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A NEW RP HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF GLECAPREVIR AND PIBRENTASVIR PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and selective LC method is described for the determination of Glecaprevir and Pibrentasvir in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 55 volumes of mixed phosphate buffer and 45 volumes of acetonitrile with detection of 253nm. Linearity was observed in the range 25-75 µg/ml for Glecaprevir (r^2 =0.998) and 50-150 µg /ml for Pibrentasvir (r^2 =0.997) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

KEY WORDS: Glecaprevir, Pibrentasvir, tablet dosage forms

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INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department . The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.

Glecaprevir is a hepatitis C virus (HCV) nonstructural (NS) protein 3/4A protease inhibitor. It is being developed as a treatment of chronic hepatitis C infection in co-formulation with an HCV NS5A

inhibitor pibrentasvir. Together they demonstrated potent antiviral activity against major HCV genotypes and high barriers to resistance. Pibrentasvir is an antiviral agent, it is approved for use with glecaprevir as the combination drug glecaprevir/pibrentasvir for the treatment of hepatitis C.

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year .very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. Basic criteria for new method development of drug analysis- The drug or drug combination may not be official in any pharmacopoeias. A proper analytical procedure for the drug may not be available in the literature due to patent regulations. Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients. Analytical methods for a drug in combination with other drugs may not be available. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. Analytical method development provides the support to track the quality of the product from batch to batch. Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase. Single dosage forms with combination of drugs are widely used today due to their advantages and their simultaneous estimation of individual component is a challenging task (1-5).

Hence aim is to develop new RP HPLC method for the simultaneous estimation of Glecaprevir and Pibrentasvir pharmaceutical dosage form

MATERIALS AND METHODS

Determination Of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of glecaprevir (6, 7)

10 mg of Glecaprevirwas weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of pibrentasvir

10mg of Pibrentasvirwas weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of mixed standard solution

Weigh accurately 10mg of glecaprevir and 10 mg of pibrentasvir in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.From above stock solution 50 µg/ml of Glecaprevir and 100 µg/mlof pibrentasvir is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of samples for Assay

10 tablets (each tablet contains Mavyret - Glecaprevir – 100mg and Pibrentasvir-40mg)were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of glecaprevir and pibrentasvir (µg/ml) were prepared by dissolving weight equivalent to 10 mg of glecaprevir and pibrentasvir and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of Glecaprevir and 50µg/ml of Pibrentasvir was made by adding 1.5 ml of stock solution to 10 ml of mobile phase

RESULTS AND DISCUSSION

The amount of Glecaprevir and pibrentasvir present in the taken dosage form was found to be 100.04% and100.36% respectively (Fig-1 and Table-1).

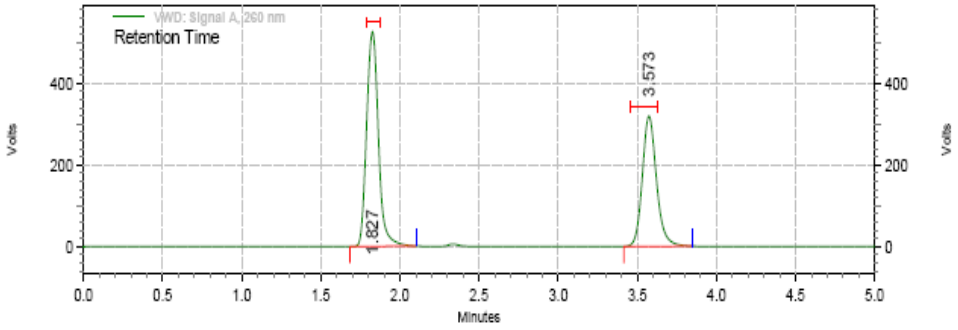

Fig-1 Chromatogram of Assay

Table-1 Assay Results

Glecaprevir			**Pibrentasvir**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	558996	556320	640249	648536
Injection-2	560893	559691	647102	645717
Injection-3	560837	563445	642088	648013
Injection-4	557645	559365	648819	647341
Injection-5	556714	557488	642237	642566
Average Area	559017	559261.8	644099	646434.6
Standard deviatuion	22.75		30.35	
%RSD	0.33		0.56	
Assay(%purity)	100.04		100.36	

It is observed from the above data, diluent or excipient peaks are not interfering with the glecaprevir and pibrentasvir peaks (Fig-2).

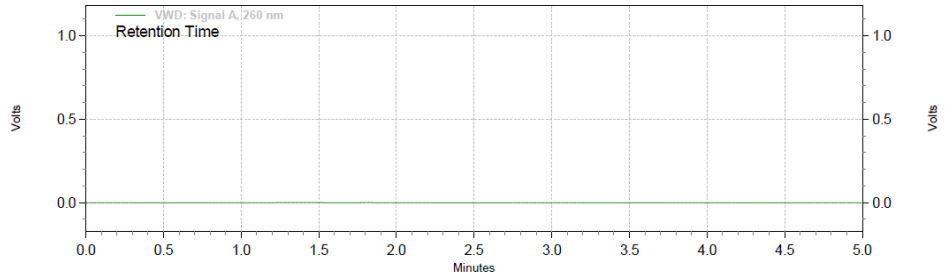

Fig-2 Chromatogram for Specificity of glecaprevir and pibrentasvir standard

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of glecaprevir and pibrentasvir is 0.998 and 0.997. The relationship between the concentration of glecaprevir and

pibrentasvir and area of glecaprevir and pibrentasvir is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Fig-3 and 4).

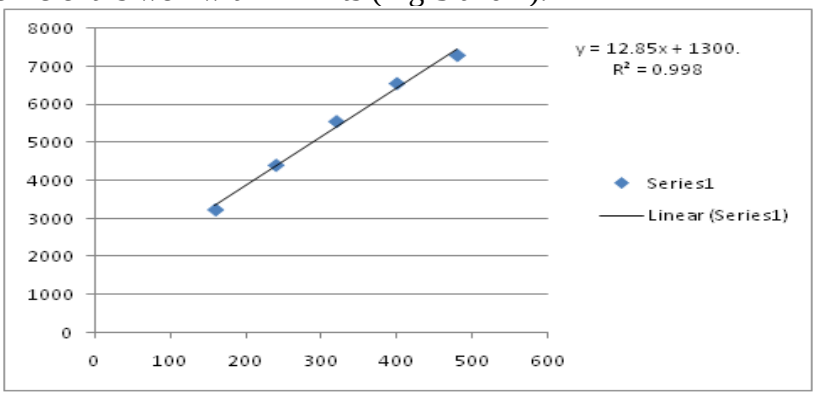

Fig-3 Linearity graph of glecaprevir

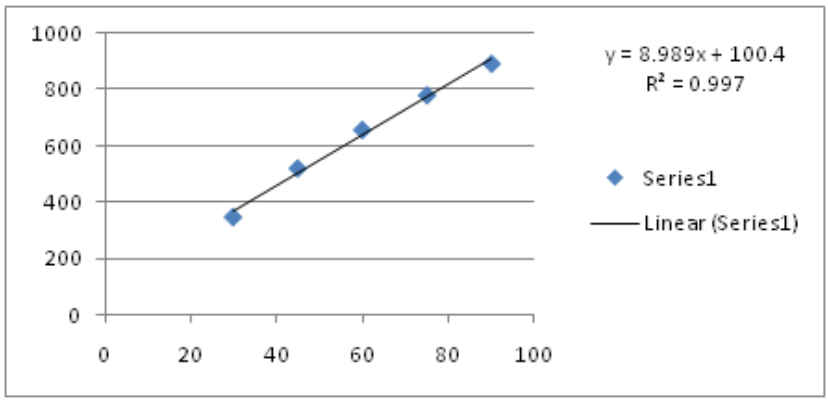

Fig-4 Linearity graph of pibrentasvir

The percentage mean recovery of glecaprevir and pibrentasvir is 101.93% and 100.5% respectively. Test results for glecaprevir and pibrentasvir are showing that the %RSD of Assay results are within limits. The LOD for this method was found to be 2.69µg/ml for glecaprevir and 1.10µg/ml for pibrentasvir. The LOQ for this method was found to be 8.16µg/ml for glecaprevir and 3.33µg/ml for pibrentasvir.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Glecaprevir and Pibrentasvir was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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