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A SYSTEMATIC ANALYTICAL QBD APPROACH FOR ESTIMATION OF VELPATASVIR PHARMACEUTICAL FORMULATION USING UPLC METHOD

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ABSTRACT

The aim of the present study was to develop simple, accurate, rapid and precise method to evaluate Velpatasvir using Ultra Performance Liquid Chromatography (UPLC) employing QbD in various residual solvents. A new analytical method was developed for the estimation of Velpatasvir using UPLC. The instrument used was WATERS UPLC Auto Sampler, Separation module Acquity, photo diode array detector 2996, Empower-software version-2. The chromatographic separation was done using BEH C18 Column (2.7×50mm), flow rate was 0.3 ml/min, and mobile phase ratio was (70:30 v/v) acetonitrile: 0.1 % OPA buffer pH 3 (pH was adjusted with sodium hydroxide), detection wavelength was 281 nm. The developed method was validated as per the ICH analytical method validation guidelines. All the validation parameters were within the acceptable range. The optimization method was done and chromatograms showed good resolution, the retention time was 0.516 and the % purity of Velpatasvir was found to be 99.82% respectively. It was concluded that the proposed new UPLC method developed for the quantitative determination of Velpatasvir by using QbD approach using Design Expert@ software and the results showed that the developed method was found to be suitable for the routine analysis of Velpatasvir in bulk and in its tablet dosage form. It was concluded that the developed method was accurate, precise, linear, reproducible, robust and sensitive.

Keywords: Chromatography, Design, ICH, Velpatasvir, Validation.

1. INTRODUCTION

Velpatasvir is chemically, (2S)-2-{[hydroxy(methoxy) methylidene] amino}-1-[(2S,5S)-2-(17-{2-[(2S,4S)-1-[(2R)-2-{[hydroxy (methoxy) methylidene] amino}-2-phenylacetyl]-4 (methoxymethyl) pyrrolidin-2-yl]-1H-imidazol-5-yl}-21-oxa-5,7 diazapentacyclo [11.8.0.0^{3,11}.0^{4,8}.0^{14,19}] henicosa-1(13),2,4(8),6,9,11,14(19),15,17-nonaen-6-yl)-5-methyl pyrrolidin-1-yl]-3-methylbutan-1-one. The chemical formula is $C_{49}H_{54}N_8O_8$. The molecular formula is 883.019 g/mol. Velpatasvir is a Direct-Acting Antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). Velpatasvir is likely similar to other selective NS5A inhibitors (Non-structural Protein 5A) which bind domain I of NS5A consisting of amino acids 33-202. Inhibition of NS5A is also known to produce redistribution of the protein to lipid droplets. Figure1 shows the chemical structure of the drug. Different analytical methods have been reported in the literature for the estimation of Velpatasvir[1,2]. However, there are no

analytical method has been reported for its estimation by using Ultra Performance Liquid Chromatography (UPLC) method by using the below mentioned methodology and validated as per ICH guidelines[3,4]. The present study was to establish a simple, sensitive, low cost UPLC method for the estimation of Velpatasvir in bulk as well as in tablet dosage forms. The developed method was validated as per ICH guidelines[5,6].

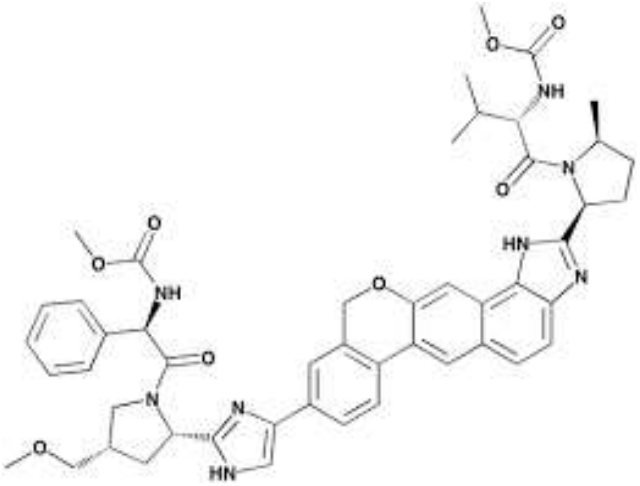


Figure 1: Chemical structure of Velpatasvir

2. MATERIALS AND METHOD

2.1. Chemicals and Reagents

The following chemicals such as pure form of Velpatasvir obtained from Natco pharma (Hyderabad) AR Methanol, Sodium hydroxide and Phosphate buffer (pH: 3.0) were obtained from Standard solutions, India. UPLC grade water used throughout analysis was obtained from Merck milli-Q purification unit.

2.2. Optimized Chromatographic Parameters:

Equipment	:	Ultra performance liquid chromatography equipped with Auto Sampler and PDA detector (Waters Acquity Model)
Column	:	Waters BEH C18 (50*2.6mm, 1.7µm)
Flow rate	:	0.3 mL per min
Wavelength	:	270 nm
Injection volume	:	4 µl

Column temperature: Ambient

Run time : 3 min

2.3. Wave length selection:

UV spectrum of 10 µg/ml Velpatasvir in methanol was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 270 nm. At this wavelength both the drugs show good absorbance.

2.4. Preparation of Phosphate buffer pH 3:

To prepare phosphate buffer solution, by adding 6.8gm of phosphate buffer in a 1000ml water. Adjust this solution to pH 3 by using sodium hydroxide.

2.5. Preparation of mobile phase:

Mix a mixture of phosphate buffer 100 ml (10%) and 900 ml Methanol UPLC (90%) and degas in ultrasonic water bath for 5 minutes. Filter through 4.5 µ filter under vacuum filtration.

2.6. Diluents Preparation:

Phosphate buffer: Methanol (10:90) ratio

2.7. Preparation of the Velpatasvir Standard & Sample Solution:

2.7.1. Standard Solution Preparation:

Accurately weigh and transfer 25 mg of Velpatasvir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.8 ml of Velpatasvir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

2.7.2. Sample Solution Preparation:

Accurately weigh and transfer equivalent to 25 mg of Velpatasvir sample is taken into a 25ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)**.** Further pipette 0.8 ml of Velpatasvir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

3. METHOD DEVELOPMENT[7,8]:

Method development was done changing ratios of mobile phase, column and other chromatography Parameter and Condition initially five trials were conducted.

Inject 5 µL of the standard, sample into the chromatographic system and measure the areas for the Velpatasvir peaks and calculate the % Assay by using the formula.

3.1. System Suitability:

Tailing factor for the peaks due to Velpatasvir in Standard solution should not be more than 2.0

Theoretical plates for the Velpatasvir peaks in Standard solution should not be less than 2000

Assay calculation for Velpatasvir:

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label Claim mg/ml.

System Suitability Results for velpatasvir:

1) Tailing factor Obtained from the standard injection was **1.65**

2) Theoretical Plates Obtained from the standard injection was **3477.23**

Assay Results for Velpatasvir:

$$\frac{554669}{545755} * \frac{25}{25} * \frac{0.8}{10} * \frac{25}{41.25} * \frac{10}{0.8} * \frac{330}{200} * \frac{99.8}{100} * 100 = \mathbf{101.43}\%$$

Chromatograms

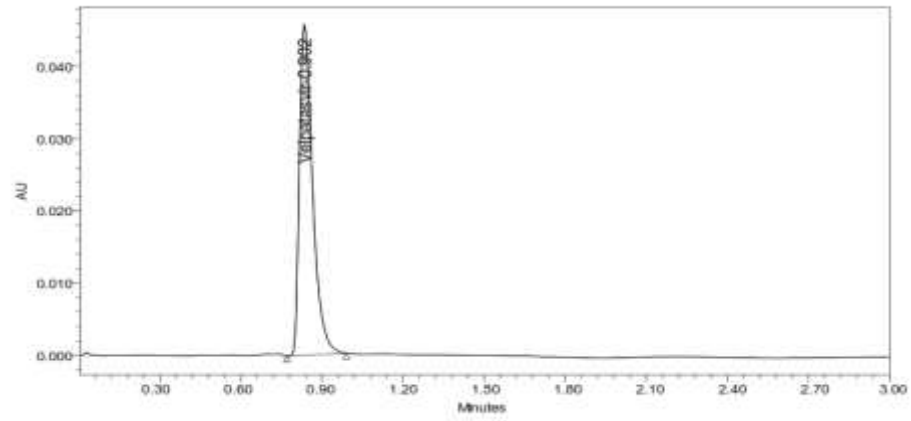


Figure 2: chromatogram of standard (Velpatasvir)

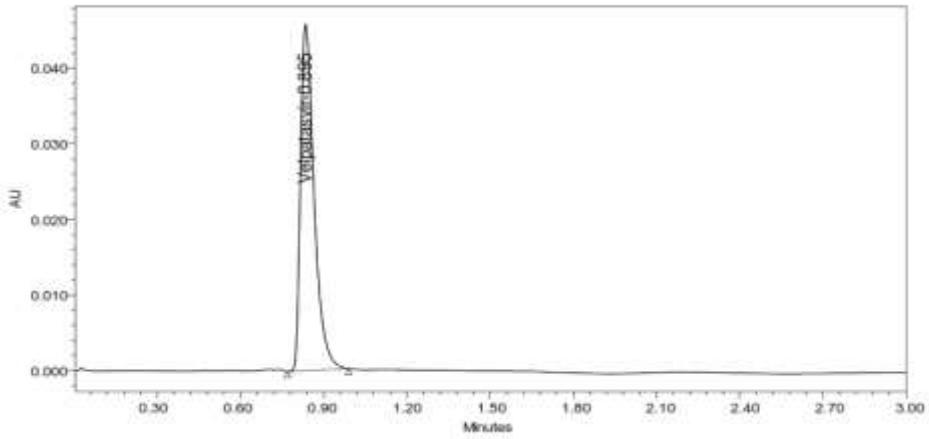


Figure 3: chromatogram of sample (Velpatasvir)

4. Validation Development

Validation of the analytical method was carried out in accordance with the ICH guidelines. The parameters assessed are accuracy, precision, specificity and linearity.

4.1. Precision

It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The results are summarized in table 1.

Table 1: Velpatasvir Precision and ID Precision

Injection	**Area for Precision**	**Area for ID Precision**
Injection-1	570667	606273
Injection-2	571067	601993
Injection-3	563139	608740
Injection-4	573794	599512
Injection-5	561645	591747
Injection-6	568453	598239
Average	568127.5	601084.0
Standard Deviation	4779.4	6068.1
%RSD	0.8	1.0

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

4.2. Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The results are summarized in table no. 1.

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%

4.3. 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 value found. The standard solution with Accuracy -50%, Accuracy -100% and Accuracy

-150% were injected into chromatographic system and calculated the amount found and amount added for Velpatasvir and further calculated the individual recovery and mean recovery values. The results were represented in table no. 2.

Table 2: Accuracy results for Velpatasvir

Concentration (at specification Level)	**Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	273183	12.5	12.49	99.91	103.38
100%	549201	25	25.11	100.43	
150%	826898	37.5	37.80	100.81	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%

4.4. Linearity

It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line. It is determined by series of three to six injections of six of more standards. Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The calibration curve was represented in fig. no. 4. (Table no. 3).

Table 3: Linearity Results for Velpatasvir

S. No	**Linearity Level**	**Concentration**	**Area**
1	I	20	135891
2	II	40	273919

3	III	60	405018
4	IV	80	541386
5	V	100	660956
6	VI	120	783352
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.999.

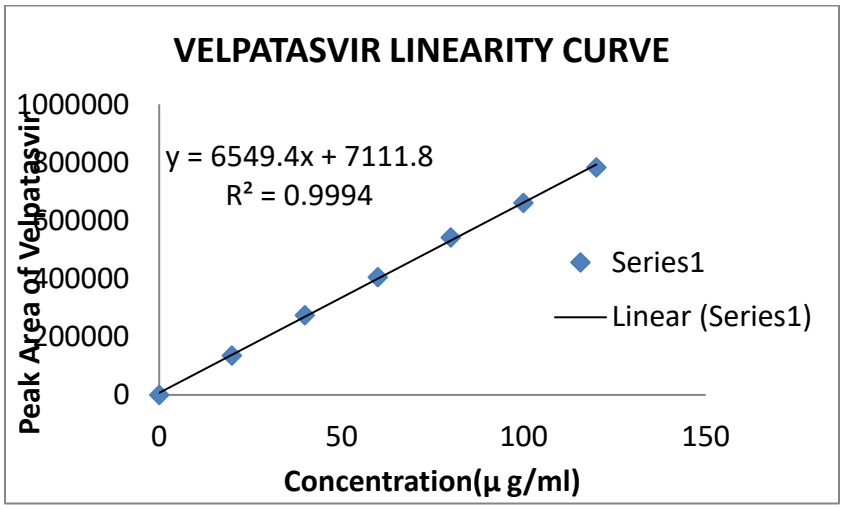


Figure 4: Linearity curve of Velpatasvir

4.5. DETECTION LIMIT

4.5.1. Limit of detection:

Preparation of 0.36 µg/ml solution:

Accurately weigh and transfer 25 mg of Velpatasvir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution). Further pipette 0.8 ml of Velpatasvir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent, and then pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 0.45ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 66 µV

Signal Obtained from LOD solution : 197 µV

S/N = 197/66 = **2.98**

Acceptance Criteria: S/N Ratio value shall be 3 for LOD solution.

4.5.2. Limit of quantification:

It is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio. The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Limit of Quantification is the lowest concentration of the substance that can be estimated quantitatively.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 66 µV

Signal Obtained from LOQ solution : 660µV

S/N = 660/66 = **10.00**

Acceptance Criteria: S/N Ratio value shall be 10 for LOQ solution.

4.6. Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, temperature variation was made to evaluate the impact on the method. The standard and sample of Velpatasvir were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

A. The flow rate was varied at 0.9 ml/min to 1.1 ml/min.

The Standard solution of Velpatasvir was prepared and analysed using the varied flow rates along with method developed flow rate. On evaluation of the above results, it was concluded that the variation in flow rate does not affected the method significantly.

Hence it was indicated that the method was robust even by change in the flow rate. Hence it indicates that the method is robust even by change in the flow rate ±10%.

4.7. System suitability results for Velpatasvir

The system suitability parameters were determined by preparing standard solutions of Velpatasvir and the solutions were injected six times and the parameters like peak tailing,

resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2% and the results were mentioned in table 4.

Table 4: System Suitability

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.27	3259.15	1.69
2	0.3	3477.23	1.65
3	0.33	3596.99	1.59

***** Results for actual flow (0.3 ml/min) have been considered from Assay standard.

B. The Wavelength was varied from 268nm to 272nm

Standard solution 80µg/ml of Velpatasvir was prepared and analysed using the varied Wavelength along with the actual wavelength in the method. The results were summarized in table no. 5.

Table 5: System suitability results for Velpatasvir

S. No.	Change in Wavelength	System Suitability Results	
		USP Plate Count	USP Tailing
1	268	3398.96	1.60
2	270	3477.23	1.65
3	272	3547.44	1.66

5. DEGRADATION STUDIES [9,10]

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Velpatasvir using the proposed method.

Preparation of stock:

Accurately weigh and transfer 25 mg of Velpatasvir is taken into a 25ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution).

5.1. Hydrolytic degradation under acidic condition

Pipette 0.8 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

5.2. Hydrolytic degradation under alkaline condition

Pipette 0.8 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns' syringe filters and place in vials.

5.3. Thermal induced degradation

Sample was taken in Petri dish and kept in Hot air oven at 110^0 C for 3 hours. Then the sample was taken and diluted with diluents and injected into UPLC and analyzed.

5.4. Oxidative degradation

Pipette 0.8 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns' syringe filters and place in vials.

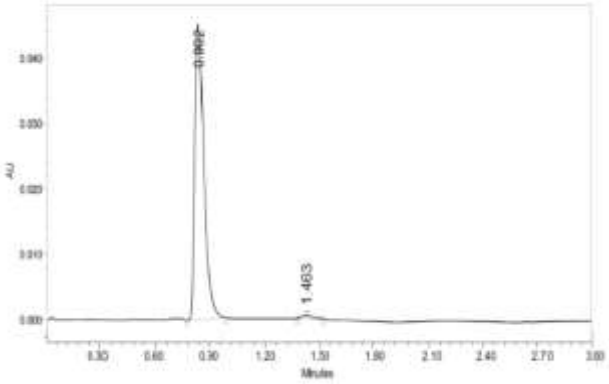
5.5. Photo degradation: Pipette 0.8 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns' syringe filters and place in vials.

Table 6: Results for Degradation studies

Sample Name	**Velpatasvir**	
	Area	**% Degraded**

Standard	545755	
Acid	510362	6.49
Base	529039	3.06
Peroxide	533748	2.20
Thermal	516206	5.41
Photo	506476	7.20

ACID:



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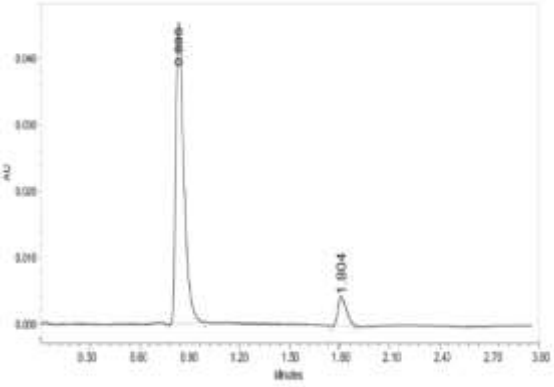


PEROXIDE:

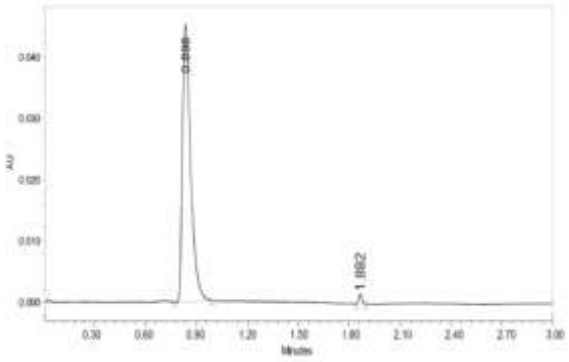


Figure 5: Degradation Graphs of Velpatasvir in Acid, Base and Peroxide conditions

6. UPLC METHOD BY QBD APPROACH [11-14]

6.1. Selection of quality target product profile

The QTPP is critical for identifying the variables that influence the QTPP parameters. For the proposed UPLC method, the retention time, theoretical plates, and peak asymmetry were identified as QTPP.

6.2. Determine critical quality attributes

The CQAs are method parameters that have a direct impact on the QTPP. The mobile phase composition and buffer pH were two critical method parameters that needed to be controlled in order to maintain QTPP's acceptable response range.

6.3. Factorial design

After defining the QTPP and CQAs, the factorial design was used to optimise and select the key components of the UPLC method: mobile phase, flow rate, and pH. Using response surface design, the various interaction effects and quadratic effects of mobile phase composition, flow rate, and pH of buffer solution on retention time, theoretical plates, and peak asymmetry were investigated and shown in figure no. 6 & 7.

Design Expert® (Version 11.0, Stat-Ease Inc., and M M) was used to create the best suited response for factorial response surfaces [20].

6.4. Evaluation of experimental results and selection of final method conditions

Design of Experiments (DoE) has been widely used to understand the effects of multidimensional and interaction of input factors on analytical method output responses. The Design Expert tools will be used to optimise the best chromatographic conditions.

6.5. Risk assessment

The optimised final method is chosen based on the method's characteristics, such as the fact that the developed method is efficient and will remain operational throughout the product's lifetime. A risk-based approach based on the QbD principles outlined in the ICH Q8 and ICH Q9 guidelines was used to evaluate the robustness and ruggedness of the method. For robustness and ruggedness studies, the parameters of the method or its performance were evaluated under various conditions, such as different laboratories, chemicals, analysts, instruments, reagents, and days.

6.6. Implement a control strategy

The analytical control strategy is a planned set of controls derived from an understanding of the various parameters, which include fitness for purpose, analytical procedure, and risk management. All of these parameters ensure that the method's performance and quality outputs are within the analytical target profile. For sample preparation, measurement, and replicate control operations, an analytical control strategy was devised.

6.7. Quality target product profile

The QTPP selected were retention time, theoretical plates, and peak asymmetry for optimization of UPLC chromatographic condition.

6.8. Critical quality attributes

The mobile phase composition, 0.1% OPA: Methanol, 45:55 v/v with good peak shape and good resolution were identified.

6.9. Factorial design

The box plot factorial design was selected for proposed UPLC method development. The optimization of various parameters is shown in (fig. 8 & 9) table 7 & 8.

Table 7: Box plot factorial design

Factor	Name	Units	Type	Sub Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	FLOW RATE	ml/min	Numeric	Continuous	0.7318	1.07	-1 ↔ 0.80	+1 ↔ 1.00	0.9000	0.0854
B	MOBILE PHASE RATIO		Numeric	Continuous	20.00	46.82	-1 ↔ 20.00	+1 ↔ 40.00	32.17	7.08
C	BUFFER PH		Numeric	Continuous	2.32	5.68	-1 ↔ 3.00	+1 ↔ 5.00	4.00	0.8536

Table 8: Responses of tailing factor of Velpatasvir

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	Tailing Factor of Velpatasvir		17.00	0.97	2.54	1.29	0.3911	2.62

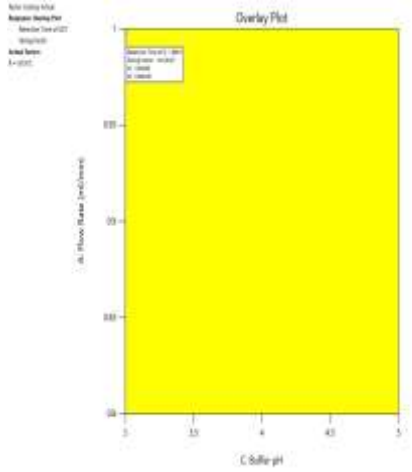
6.10. Coefficients in Terms of Coded Factors

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable and results were mentioned in table 9.

Table 9: Results of coefficients in terms of coded factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	1.03	1	0.0710	0.8657	1.20	
A-FLOW RATE	-0.0634	1	0.0615	-0.2090	0.0821	1.46
B-MOBILE PHASE RATIO	-0.3925	1	0.0868	-0.5978	-0.1872	2.00
C-BUFFER PH	-0.0188	1	0.0615	-0.1644	0.1267	1.46

AB	0.1435	1	0.0914	-0.0727	0.3598	1.66
AC	0.1271	1	0.0860	-0.0763	0.3305	1.41
BC	0.0840	1	0.0914	-0.1323	0.3002	1.66
A^2	0.0790	1	0.0472	-0.0325	0.1906	1.03
B^2	0.2902	1	0.0755	0.1117	0.4687	1.77
C^2	0.1833	1	0.0472	0.0718	0.2949	1.03



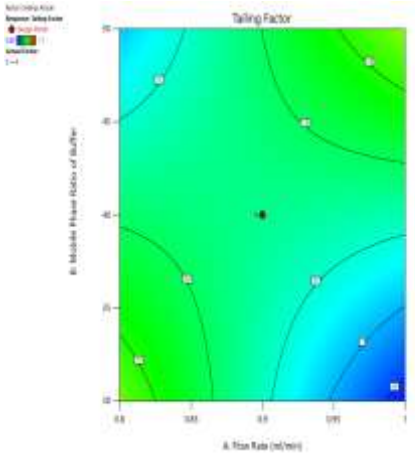


Figure 6 & 7: Overlay Graph for Optimization of the Factor; Counter Plot of tailing Factor

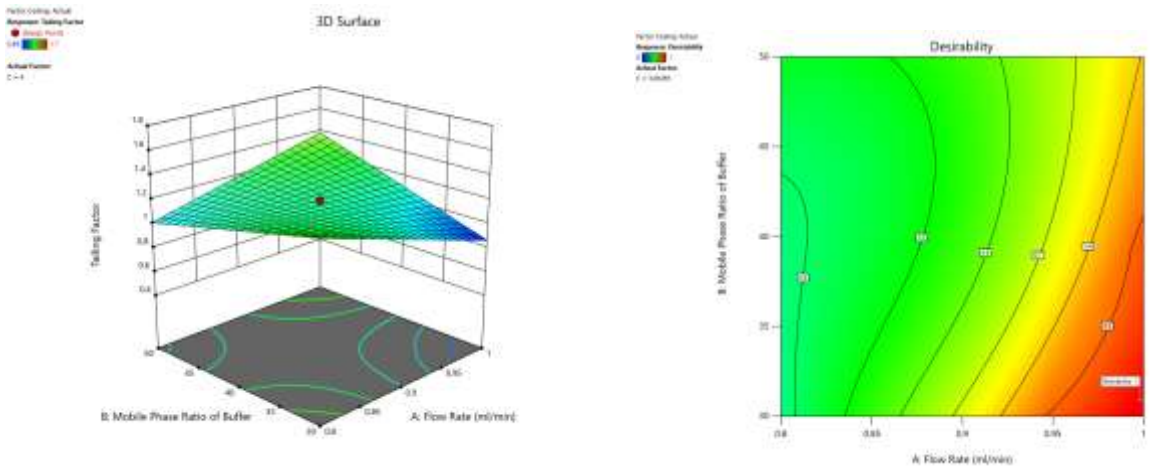


Figure 8&9: 3D Surface Graph of Tailing factor; Desirability graph for Tailing Factor of Velpatasvir

7. CONCLUSION

Development of new analytical methods for the determination of drugs in pharmaceutical dosage is important in pharmacokinetic, toxicological biological studies. Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs. It was concluded that the proposed new UPLC method developed for the quantitative determination of Velpatasvir by using QbD approach using Design Expert$^{@}$ software. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine determination of Velpatasvir depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies for the drug Velpatasvir.

8. ACKNOWLEDGEMENT

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9. CONFLICT OF INTEREST: None

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