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Development and Validation of UV-Spectrophotometric Method for Estimation of Velpatasvir in Bulk Form by Absorbance Maxima Method

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ABSTRACT

Recent study was conducted to develop a simple UV spectrophotometric method to determine an HCV inhibitor, Velpatasvir in bulk form according to official requirement and validate as per ICH guidelines. λmax of Velpatasvir was found 303 nm. Linearity existedperceived in the concentration assortment 5-40 μg/ml (r^2=0.999) for the method. The method was validated pertaining to linearity, precision and accuracy studies, LOD and LOQ consistent with ICH guidelines. Recovery studies for absorption maxima method was found to be 100.35%, 100.0% and 100.08% respectively. The existent method was establish to be simple, linear, precise, accurate as well as sensitive and can be applied for routine quality control enquiry for the analysis of Velpatasvir in bulk form.

Keywords: Hepatitis C virus (HCV), Velpatasvir (VEL), λmax and ICH guidelines.

INTRODUCTION

The hepatitis C virus (HCV), a virus of the family Flaviviridae, having single-stranded RNA as well as six major genotypes, communicate a disease to 150 million people in global. Chronic HCV infection leads to progressive liver fibrosis, which can also cause cirrhosis, hepatic decompensation, in addition to hepatocellular carcinoma. As numerous as half a million individualsbreathe their last breathyear after year from liver disease concomitant with chronic HCV infection [1]. Velpatasvir is an investigational inhibitor of the HCV NS5A protein having antiviral activity alongsidealtogether HCV genotypes [2]. The novel pan-

genotypic course of therapy [sofosbuvir (SOF) as well as velpatasvir (VEL)] intended for hepatitis C virus (HCV) has been concomitant with greatefficiency [3]. In phase 2 trials, 12 weeks of management with the amalgamation of sofosbuvir as well as velpatasvir stemmed in high proportions of sustained virologic effect in patients suffering from HCV genotype 2 or 3 [4]. The availability of this pangenotypic pill holds assurance for providing extremely effective treatment with trifling laboratory testing for chronic HCV all-inclusive [5]. Velpatasvir is HCV NS5A replication complex inhibitors [6]. Velpatasvir is freely soluble in ethanol, methanol and sparingly soluble in water [7]. Chemical structures of velpatasvir are given in Figure 1.
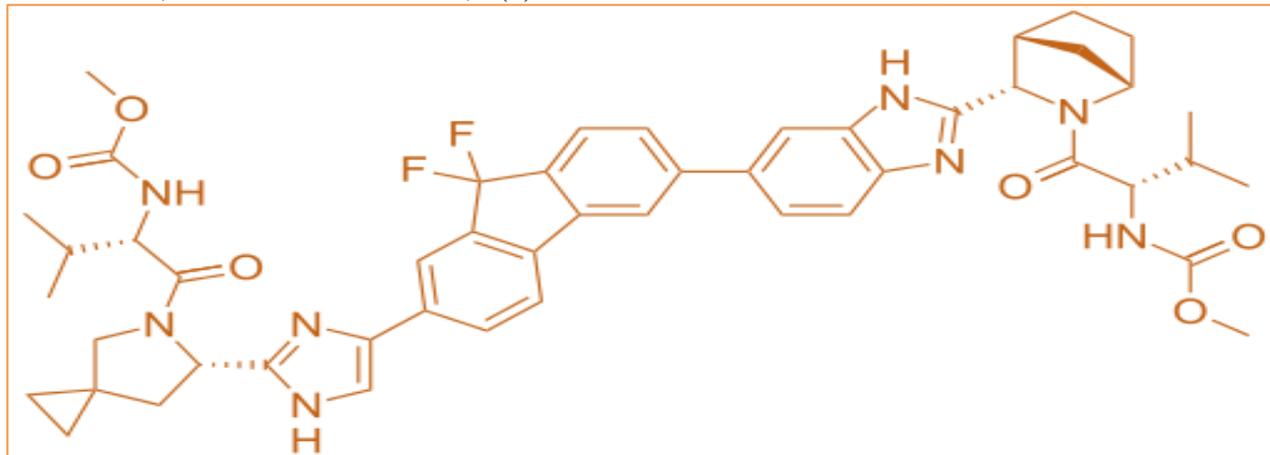
Figure 1: Chemical structure of Velpatasvir.

Diverse HPLC method for simultaneous determination of velpatasvir with sofosbuvir has been developed till the date. A RP-HPLC method was established for the quantitative assessment of Sofosbuvir in addition to Velpatasvir in bulk drug as well as pharmaceutical dosage forms. Methanol: TEA pH 4.2 (40:60) was selected as the mobile phase. The %RSD values remained within 2 also the method was designate precise [7]. A novel stability indicating RP-HPLC method was developed as well as validated for simultaneous assessment of velpatasvir plus sofosbuvir in tablet form. The offeredmethod is capable of giving faster elution of the analytes with good resolution. Forced degradation studies were conducted to know the stability of the analytes under specified conditions. During forced degradation study, the analytes were stable in all the conditions and no interferences were found, both drug peaks have purity angles lesser than their purity threshold indicating peak purity. The percentage recovery and precision studies showed that the method designate as accurate besides precise [6]. An specific and sensitive reverse phase-HPLC method has been established as well as authenticated for the concurrent valuation of Sofosbuviras well as Velpatasvir in bulk drug plus pharmaceutical dosage form [8]. A RP-HPLC method has been establishedandauthenticateddesigned for Sofosbuvir plus Velpatasvir in bulk and combined Tablet dosage form. Separation was conceded out on a Primesil C18 (4.6 x 250 mm, 5 µm) column consuming a mixture of Acetonitrile: 0.1% perchloric acid (50:50 v/v) as the mobile phase on a flow rate of 1.2 mL/min, the recognition was conceded at 262 nm. The retention time of the Sofosbuvir and Velpatasvir

4.25, 6.05 min correspondingly. The method yield linear retorts in the concentration series of 25-150 µg/mL for Velpatasvir, and 100-600 µg/ml of Sofosbuvir [9]. But according to our knowledge no UV method is available for determination of VEL alone in bulk form.

The present research work describes the estimation of assay content of VEL in active pharmaceutical ingredient (API) form using ultraviolet-Visible (UV-Vis) spectrophotometry technique. The work provides a sensitive, specific, as well as economical method for the determination of VEL in very short time by the UV-Vis spectrophotometer. 50% ACN is used as a diluent established on the drug solubility properties. Developed UV-Vis spectrophotometric method was validated with respectto Official guidelines.

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EXPERIMENTAL

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Required materials

Velpatasvir, Acetonitrile, distilled water. Glass wares of Pyrex material were incorporated.

Instruments

Weighing Balance 'Shimadzu Japan' and Spectrophotometer 'UV-1601, UV / Visible spectrophotometer, Shimadzu Japan' were incorporated.

Method development

Selection of wavelength detection: Velpatasvir was run on UV spectrophotometer at 200-400 nm and the λmax was found 303 nm.

Preparation of standard stock solution

Standard stock solution of Velpatasvir was organized by thawing 50 mg of VEL in a 100 ml volumetric flask. Final volume was made up to 100 ml with acetonitrile and distilled water (1:1) to get working standard stock solution containing 500 μg/ml of Velpatasvir and further dilutions were made by using combination of acetonitrile and distilled water (1:1).

Calibration curve for velpatasvir

Serial dilutions were prepared with acetonitrile and distilled water (1:1) to obtain concentration in range 5-40 μg/ml. The spectrum was recorded, absorbance was measured at 303 nm and a calibration curve was plotted.

Validation parameters

According to ICH guidelines the aforementioned method was validated [10].

Linearity

The linearity of method was appraised by evaluating diverse concentrations of the standard solution of Velpatasvir. Beer's law was obeyed in the concentration range 5-40 μg/ml.

Accuracy

A series of Samples were prepared for the range 50 to 150% for accuracy testing and the results were estimated by checking absorbance at 303 nm.

Precision

50 mg of Velpatasvir was deliberated precisely then dissolved in 100 ml of acetonitrile: distilled water, 1:1. Since the standard stock solution suitableamount of solution was occupied to make auxiliary dilutions using acetonitrile and distilled water (1:1) to give 10 μg/ml. Absorbance was measured at 303 nm against standard solution. This

procedure was carried out 3 times.

Limit of detection (LOD) and Limit of quantitation (LOQ)

Limits of detection (LOD) can be defined as the lowest concentration of the analyte that the analytical method canreliably differentiate from the background. Limits of quantification (LOQ) can be defined as the lowest concentration thatcan be quantified with acceptable accuracy and precision.

Specificity

The specificity of the method was documented by preparing Placebo and sample. Both scanned at the wavelength of active.

RESULT AND DISCUSSION

Optimization of UV-vis spectrophotometric method conditions

The main purpose of the current method is to develop a simple, sensitive, and precise UV-Vis spectrophotometric method for the assessment of VEL for the routine quantitative estimation of samples which will reduce dreary sample preparations, cost of resources and manpower obligatory to perform the analysis. The spectral analysis indicated that the λmax of VEL is 303 nm. Simple diluent 50% ACN was selected for the standard and sample solutions of VEL drug substance. Thus, the established UV-Vis spectroscopic method for the analysis of VEL in its API form enables analysis of several samples at the same time due to its simplicity in performing the analysis.

Method validation

According to ICH guidelines the fore mentioned method was validated.

Linearity

The linearity graphs were plotted between the absorbance versus concentration to obtain the calibration curve. The data is recorded and shown in Table 1.

Table 1: Calibration data of velpatasvir for absorbance maxima.

Concentration (μg/mL)	Absorbance
5	0.1279
10	0.2602
20	0.5136
30	0.7633
40	1.0091

The correlation coefficient was found to

be 0.9999. Linearity graph is shown in Figure 2.
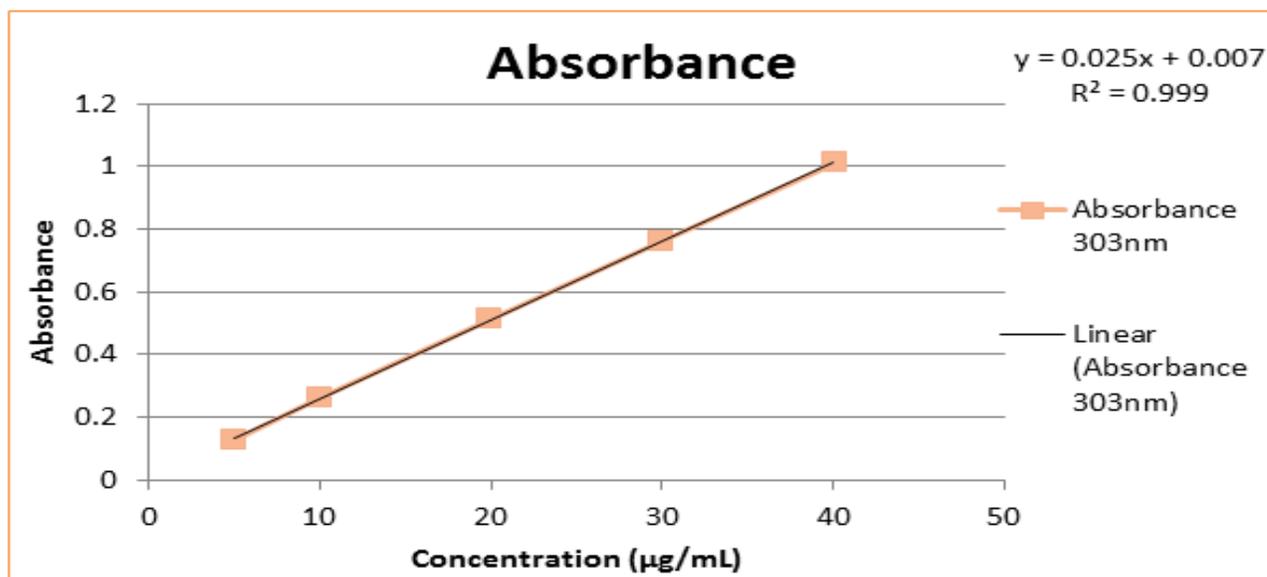
Figure 2: Calibration curve for velpatasvir.

Results demonstrate that an excellent correlation between the absorbance and concentration of VEL drug substance and shown in Table 2.

Table 2: Parameters from the calibration curve.

Parameters	Observation at 303nm
Calibration Curve	Linear
Expression	y = mx+c
Factor (y intercept)	0.007
Factor (slope)	0.063
Coefficient (r^2)	0.999

Accuracy

The percentage recovery results for VEL were varied from 100.00% to 100.35% at three different concentration levels, and the results were shown in Table 3.

Table 3: Accuracy and recovery of the developed method.

S. No	Level	Amount recovered	% recovery
1	50	50.18	100.35
2	100	100	100
3	150	150.12	100.08

Based on the % recovery data, it was concluded that the developed method is capable for the estimation of VEL drug substance and is adequate for routine analysis.

Precision

The percent assay value difference was determined for

solutions stored at room temperature for 24 hrs. VEL solution

found to be stable up to 24 hrs at room temperature. Solution stability results at room temperature are shown in Table 4.

Table 4: Statistical evaluation of inter-day and intra-day precision studies.

	Inter-day	Intra-day
MEAN ABSORBANCE	0.5137	0.5205
SD	0.001	0.001
%RSD	0.011	0.019

Limit of Detection (LOD) & Limit of Quantitation (LOQ)

LOD in addition to LOQ were appraised via the regression equation. Results are shown in Table 5.

Table 5: LOD and LOQ of Velpatasvir.

Parameters	Absorbance Maxima Method
LOD	0.351 μg/mL
LOQ	1.065 μg/mL

Specificity

The developed method is specific for the aforementioned drug and no hindrance was observed in placebo and in sample.

CONCLUSION

In present work, UV-spectrophotometric method was established and was validated consistent with ICH guidelines for VEL in bulk powder. Coefficient of correlation was found

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in the range of 0.999 for the drug as well as %RSD was <2%.Therefore, it can be clinched that the developed method is accurate and precise and can be employed effectively for the assessment of VEL.

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