Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

SIMULTANEOUS ESTIMATION OF GLECAPREVIR AND PIBRENTASVIR IN BULK AND MARKETED FORMULATION BY USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

M. Vineela*[1], Kavitha Waghray[1], S. Ramya Sri[1], B. Sri Latha[1]

[1*]Department of Pharmacy, University College of Technology, Osmania University, Hyderabad - 500 007, Telangana, India.

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Glecaprevir and Pibrentasvir, in its pure form as well as in tablet dosage form. Chromatography was carried out on Sunfire C18 (4.6×250mm) 5µ column using a mixture of Water and Acetonitrile (60:40% v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 220nm. The retention time of the Glecaprevir and Pibrentasvir was 3.0, 3.8±0.02min respectively. The method produce linear responses in the concentration range of 25-125µg/ml of Glecaprevir and 10-50µg/ml of Pibrentasvir. The method quality for the determination of assay was below 2.0%RSD. The method is important in the quality control of bulk and pharmaceutical formulations.

KEYWORDS

Glecaprevir, Pibrentasvir, RP-HPLC and Validation.

Author for Correspondence:

Vineela M,
Department of Pharmacy,
University of Technology,
Osmania University,
Hyderabad, Telangana, India.

Email: rajinisuralabs@gmail.com

INTRODUCTON

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while

others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics.

DRUG PROFILE

Drug : Glecaprevir
Synonym : Glecaprevirum
Drug category : Cytochrome P-450 CYP1A2 Inhibitor
Structure :

Chemicalname/ Nomenclature / IUPAC Name

(1R, 14E, 18R, 22R, 26S, 29S)-26-tert-butyl-N-[(1R, 2R)-2-(difluoromethyl)-1-{[(1-methylcyclopropyl)sulfonyl]carbamoyl}cyclopropyl]-13, 13-difluoro-24, 27-dioxo-2, 17, 23-trioxa-4, 11, 25, 28-tetraazapentacyclo[26.2.1.0^{3, 12}.0^{5, 10}.0^{18, 22}]hentriaconta-3, 5(10), 6, 8, 11, 14-hexaene-29-carboxamide

DRUG PROFILE

Drug : Pibrentasvir
Drug category : Cytochrome P-450 CYP1A2 Inhibitor
Structure :

Chemical name/ Nomenclature / IUPAC Name

(2S, 3R)-1-[(2S)-2-{5-[(2R, 5R)-1-{3, 5-difluoro-4-[4-(4-fluorophenyl) piperidin-1-yl] phenyl}-5-{6-fluoro-2-[(2S)-1-[(2S, 3R)-2-{[hydroxyl (methoxy) methylidene] amino}-3-methoxybutanoyl] pyrrolidin-2-yl]-1H-1, 3-benzodiazol-5-yl} pyrrolidin-2-yl]-6-fluoro-1H-1, 3-benzodiazol-2-yl} pyrrolidin-1-yl]-2-{[hydroxy(methoxy) methylidene] amino}-3-methoxybutan-1-one.

EXPERIMENTAL WORK

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Pibrentasvirand 10mg of Glecaprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the samesolvent. (Stock solution)

Furtherpipette 0.15ml of the Pibrentasvir and 0.3ml of the Glecaprevirstock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Pibrentasvir and Glecaprevir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the samesolvent.

Furtherpipette 0.3ml of the Sample stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

PREPARATION OF DRUGSOLUTIONS FOR LINEARITY

Accuratelyweigh and transfer 10 mg of Pibrentasvir and 10mg of Glecaprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the samesolvent. (Stock solution)

Preparation of Level - I (10µg/ml of Pibrentasvir and 25 µg/ml of Glecaprevir)

Pipetteout 0.1ml of Pibrentasvir and 0.25ml of Glecaprevir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level - II (20 µg/ml of Pibrentasvir and 50µg/ml of Glecaprevir)

Pipetteout 0.2ml of Pibrentasvir and 0.5ml of Glecaprevir stock solutions wastake in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level - III (30µg/ml of Pibrentasvir and 75µg/ml of Glecaprevir)

Pipetteout 0.3ml of Pibrentasvir and 0.75ml of Glecaprevir stock solutions wastake in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level - IV (40µg/ml of Pibrentasvir and 100µg/ml of Glecaprevir)

Pipetteout 0.4ml of Pibrentasvir and 1.0ml of Glecaprevir stock solutions wastake in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level - V (50µg/ml of Pibrentasvir and 125µg/ml of Glecaprevir)

Pipetteout 0.5ml of Pibrentasvir and 1.25ml of Glecaprevir stock solutions wastake in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure

Injecteachlevel in to the chromatographic system and measurethepeakarea.

Plot a graph of peakareaversusconcentration (on X-axisconcentration and on Y-axis Peakarea) and calculatethecorrelationcoefficient.

Optimized Chromatogram (Standard)

Mobilephase ratio : Acetonitrile: Water (40:60v/v)
Column : Sunfire C18 (4.6×250mm) 5µ
Column temperature : 35ºC
Wavelength : 220nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 6minutes

Acceptancecriteria

- The oretical plates must be not less than 2000
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

SPECIFICITY

The ICH documents define specificity as the ability to assessunequivocallytheanalyte in the presence of components that may beexpected to bepresent, such asimpurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantities S-Pibrentasvir and Glecaprevir in drug product.

$$\%ASSAY = \frac{\text{Samplearea}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Pibrentasvir and Glecaprevir in pharmaceutical dosage form was found tobe100.5%

LINEARITY

LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Pibrentasviris a straight line.

Y = mx + c
Slope (m) =43950
Intercept (c) = 8388
Correlation Coefficient (r) = 0.999

Validation Criteria

The responselinearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion

Correlation Coefficient (r) is 0.99, and the intercept is8388. The sevaluesmeet the validation criteria.

LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Glecaprevir is a straight line.

Y = mx + c
Slope (m) =9933
Intercept (c) = 10151
Correlation Coefficient (r) = 0.999

Validation Criteria

The responselinearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion

Correlation Coefficient (r) is 0.99, and the intercept is10151. The sevaluesmeet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the samehomogeneoussampleunder the prescribed conditions.

Acceptancecriteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limit shence method is precise.

Acceptancecriteria

- %RSD for sample should be NMT 2

- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Acceptancecriteria

- %RSD of Six different sample solutions should not more than 2

Acceptancecriteria

- %RSD of Six different sample solutions should not more than 2.

Acceptancecriteria

- %RSD of Six different sample solutions should not more than 2Table:

Acceptancecriteria

- %RSD of Six different sample solutions should not more than 2

Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Acceptance Criteria

- The percentage recovery was found to be within the limit (98-102%).
- The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Acceptance Criteria

- The percentage recovery was found to be within the limit (98-102%).
- The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION FOR S-PIBRENTASVIRAND GLECAPREVIR

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

where

σ = Standard deviation of the response

S = Slope of the calibration curve

RESULTS

Pibrentasvir

=3.3×9373/43950

=0.7µg/ml

Glecaprevir

=3.3×5548/9933

=1.8µg/ml

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

RESULTS

Pibrentasvir

=10×9373/43950

=2.1µg/ml

Glecaprevir

=10×5548/9933

=5.5µg/ml

Robustness

Acceptancecriteria

The tailing factor should beless than 2.0 and the number of the oretical plates (N) should bemore than 2000.

INSTRUMENTS USED

Table No1: Instruments used

S.No	Instruments and Glasswares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
2	pH meter	LabIndia
3	Weighingmachine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

Table No.2: Chemicals used

S.No	Chemical	Brand names
1	Pibrentasvir	Sura labs
2	Glecaprevir	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Triethylamine	Merck

Table No.3: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Pibrentasvir	3.006	731322	61677	1.2	8574
2	Glecaprevir	3.853	3421257	319786	1.1	9664

Table No. 19: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Pibrentasvir	3.005	658995	61772	1.1	7442
2	Glecaprevir	3.848	3096188	324054	1.2	7331

Table No.4: Chromatographic Data for Linearity Study For PIBRENTASVIR

S.No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	33.3	5	230247
2	66.6	10	462332
3	100	15	659905
4	133.3	20	892989
5	166.6	25	1101075

Table No.5: Chromatographic Data for Linearity Study For GLECAPREVIR

S.No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	33.3	10	1215225
2	66.6	20	2135937
3	100	30	3020839
4	133.3	40	4078841
5	166.6	50	5058145

REPEATABILITY

Table No.6: Results of repeatability for Pibrentasvir

S. No	Peakname	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pibrentasvir	3.003	654426	61521	8474	1.1
2	Pibrentasvir	3.005	659862	61937	8262	1.2
3	Pibrentasvir	3.007	650837	62018	8117	1.1
4	Pibrentasvir	3.008	651433	61893	7917	1.2
5	Pibrentasvir	3.005	652752	61867	8011	1.1
Mean			653862			
Std.dev			3626.323			
%RSD			0.554601			

Table No.7: Results of repeatability for Glecaprevir

S. No	Peakname	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Glecaprevir	3.851	3028371	381736	6881	1.1
2	Glecaprevir	3.852	3009188	380138	9363	1.2
3	Glecaprevir	3.854	3067464	386615	7844	1.1
4	Glecaprevir	3.853	3076611	380183	9746	1.2
5	Glecaprevir	3.851	3011912	379471	7883	1.2
Mean			3038709			
Std.dev			31463.69			
%RSD			1.035429			

INTERMEDIATE PRECISION

Day 1

Table No.8: Results of Intermediate precision day1 for Pibrentasvir

S.No	Peak Name	RT	Area(µV*sec)	Height(µV)	USP Plate count	USP Tailing
1	Pibrentasvir	3.007	658911	60173	9141	1.1
2	Pibrentasvir	3.005	650383	61936	9662	1.2
3	Pibrentasvir	3.005	658813	60383	9746	1.1
4	Pibrentasvir	3.005	651138	60774	7746	1.1
5	Pibrentasvir	3.005	659937	61947	8264	1.2
6	Pibrentasvir	3.010	653715	61893	7836	1.1
Mean			655482.8			
Std.Dev.			4258.945			
%RSD			0.649742			

Table No.9: Results of Intermediate precision day1 for Glecaprevir

S.No	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Glecaprevir	3.851	3021731	369771	8564	1.1
2	Glecaprevir	3.848	3019183	372746	9227	1.1
3	Glecaprevir	3.848	3029847	371866	7565	1.2
4	Glecaprevir	3.850	3028471	369017	7726	1.1
5	Glecaprevir	3.849	3088641	376453	6746	1.2
6	Glecaprevir	3.860	3056633	386621	5977	1.1
Mean			3040751			
Std.Dev.			26990.09			
%RSD			0.887613			

Day 2

Table No.10: Results of Intermediate precision Day 2 for Pibrentasvir

S.No	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Pibrentasvir	3.006	648822	61847	6983	1.1
2	Pibrentasvir	3.008	640863	59882	7728	1.2
3	Pibrentasvir	3.008	643382	60774	9576	1.1
4	Pibrentasvir	3.007	641884	58928	8275	1.2
5	Pibrentasvir	3.007	647822	61483	9837	1.1
6	Pibrentasvir	3.005	649181	60928	8744	1.2
Mean			645325.7			
Std.Dev.			3711.009			
%RSD			0.57506			

Table No.11: Results of Intermediate precision Day 2 for Glecaprevir

S.No	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Glecaprevir	3.853	3075833	389911	7039	1.1
2	Glecaprevir	3.857	3029583	379019	9857	1.2
3	Glecaprevir	3.854	3021991	381875	7881	1.1
4	Glecaprevir	3.855	3022485	391099	7902	1.2
5	Glecaprevir	3.854	3085833	389222	9285	1.1
6	Glecaprevir	3.853	3019482	391184	8955	1.2
Mean			3042535			
Std.Dev.			30022.42			
%RSD			0.986757			

Accuracy50%

Table No.12: Results of Accuracy for concentration-50%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Pibrentasvir	3.006	335352	31861	1.1	8573
2	Pibrentasvir	3.022	336153	39371	1.1	5891
3	Pibrentasvir	3.006	330183	37857	1.2	6573
4	Glecaprevir	3.855	1593716	179472	1.1	9164
5	Glecaprevir	3.877	1583631	178947	1.2	8264
6	Glecaprevir	3.854	1579482	176534	1.1	7248

Accuracy100%

Table No.13: Results of Accuracy for concentration-100%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Pibrentasvir	3.007	657351	61655	1.1	7842
2	Pibrentasvir	3.006	657874	61948	1.1	6018
3	Pibrentasvir	3.005	658292	61183	1.1	7544
4	Glecaprevir	3.855	3078171	386641	1.2	8922
5	Glecaprevir	3.853	3076144	378656	1.1	9355
6	Glecaprevir	3.850	3097262	386521	1.2	8456

Accuracy150%

Table No.14: Results of Accuracy for concentration-150%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Pibrentasvir	3.004	974626	89388	1.1	8462
2	Pibrentasvir	3.006	975411	89749	1.2	9771
3	Pibrentasvir	3.008	970815	88937	1.2	8947
4	Glecaprevir	3.847	4598264	436613	1.1	7917
5	Glecaprevir	3.851	4589462	439282	1.1	9364
6	Glecaprevir	3.853	4501948	437167	1.2	8462

Table No.15: The accuracy results for Pibrentasvir

S.No	%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
1	50%	331938	7.5	7.3	99.88	100.166
2	100%	658274	15	14.7	98.89	
3	150%	970963	22.5	22.2	101	

Table No.16: The accuracy results for Glecaprevir

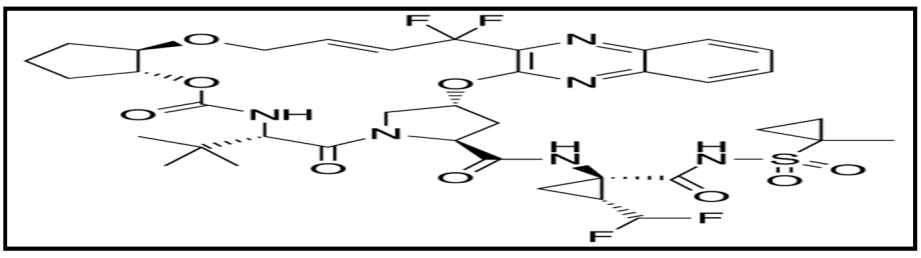
S.No	%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
1	50%	209357	7.5	7.49	99.7%	99%
2	100%	420697.7	15	14.9	99%	
3	150%	631550.7	22.5	22.48	99%	

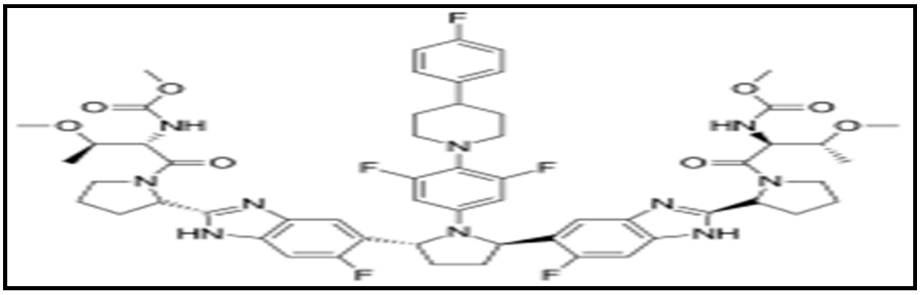
Table No.17: Results for Robustness Pibrentasvir

S.No	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
1	Actual Flow rate of 0.9mL/min	658211	3.006	8793	1.2
2	Less Flow rate of 0.8mL/min	621077	3.441	7269	1.3
3	More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	642190	2.663	9446	1.2
4	Less organic phase	542402	3.185	8126	1.1
5	More organic phase	642112	2.867	5854	1.3

Table No.18: Results for Robustness-Glecaprevir

S.No	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
1	Actual Flow rate of 0.9mL/min	429069	3.853	5224	1.59
2	Less Flow rate of 0.8mL/min	472673	4.426	6328	1.58
3	More Flow rate of 1.0mL/min	392497	3.415	6217	1.54
4	Less organic phase	391379	4.291	6996	1.61
5	More organic phase	391703	3.583	6120	1.50





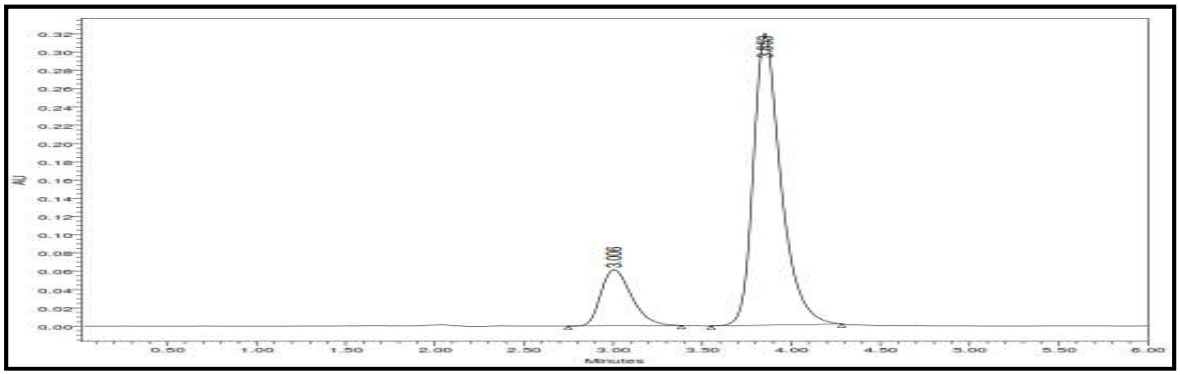


Figure No.1: Optimized Chromatogram (Standard)

Optimized Chromatogram (Sample)

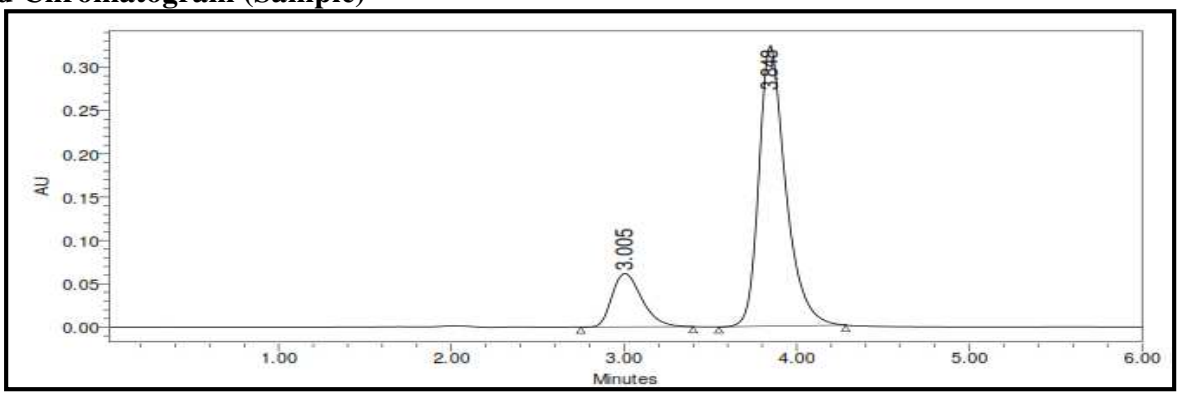


Figure No.2: Optimized Chromatogram (Sample)

VALIDATION

Blank

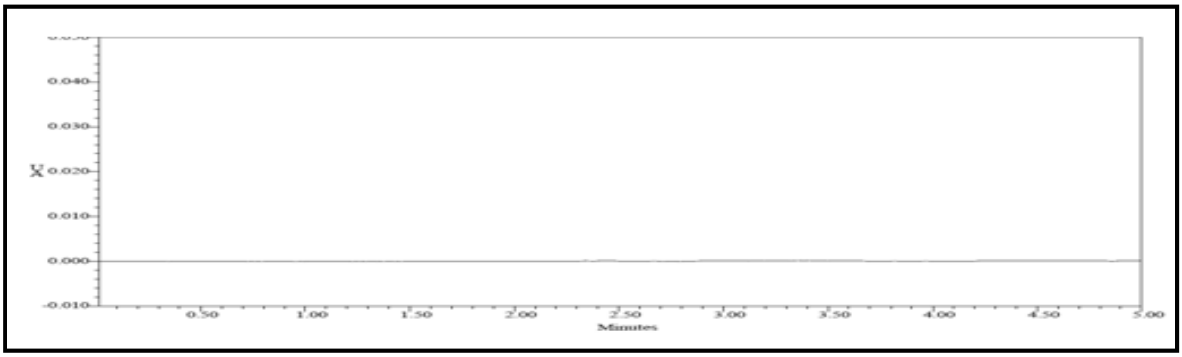


Figure No.3: Chromatogram showing blank (mobile phase preparation)

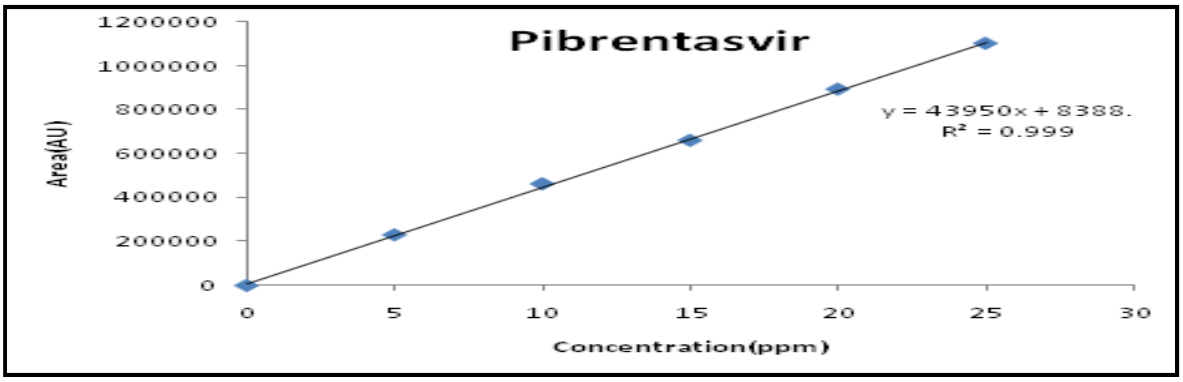


Figure No.4: Chromatogram showing linearity level

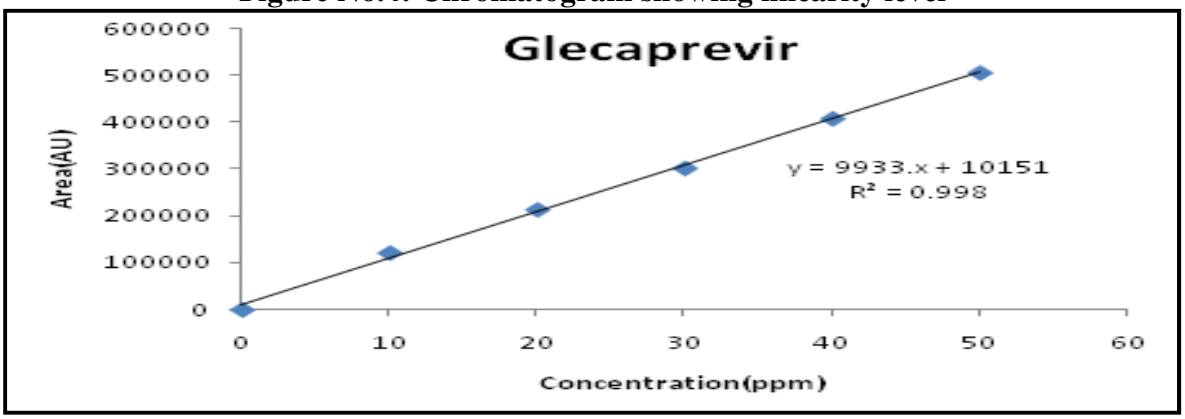


Figure No.5: Chromatogram showing linearity level

CONCLUSION

- In the presentinve stigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Glecaprevir and Pibrentasvir in bulk drug and pharmaceutical dosage forms.
- This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.
- Glecaprevir and Pibrentasvir was freely soluble in ethanol, methanol and sparingly soluble in water.
- Water and Acetonitrile (60:40% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.
- The %RSD values were within 2 and the method was found to beprecise.
- The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precis ecompared to the Spectrophotom etricmethods.
- This method can be used for the routine determination of Glecaprevir and Pibrentasvir in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to Sura Labs, Dilshukhnagar, Hyderabad for providing the necessary facilities for the research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Kealey and Haines P J. Analytical Chemistry, *Bios Publisher,* 1st Edition, 2002, 1-7.

2. Braith Wait A and Smith F J. Chromatographic Methods, *Kluwer Academic Publisher,* 5th Edition, 1996, 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, *Academic press,* 1st Edition, 1997, 24-37.
4. Yuri Kazakevich and Rosario Lobrutto. HPLC for Pharmaceutical Scientists, *Wiley Interscience A John Wiley and Sons, Inc., Publication,* 1st Edition, 2007, 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V R. Practical High-Performance Liquid Chromatography, England, *John Wiley and Sons Ltd*, 4th Edition, 2004, 7-8.
7. Sahajwalla C G. A new drug development, *Marcel Dekker Inc.,* New York, 141, 2004, 421-426.
8. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
9. Detectors used in HPLC (online)URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
10. Detectors (online),URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
11. Detectors (online),URL:http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf
12. Detectors (online),URL:http://www.ncbi.nlm.nih.gov/pubmed/8867705
13. Detectors (online),URL:http://www.chem.agilent.com/Library/applications/59643559.pdf
14. Detectors (online),URL:http://hplc.chem.shu.edu/new/hplcbook/detector
15. Draft I C H Guidelineson Validation of Analytical Procedures Definitions and terminology, *Federal Register, IFPMA, Switzerland,* 60, 1995, 1126.
16. Code Q2B, Validation of Analytical Procedures; Methodology, ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 1996, 1- 8.
17. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
18. Data elementsrequired for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
19. Snyder L R. practical HPLC method development, John Wiley and sons, New York, 2nd Edition, 1997, 180-182.
20. Skoog D A, West D M, Holler F J. Introduction of analytical chemistry, Sounder college of publishing, *Harcourt Brace College Publishers,* 1994, 1-5.

Please cite this article in press as: Vineela M *et al.* Simultaneous estimation of glecaprevir and pibrentasvir in bulk and marketed formulation by using high performance liquid chromatography, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 7(1), 2019, 26-36.