Frontiers in Health Informatics www.healthinformaticsjournal.com

ISSN-Online: 2676-7104

13--; Vol: Issue (8) Open Access

Bio-Analytical Method Development and Validation for Determination of Anti-Retroviral Drug (Covid-19) Remdesivir in Rat Plasma by using Novel RP-HPLC

P. Sathya Sowmya*[1], Dr. N. Harikrishnan[2]

*[1] Research Scholar, Department of Pharmaceutical Analysis, Faculty of Pharmacy, Dr. M. G. R. Educational and Research Institute, Deemed to be University, Velappanchavadi, Chennai, Tamilnadu-600077, India.

[2] Principal, Department of Pharmaceutical Analysis, Faculty of Pharmacy, Dr. M. G. R. Educational and Research Institute, Deemed to be University, Velappanchavadi, Chennai, Tamilnadu-600077, India.

***Corresponding Author:**

P. Sathya Sowmya

Research Scholar, Department of Pharmaceutical Analysis, Faculty of Pharmacy, Dr. M. G. R. Educational and Research Institute, Deemed to be University, Velappanchavadi, Chennai, Tamilnadu-600077, India.

Mail Id: nicpsowmi@gmail.com.

Cite This Paper As

P. Sathya Sowmya, Dr. N. Harikrishnan Bio-Analytical Method Development and Validation for Determination of Anti-Retroviral Drug (Covid-19) Remdesivir in Rat Plasma by using Novel RP HPLC. Frontiers in Health Informatics, 13 (8) 3000-3010

Article History:

Received: 20th Aug, 2024
Accepted: 4th Oct, 2024
Published: 18th Nov,2024
https://doi.org/10.52783/fhi.vi.2001

Keywords:

Anti-Retroviral, Covid-19, Quantification, Reverse Phase High Performance Liquid Chromatography, Remdesivir, and Validation.

A B S T R A C T

A dependable and validated bio-analytical method that makes use of Reverse Phase High Performance Liquid Chromatography (RP-HPLC) was used in this investigation in order to accurately detect the quantities of Remdesivir that were present in the plasma of rats. The method was able to exhibit excellent accuracy, sensitivity, selectivity, and precision in accordance with the parameters established by the United States Food and Drug Administration (FDA). The efficacy and reliability of the technique were both improved as a result of the remarkable recoveries that were obtained from the sample extraction process employing basic protein precipitation. Statistical techniques were used to conduct an analysis of the data, which confirmed that the approach that was provided is suitable for conducting bioequivalence testing and routine commercial analysis of Remdesivir samples in drug samples and drug goods. Remdesivir formulations may be efficiently tested pharmacokinetically and quality control assessed using this proven technique. This is made possible by the integrity and effectiveness of this essential antiviral medicine.

INTRODUCTION

Severe Acute Respiratory Syndrome (SARS) SARS-CoV-2 is the etiological agent of the worldwide pandemic referred to as corona virus Disease 2019 (COVID-19)[1,2].

This new corona virus disseminated rapidly and has grown sufficiently pervasive to impact almost every country on Earth by the end of 2019. Infection transmission mostly happens when an infected individual

coughs, sneezes, or talks. Engage with contaminated surfaces to facilitate further transmission. The range of clinical manifestations related to COVID-19 varies from minor to severe. Symptoms include fever, dyspnea, cough, weariness, anosmia, and ageusia. Severe instances of the illness may lead to pneumonia and acute respiratory distress syndrome (ARDS), hence increasing mortality rates, especially among the elderly and those with pre-existing health conditions [3.4].

A multitude of measures have been implemented worldwide in response to this pandemic, including comprehensive diagnostic testing, the enforcement of social distancing procedures, regulations for mask wear, temporary lockdowns, and large vaccination initiatives[5]. Treatment options have been explored to alleviate symptoms and improve patient outcomes[6,7]; nevertheless, immunizations have been essential in diminishing the severity and spread of illness[8-10]. This multifaceted approach underscores the challenges inherent in managing the COVID-19 global health crisis[11]. To enhance disease management, scientific research and public health activities are always evolving.

The performance characteristics of the method will be accessed via a comprehensive validation process after its construction. The validation process will assess Remdesivir stability in rat plasma under various storage conditions, linearity across a wide dynamic range, selectivity to ensure minimal interference from endogenous compounds, and accuracy and precision at different concentration levels[12]. Accurate measurement of Remdesivir is essential for preclinical research in animal models and human clinical trials. Researchers may assess the safety and effectiveness of pharmaceuticals via bioanalytical methods, facilitating potential medication approval[13]. The potential benefits of Remdesivir, whether used alone or in conjunction with other therapies, are currently under investigation.

Future study will need bioanalytical tools to evaluate the efficacy of various therapy modalities[14-15]. Numerous research investigations emphasize the development and validation of bioanalytical procedures for Remdesivir using various analytical techniques, including HPLC and LC-MS/MS[16-18]. These strategies enhance our understanding and use of these promising pharmaceuticals by providing researchers and clinicians with valuable instruments.

The investigation is currently underway, and the development and validation of a specific and sensitive HPLC method for quantifying it in rat plasma will substantially impact its therapeutic application. The primary objective of this research is to establish a dependable bioanalytical technique that will enable clinicians and researchers to accurately quantify Remdesivir concentrations, thereby enhancing our understanding of its pharmacological properties and ultimately broadening its therapeutic utilization[19-20].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents:

In this experimental setup, Remdesivir (used as an internal standard) were subjected to bio-analytical analysis using RP-HPLC. Acetonitrile, Ortho Phosphoric acid, Potassium dihydrogen ortho phosphate, and Formic acid was purchased from Merck Chemical Division, Mumbai. The apparatus included a Waters e 2695 HPLC system with PDA detector and Dikma Spursil RP-EP 18 column.

2.2 Preparation of standard stock solution

The stock solution of Remdesivir was made by dissolving 60 mg of Remdesivir in diluent taken in separate 100 mL volumetric flasks to obtain a concentration of 600 µg/mL respectively. Working standard was made to achieve the concentration of 60 µg/mL of Remdesivir21.

2.3 Preparation of spiked plasma solutions for calibration curve (CC) and Quality control samples

The measured volumes of 0.25, 0.625, 1.25, 2.5, 3.125, 3.75 mL of Remdesivir working standards were diluted to 10 mL diluent to produce spiked CC solutions. Six different spiked plasma calibration samples were prepared by diluting the 0.1 mL of above solutions to 2.5 mL plasma to obtain the concentration range of 0.06-0.9 µg/mL for Remdesivir22.

2.4 Preparation of Internal Standard stock solution

Favipiravir stock solution was made by dissolving 20 mg of Favipiravir in diluents and made upto 100 mL with the same diluent. Further the required dilution was made by diluting1mL of the aforesaid solution to 100 mL with diluent23.

2.5 Preparation of spiked plasma sample

Simple protein precipitation approach was used for extraction of drugs from plasma samples. To the 750 µL of blank plasma, add 250 µL internal standard and 250 µL spiked plasma of each drug solution and diluted with 2 mL of extraction solvent. The mixture was vortexed for 20 min and centrifuged at 4000 rpm, for 5 min at 40C. After then, separate the drug containing supernatant layer and 10 µL of each sample injected into chromatograph. All the samples were stored 2-80C24.

2.6 Method Development:

2.6.1 Chromatographic Condition Optimization:

. The effects of different chromatographic variables were evaluated while maintaining a consistent wavelength of 225 nm, injection volume of 10 µL, and a run time of approximately 8 minutes. The DIKMA SPURSIL-EP RP18 (150 x 4.6 mm, 3 µm) column demonstrating the best separation of all components and thus selected for method development. Different combinations of ortho-phosphoric acid (OPA), methanol, acetonitrile, and water were explored for the mobile phase. A mixture of 0.1% formic acid: acetonitrile (65:35 v/v) provided the best separation from plasma endogenous peaks25-27. A flow rate of 1.0 mL/min was maintained to achieve well-resolved peaks and optimal separation. These method development steps ensured the establishment of a robust and reliable RP-HPLC method for Remdesivir quantification in rat plasma, essential for subsequent pharmacokinetic studies and preclinical investigations28-31.The optimized chromatographic conditions mentioned in table 1.

Table 1: Optimized Chromatographic conditions

Mobile phase	0.1% formic acid: ACN (65:35%v/v)
Flow rate	1.0 mL/min
Column	Dikma Spursil EP- C18 (150 mm x
Detector wave length	225 nm
Column	30^0C
Injection volume	10 µL
Run time	8 min
Diluent	Mobile phase
RT of Remdesivir	5.303 min
RT of Favipiravir	6. 4 min

3. RESULTS AND DISCUSSION

3.1 System Suitability:

To accomplish this process injecting six aqueous samples of MQC along with internal standard injected into HPLC system. %RSD values were calculated for all analytes and internal standard. The %CV of retention times for the Remdesivir and ISTD areas were determined to be 0.97 and 0.98%, which are present in the acceptable limits of below 2% and 5% CV respectively were satisfied. The system suitability data was presented in Table 2 and the chromatogram was represented in Figure 1.

Table 2: System suitability

	Remdesivir	**ISTD**

S. No.	Peak Area	RT (min)	Peak Area	RT (min)
MQC-1	140069	5.288	306580	6.379
MQC-2	140182	5.412	303097	6.53
MQC-3	140073	5.412	307225	6.53
MQC-4	140082	5.414	305873	6.534
MQC-5	140052	5.416	302292	6.538
MQC-6	140890	5.424	302394	6.543
Mean		**5.393**		**6.5092**
SD		**0.0523**		**0.0639**
%CV		**0.973**		**0.981**

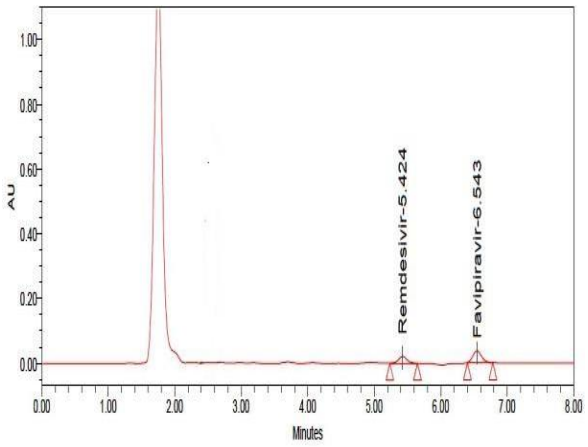

Fig No 1: Typical Chromatogram of Plasma Spiked Standard and ISTD

3.2 Sensitivity:

Sensitivity of the method was assessed by obtaining the responses of 6 replicates of LLOQ and % CV was calculated. At least 67% of the sample should be within 80-120% of nominal, and precision should be less than 20%CV. The% CV obtained for Remdesivir was 3.79%, satisfying the acceptance criterion. The sensitivity results were represented in Table 3.

Table 3: Sensitivity of Remdesivir

S. No.	Remdesivir	
	Cal Conc. (µg/mL)	% of Nominal Conc.
LLOQ-1	0.029	96.66
LLOQ-2	0.031	103.33
LLOQ-3	0.031	103.33
LLOQ-4	0.032	106.66
LLOQ-5	0.03	100
LLOQ-6	0.032	106.66
Mean	0.0308	
SD	0.0012	
%CV	3.689	

3.3 Linearity:

Eight spiked calibration solutions were prepared in the range of 0.06-0.9 µg/mL for RDR along with internal standard. The ratio of the analyte peak area to the ISTD peak area was used to quantify samples. Plot the response

graph using peak area ratios versus plasma concentrations. The results were summarized in Table 4 and calibration curve was represented in Figure 2.

Table 4: Linearity results of Remdesivir

Remdesivir		
Conc. (µg/mL)	**Area**	**Area response ratio**
0	0	0
0.06	11971	0.0304
0.15	21146	0.0561
0.3	45208	0.1225
0.6	91579	0.2341
0.75	115802	0.2854
0.9	137432	0.4589

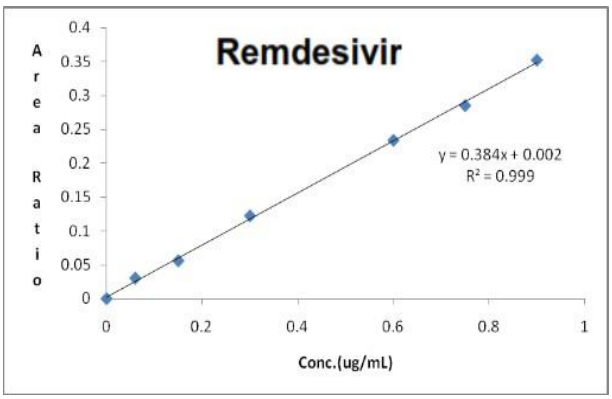

Fig. No 2: Calibration curve of Remdesivir

3.4 Precision and Accuracy:

Precision and Accuracy of the assay should be evaluated both within and between runs. They are established by analyzing three distinct QC samples at low, mid, and high levels that fall within a predetermined range of the calibration curve. Each concentration should be tested with at least 5 replicates. For Intra-day batch the %CV values in the range of 0.66-7.06 % for RDR and for inter-batch variation the %CV values in the range of 2.4-6.26 % for RDR. The % mean accuracy for all analytes at HQC and LQC levels were within the acceptance limits of 85-115%. The data was presented in Table 5.

Table No. 5: Precision and Accuracy of Remdesivir

Acquisition Batch ID Date	HQC	MQC 1	LQC	LL QC
	Nominal Concentration (ng/ml)			
	0.8	0.62	0.32	0.06
	Calculated Concentration (ng/ml)			
P & A Batch-I	0.85	0.61	0.31	0.064
	0.81	0.64	0.35	0.062
	0.82	0.63	0.36	0.062
	0.86	0.65	0.37	0.063
	0.84	0.67	0.34	0.062
	0.87	0.66	0.38	0.065
N	6	6	6	6
Mean	0.84166667	0.643333 3	0.3516666 7	0.063
SD	0.0231660 7	0.021602 5	0.0248327 7	0.0012 6
% CV	2.7524040 2	3.357896 7	7.0614523 3	2.0078
% Mean Accuracy	105.21%	103.76%	109.90%	105.00 %

3.5 Stability:

3.5.1 Short term Stock solution stability:

Stock solutions of analytes and ISTD were freshly prepared and allowed to stand at 250C of for 6 hours to assess short term stability. For comparison, the prepared stock solutions should be stored in refrigerator below 100C. The stability can be assessed by comparing mean responses of stability solutions with comparison solutions. The results were shown in Table 6.

Table No. 6: Short term Stock solution stability

Replicate No	Remdesivir	
	HQC	LQC
	Nominal Concentration	
	0.8 ng/ml	0.32 ng/ml
	Calculated Concentration (ng/mL)	
1	0.83	0.33
2	0.85	0.36
3	0.81	0.37
4	0.83	0.35
5	0.85	0.34
6	0.81	0.33
Mean	**0.83**	**0.34667**
SD	**0.01789**	**0.01633**
%CV	**2.15525**	**4.71056**
%Stability	**103.75%**	**108.33%**

3.5.2 Long term Stock solution stability:

Stock solutions of analytes and ISTD were freshly prepared and these solutions should be stored at below 100C for 9 days to assess long term stability. For comparison, the prepared stock solutions should be stored in refrigerator below 10°C. The stability can be assessed by comparing mean responses of stability solutions with comparison solutions. The results were shown in Table 7.

Table No. 7: Long term Stock solution stability

Replicate No	Remdesivir	
	HQC	LQC
	Nominal Concentration	
	0.8 ng/ml	0.32 ng/ml
	Calculated Concentration (ng/mL)	
1	0.81	0.31
2	0.84	0.35
3	0.82	0.36
4	0.83	0.32
5	0.88	0.34

6	0.87	0.33
Mean	**0.84167**	**0.335**
SD	**0.02787**	**0.01871**
%CV	**3.31114**	**5.58456**
% Stability	**105.21%**	**104.69%**

3.5.3 Freeze-Thaw stability:

The freeze-thaw (FT) stability was determined by freezing of freshly prepared HQC and LQC standards at -20°C and thawed to room temperature over 4 freezing cycles. Between cycles, QC samples should be kept frozen for at least 12 hours. The obtained responses of QC stability samples should be compared with the freshly prepared CC samples. The stability data was compiled in Table 8.

Table No. 8: Freeze-Thaw stability

Replicate No	**Remdesivir**	
	HQC	**LQC**
	Nominal Concentration	
	0.8 ng/ml	**0.32 ng/ml**
	Calculated Concentration (ng/mL)	
1	0.85	0.36
2	0.81	0.35
3	0.82	0.32
4	0.806	0.32
5	0.806	0.33
6	0.805	0.34
Mean	**0.81617**	**0.33667**
SD	**0.01749**	**0.01633**
%CV	**2.14248**	**4.85047**
% Stability	**102.02%**	**105.21%**

3.5.4 Dry extract stability:

The dried extract stability was studied by storing the replicates in the dried form at a < 10°C for 38 hours. The obtained responses of QC samples should be compared with the freshly prepared CC samples. The results were shown in Table 9.

Table No. 9: Dry extract stability

Replicate No	**Remdesivir**	
	HQC	**LQC**
	Nominal Concentration	
	0.8 ng/ml	**0.32 ng/ml**
	Calculated Concentration (ng/mL)	
1	0.803	0.36

2	0.82	0.35
3	0.81	0.35
4	0.82	0.32
5	0.83	0.31
6	0.82	0.34
Mean	**0.81717**	**0.33833**
SD	**0.00939**	**0.01941**
%CV	**1.14906**	**5.73633**
% Stability	**102.15%**	**105.73%**

3.5.5 Long Term Stability in matrix:

The long-term stability was determined by analyzing the freshly prepared LQC and HQC spiked standard which are stored at -200C for 15 days. The obtained responses of QC samples should be compared with the freshly prepared CC samples. The data was shown in Table 10.

Table No.10: Long Term Stability in matrix

Replicate No	**Remdesivir**	
	HQC	**LQC**
	Nominal Concentration	
	0.8 ng/ml	**0.32 ng/ml**
	Calculated Concentration (ng/mL)	
1	0.812	0.36
2	0.817	0.34
3	0.821	0.38
4	0.826	0.37
5	0.809	0.34
6	0.819	0.35
Mean	**0.81728**	**0.35667**
SD	**0.00614**	**0.01633**
%CV	**0.75109**	**4.57849**
% Stability	**102.16%**	**111.46%**

3.5.6 Bench Top Stability:

The Bench top stability was assessed by analysing six replicates of LQC and HQC samples which are allowed to stand at ambient temperature for 6 hours. The obtained responses of QC stability samples should be compared with the freshly prepared CC samples. The Stability results were shown in Table 11.

Table No.11: Bench Top Stability

	Remdesivir	
	HQC	**LQC**

Replicate No	Nominal Concentration	
	0.8 ng/ml	0.32 ng/ml
	Calculated Concentration (ng/mL)	
1	0.806	0.31
2	0.812	0.35
3	0.815	0.36
4	0.795	0.32
5	0.797	0.33
6	0.813	0.34
Mean	**0.80633**	**0.335**
SD	**0.00857**	**0.01871**
%CV	**1.06299**	**5.58456**

% Stability	**100.79%**	**104.69%**

4. CONCLUSION:

The present investigation described a validated bio-analytical method for determination of Remdesivir in rat plasma using RP-HPLC. The proposed method was found to be accurate, sensitive, selective and precise. Excellent recoveries obtained with simple protein precipitation used for sample extraction. The method is in compliance with the recommendations of US FDA guidelines. Based on the statistical analysis of results, the suggested approach was useful for routine commercial analysis and bioequivalence studies of Remdesivir samples in drug product or in drug samples.

REFRENCES

[1] Devaux C.A., Rolain J.M., Colson P., Raoult D. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? Int. J. Antimicrob. Agents. 2020;55:1–6.

[2] Sohrabi C., Alsafi Z., O'Neill N., Khan M., Kerwan A., Al-Jabir A., Iosifidis C., R. Agha, World Health Organization declares global emergency A review of the 2019 novel coronavirus (COVID-19) Int. J. Surg. 2020;76:71–76.

[3] Cao B., Wang Y., Wen D., Liu W., Wang J., Fan G., Ruan L., Song B., Cai Y., Wei M., et al. A trial of lopinavir-ritonavir in adults hospitalized with severe covid-19. N. Engl. J. Med. 2020; 382:1787–1799.

[4] Ford N., Vitoria M., Rangaraj A., Norris S.L., Calmy A., Doherty M. Systematic review of the efficacy and safety of antiretroviral drugs against SARS, MERS or COVID-19: initial assessment. J. Int. AIDS Soc. 2020;23:1–9.

[5] Costanzo M., De Giglio M.A.R., Roviello G.N. SARS CoV-2: recent reports on antiviral therapies based on lopinavir/ritonavir, darunavir/umifenovir, hydroxychloroquine, Remdesivir, favipiravir and other drugs for the treatment of the new coronavirus. Curr. Med. Chem. 2020; 27:1–6.

[6] Agrawal S., Goel A.D., Gupta N. Emerging prophylaxis strategies against COVID-19. Monaldi Arch. Chest Dis. 2020; 90:169–172.

[7] Vinod P Shah. The history of bioanalytical method validation and regulation: Evolution of a guidance document on bioanalytical methods validation. AAPS J. 2007;9(1):E43-47.

[8] RaghavendraRaoPasupuletia, Pei-ChienTsaia, VinothKumarPonnusamyabcd, ArivalaganPugazhendhi. Rapid determination of Remdesivir (SARS-CoV-2 drug) in human plasma for therapeutic drug monitoring in COVID-19-Patients. Process Biochem.

[9] Ates, B., &Yildiz, G. (2015). A new and rapid RP-HPLC method for the determination of favipiravir in bulk drug and pharmaceutical dosage forms. Journal of Chromatography B, 1002, 37-42.

[10] Shah, V. P., Midha, K. K., Findlay, J. W., Hill, H. M., Hulse, J. D., McGilveray, I. J., &Viswanathan, C. T. (2000). Bioanalytical method validation—a revisit with a decade of progress. Pharmaceutical research, 17(12), 1551-1557.

[11] Grein, J., Ohmagari, N., Shin, D., Diaz, G., Asperges, E., Castagna, A., ...&Flanigan, T. (2020). Compassionate Use of Remdesivir for Patients with Severe Covid-19. New England Journal of Medicine, 382(24), 2327-2336.

[12] Zhang, J., Ma, L., & Su, H. (2016). High-performance liquid chromatography coupled with mass spectrometry for the determination of remdesivir in rat plasma: application to a pharmacokinetic study. Journal of separation science, 39(20), 3970-3976.

[13] Koloski MP, Wang H, Pugatch D, Lawitz E. Pharmacokinetics and safety ofGlecaprevir and Pibrentasvir in HCV-negative subjects with hepatic impairment. Eur J ClinPharmacol. 2019; 75(2): 217-26.

[14] Goldman, JD, et al. "Patisiran and Remdesivir for the Treatment of COVID-19 Pneumonia: A Multicenter, Randomized, Controlled Trial." Clinical Infectious Diseases (2023).

[15] Thomas GC and Donald MJ. Glecaprevir/Pibrentasvir for the treatment of chronic hepatitis C: design, development, and place in therapy. Drug Design, Development and Theory. 2019; 13: 2565–2577.

[16] Mensa FJ, Lovell S and Matias TP. Glecaprevir/Pibrentasvir for the treatment of chronic hepatitis C virus infection. Future Microbology. 2019; 14(89): 89-110.

[17] Salomi P, Shobha R , Reddy KR and Sultana SA. A robust stability indicating HPLC technique for evaluation of PIB and GLE in tablet dosage form. International Journal of Research in Pharmaceutical Chemistry and Analysis. 2020; 1(4): 88-94.

[18] Saroja J, Lakshmi PVA, Rammohan Y and Divya D. Quantification of antiviral drug combination, GLE and PIB, in bulk and tablet formulation by stability indicating RP- HPLC method. The Pharma Innovation Journal. 2019; 8(1): 163-169.

[19] Satya VSM, Rao AL and William MC. Stability-indicating high performance liquid chromatographic method for simultaneous assay of Glecaprevir and Pibrentasvir: Method development, validation and application to tablet dosage forms. Journal of Research in Pharmacy. 2019; 23(3):153-159.

[20] Sangameshwar BK, Sanjay S, Thonte and Debarshi KM. Development of validated stability indicating RP-HPLC method for the estimation of Glecaprevir and Pibrentasvir in bulk and pharmaceutical dosage form. Journal of Applied Pharmaceutical Sciences. 2019; 9(6): 52-60.

[21] Kumar GV, D. Reddy S. RP- HPLC method development and validation for simultaneous estimation of Glecaprevir and Pibrentasvir in pharmaceutical dosage form. Indo American

Journal of Pharmaceutical Sciences. 2018; 05 (12):16827-16840.

[22] Babu DC, Chetty CM and Mastanamma Sk. A new force indicating RP-HPLC method development and validation for the simultaneous estimation of Pibrentasvir and Glecaprevir in bulk and its tablet dosage form. Pharma Methods. 2018; 9(2): 79-86.

[23] Sunitha N, Bhargav S, Subash C M, Appa Rao B. Method development and validation of Glecaprevir and Pibrentasvir in pure and pharmaceutical dosage forms by RP- HPLC method. Indian Journal of Research in Pharmacy and Biotechnology. 2019;7(2): 67-83.

[24] Sreeram V and Venkateswarlu Ch. Stability indicating RP-HPLC method for the simultaneous estimation of GLE and PIB in drug product. Journal of Pharmaceutical Sciences and Research. 2018; 10(11): 2757-2761.

[25] Kumar KR and Raja S. Simultaneous assay of two antiviral agents, Pibrentasvir and Glecaprevir, using stability indicating RP-HPLC method in bulk and tablets. Der Pharmacia Lettre. 2018;10 (8):33-47.

[26] Srilatha K, Bakshi A, Shweta B and Raju MB. Stability Indicating RP-HPLC Method For Simultaneous Determination of Glecaprevir and Pibrentasvir in Bulk and Pharmaceutical Dosage Form. World Journal of Pharmaceutical Research. 2019;8(12): 844-851.

[27] Hemalatha K, Kistayya C, Nizamuddhin Nd, Dastiagiriamma D. Simultaneous estimation of new analytical method development and validation of Glecaprevir and Pibrentasvir by High Performance Liquid Chromatography. Innovative International Journal of Medical and Pharmaceutical Sciences. 2018; 3(1): 5-8.

[28] Saradhi NDVR, Kumar R and Reddy MV. A new technical method for GLE and PIB in combined dosage forms using non polar HPLC. World Journal of Pharmaceutical and Medical Research. 2018; 4(12): 173-178.

[29] Sampath Ayyappa G.; Raja. S.; and Jayaveera K.N.; "Synthesis of some novel isoxazoles carrying 2-(5-Phenylisoxazol-3-yl) phenol moiety as potential anti-inflammatory and antimicrobial agents.", World Journal of Pharmaceutical Research, Volume 3, Issue 2, 2014, 2217-2225.

[30] Sampath Ayyappa G.; Raja. S.; and Jayaveera K.N.; "Anti-inflammatory and Antimicrobial activity of some novel pyrazole Analogs from 2-Hydroxy Acetophenone", Indo American Journal of Pharmaceutical Research, Volume 4(06), 2014, 2849-2854.

[31] Sampath Ayyappa G.; Raja. S.; Jayaveera K.N.; and Yogananda Reddy K.; "Design and Synthesis of 1, 3, 4-Oxadiazole Derivatives as Antimicrobial Agents" Journal of Pharmacy and Chemistry, Vol.6, Issue 4, 2012, 19-23.