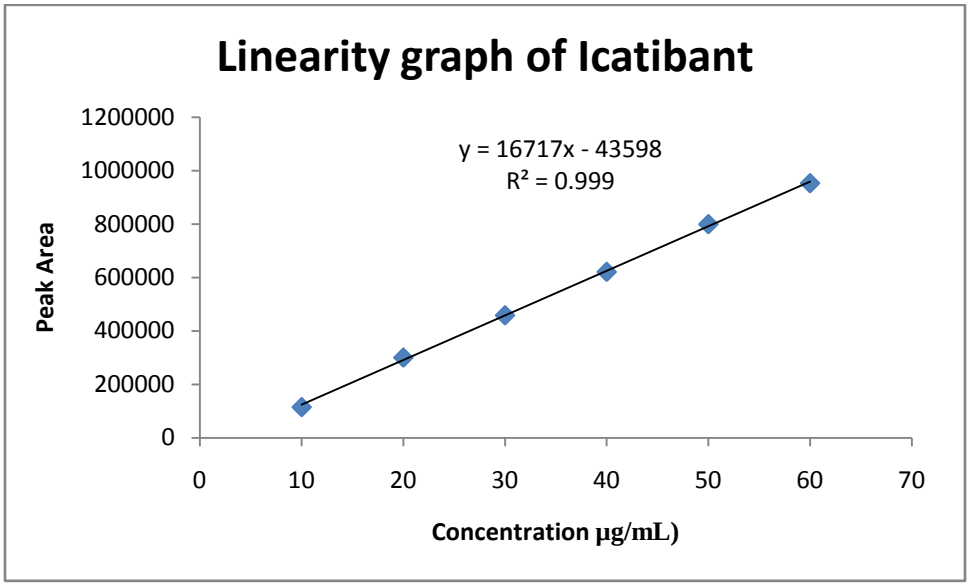


Figure 3: Linearity Plot

Precision:

To study precision, six replicate standard solutions of Icatibant(10µg/ml) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be well within the acceptance criteria of not more than 2.0%. Results of system precision ,method precision and intermediate precision studies are shown in Table.4 and Table.5.

Concentration (10µg/ml)	Intraday precision peak areas	Interday precision peak areas
1	115568	114213
2	115798	115689
3	116029	115982
4	115867	119689
5	114972	115867
6	116003	116021
%RSD	0.34	1.57

Table 4: Intraday and Inter day Precision Results of Icatibant

Intermediate precision or Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days. Ruggedness is

also expressed in terms of percentage relative standard deviation.

SAMPLE	CONC (µg/ml)	INJECTION NO	PEAKS AREA	%R.S.D (Acceptance criteria ≤ 2.0%)
Icatibant	10µg/ml	1	116421	0.17
		2	115986	
		3	116106	
		4	115938	
		5	116036	
		6	115874	

Table 5: Ruggedness results of Icatibant

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.83µg/ml dilution Peak was not clearly observed, based on which 0.83µg/ml is considered as Limit of Detection and Limit of Quantification is 2.5µg/ml.

Robustness:

The robustness study was performed by slight modification in compostion of the mobile phase, pH of the buffer and wave length. Icatibant at 10 µg/ml concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study are shown in Table 6.

Condition	%Assay	%Difference
Unaltered	100	
Wavelength at 222nm	100.74	0.74
Wavelength at 226m	100.99	0.99
Mobile phase:		
Methanol(77):acetonitrile (10): water (13) v/v/v	100.22	0.22
Methanol(67):acetonitrile (20): water (13) v/v/v	100.84	0.84
pH of buffer at 5.6	100.33	0.33
pH of buffer at 5.2	100.64	0.64

Table 6: Robustness results of Icatibant

Recovery:

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 tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 30µg/ml, 40µg/ml and 50µg/ml. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.3 to 101.7% were obtained by the proposed method. This indicates that the proposed method is accurate. Results are given in table.7.

% Recovery	Icatibant					
	Target Conc., (µg/ml)	Spiked conc, (µg/ml)	Final Conc, (µg/ml)	Conc., Obtained	% of Recovery	RSD
50%	20	10	30	29.84	99.46	1.125
	20	10	30	30.28	100.9	
	20	10	30	30.51	101.7	
100%	20	20	40	39.32	98.3	1.667
	20	20	40	40.27	100.6	
	20	20	40	40.61	101.5	
150%	20	30	50	59.28	98.8	1.146
	20	30	50	60.41	100.6	
	20	30	50	60.53	100.8	

Table7: Recovery results of Icatibant

Stability test:

To perform the Stability test the standard solution of 10µg/ml was stored at ambient temperature (±10^{0}C) for two days. After this these storage solutions and freshly prepared solution were tested with proposed method. It is noticed that assay of these results did not decreased below 98%. The results of stability test were shown in Table8.

Standard solution			**Sample solution**		
Time (hours)	**Peak area**	**%variation**	**Time (hours)**	**Peak area**	**%variation**
Initial	115233	---	Initial	115037	---
12	115014	0.20	12	114562	0.41
24	115189	0.04	24	113126	1.67

Table 8: Stability test results

System suitability:

System suitability of the method was evaluated by analyzing the repeatability, peaks symmetry (Symmetry factor),theoretical plates of the column, peak area and retention time. Result of System Suitability

data presented in Table 9.

Parameter	Tailing factor	Theoretical plates
Specificity study	1.78	25911
Linearity study	1.64	25586
Precision study	1.82	25748

Table 9: System suitability results

Assay of Formulation of Icatibant:

Commercially available lyophilized injection Firazyr of Shire US Manufacturing Inc. was chosen for this purpose**.** The contents of five vials of Firazyr each containing 10 mg of Icatibant were pooled up and reconstituted with the mobile phase in a 10 ml volumetric flask. An aliquot from this solution (1.2mg/mL) was taken in a separate 10ml volumetric flask that the concentrations of the drug and the internal standard were 120µg/ml respectively. The sample was injected into the column for five times and the mean peak of the drug was calculated from the chromatogram. The drug content was quantified using the regression equation obtained for the pure sample.

Formulation	Dosage	Concentration	Amount found	% Assay
Firazyr	10mg	120µg/ml	119.80	99.83

Table 10: Assay result of Icatibant

RESULTS AND DISCUSSION

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Icatibant tablet dosage form. The mobile phase consisted of Methanol: Acetonitrile: water 57:30:13 v/v/v. The retention time for Icatibant was 9.98mins. The %RSD for Intra dayprecision (0.34%) inter day precision (1.57%) was found to be less than 2%. Satisfactory recoveries ranging from 98.3 to 101.7% (table 7) were obtained by the proposed method The% assay of Icatibant formulation was 99.83% (table10).

CONCLUSION

The proposed method for the assay of Icatibant is very simple and rapid. It should be emphasized, that it is an isocratic method and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness.

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