Journal of Pharmaceutical and Biological Research

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Analytical Method Development and Vaidation for Ombitasvir and Paritaprevir in Combined Dosage Form by RP-HPLC

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A B S T R A C T

Ombitasvir and Paritaprevir by using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate bufferpH 3 (pH was adjusted with orthophosphoric acid), detection wavelength was 252nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Ombitasvir and Paritaprevir was found to be 101.27% and 99.97% respectively. The system suitability parameters for Ombitasvir and Paritaprevir such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Ombitasvir and Paritaprevir was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively.

Keywords: Ombitasvir and Paritaprevir, WATERS HPLC, ICH guidelines etc.

A R T I C L E I N F O

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Article History: Received 19 September 2015, **Accepted** 10 November 2015, **Available Online** 21 December 2015

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Manuscript ID: JPBR2797

PAPER-QR CODE

Citation: K. Nithiyananthan, *et al. Analytical Method Development and Vaidation for Ombitasvir and Paritaprevir in Combined Dosage Form by RP-HPLC. J. Pharm. Bio. Res.*, 2015, 3(2): 315-320.

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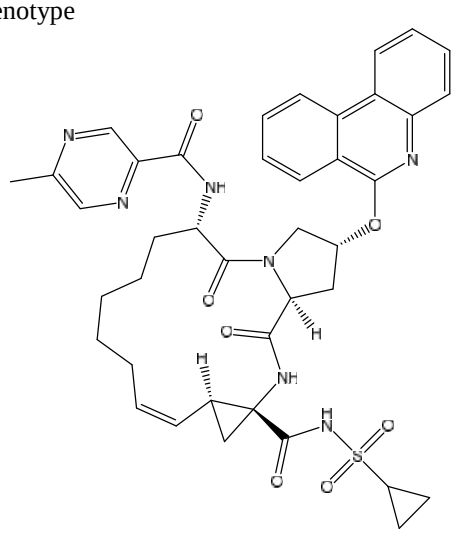
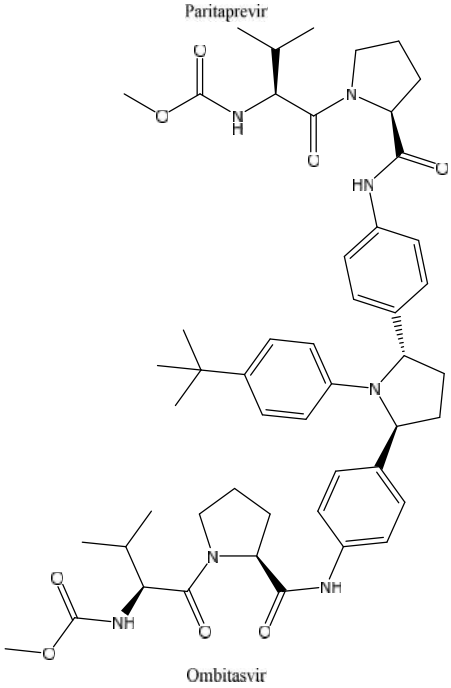
1. Introduction

Ombitasvir is an antiviral drug for the treatment of hepatitis C virus (HCV) infection by Gilead. In the United States, it is approved by the Food and Drug Administration for use in combination with paritaprevir, ritonavir and dasabuvir in the product Viekira Pak for the treatment of HCV genotype 1, and with paritaprevir and ritonavir in the product Technivie for the treatment of HCV genotype Paritaprevir (previously known as ABT-450) is an acylsulfonamide inhibitor of the NS3-4A serine protease manufactured by Abbott Laboratories that shows promising results as a treatment of hepatitis C. When given in combination with ritonavir and ribavirin for 12 weeks, the rate of sustained virologic response at 24 weeks after treatment has been estimated to be 95% for those with hepatitis C virus genotype 1. Resistance to treatment with paritaprevir is uncommon, because it targets the binding site, but has been seen to arise due to mutations at positions Velpatasvir is an NS5A inhibitor (by Gilead) which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotype



Paritaprevir



Ombitasvir

2. Material and methods

Instrument used: HPLC-auto sampler –UV detector, Separation module2695, UV.detector2487, Empower-software version-2, Waters, U.V double beam spectrometer, UV 3000+, U.V win software, Lab India. Digital weighing balance (sensitivity 5mg), pH meter, Sonicator.

Chemicals:

Ombitasvir and Paritaprevir, Ortho phosphoric acid, Acetonitrile, Methanol, Water, KH_2PO_4, K_2HPO_4.

Table 1: Optimized chromatographic conditions

Column	Thermosil C18 (4.0×125 mm) 5.0µm
Mobile phase	Methanol: Sodium acetate buffer (70: 30 % v/v)
Detection wavelength	252 nm
Flow rate	0.7 ml/min
Injection volume	10µl
Column temperature	Ambient
Auto sampler temp	Ambient
Retention time	2.449 & 3.191 mins
Run time	8 min

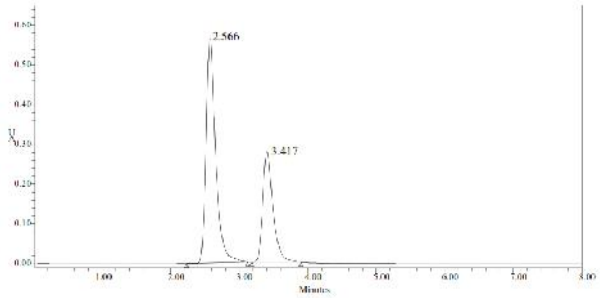


Fig 1: Optimized Chromatogram

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Method Validation

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity studies should cover the range of 0-150% of the expected level of the analyte. The data is then processed using the method of least squares regression. The resulting plot, slope, intercept and correlation coefficient provide the desired information on linearity. ICH recommends that, for the establishment of linearity, a minimum of five concentrations should normally be used

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

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 same homogeneous sample under the prescribed conditions. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

a. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

b. Intermediate precision: Intermediate precision expresses within laboratories variations: different day's different analysts, different equipment, etc.

c. Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies usually applied to standardization of methodology).

ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range of the procedure (i.e., three replicates of three concentrations) or using a minimum of six determinations at 100% of the test concentration.

Detection Limit (LOD)

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. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

Quantification Limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Several approaches for determining the Quantification limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

Range:

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range of the analytical procedure is validated by verifying that the analytical procedure provides acceptable precision, accuracy and linearity when applied to the samples containing analytes at the extremes of the range as well as within the range.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. A good practice is to vary important parameters in the method systematically and measure their effect on separation. The variable method parameters may involve temperature (±50C), buffer pH (±0.5), ionic strength of buffers, level of additives to MP, flow rate (±0.2ml/min), wavelength (±2nm).

Ruggedness:

The precision obtained when the assay is performed by multiple analysis, using multiple instruments, on multiple days, in one laboratory, different sources of reagents and multiple lots of columns should also be included in this study.

System Suitability: It is essential for the assurance of the quality performance of chromatographic system. The accuracy and the precision of HPLC data collected, which begins with a well -behaved chromatographic system.

3. Results and Discussion

The chromatographic method development for the simultaneous estimation of Ombitasvir and Paritaprevir were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Ombitasvir and Paritaprevir in API and pharmaceutical dosage form by RP-HPLC method.

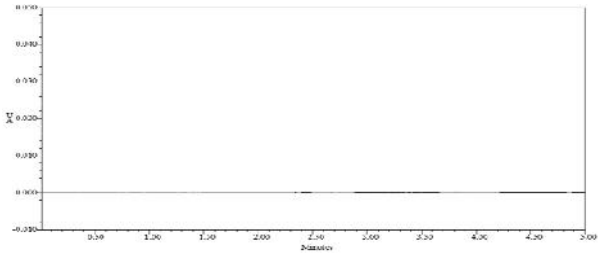


Fig 2: Chromatogram for Blank

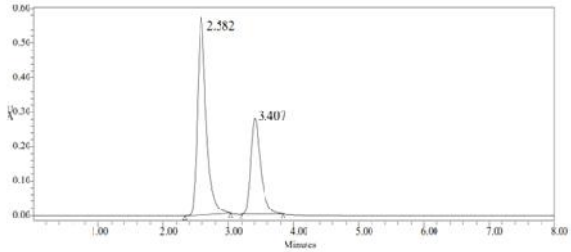


Fig 3: Chromatogram for sample

Assay calculation for Ombitasvir and Paritaprevir:

The assay study was performed for the Ombitasvir and Paritaprevir.

Table 2: Assay results

S.No	Name of compound	Amount taken	%purity
1	Ombitasvir	754.7	99.24
2	Paritaprevir	735.6	101.04

Validation Report

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak.

Linearity:

The linearity study was performed for the concentration of 50 ppm to 250 ppm and 5ppm to 25 ppm level. Each level was injected into chromatographic system.The linearity study was performed for concentration range of 5.µg-25µg

and 50µg-250µg of Ombitasvir and Paritaprevir and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999).

Accuracy:

The accuracy study was performed for 50%, 100% and 150 % for Ombitasvir and Paritaprevir succinate.The accuracy study was performed for % recovery of Ombitasvir and Paritaprevir. The % recovery was found to be 99.56% and 99.47% respectively (NLT 98% and NMT 102%).

Precision:

Repeatability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The precision study was performed for five injections of Ombitasvir and Paritaprevir.The Method precision study was performed for the %RSD of Ombitasvir and Paritaprevir was found to be 0.82 and 0.86 (NMT 2).

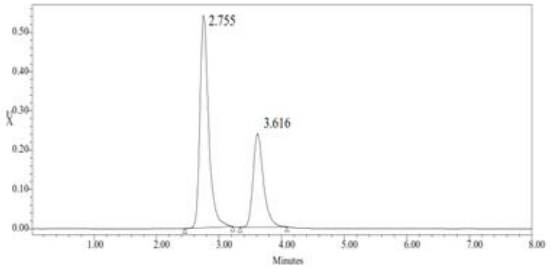


Fig 4: Chromatograms showing precision injections

Intermediate precision/Ruggedness:

The standard solution was injected for five times and measured the area for all five injections in HPLC.The intermediate precision study was performed for five injections of Ombitasvir and Paritaprevir. Each standard injection was injected into chromatographic system.The intermediate precision was performed for %RSD of Ombitasvir and Paritaprevir was found to be 0.19 and 0.44 respectively (NMT 2).

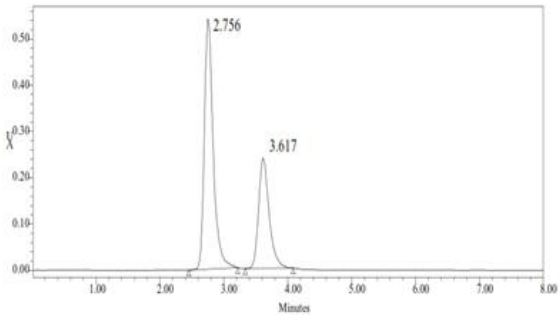


Fig 5: Chromatograms showing intermediate precision

Detection limit: The L.O.D was performed for Ombitasvir and Paritaprevir was found to be 3.17and 0.0172 respectively.

Quantification limit: The L.O.Q was performed for Ombitasvir and Paritaprevir was found to be 5.80 and 0.212 respectively.

Robustness:

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Ombitasvir and Paritaprevir. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly.

Table 3: Linearity Results for Ombitasvir

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	471543
2	II	10 ppm	656277
3	III	15 ppm	794999
4	IV	20 ppm	946124
5	V	25 ppm	1002139
Correlation Coefficient			0.999

Table 4: Linearity Results for Paritaprevir

S.No	Linearity Level	Concentration	Area
1	I	50ppm	56472
2	II	100 ppm	73841
3	III	150ppm	92655
4	IV	200ppm	111541
5	V	250ppm	130567
Correlation Coefficient			0.999

Table 5: Accuracy results for Ombitasvir

%Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	2630409	5	4.96	99.91%	99.56%
100%	5277055	10	9.98	99.18%	
150%	7514836	15	15.02	99.60%	

Table 6: Accuracy results for Paritaprevir

%Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1366666	0.5	0.99	99.53%	99.47%
100%	2777487	1.0	1.05	99.38%	
150%	4151234	1.5	1.495	99.52%	

Table 7: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Ombitasvir	373625.50	581075863	3.17
Paritaprevir	5772.40	476579210	0.0172

Table 8: Showing results for Limit of Quantification

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Ombitasvir	372727.80	574265980	5.80
Paritaprevir	5761.30	478828490	0.212

Table 9: Showing system suitability results for Ombitasvir

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	**1**	**4668**	**1.3**
3	1.2	5216	1.4

Table 10: Showing system suitability results for Paritaprevir

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	**1**	**6089**	**1.2**
3	1.2	6998	1.3

Table 11: Showing system suitability results for Ombitasvir

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	***Actual**	**4668**	**1.3**
3	5 % more	6387	1.4

Table 12: Showing system suitability results for Paritaprevir

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	***Actual**	**6089**	**1.2**
3	5 % more	4817	1.2

4. Conclusion

A recently method was established for simultaneous estimation of Paritaprevir and Ombitasvir by R.P-H.P.L.C method. The chromatographic conditions were success fully developed for the separation of Paritaprevir and Ombitasvir by using Thermosil C_{18}.1 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) metha: Sodiu acetat buffpH 3 (pH was adjusted with orthophoacid), detection wavelength was 252nm. The % purity of Paritaprevir and Ombitasvir was found to be 101.27% and 99.97% respectively.

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