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Rp-Hplc Method For Determination Of Plecanatide In Commercial Formulations.

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Abstract: In order to ensure therapeutic safety and efficacy, plecanatide estimation in pharmaceutical formulations is essential. This research presents a novel stability-indicating chromatographic approach that represents a significant advance in plecanatide analysis. The technique uses a straightforward, exact, and selective method to distinguish plecanatide from other contaminants, providing accurate assessment even in samples that have been deteriorated. The method exhibits good linerity, accuracy, precision and sensitivity. Its analytical effectiveness has been validated according with ICH requirements. This novel approach is a crucial tool for the quality control of plecanatide in commercial preparations because it is not only more efficient and quicker than current techniques but also conforms with regulatory standards. For the analysis, a HiQ Silica C18 (250x4.6mm, 5µm) column was used. Acetonitrile:Water (65:35 v/v) was the composition of the mobile phase). The retention time was found to be 3.4 minutes with well resolved and sharp peak. Flow rate was set to 0.8 mL min^{-1}. PDA detector was used and wavelength for detection was kept at 254 nm.

Keywords: Plecanatide**,** impurities, Pharmaceutical formulations, ICH guidelines, regulatory requirements.

1. Introduction

L-asparaginyl-L-alpha-aspartyl-L-alpha-glutamyl-L-cysteinyl-L-alpha-Glutamyl-L-leucyl-L-cysteinyl-L-valyl-L-asparaginyl-L-valyl-L-alanyl-L-cysteinyl-L-threonylglycyl-L-cysteinyl-L-leucine is the chemical definition of plecanatide. A guanylate cyclase C (GC-C) agonist. Plecanatide and its active metabolite interact to GC-C and exert localised effects on intestinal epithelial cells' luminal surface. Cyclic guanosine monophosphate (cGMP) is raised when GC-C is activated, and this in turn triggers the intestinal lumen to secrete bicarbonate and chloride. The main mechanism underlying this process is the activation of the CFTR ion channel, which results in an increase in intestinal fluid and accelerated transit. Plecanatide has been shown in animal models to accelerate intestinal transit, improve fluid secretion into the gastrointestinal (GI) tract, and encourage changes in stool consistency. In an animal model of visceral discomfort, plecanaide reduced contractions of the abdominal muscles, a measure of intestinal pain.[1]. The literature study demonstrates that plecanatide bodily fluids and pharmacological preparations can be measured using a variety of techniques[2]. GC-MS[3], HPTLC [4], RP-HPLC [5], UV spectrophotometric method [6-8], slow injection chemiluminescence [9], voltammetry[10], and LC-MS [11,12] are some of these techniques. HPLC with electrochemical detection is one of them. No research has been done on the mechanism.

The Chemical structure of Plecanatide and drug profile are depicted in figure 1 and table 1 respectively.

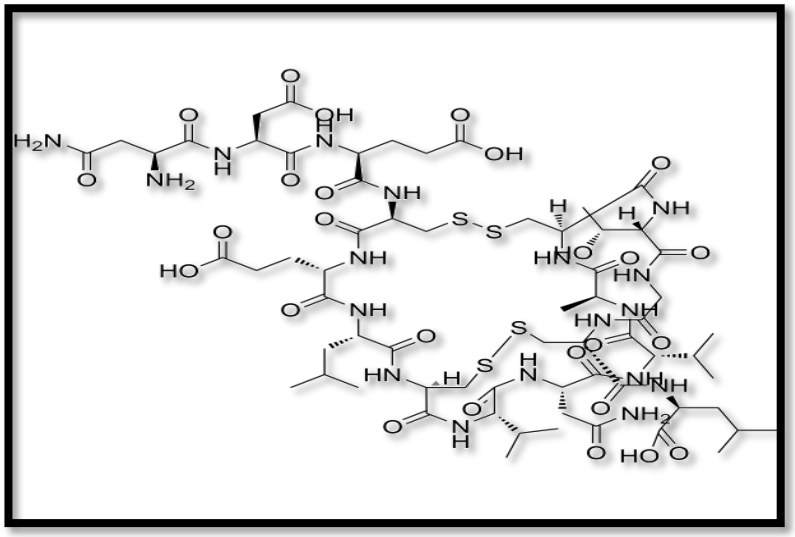

Figure 1: Chemical Structure of Plecanatide.

Table 1: Drug Profile of Plecanatide

ATTRIBUTE	DESCRIPTION
NAME OF DRUG	PLECANATIDE
FUNCTIONAL CATEGORY	Guanylate Cyclase-C Receptor Agonists.
MELTING POINT	221-225^{0}c
MOLECULAR FORMULA	$C_{65}H_{104}N_{18}O_{26}S_4$
CHEMICAL NAME	L-asparaginyl-L-alpha-aspartyl-L-alpha-glutamyl-L-cysteinyl-L-alpha-Glutamyl-L-leucyl-L-cysteinyl-L-valyl-L-asparaginyl-L-valyl-L-alanyl-L-cysteinyl-L-threonylglycyl-L-cysteinyl-L-leucine
MOLECULAR WEIGHT	1681.89 g/mol
SOLUBILITY	It is soluble in acetonitrile, water, and organic solvents like dimethyl formamide and DMSO.

2.MATERIALS AND METHODS

2.1 Chemicals

Synergy Pharmaceuticals provided a complimentary sample of plecanatide. We bought 3 mg of Trulance from an adjoining pharmacy. All solvents employed in the experiment were of analytical grade.[13–16]

3.Chromatographic conditions

3.1 Tools

The Jasco HPLC device was utilised to capture the study's chromatograms. To weigh the materials, an electronic analytical balance was utilised.

3.2 Diluent Selection

Since acetonitrile produces good and well-resolved peaks with the mobile phase composition set ,it was chosen as the diluent.[17]

3.3 Standard solution preparation

1000µg mL-1 was produced by dissolving 10 mg of plecanatide in 10 mL of acetonitrile. This solution was further diluted in accordance with requirement [18].

3.4 Chromatographic conditions optimised

3.4.1 Mobile Phase

Numerous mobile phase trials were conducted in order to identify the optimal composition that generates crisp, symmetric, and well-resolved peaks. The ideal chromatographic settings for this investigation are shown in Figure 2.

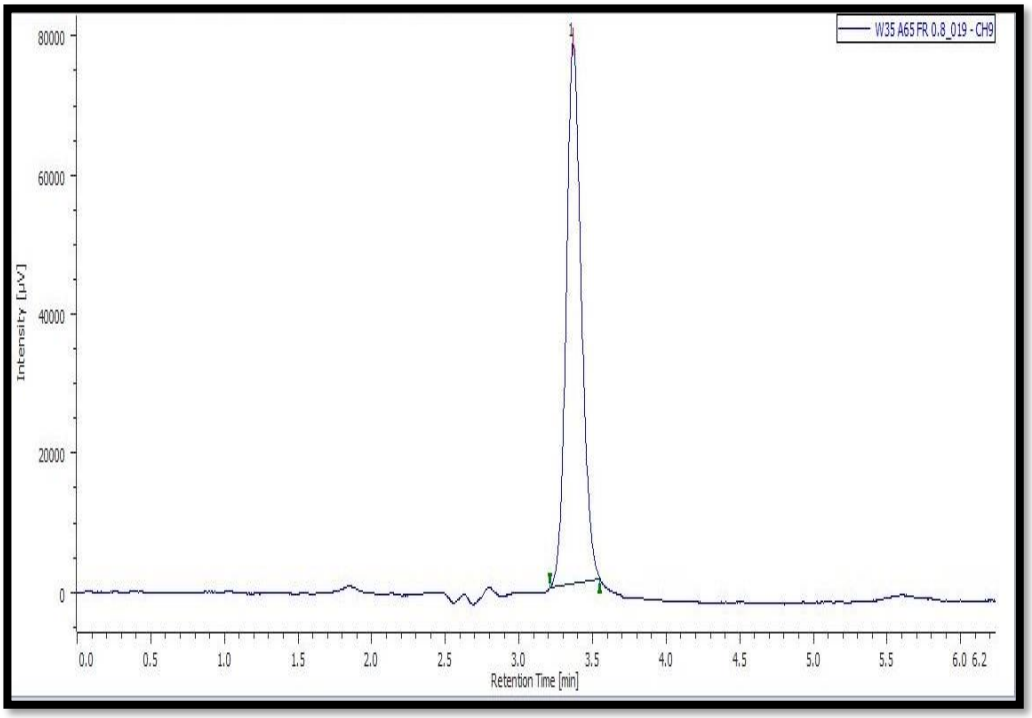

Figure 2: Chromatogram of Plecanatide.

4. Construction of calibration curve
Dilution of the standard solution produced concentrations ranging from 10 to 60 µg mL-1.The calibration curve was created by conducting the analysis at 254 nm. The absorbance of each solution was measured at its 254 nm λmax.

5.Preparation of tablet solution
The tablet solutions were made using the TRULANCE (3 mg) tablets. After dissolving 80 mg of capsule powder in 100 mL of double-distilled water, determine the tablet's average weight. The solution was filtered and then sonicated to get it ready for more evaluation. The filtered formulation solution was diluted to produce a 100µg mL-1solution in order to calculate accuracy or recovery percentage.[24]

6.Validation of analytical methods

An analytical method is validated in a laboratory to ensure that its performance meets the requirements of the intended application. We assessed the recommended spectrophotometric method's linearity, linear range, accuracy, precision, LOD, LOQ, robustness, and roughness.

6.1Linerity
The linearity was evaluated using various doses of Plecanatide reference standard solutions. The concentration range used to create the curve was 10-60 µg mL-1. The calibration curve was created by plotting the concentration of reference solutions against absorbance, which yields a straight line. The coefficient of determination was by regression analysis.

6.2Precision
The usual addition procedure was utilised to determine the accuracy of the test method. There were prepared solutions for 80%, 100%, and 120%. Each solution was made three times, and from each one, the mean recovery % was calculated. These samples were measured at 254 nm.

6.4 LOD and LOQ
The precision of intraday and interday data was used to establish the limit of detection (LOD) and the limit of quantification. These were computed using the following formulas: LOQ=10σ/s and LOD=3.3σ/s.[23]

7. RESULTS AND DISCUSSION

The results of an extensive technique validation investigation for an HPLC method are presented in this article. Key performance metrics, such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ), were used to validate the procedure. When evaluating the HPLC method's appropriateness and reliability for quantitative analysis, several parameters are essential.

7.1Linerity
By utilising standard solutions with known concentrations to create a calibration curve, the linearity of the HPLC method was assessed. The calibration curve yielded a correlation coefficient (r2) of 0.9997, suggesting a robust linear association between the analyte concentration and the absorbance measurement. Table 2 and Figure 3 show how linear the procedure is. This indicates that the target analyte can be precisely quantified using the HPLC method across a broad concentration range.

Table 2: Calibration Plot of Plecanatide.

Concentration (µg mL-1)	Peak Area
10	231811
20	551402
30	863613
40	1198958
50	1545669
60	1868482

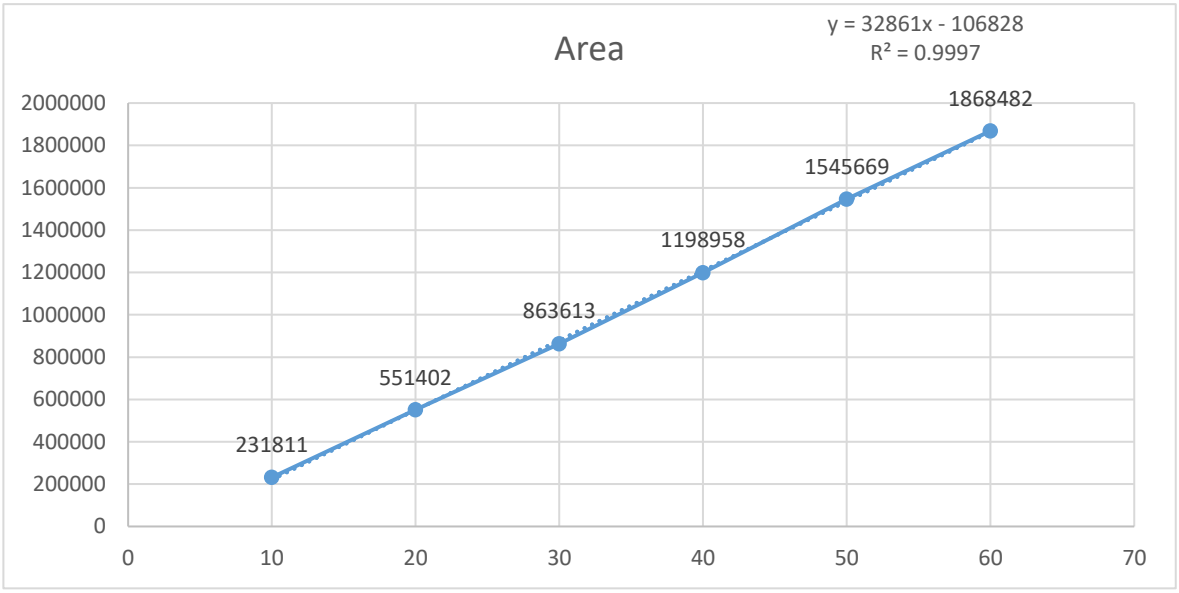

Figure 3: Calibration Plot of Plecanatide.

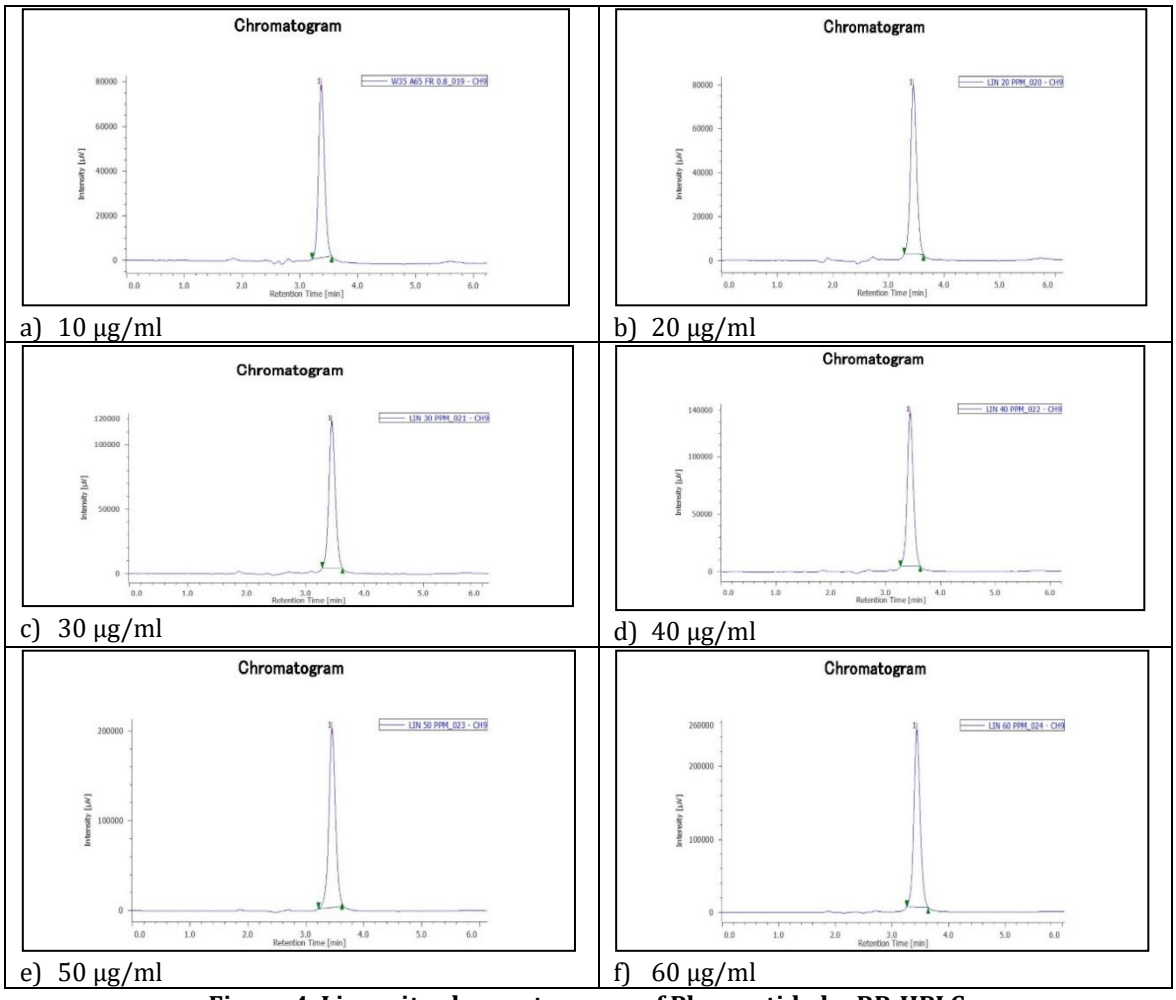

Figure 4. Linearity chromatograms of Plecanatide by RP-HPLC

7.2 Accuracy

Analyte concentrations were spiked into various matrices in recovery tests to evaluate the accuracy of the HPLC approach. The range of average recovery values, which showed a good degree of accuracy, was 98% to 102%. These findings imply that matrix interferences do not considerably impact the HPLC method, which can yield accurate and dependable results for a wide range of sample types. Table 3 presents the findings.

Level	Peak area	calculated concentration	% recovery	mean concentration	standard deviation	%RSD
0.8	690142	28.2425829	100.70%	16.056657	1.723311303	10.7326905
	690051	28.23883419				
	762554	31.22556952				
1	762554	31.22556128	98.91%	19.89124476	1.447625697	7.2777029
	800101	32.77230484				
	729875	29.87936972				
1.2	836965	34.29090422	101.83%	24.2197748	0.557304371	2.30103036
	812010	33.26289186				
	815432	33.40385994				

Table 3: Accuracy of Plecanatide.

7.4 Precision

The accuracy of the HPLC method was evaluated by doing multiple analyses on the same sample. It was discovered that the intra-day precision had a relative standard deviation (RSD) of less than 2% after analysing numerous aliquots of the same sample on the same day. The inter-day precision, which was determined by analysing the same sample across many days, likewise showed acceptable variability, with an RSD < 2%. The Precision results are presented in Tables 4 and 5. These results show how the UV approach is highly precise and reproducible, ensuring reliable and consistent observations.

Table 4: Intraday Precision Results (n=6±S.D)

Concentration (µg mL-1)	Peak Area	Obtained Concentration	S.D	% RSD
50	1052233	1052230	12792.91	1.20%
50	1073976	1073973		
50	1053170	1053167		
50	1078230	1078227		
50	1053325	1053322		
50	1076173	1076170		

Table 5: Interday Precision Results

Concentration (µg mL-1)	Abs	Obtained concentration	S.D	RSD
50	548557	548553.7	1089.849	0.19%
50	547213	547209.7		
50	549921	549917.7		
50	549821	549817.7		
50	548233	548229.7		
50	547798	547794.7		

7.5 Detection Limit (LOD) and Quantification Limit (LOQ)

The limit of detection and limit of quantification were found to be 1.06 and 3.21 µg mL $^{-1}$ respectively.

7.6 Robustness:

Table 6 displays the results, which indicate that the modest modification in the examined parameters had no effect on the selected factors. Additionally, it was discovered that these parameters had no apparent effect on the Plecanatide choice. Peak area differences were not statistically significant, and retention time variability was not as extensive. As such, it guarantees the robustness of the created procedure.

Table 6: Robustness evaluation by RP-HPLC

Sr.no.			1.	2.	3.	MEAN	SD	%RSD
Flow rate	0.5ml/min	Area	476036	479457	478855	**478116**	**1826.30**	**0.38%**
		R_1	5.500	5.490	5.493	**5.495**	**0.0050**	**0.09%**
		NTP	6616	6494	6616	**6575**	**70.43**	**1.07%**
	1.1ml/min	Area	220781	216358	215075	**217404**	**2993.53**	**1.38%**
		R_1	2.500	2.503	2.503	**2.502**	**0.0017**	**0.069%**
		NTP	4003	3938	3839	**3926**	**82.58**	**2.1%**
	251nm	Area	357588	370482	374441	**367503**	**8812.42**	**2.4%**
		R_1	3.427	3.430	3.433	**3.43**	**0.003**	**0.087%**
		NTP	5712	5758	5902	**5790**	**99.12**	**1.71%**

Wavelengh	**258nm**	**Area**	195510	206335	210525	**204123**	**7747.97**	**3.8%**
		R_1	3.433	3.426	3.430	**3.429**	**0.0035**	**0.102%**
		NTP	4887	4680	4905	**4824**	**125.03**	**2.59%**
Temperature	**20°C**	**Area**	291836	300849	301927	**298204**	**5541.12**	**1.86%**
		R_1	3.433	3.430	3.430	**3.431**	**0.0017**	**0.05%**
		NTP	5623	5339	5401	**5454**	**149.3**	**2.74%**

8. Conclusion

The HPLC method's outstanding linearity, accuracy, precision, sensitivity, and quantification capacity are demonstrated by the method validation results. These results confirm that the HPLC method is appropriate for quantitative analysis of the target analyte, guaranteeing solid and trustworthy data collection. The HPLC method is used for precise determination of analyte concentrations in research, quality control, and other analytical applications.

REFERENCES:

1. Sharma, S.; Goyal, S.; Chauhan, K. A Review on Analytical Method Development and Validation. Int. J. Appl. Pharm.**2018**, 10(6), 8.
2. Maurya, U. S.; Goyal, A. Approaches of Analytical Method Development and Validation: A Review. Int. J. Res. Pharm. Chem.**2021**,11(1).
3. Doltade, M.; Saudagar, R. Analytical Method Development and Validation: A Review. J. Drug Delivery Ther.**2019**,9 (3), 563–570.
4. Ravisankar, P.; Gowthami, S.; Devlala Rao, G. A Review on Analytical Method Development. Indian J. Res. Pharm. Biotechnol.**2014**, 2 (3),1183-1195.
5. Bhagat, R.; Saudagar, R. B. A Review on Analytical Method Development and Validation. J. Drug Delivery Ther.**2019**, 9(3-s), 1064-1067. http://dx.doi.org/10.22270/jddt.v9i3-s.2957.
6. Patil, S. T.; Aherrao, R. A.; Pawar, S. P. A Sho rt Review on Method Validation. J. Pharm. Biosci,**2017**, 5 (4), 30-37.
7. Chauhan, A.; Mittu, B.; Chauhan, P. Analytical Method Development and Validation: A Concise Review. J. Anal. Bioanal. Tech.,**2015**, 6 (1), 233.
8. Riddhiben, M.; Piyushbai, M.; Stability Indicating HPLC Method Development–A Review. Int. Res. J. Pharm.**2014**, 2(5), 79-87.
9. Aryal, S. UV Spectroscopy-Principle, Instrumentation, Applications. Online Microbiol.https://microbenotes.com /uv-spectroscopy-principle-instrumentation-applications/
10. Thakur, A. Principle, Working and Applications of UV Spectroscopy. India Study Channel. https://www.indiastudychannel.com/resources/146681-Principle-working-and-applications-of-UV-spectroscopy.aspx.
11. Aryal, S. High-Performance Liquid Chromatography (HPLC). Microbiol. Notes. https://microbenotes.com/ high-performance-liquid-chromatography-hplc/.
12. Charde, M. S.; Welankiwar, A. S.; Kumar, J. Method Development Chromatography with by Liquid Validation. Int. J. Pharm. Chem.**2014**, 04 (02), 57-61.
13. Sood, S.; Bala, R.; Gill, N. S. Method Development and Validation using HPLC Technique–A Review. J. Drug Discov. Ther. **2014**, 2(22), 18-24.
14. Geetha, G.; Naga, K.; Raju, G.; Sudheer Kumar, B. V.; Gnana Raja, M.; Bhuvaneswari, S. Analytical Method Validation: An Updated Review**.2012**.
15. Maheshwari, S.; Khandhar, A. P.; Jain, A. Quantitative Determination and Validation of Ivabradine HCL by Stability Indicating RP-HPLC Method and Spectrophotometric Method in Solid Dosage Form. Eur. J. Anal. Chem.**2010**, 5(1), 53–62.
16. PubChem. *Plecanatide*. pubchem.ncbi.nlm.nih.https://pubchem.ncbi.nlm.nih.gov/compound/Plecanatide.
17. Dayaramani, R. A.; Patel, P. U.; Patel, N. J. Development and Validation of RP-HPLC Method for Estimation of Stavudine in Bulk and in Capsule Formulation. *Res. J. Pharm. Tech.**2020**, 13(1), 15-21. doi: 10.5958/0974-360X.2020.00003.7.
18. Watson, D. G. Pharmaceutical Analysis: A Textbook for Pharmaceutical Chemists. Churchill Livingstone: London,**2012**.
19. Anwer, E. T.; Porwal, O.; Dudhe, R. Development and Validation of RP-HPLC Method for Estimation of Cefotaxime Sodium in Bulk and Formulation. Res. J. Pharm. Tech..**2022**, 15(7), 3114-8. doi: 10.52711/0974-360X.2022.00521.
20. Mendhan, J.; Denney, R. C.; Barnes, J. D.; Thomas, M.; Sivasankar, B. Vogel's Textbook of Quantitative Chemical Analysis, 5th ed.; ELBS Longman: London, **1997**, 216-217.
21. Waghmode, R.; Tegeli, V.; Dalal, A. Analytical Method Development and Validation of RP-HPLC Method for the Estimation of Sofosbuvir in Bulk and Formulation.J.Adv.Sci.Res.**2022**,3(07),4045 https://doi.org/10.55218/JASR.202213704.

22. Kamuda, J. A.; Mazzola, N. Plecanatide (Trulance) for Chronic Idiopathic Constipation and Irritable Bowel Syndrome with Constipation. Pharm. Ther.**2018**, 43(4), 207.
23.Smith, J. D.; Johnson, A. B.; Parker, C. Method Development and Validation for the Analysis of Plecanatide Drug in Biological Samples. *J. Pharm. Anal.* [Online] **2023**, 13, 123-135. DOI: 10.1016/j.jpha.**2023**.