



Research Paper

Development and validation of Ombitasvir In RP- HPLC

A. Raja shekar*, M. Chaithanya¹, D.Bhandhavi¹

* Department of Pharmaceutical analysis, Mother Teresa college of Pharmacy, Ghatkesar, Medchal Telangana-501301

¹ Assistant Professor, Department of Pharmaceutical analysis, Mother Teresa college of Pharmacy, Ghatkesar, Medchal Telangana-501301

ARTICLE INFO

ARTICLE HISTORY

Received 08 September 2023
 Received in revised form 10 September 2023
 Accepted 19 September 2023
 Available online 30 September 2023

Key words:

RP- HPLC, Ombitasvir

ABSTRACT

Ombitasvir (OMB) in mass and measurement structure now have a reliable, specific, rapid, and actual Streams stage - elite execution fluid chromatographic method. At a circulation charge per unit of 1.0 mL/min, a mixture of support arrangement (1 ml of Orthophosphoric corrosive changed into taken in a 1000 ml volumetric flagon and alternate the PH with Weakened NaOH upto three) and Acetonitrile as a transportable degree were used to attain chromatographic approval on Inertsil ODS C-18 insightful phase (4.6250 mm; five m). The discovery of ultraviolet light occurred at 252 nanometers. Ombitasvir had respective storage durations of 2.315 minutes, 2.89 minutes, and 5.483 minutes. Over the fixation range of 6.25-37. five g/mL, 37.5-225 g/mL, and 25-150 g/mL for Ombitasvir, the adjustment plot became direct ($r^2=0.999$). The approach had been permitted almost around ICH rules and carried out to the measurement of Ombitasvir, Paritaprevir and Ritonavir in unadulterated shape and dose shape

Corresponding author :

A. Raja shekar, Department of Pharmaceutical analysis, Mother Teresa college of Pharmacy, Ghatkesar, Medchal Telangana-501301

EMAIL: tejajilla21061999@gmail.com



1. INTRODUCTION

HPLC is able to separate macromolecules and ionic species labile natural products, polymeric materials, and a wide variety of other high – molecular weight poly functional group. HPLC is the fastest growing analytical technique for the analysis of the drugs. It's simplicity, high specificity, and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids¹.

In this the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3-10 μ m. Modern LC uses very small particles for packing. The small particle size results in more rapid approach to the distribution equilibrium and consequently smaller plate height, so that a given length of column includes large number of plates which makes the column efficient and the peak narrow. But close packing of these small particles reduces the flow rate of the mobile phase through the packed bed (the packing said to develop high back pressure) and in order to achieve a reasonable flow rate it is necessary to apply pressure to the mobile phase. So the designation, put forth as high pressure liquid chromatography. Thus HPLC is having advantages of improved

resolution, faster separation, improved accuracy, precision and sensitivity².

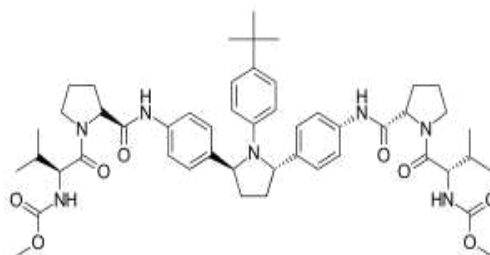


Figure no. 1 Chemical structure of Carvedilol

Ombitasvir is an antiviral drug for the treatment of hepatitis C virus (HCV) infection. In the United States, it is approved by the Food and drug administration for use` in combination with , and Dasabuvir, in the product Viekira Pak for the treatment of HCV genotype 1, and with and in the product Technivie for the treatment of HCV genotype 4³. Ombitasvir is an NS5A inhibitor that acts by inhibiting the HCV protein NS5A .

2. MATERIALS AND METHOD

Instruments

Shimadzu 2600 double beam UV-Visible spectrophotometer, Waters HPLC, software: Empower, 2695 separation module, PDA detector. pH meter Adwa-AD 1020 and Weighing machine Afcoset ER-200A.

Materials

Ortho phosphoric acid, water and methanol for HPLC and acetonitrile. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India. The drugs used in present study were procured from Hetero drugs pvt.Ltd. Hyderabad. Commercially available Technivie tablets claimed to contain 12.5 mg Ombitasvir; 75 mg and 50 mg have been utilized in the present work⁴⁻⁵.

Optimized chromatographic conditions

Instrument used :	Waters HPLC with auto sampler and PDA detector.
Temperature :	Ambient
Column :	Inertsil ODS C18 (4.6 x 250mm, 5 μ m)
Buffer :	0.1% of Orthophosphoric acid
pH :	3.0
Mobile phase :	40% buffer 60% Acetonitrile
Flow rate :	1 ml per min
Wavelength :	252 nm
Injection volume :	10 μ l
Run time :	10 min.

Method development

Mobile Phase Optimization:

Initially the mobile phase tried was methanol:OPA buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to 0.1 % OPA buffer with (pH 3.0) Acetonitrile in proportion 40: 60 v/v respectively.

Wave length selection:

UV spectrum of 100 μ g/ml Ombitasvir, and in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 252nm. At this wavelength all these three drugs show good absorbance⁶.

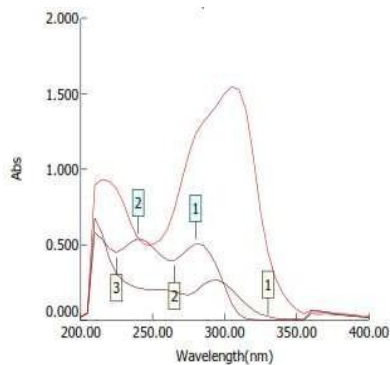


Figure no. 2 UV Spectrum of Ombitasvir

Optimization of Column

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and thermosil column. Inertsil ODS C 18 (4.6 x 250mm, 5 μ m) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Preparation of buffer and mobile phase

Preparation of 0.1 % OPA buffer

1 ml of Orthophosphoric acid was taken in a 1000ml volumetric flask and adjust the pH with Diluted NaOH upto 3, finally the solution was filtered by using 0.45 Micron membrane filter, sonicated it for 10 mins.

Preparation of mobile phase

Accurately measured 400 ml (40%) of above buffer and 600 ml of Acetonitrile HPLC (60%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration. The Mobile phase was used as the diluent⁷⁻⁸.

Preparation of Standard stock Solution

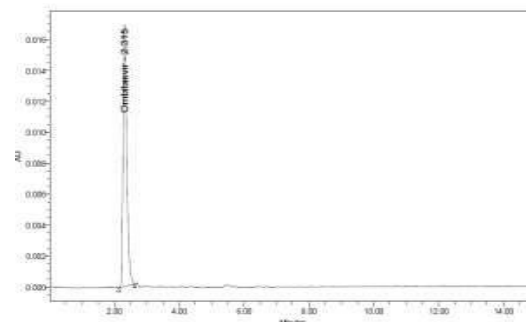
Accurately weighed and transferred 25mg of Ombitasvir, 150mg of and 100mg of working standard into a 100 ml clean dry volumetric flask added about 70 mL of Diluent and sonicated to dissolve it completely and diluted up to the mark with the same solvent.

Further pipette out 1 ml of the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent. (25ppm of OMB, 150ppm of PAR and 100ppm of RIT).

Sample Solution Preparation

Accurately weighed 10 tablets crush in mortar and pestle and transferred equivalent to 25mg of Ombitasvir, 150mg of and 100mg of sample into a 100mL clean dry volumetric flask added about 70 mL of Diluent and sonicate it up to 30 mins to dissolve it completely and diluted up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter⁹.

Further pipette 1 ml of Ombitasvir, and from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. (25ppm of OMB, 150ppm of PAR and 100ppm of RIT).



Inject 10 μ l of the standard, sample into the chromatographic system and measured the areas for Ombitasvir, and peaks and calculated the %Assay by using the formulae.

Figure no. 3 Chromatogram of Ombitasvir

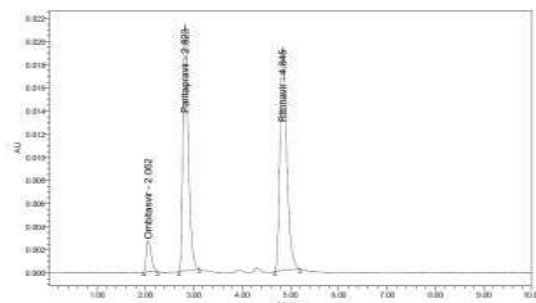


Figure no. 4 Chromatogram of Ombitasvir

3.RESULT AND DISCUSSION

System suitability

To ensure that the analytical method is working satisfactory and capable to give accurate and precise results, system suitability test has been

Injection	Ombitasvir
Injection-1	16747
Injection-2	16557
Injection-3	16734
Injection-4	16707
Injection-5	16601
Injection-6	16963
Average	16718
Standard Deviation	141.9
%RSD	0.8

Table no. 1 The results are summarized for Ombitasvir

% Conce	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	9677	12.5	12.32	98.52	99.24
100%	19605	25	24.95	99.80	
150%	29287	37.5	37.27	99.39	

Table no.2 Accuracy results for Ombitasvir

S. No	Linearity Level	Conce (µg/ml)	Area
1	I	6.25	5622
2	II	12.5	9950
3	III	18.75	14690
4	IV	25	19801
5	V	31.25	24369
6	VI	37.5	29177
Slope			759.51
Intercept			653.87
Correlation Coefficient			0.9996

Table no.3 Linearity Results for Ombitasvir

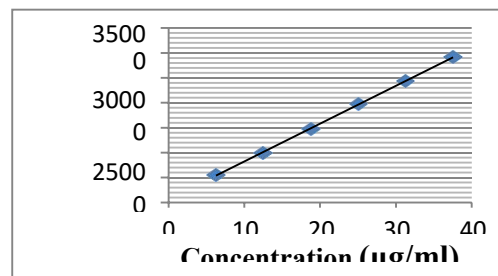


Figure no. 5 Calibration curve of Ombitasvir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	1936.19	1.52
2	1.0	2200.68	1.91
3	1.2	1809.91	1.45

Table no.4 System suitability results for Ombitasvir

	Area	% Degraded
Standard	18441	
Acid	17423	5.52
Base	16230	11.99
Thermal	17632	4.39
Photo	16581	10.09

Table no.5 Degradation results for Ombitasvir

4.CONCLUSION

The developed RP-HPLC method is simple, specific, accurate and precise for the simultaneous determination of Ombitasvir, and from pharmaceutical formulations and dosage form¹⁰⁻¹¹. The developed method provides good resolution between, OMB. It was successfully validated in terms of system suitability, linearity, range, precision, accuracy, specificity, LOD, LOQ and robustness in accordance with ICH guidelines. Thus, the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination¹²⁻¹³.

5.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

We are highly grateful to our Honorable Chairman, Mother Terasa college of Pharmacy, Telangana state for providing the necessary facilities to carry out this research work.

7. REFERENCE

- Vanderhoff BT, Ruppel HM, Amsterdam PB. Carvedilol: The new role of beta blockers in congestive heart failure. Am FAM Physician, 1998; 58(7):1627-34.

2. Kunchanur MG, Kinhal R, Patil S, development and validation of high performance liquid chromatographic method for estimation of Temozolomide in pure and pharmaceutical dosage form. *Journal of global trends in pharmaceutical sciences* 2020;11(3):8057-64.
3. Sethi PD. High performance liquid chromatographyQuantitative analysis of pharmaceutical formulations. CBS Publishers and Distributors, New Delhi 2001; 1(3-11):116-20.
4. Bagaglio S, Uberti-Foppa C, Morsica G. Resistance Mechanisms in Hepatitis C Virus: implications for Direct-Acting Antiviral Use. *Drugs*. 2017; 12:124-128.
5. Viekira Pak (Ombitasvir, Paritaprevir And Ritonavir Tablets; Dasabuvir Tablets), For Oral Use. Full Prescribing Information" (PDF). AbbVie Inc., North Chicago, IL 60064. Retrieved 30 July 2015.
6. Kowdley KV, Lawitz E, Poordad F, Cohen DE, Nelson DR, Zeuzem S, Everson GT, Kwo P, Foster GR, Sulkowski MS, Xie W, Pilot-Matias T, Lioussis G, Larsen L, Khatri A, Podsadecki T, Bernstein B. Phase 2b trial of interferon-free therapy for hepatitis C virus genotype 1. *N Engl J Med*. 2014 Jan 16;370(3):222-32
7. Mauss, et al., eds. (2013). *Hepatology 2013 a clinical textbook* (PDF) (4th ed.). Düsseldorf: Flying Publisher. ISBN 978-3-924774-90-5.
8. Papaseit E, Vázquez A, Pérez-Mañá C, Pujadas M, De La Torre R, Farré M, Nolla J. Surviving life-threatening MDMA (3,4-methylenedioxymethamphetamine, ecstasy) toxicity caused by ritonavir (RTV). *Intensive Care Medicine*. 2012; 38 (7):1239–1240.
9. Mensing S, Eckert D, Sharma S, Polepally AR, Khatri A, Podsadecki TJ, Awni WM, Menon RM, Dutta S. Population pharmacokinetics of paritaprevir, ombitasvir, dasabuvir, ritonavir and ribavirin in hepatitis C virus genotype 1 infection: analysis of six phase III trials. *British J Clin Pharmacol*. 2017; 83: 527–539.
10. Ocque AJ, E Hagler CE, Difrancesco R, Woolwine-Cunningham Y, Bednasz CJ, Morse GD, Talal AH. Development and validation of a UPLC–MS/MS method for the simultaneous determination of paritaprevir and ritonavir in rat liver. *Bioanalysis*. 2016; 8(13):1353-63.
11. Baje SI, Jyothi B, Madhavi N. RP-HPLC Method for simultaneous estimation of ritonavir, ombitasvir and paritaprevir in tablet dosage forms and their stress degradation studies. *Int J App Pharm*. 2019; 11(2):193-210.
12. Wadie MA, Mostafa SM, El SM, Elgawish MS. Development and validation of a new, simple-Hplc method for simultaneous determination of ombitasvir, paritaprevir, ritonavir and ribavirin in tablet dosage form. *Iosr journal of pharmacy and biological sciences*. 2017; 12(6): 28-35.
13. Jahnavi B, Ganapaty S. Stability indicating RP-HPLC method development and validation for the simultaneous determination of ombitasvir, paritaprevir and ritonavir in tablet dosage forms. *Asian Journal of Pharmaceutical Education and Research*. 2018;7(1):90-101.