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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF PLECANATIDE FROM BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract

A novel, simple, efficient, rapid, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the estimation of Plecanatide in the bulk and pharmaceutical dosage form. The currently developed method was subsequently validated according to ICH guidelines in terms of linearity, accuracy, precision, the limit of detection, the limit of quantification, robustness, etc. The separation of the selected drugs was optimized after several trials including change of mobile phase and its composition, stationary phase, flow rate, column temperature, etc. The separation was performed by using a Develosil ODS HG-5 and UV absorption was measured at 246 nm. Ortho phosphoric acid, Triethylamine buffer: Methanol [520: 480] was selected as the mobile phase at a flow rate of 1 mL/min. As per International Conference on Harmonization (ICH) Q2 R1 guidelines, several validation parameters were evaluated which include specificity, linearity, precision, accuracy, the limit of detection (LOD), and the limit of quantitation (LOQ). The acceptable degree of linearity range was found to be 10 to 60 µg/mL. The percent recovery was found to be 98.55 to 101.88%. Hence, the proposed method is simple, selective, and specifically meets the requirements of ICH guidelines for the validation of the analytical method.

Keywords: Plecanatide; RP-HPLC; flow rate; linearity; mobile phase; validation.

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Introduction

A simple and sensitive reverse phase HPLC method has been developed for the analysis of Plecanatide. The method utilizes sample preparation followed by separation on a Develosil ODS HG-5, column 150 mm lengths, 4.6mm inner diameter, with 5µm particle size. The analyte was monitored by UV detection at 246nm using an isocratic mode with buffer and methanol in the ratio 520:480v/v as mobile phase. The flow rate was set at 1.0ml/min [1]. The retention time for the drug was at 4.543min. Calibration curve for Plecanatide was recorded. The objective of this study was to optimize the assay method for the estimation of Plecanatide. The trials were done to optimize the chromatographic conditions. Plecanatide works as a laxative by drawing water in to the gastrointestinal tract thereby softening stool and encouraging its natural passage [2]. Plecanatide is soluble in organic solvents such as DMSO and dimethyl formamide. The solubility of Plecanatide in these solvents is approximately 3 and 1 mg/ml, respectively.

Experimental

Materials and methods

Active pharmaceutical ingredient Plecanatide was obtained as a gift sample from Anthem Biosciences Pvt Ltd, Bengaluru. The pharmaceutical dosage form (Trulance) was purchased from local pharmacy. The solvents used in this work were of HPLC grade and obtained from Bross chemicals.

Buffer preparation

The buffer solution was prepared by mixing 2 ml of triethylamine and 2 ml of orthophosphoric acid in HPLC grade water and the volume was made up to 1000 ml and pH was adjusted with orthophosphoric acid. It was filtered through 0.45 lm nylon membrane filter and degassed. It was used as a diluent for the preparation of sample and standard solution [3].

Method

Preparation of mobile phase and standard solution of the drug for trails

The mobile phase was prepared for the purpose of different trails. The mobile phase was filtered through 0.4µ filter and sonicated. The mobile phase was used as a diluent to prepare the standard solution. Standard solution was prepared for each trail in the respective mobile phase.

Working standard solution of Plecanatide

About 50 mg of working standard of Plecanatide was weighed and transferred into a clean and dry 50 ml standard flask. The sample was dissolved in a small volume of mobile phase by sonication for about 10 min and the volume was made up with the mobile phase. (1000µg/ml).0.5 ml of the stock solution was pipetted into a 10 ml standard flask and diluted to mark with mobile phase (concentration-50 mcg/ml) [4].

The standard solution was injected into the column in each trail. The Retention time at each trail was determined. The column, mobile phase and results obtained in the trails have been indicated in the table 6. The table also reveals the result of the chromatograms in term of retention time. The trail no 5 employed was found to be satisfactory in which column Develosil ODS HG-5, mobile phase orthophosphoric acid, and triethylamine buffer: methanol (520:480) were used to obtain adequate results.

Method Validation

System Suitability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The Chromatogram is shown in Fig.1.

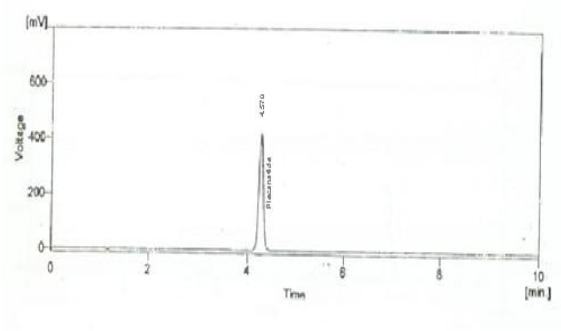


Fig.1. Chromatogram of Standard

Specificity

About 50 mg of Plecanatide working standard was weighed accurately and transferred into 100 ml standard flask, dissolved in small volume of the mobile phase. 100 mg of placebo was mixed with above solution and made up the volume with mobile phase, filtered through millipore filter, 10µl of this solution was injected and reports were shown in fig 2.

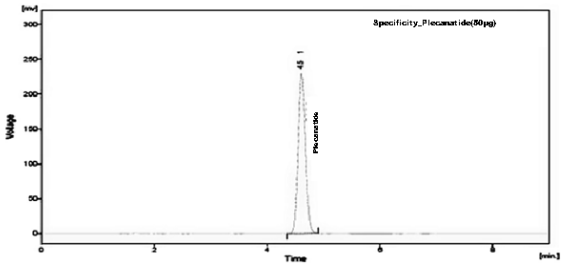


Fig 2. Chromatogram for Specificity

Linearity

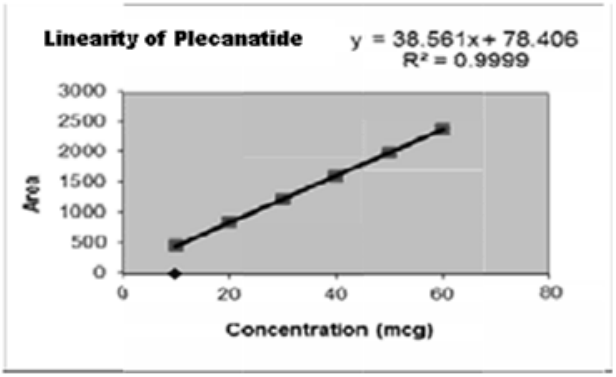
Linearity was assessed by performing measurement at several analyte concentrations. A minimum five concentrations were recommended for linearity studies [5].

Preparation of working standard solution

Plecanatide was weighed accurately and stock solution was prepared. Different volumes of stock solution were diluted to get a concentration range of 10 to 60 µg/ml. 10µl of working standard solution were injected in duplicate and the chromatograms were recorded. The correlation co-efficient and percentage curve fitting were calculated from the following formula.

$$R = \frac{3\,(x - \bar{x})^{2}(y - \bar{y})^{2}}{(n - 1)\,Sx\,S_{y}}$$

Fig.3. Calibration curve of Plecanatide



Precision

The precision of an analytical method was determined by assaying sufficient number of sample and relative standard deviation is calculated [6].

Method Precision

Average weight of the tablet was computed from the weight of 20 tablets. The tablets were powdered. The tablet powder equivalent to 100 mg of Plecanatide was accurately weighed and transferred into a clean and dry 100 ml standard flask. The sample was dissolved in a small volume of mobile phase by sonication for about 10 min and the volume was made up with the mobile phase. The solution was filtered by using Whatmann filter paper (Concentration 1000µg/ml).0.5 ml of the stock solution was pipetted into a10 ml standard flask and diluted to mark with mobile phase and filtered through 0.45 µ filter (concentration-50 mcg/ml). The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The Chromatograms were shown in the Fig 4.

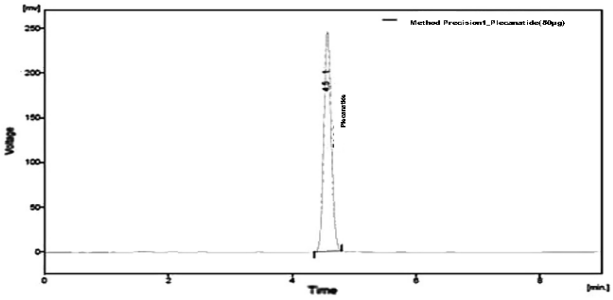


Fig 4. Chromatogram for Precision

Ruggedness

The ruggedness of an analytical method is degree of reproducibility of test result obtained by the analyst under a variety of normal test condition [7]. Working standard solution and working sample solution of Plecanatide were prepared by different analyst and on different days and 10 µl of working sample solution was injected and chromatograms were recorded shown in Fig 5.

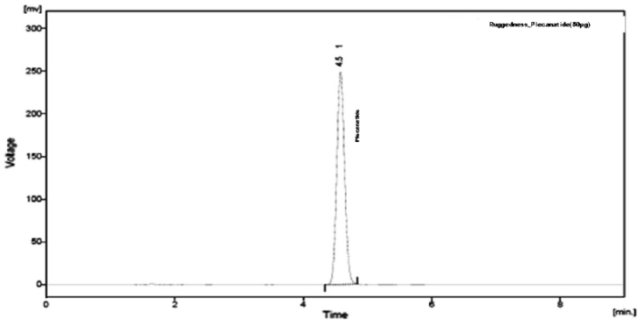


Fig 5. Chromatogram for Ruggedness

Accuracy

Accuracy is measured as the percentage of the analytes recovered by the assay.

Preparation of Sample Stock Solution

Average weight of the tablet was computed from the weight of 20 tablets. The tablets were powdered. The tablet powder equivalent to 100 mg of Plecanatide was accurately weighed and transferred into a clean and dry 100 ml standard flask. The sample was dissolved in a small volume of mobile phase by sonication for about 10 min and the volume was made up with the mobile phase. The solution was filtered by using whatmann filter paper (Concentration 1000µg/ml). 0.5 ml of the stock solution was pipetted into a10 ml standard flask and diluted to mark with mobile phase and filtered through 0.45 µ filter (Concentration-50 mcg/ml) [8]. The stock solution was diluted with mobile phase. Further to obtain a concentration ranging from 45mcg to 65 mcg/ml (Table 1).

Table 1. Percentage Recovery data for Plecanatide

S.No	Spike Level	Amount (µg / ml) added	Amount (µg / ml) found	% Recovery	Mean % Recovery
1	100 %	45	45.85	101.88	101.78
	100 %	45	45.77	101.77	
	100 %	45	45.78	101.75	
2	125 %	55	54.8	99.68	99.77
	125 %	55	54.84	99.72	
	125 %	55	54.87	99.77	
3	150 %	65	64.05	98.55	99.86
	150 %	65	64.23	98.81	
	150 %	65	64.12	98.70	

Robustness

Effect of variation of Flow rate:

A study was conducted to determine the effect of variation in flow rate by injecting 0.9 ml/min and 1.1ml/min. Sample solution was prepared and injected into the HPLC system [9]. The retention time values were measured. The chromatograms were shown in the Fig. 6.

Effect of variation of wavelength

A study was conducted to determine the effect of variation in wavelength [10]. Standard solution was prepared and injected into the HPLC system at 248nm and 244nm. The effects of variation in wavelength were measured. The chromatograms were shown in the Fig.7.

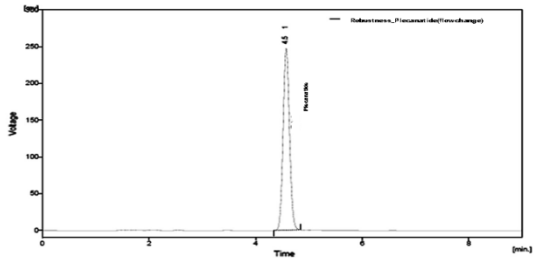


Fig 6. Chromatogram for robustness (change in flow rate).

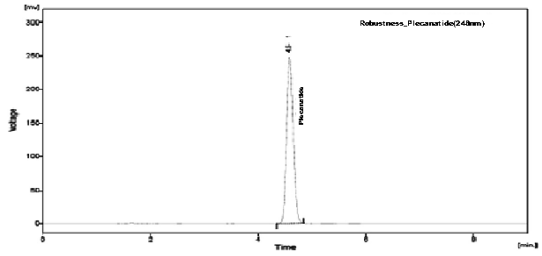


Fig 7. Chromatogram for robustness (change in wavelength).

Results and Discussion

Validation of analytical method for determination of assay of Plecanatide 3 mg tablets was performed for the parameters including – Specificity, Linearity and Range, Precision (System precision, Method precision), Intermediate precision (Ruggedness), Accuracy and Robustness. The summary of results obtained is appended below.

Parameter	Acceptance Criteria	Results
Specificity	There should not be any interference from placebo, blank and main peak. (Active)	There is no interference from blank, placebo and sample peak.
Linearity and Range	Correlation coefficient should be not less than 0.995 over working range.	Correlation coefficient = 0.9999.
Precision Repeatability System precision	%RSD should not be more than 2.0%	SD=0.1789 %RSD=0.18
Repeatability Method precision	%RSD should not be more than 2.0%	SD=0.1519 %RSD=0.15

Intermediate precision Ruggedness	%RSD should not be more than 2.0% The difference between assay of method precision and intermediate precision should not be more than 2.0%	Day 1 and Analyst 1 % Assay =99.68 % RSD =0.43 Day 2 and Analyst 2 % Assay =100.865 % RSD =0.26
Accuracy	Recovery at each level and % mean recovery should be between 100% to 150% with % RSD should not be more than 2.0%	Recovery at each level 98.55 to 101.88. Mean Recovery 99.86 to 101.78. % RSD = 0.18
System suitability	% RSD should not be more than 2.0%	SD= 3.76352 %RSD=0.18
Robustness :	**By change in flow rate**	
a) 0.9 ml/min b) 1.1 ml/min	%RSD should not be more than 2.0% Asymmetry factor should not be more than 2.0%. %RSD should not be more than 2.0%. Asymmetry factor should not be more than 2.0%	%RSD = 0.019 Asymmetry factor =1.105 %RSD = 0.21 Asymmetry factor =1.161
By change in wavelength		
a) 248 nm b) 244 nm	% RSD should not be more than 2.0% A symmetry factor should not be more than 2.0% % RSD should not be more than 2.0% A symmetry factor should not be more than 2.0%	% RSD =0.015 Asymmetry factor = 1.114. % RSD =0.043 Asymmetry factor = 1.167.

The observations and results obtained for each parameter including Specificity, Linearity and Range, Precision (System precision, Method precision), Intermediate precision (Ruggedness), Accuracy and Robustness lie well within the acceptance criteria.

Conclusion

The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-60 µg / ml. The % recovery of Plecanatide was found to be in the range of 99.86 % - 101.78 %. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and wave length separately and analysis being performed by different analysts. Good agreement was seen in the assay results of pharmaceutical formulation by developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Plecanatide in the pharmaceutical formulation.

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Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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