Asian Journal of Chemical and Pharmaceutical Research
CODEN (USA):AJCPHP | ISSN: 2347-8322 |Publisher: Pharma Research Library
Home Page: https://pharmaresearchlibrary.org/journals/index.php/ajcpr
DOI: https://doi.org/10.30904/j.ajcpr.2025.4810
A. J. Chem. Pharm, Res., 2025, 13(1): 41-46

Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Plecanatide from Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

The quantification of Plecanatidein both its pure and pharmaceutical formulation forms has been proposed using the straightforward, sensitive, and selective Reverse Phase High Performance Liquid Chromatography (RP-HPLC) approach. The Phenomenex Luna C_{18} (250 x 4.6mm x 5µm) column was used for the chromatography. The mobile phase consisted of 0.1% orthophosphoric acid, acetonitrile, in the ratio of 60:40% v/v. At 254 nm, the flow rate was detected, and it was 0.9 ml/min. 2.892 minutes was determined to be the retention time. The suggested approach was verified in compliance with ICH regulations. The linearity was discovered within the 5-30 µg/mL range, correspondingly. Every validation parameter fell within the permissible bounds. Excipients did not interfere with the medication, according to the recovery trials, and the percentage of recovery was 99.70. In order to establish the drug's sensitivity, it was put through stress tests that involved exposing it to a variety of conditions, including acidic, basic, oxidative, thermal, and photolytic ones. In accordance with ICH recommendations, the proposed approach was successfully used to estimate the amount of medicine in tablet dosage form and to investigate the stability of the product under various stress situations.

Keywords: Plecanatide, Validation, Stress studies.

A R T I C L E I N F O

Article History:
Received : 15 Mar 2025
Revised : 30 Mar 2025
Accepted : 19 May 2025
Published : 07 June 2025

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Citation: *K.Rajasekhar,et al. Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Plecanatide from Bulk and Pharmaceutical Dosage Form. A. J. Chem. Pharm, Res., 2025, 13(1): 41-46.*

C o n t e n t s

1. Introduction

The inability or difficulty passing stool is known as constipation; it is frequently accompanied by straining or a feeling that the evacuation is not complete. Constipation-like symptoms characterize chronic idiopathic constipation (CIC), a functional gastrointestinal (GI) condition that lasts for at least three months but typically has no obvious underlying physiological abnormality[1].Plecanatide IUPAC name (4S)-4-{[(2S)-2-{[(2S)-2-amino-1-hydroxy-3-(C-hydroxy carbonimidoyl) propyl idene]amino}-3-carboxy-1-hydroxypropylidene]amino}-4-{[(1R,4S,7S,10S,13S,16R, 19S,22S,25R,32S,38R)-38-{[(1S)-1-carboxy-3-methyl butyl]-C-hydroxycarbonimidoyl}-22-(2-carboxy ethyl)-3,6, 9,12,15,18,21,24,30,33,36-undecahydroxy-10-[(C-hydroxy carbonimidoyl)methyl]-32-[(1R)-1-hydroxyethyl]-4-methyl -19-(2-methylpropyl)-7,13-bis (propan-2-yl)-27,28,40,41-tetra thia-2,5,8,11,14, 17,20,23, 31,34, 37-undecaazabicycle [14.13.13] dotetraconta-2,5,8,11,14,17,20,23,30,33,36-undecaen-25-yl]-C-hydroxy carbonimidoyl} butanoicacid[2]. Plecanatide, an active metabolite of guanylate cyclase C (GC-C) agonist, binds to GC-C and acts locally on the

luminal surface of intestinal epithelial cells. Activation of GC-C causes an increase in cyclic guanosine monophosphate (cGMP), which in turn stimulates the secretion of bicarbonate and chloride into the intestinal lumen. This process is primarily responsible for activating the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel, which causes an increase in intestinal fluid and speeds up transit.

Despite a thorough review of the literature, only one assay techniques for estimating Plecanatide using HPLC in bulk, its pharmaceutical formulation separately[3], and in its combinations [4] with other medications were reported. In order to estimate Plecanatide using RP-HPLC, the current study aims to create a novel, quick, easy, accurate, efficient, and repeatable stability indicating [5–18] analytical method. Additionally, it validates[19–25] the analytical procedures Q2 (R1) and Stability Testing Q1A (R2) in accordance with ICH guidelines.

2. Materials and Methods

2.1Materials

Acetonitrile, Methanol and Water HPLC-grade solvents-were purchased from Merck Pvt.Ltd., Mumbai, Indiafor use in the current investigation. The following high-grade analytical reagent solvents were purchased from SD Fine-Chem Limited, Mumbai, India: hydrochloric acid, sodium hydroxide pellets, orthophosphoric acid, and hydrogen peroxide (H_2O_2) 30% w/v. The pharmaceutical dosage form (Trulance) was purchased from the local pharmacy.

2.2 Instruments:

The chromatographic technique was performed on a Shimadzu HPLC separation module LC20AD with Photo diode array detector and a Rheodyne manual injector model 7725i with 20µL sample loop connectedto LC solutions chemstation. Chromatographic separation was carried using Phenomenex Luna C18 (250x4.6mm, 5µm) column. Mobile phase was filtered through a 0.45µ membrane filter (Millipore Pvt. Ltd., Bangalore, India) and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai, India).

2.3 Preparation of Solutions:

Selection of mobile phase:

Various mobile phase compositions and ratios, such as methanol: water and acetonitrile: water, are tested during optimization. The system's appropriateness for these mobile phase testing needed to be revised. Acetonitrile-containing mobile phase with 0.1% OPA in a 40:60 v/v ratio and a flow rate of 0.9 ml/min provided all system suitability parameters. Plecanatide analysis was conducted on this mobile phase due to the satisfactory results.

Stock solution of Plecanatide

To attain a concentration of 1000µg/ml, precisely weighed 10 mg of pure Plecanatide, transfer it into a 10 ml volumetric flask, and then added 10 ml of mobile phase. This stock solution is sonicated in an ultrasonic water bath to eliminate air bubbles. With a volumetric flask, remove 1 millilitre of this solution and add 10 millilitres of mobile phase to achieve a 100µg/ml concentration. A series of aliquots were made from this solution to advance the method's development.

Preparation of working standard solution

The stock solution mentioned above was diluted to yield a 5 to 30 µg/ml concentration range. The chromatograms were obtained, 20 µl of the working standard solution was injected, and the correlation coefficient and % curve fitting were computed using the given formulas.

2.4 Methodology

The mobile phase conjunction for chromatography is Acetonitrile : 0.1% OPA (40:60%, v/v). The separation was obtained on column C18 Phenomenex Luna (250X4.6mm; 5µ), temperature ambient, injection volume 20µl, and wavelength 224nm. The flow rate of 0.9 ml/min was maintained throughout the analysis. The ICH Q2 (R1) recommendations state that these chromatographic conditions must be applied to all validation parameters. Chromatogram satisfies the requirements for acceptance, including injection reproducibility, theoretical plates, capacity factor, and tailing factor. Improved chromatogram, as seen in Figure 2.

Method Validation

The analytical method was validated as per ICH Q2 (R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, LOD, and LOQ.

System suitability: In order to measure system suitability appropriateness characteristics, such as theoretical plates, retention time, tailing factor, and percentage RSD, the prepared standard solution is injected six times.

Accuracy

By adding three distinct concentration levels—50%, 100%, and 150%, respectively recovery tests were conducted to ensure accuracy for this approach. RSD was computed as a percentage of recovery.

Specificity

By comparing the data from the drug solution before and after spiking, it was possible to conclude that the approach was specific because there was no discernible interference from the blank with the recovery of plecanatide.

Precision

The estimated sample analysis, performed using six replicates of a fixed concentration from the standard stock solution, demonstrates the method's repeatability and reproducibility. At confidence intervals, it was executing intraday and interday precision. It was discovered that the method's percentage RSD was less than 2%.

Linearity and range

By creating various standard solutions of Plecanatide at various concentration levels, linearity was tested. The standard solutions were made with plecanatide concentrations ranging from 5 to 30µg/ml. The HPLC equipment was used to inject each concentration, and the areas that were acquired were recorded. Draw a graph with the concentration on the X-axis and the area taken on the Y-axis.

LOD and LOQ

LOD was determined by diluting the Plecanatide standard solution and calculating the concentration at which the sample peaks' responses are three times greater than the noise peak. To calculate the LOQ, the standard Plecanatide solution was diluted, and the concentration was found to be

the sample peaks' response multiplied by ten times that of the noise peak. **Robustness**

Robustness was assessed by slightly modifying the parameters of the approach and tracking the impact on the method through the results of system compatibility tests.

Forced Degradation Study:

The undamaged tablets were transferred to create the degradation samples, which were then used in media that were acidic, alkaline, oxidant, thermal, and photolytic. Following the completion of the degradation treatments, diluent was added to the stress content solutions to dilute them to a concentration of approximately 100 µg/ml.

a) **Acidic Degradation:**

A 1mg of drug was weighed, diluted in 10 ml of mobile phase, and heated to 100° C for an hour before being cooled to room temperature to conduct an acidic degradation study. 0.1 N HCl was added to the solution to dilute it even more and get 100 µg/ml solution.

b) **Alkali Degradation:**

1mg drug was weighed, dissolved in 10ml of mobile phase, and heated to100° C for an hour before being cooled to room temperature to achieve alkaline degradation.For the solution to have a concentration of 100 µg/ml, it was further diluted with 0.1 N NaOH.

c) **Oxidative Degradation Study:**

The sample was heated to 100° C for an hour and then cooled to room temperature as part of the oxidative degradation investigation. To get 100µg/ml of concentration, 10% v/v H_2O_2 was added to the solution.

d) **Thermal Degradation Study:**

In order to conduct a thermal degradation research, 1mg drug was weighed, diluted in ten millilitres of mobile phase, and heated to 100° C for an hour before being cooled to room temperature. To get 100µg/ml, dilution of this solution is utilized.

3. Results and Discussion

Using HPLC, the area was determined after each of the five injections of the standard solution. The five replicate injections' peak areas' percent relative standard deviation (%RSD) was computed, and it was discovered to be within the predetermined bounds. In Figure 1, the chromatogram with the five injections is displayed.

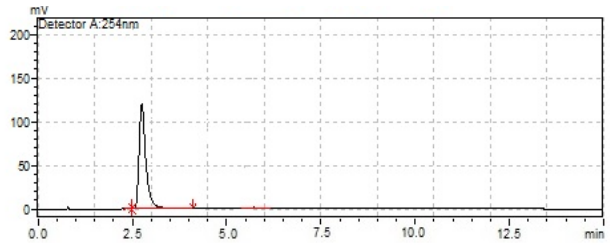

Fig.1. Optimized chromatogram of Plecanatide

Specificity

A 50 mg sample of Plecanatide working standard was accurately weighed and transferred to a 100 ml standard flask. The standard was dissolved in a small volume of mobile phase. Then, 100 mg of placebo was added to the flask and the volume was brought up to the mark with mobile phase. The solution was filtered through a Millipore filter before injecting a 20µl aliquot. The results were shown in table 1 and chromatogram shown figure 2.

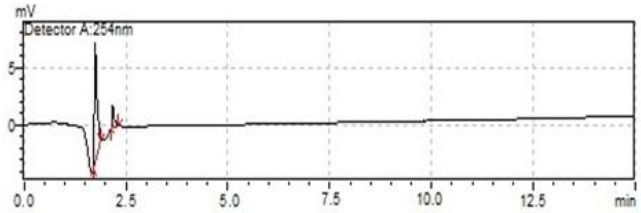

Fig.2. Chromatogram of Blank

System Suitability

System suitability parameters are performed by injecting prepared standard solution in six times and measured the parameters like theoretical plates, retention time, tailing factor and %RSD. All the results shown in table 2

Linearity

A 50 mg sample of Plecanatide working standard was accurately weighed and transferred to a 100 ml standard flask. The standard was dissolved in a small volume of mobile phase. Then, 100 mg of placebo was added to the flask and the volume was brought up to the mark with mobile phase. The solution was filtered through a Millipore filter before injecting a 10µl aliquot. The resulting chromatogram is shown in the figure 3.

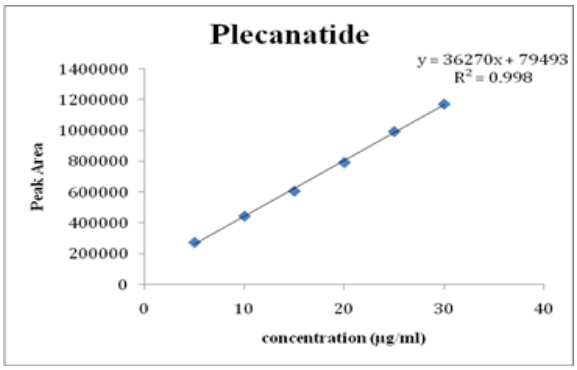

Fig 3. Calibration curve of Plecanatide

Precision

By weighing twenty tablets, the average weight of the tablets was determined. The tablets were ground into a powder, and the resulting material—which contained 100 mg of plecanatide was precisely weighed before being added to a dry, clean 100 ml standard flask. After roughly ten minutes of sonication, the sample was dissolved in a tiny amount of mobile phase, and the volume was subsequently increased with mobile phase. Whatman filter paper (concentration 1000 µg/ml) was used to filter the mixture. Pipetted into a 10 ml standard flask, 0.5 ml of the stock solution was diluted with mobile phase to mark the concentration and then filtered through a 0.45 µm filter (50 µg/ml). Using HPLC, the area was determined after each of the five injections of the sample solution. The percentage RSD for each of the six replicated injections fell between predetermined bounds and shown in table 4.

Accuracy

By creating solutions with varying concentrations 50%, 100%, and 150% in which the amount of pure medication 10 mg, 20 mg, and 30 mg for 50%, 100%, and 150%, respectively was adjusted while the amount of marketed formulation remained constant, the accuracy of an analytical approach was ascertained. The solutions were made three times, and the accuracy was shown by the percentage of recovery. The findings are presented in the table 5 and chromatograms shown in figure 4& 5.

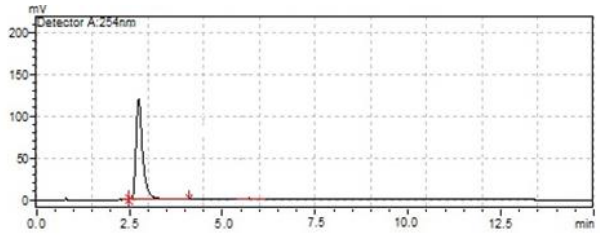

Fig.4. 100% Accuracy chromatogram of Plecanatide

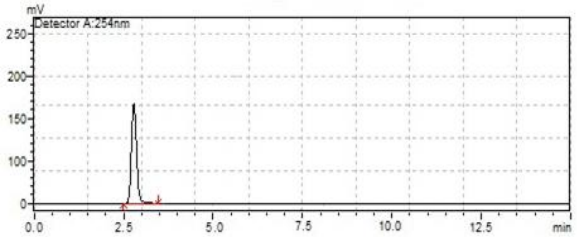

Fig.5.150% Accuracy chromatogram Plecanatide

Forced Degradation studies

After degradation, each sample obtained under each forced degradation condition was diluted appropriately with mobile phase to get a final concentration of 20μg/mL; the resulting solution was injected in the column under described chromatographic condition. The chromatogram obtained was studied for area of drug peak and appearance of secondary peaks. The decrease in the area of the drug peak and the occurrence of secondary peaks was considered as indication of degradation. The results were tabulated in table 7 and chromatogram shown in figure 6,7 & 8.

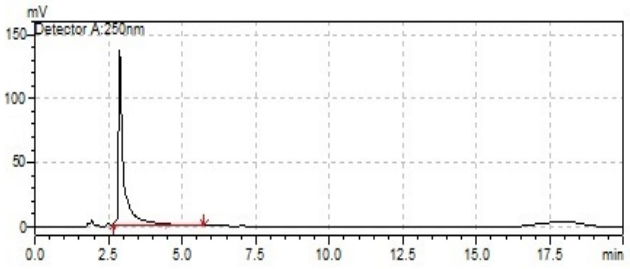

Fig.6. Acidic condition chromatogram of Plecanatide

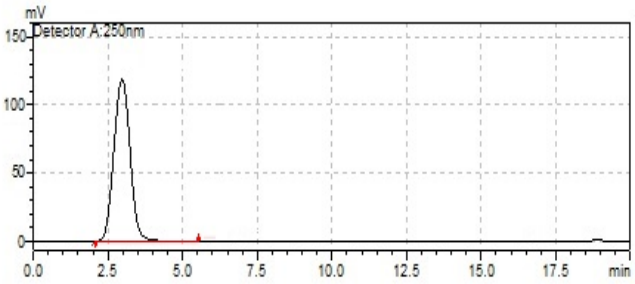

Fig.7. Basic condition chromatogram of Plecanatide

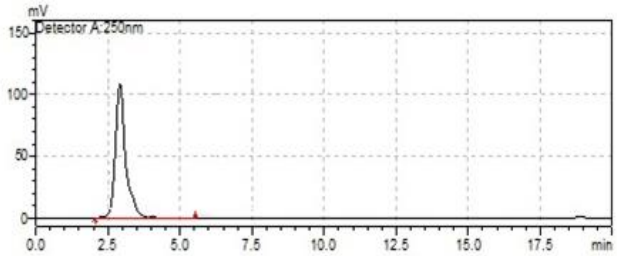

Fig.8. Oxidation condition chromatogram of Plecanatide

Table 1: Results for Retention time of blank, placebo, Standard, Sample

Solution	Blank	Placebo	Standard	Sample
Retention Time (min)	NIL	NIL	2.892	2.931

Table.2: Results of System suitability

Parameter	Results	limits
Peak area response	RSD - 0.87%	RSD <2%
Theoretical plates (N)	5391	N > 2000
Tailing factor (T)	1.13	T ≤ 2

Table 3: Results of Linearity by RP-HPLC method

Sr. No.	Concentration (µg/ml)	Peak Area response (mV)
1	5	172531
2	10	343657
3	15	506743
4	20	693667
5	25	894764
6	30	1073926
Regression Equation		Y= 36270x +79493
R^2		0.998

Table.4: Results for Comparison of method precision and intermediate precision studies

Sr. No	Intra day	Inter day
	Peak Area (mV)	**Peak Area (mV)**
1	789756	790867
2	792978	782978
3	775612	794198
4	769879	781290
5	788741	766268
6	762789	771346
Average	779959.17	781157.83
Std Dev	12311.871	10822.417
% RSD	1.58	1.39

Table 5: Results for Sensitivity of

Sr. No	Limit of Detection (LOD)			Limit of Quantification (LOQ)		
	Std. Dev	**Slope**	**LOD (µg ml)**	**Std. Dev**	**Slope**	**LOQ (µg ml)**
1	10822.417	36270	0.985	10822.417	36270	2.984

Table 6: Recovery results

Sr.No	Levels	Peak Area Response	ppm spiked (µg/ml)	ppm Recovered (µg/ml)	% Recovery	Avg. Recovery
1	50%	434877	10.066	9.918	98.52	99.44
2		441437	10.066	10.067	100.01	
3		442871	10.066	10.044	99.78	
4	100%	793852	20.013	20.005	99.96	99.76
5		791319	20.013	19.941	99.64	
6		791531	20.013	19.946	99.67	
7	150%	1195354	30.119	30.1223	100.01	99.92
8		1193142	30.119	30.0666	99.83	
9		1194323	30.119	30.0963	99.92	

Table 7: Results of Robustness studies

Sr. No	As such	Flow rate		Temperature	
	Standard	**-0.1 ml/min**	**+0.1 ml/min**	**-5°C**	**+5°C**
	Area response				
1	772183	761376	778168	787379	769987
2	759279	776259	793176	792547	755881
3	778373	751572	767189	766983	763974
Mean	769945	763069	779511	782303	763280.7
Std. Dev	9741.75	12430.27	13045.45051	13516.8	7078.513
%RSD	1.27	1.63	1.67	1.73	0.93

Table 8: Results of Forced Degradation studies

S.No	Stress degradation condition	% Degraded
1	Basic hydrolysis	2.01
2	Acid hydrolysis	2.16
3	Oxidative	3.37
4	Thermal	1.19

4. Conclusion

The proposed research outlines a brand-new RP-HPLC technique that uses a straightforward mobile phase to estimate bulk levels of Plecanatide. With a quick analytical period of less than three minutes, the approach yields good resolution for the chemical. Compared to the published approaches, the verified method was found to be simple, quick, accurate, selective, and exact. The percentage of recovery indicates that the excipients employed in the formulation do not interfere with the procedure. In terms of solvent use, the procedure is also economical. As a result, regular analysis of Plecanatide in its dose form can be performed using the suggested methodology.

Acknowledgement
I am very thankful to Director, JNTUA-OTPRI, Ananthapuramu for providing the laboratory facilities, chemicals to carryout entire research work.

Conflict of Interest
We declare that we have no conflict of interest

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