International Journal of Engineering Science Invention (IJESI)
ISSN (Online): 2319 – 6734, ISSN (Print): 2319 – 6726
www.ijesi.org || PP. 36-41

Development And Validation Of Rp-Hplc Method For Determination Of Velpatasvir In Bulk

G. Devi Sirisha[1], B. Jansi Rani[2]

[1]*Department of Pharmaceutical Analysis, Sir C.R.Reddy College Of Pharmaceutical Sciences, Eluru-534007, A.P., India.*

[2]*Department of Pharmaceutical Analysis, Sir C.R.Reddy College Of Pharmaceutical Sciences, Eluru-534007, A.P., India.*

Abstract: *Velpatasvir is an antiviral drug used for the treatment of Hepatitis-C.A rapid and sensitive stability indicating novel reversed phase-HPLC method with UV detection at 296 nm was developed for routine analysis of Velpatasvir in bulk form.The method was optimized selecting chromatographic conditions of 65 : 35 acetonitrile : water using Zodiac column (C_{18} 250 mm × 4.6 mm 5 µm), 20 µL injection volume, flow rate of 1 mL/min at ambient temperature (30°C), and 296 nm. The retention time of velpatasvir was found to be 5.06min. Another column of C_{18}Spolar (4.6 × 250 mm, 5 µm) was tested showing no big difference in the method results. The calibration curve was linear in the concentration range of10–50µg/ ml. The method was validated giving good precision (RSD% < 1), acceptable linearity (≥ 0.999), and low LOD and LOQ (0.76 and 2.3 µg/mL, resp.) on both columns. This method is simple, specific, linear, and rugged. Hence it can be applied for routine analysis. Forced degradation studies were also conducted by adopting proposed method to access the stability of analytes under acid, base, peroxide and photolytic condition and the method is suitable to resolve the degradation products*

Keywords: *Forced degradation,Method validation ,RP-HPLC,Velpatasvir.*

I. Introduction:

Molecular formula: $C_{49}H_{54}N_8O_8$ Molecular weight: 529.45g/mol. Solubility: soluble in acetonitrile, methanol, water. Velpatasvir (figure:1)is chemically Methyl{(2S)-1-|(2S,5S)-2-(9-{2-|(2S,4S)-1-{(2R)|(methoxycarbonyl)amino|-2-phenylaacetyl}-4(methoxymethyl)-2-pyrrolidinyl|-1H-imidazol-4-yl}-1,11-dihydroisochromeno|4',3',6',/|naphthol|1,2-d|imidazol-2-yl)-5-methyl-1-pyrrolidinyl|-3-methyl-1-oxo-2-butanyl}carbamate. It is white to tan or yellow hygroscopic solid with pka values of 3.72 and 5.98. velpatasvir is an NS5A inhibitor which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP method with UV detection for the determination of Velpatasvir in bulk form. The important features and novelty of the proposed method included simple sample treatment with sonicator of small amount of powder sample at ambient temperature, short elution time(less than 7min), good precision (R.S.D. less than 2%).

II. Materials And Method

Material: Velpatasvir was obatained from hetero pharmaceuticals. Solvents: Acetonitrile (Hplc grade), Water (Hplc grade) Preparation of mobile phase: Mix a mixture of above buffer solvents acetonitrile and water in 65:35 ratio and degas in ultrasonic water bath for 3minutes.Filter through 0.45µ filter under vacuum filtration. Preparation of standard solution:Stock solution of VPS (1mg/mL) was prepared by weighing 10mg and dissolving in 10ml mobile phase. Standard solutions of VPS were prepared in the range of 10 µg/mL to 50 µg/mL by diluting the stock solution with mobile phase. Selection of Analytical Wavelength

For HPLC method, analytical wavelength was determined from the absorption spectra of velpatasvir obtained by using UV-Visible spectrophotometer. From the solution of velpatasvir was scanned in the range of 200 – 400 nm. Wavelength of maximum absorption was determined for drug. Velpatasvir showed maximum absorbance at 261 nm. Fig.2

Instrument and chromatographic conditions: Schimadzu HPLC system with Discovery C18 (250mm x 4.6mm, 5µ) column, manual injector and PDA detection mode running on LC solutions software was used.

An isocratic mode with acetonitrile and water in 65:35 as mobile phase at 1.0ml/min flow rate was used for separation of drugs. The detection of drugs was done at 296nm with column oven temperature maintained at 30ºC. The other instruments used were pH meter (EI), Digital Balance (Infra Instruments).

III. Method Validation

The developed method was validated for precision, specificity, accuracy (recovery), linearity and robustness as per the ICH guidelines

3.1. System suitability: The typical values for evaluating system suitability of a chromatographic procedure are relative standard deviation (RSD) <2%, tailing factor <1.5, and theoretical plates >1500. The retention time, peak area, theoretical plates, and tailing factor were evaluated for the system. The results were presented in table-2.

3.2. Linearity: Linearity was studied by analyzing five standard solutions covering the range of 10–50 µg/ml of velpatasvir. From the primary stock solution 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml of velpatasvir. A calibration curve (figure-5) with concentration versus peak areas was plotted by injecting the above concentrations. Linearity values were given in table-2

3.3.Accuracy : The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added. The accuracy is calculated from the test results as the triplicates sample preparation.

Procedure : The triplicates of stock solution equivalent to 10 µg/ml,30 µg/ml, and 50 µg/ml were prepared by using standard stock solution. Each preparation was injected into the HPLC system. Accuracy values were shown in table-3.

3.4. Precision: The precision of the method was checked by repeated preparations. The measurement of peak areas of repeated solutions (n=6) for 10 µg/ml sample.precision values were given in table-4

3.5. Specificity: The specificity of the method was determined by injecting the placebo solution and comparing with standard solution for the interference with Velpatasvir peak.

3.4. Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ are determined by standard deviation (SD) and slope of the calibration curve. The limiting values are calculated as per the following equations: LOD = (3.3 × SD)/ Slope and LOQ = (10 × SD)/ Slope

3.5. 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. E.g. flow rate, concentration, run time etc.

IV. Figures And Tables

4.1.Chemical structure of velpatasvir

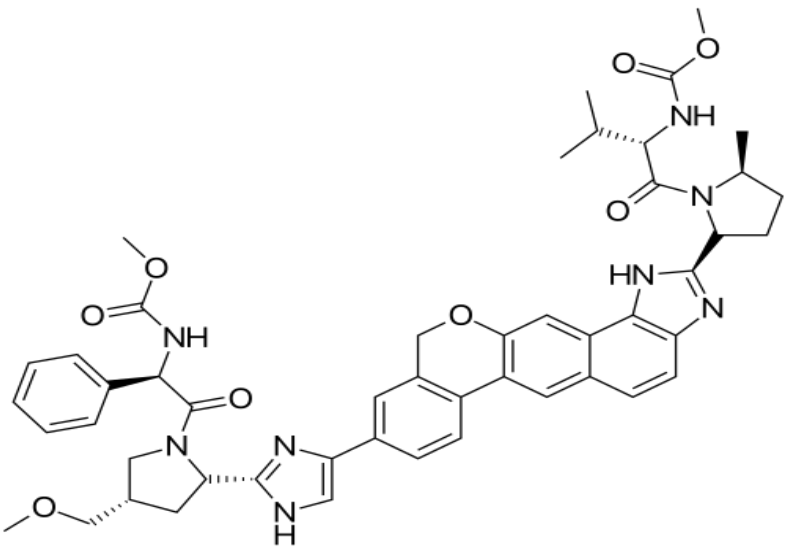

Figure-1: chemical structure of velpatasvir

4.2.Selection of wavelength

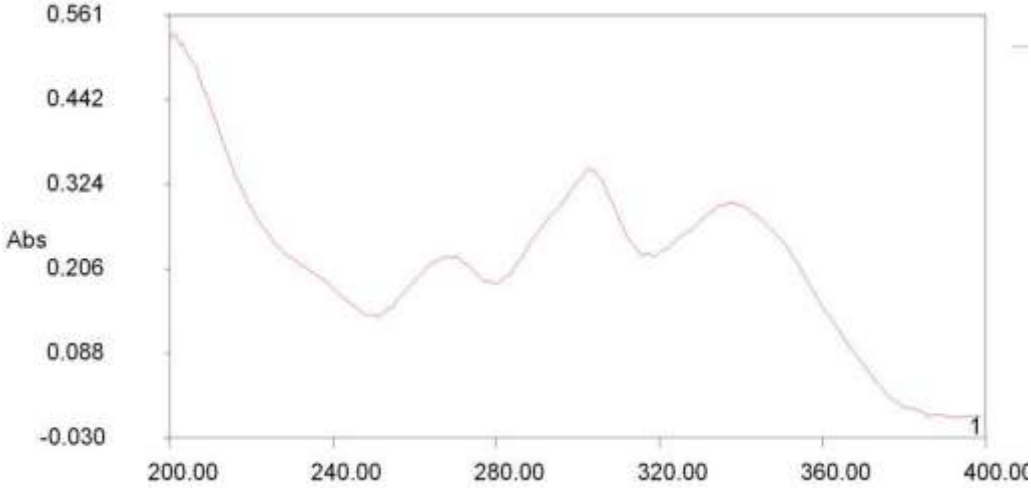

Figure-2:uv spectra of velpatasvir for detection wavelength (□max) = 296 nm

4.3. Chromatograms

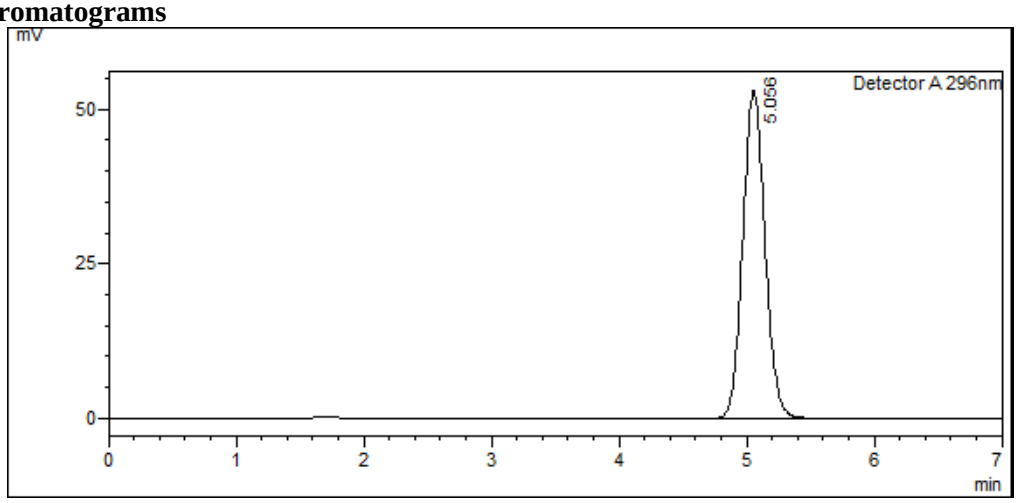

Figure.3: standard chromatogram of velpatasvir

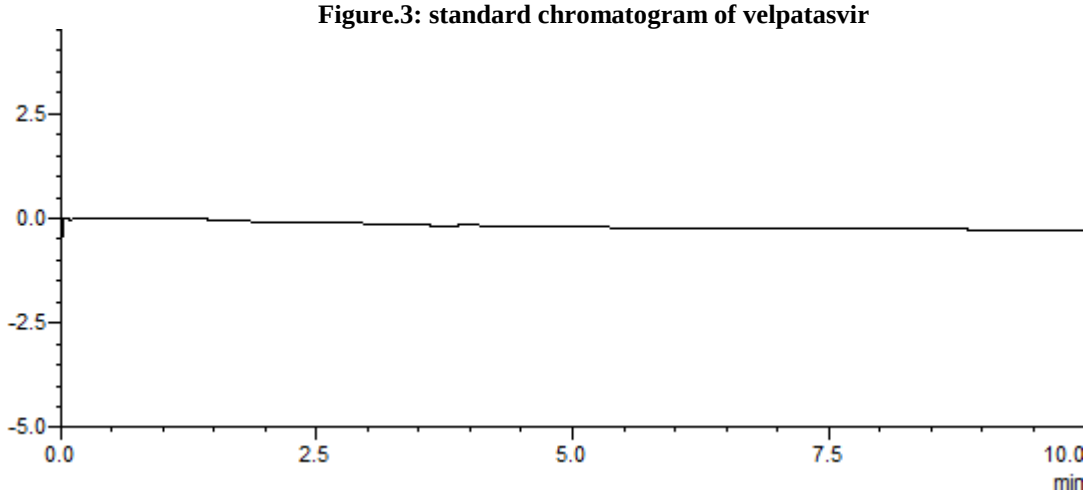

Figure.4: blank chromatogram

Tables:

Table 1. Optimized method parameters

Column C_{18}	Spolar C18 (4.6×150mm) 5µ
Mobile Phase	Acetonitrile:Water(65:35)
Flow Rate	1.0 mL /min.

Run Time	6 min.
Column Temp.	Ambient
Volume Of Injection Loop	20µL
Detection Wave Length	296 nm
Linearity Range	10-50 µg/mL

Table 2.Results of system suitability forvelpatasvir

S.No	Peak Name	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	**velpatasvir**	5.0	2947505	7462	1.1
2	**velpatasvir**	5.0	2958475	7462	1.1
3	**velpatasvir**	5.0	2965847	6472	1.1
4	**velpatasvir**	5.0	2952642	7183	1.1
5	**velpatasvir**	5.0	2951645	7428	1.1
Mean			2955223		
Std. Dev.			7114.704		
% RSD			0.24075		

Table 3. Calibration data of velpatasvir

CONC (µg/ml)	AMOUNT FOUND (µg/ml)	AMOUNT ADDED (µg/ml)	% RECOVERY	MEAN
10	10.07	10	100.07	100.05
10	10.05	10	100.52	
10	9.95	10	99.56	
20	19.99	20	99.399	100
20	20.00	20	100.02	
20	19.99	20	99.99	
30	30.00	30	100.00	99.98
30	29.99	30	99.97	

Table 4. Accuracy of velpatasvir

Concentration µg/ml	**Average Peak Area**
10	172340
20	321543
30	483214
40	641896
50	793876
Slope (m)	15684.47
Intercept (C)	16821
r^2	0.999

30	29.99	30	99.99	

Table 5. Precision studies ofvelpatasvir

S. No	Peak name	Area (µV*sec)	USP Plate Count	USP Tailing
1	**velpatasvir**	292070	7583	1.1
2	**velpatasvir**	290052	7593	1.1
3	**velpatasvir**	295785	8674	1.1
4	**velpatasvir**	300121	7958	1.1
5	**velpatasvir**	292576	9745	1.1
Mean		29463.7		
Std.dev		3617.9		
%RSD		1.2		

Table 6. LOD and LOQ ofvelpatasvir

LOD	LOQ
0.76	2.30

Table 7.Robustness ofvelpatasvir

S.No	Flow rate(ml/min)	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	1.2	3.6	2977505	7462	1.1
2	1	5.0	2958475	7462	1.1
3	0.8	6.2	2865847	6472	1.1

V. Result And Descussion

In this paper we developing the reverse phased column procedure for a suitable method for the pharmaceutical analysis of Velpatasvir drug. Atypical Chromatogram obtained by using the mobile phase (Figure No 3). The precision and Accuracy of the method was determined.The method was validated for linearity, precision and accuracy parameters [9]. Linearity of the method was studied by injecting six concentrations of drug prepared in the mobile phase in the range 10-500 µg/mL and solutions are analyzed through the high pressure liquid chromatographic technique (Figure No. 3). The peak area were plotted against concentration was subjected to linear plot and the results present in table (Table no.3). Precision of this method was studied in inter day and intraday variation [12]. The precision of intraday studies was repeated on two consecutive days. The developed method was found to be precise as the percentage of RSD values for inter-day and intra –day precision studies were found to be less than 2%.

VI. Conclusion

A simple, rapid, accurate and precise RP-HPLC analytical method has been developed for the determination of Velpatasvir in active pharmaceutical ingredient. The method was validated in accordance with ICH guidelines. First, the method goals are clarified based on process understanding. Here a better understanding of the factors influencing chromatographic separation and greater confidence in the ability of the methods to meet their intended purposes is done. Specificity of the method was determined by analyzing samples containing a mixture of the drug product and excipients. It givessymmetric peak shape, good resolution and reasonable retention for it. The method was validated accordance to ICH guidelines. All the validated parameters were found within acceptance criteria. The validated method is specific, linear, precise, accurate, robust for determination. RP-HPLC method resulted in more robust methods which can produce consistent, reliable, and quality data throughout the process and also save time and money.

References

[1] www.drugbank.ca
[2] United States Food and Drug Administration. Guidance for Industry: Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls Documentation. Rockville, MD: Draft Guidance USFDA; 2001
[3] Spina, A., Eramova, I. and Lazarus, J.V. Policy Responses to Viral Hepatitis B and C
[4] among People Who Inject Drugs in Member States of the WHO European Region: A
[5] Sub-Analysis of the WHO 2013 Global Hepatitis Policy Survey. BMC Infectious
[6] Diseases, 2014; 14(1).http://dx.doi.org/10.1186/1471-2334-14-S6-S15
[7] ICH Q2R1 Guideline, Validation of Analytical Procedures: Text and Methodology,
[8] Current Step 4 Version Parent Guideline dated 27 October 1994 (Complementary
[9] Guideline on Methodology dated 6 November 1996 incorporated in, November 2005;
[10] ICH Q2B guideline, Validation of Analytical Procedures: Methodology, Guidance for
[11] Industry, 1-10.