ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of Sofosbuvir and Velpatasvir in pharmaceutical dosage form. The column used was Kromosil C18 (150mm x 4.6 mm, 5µm) in isocratic mode, with mobile phase containing phosphate buffer and acetonitrile (70:30 %v/v). The buffer is prepared by adding 1.41gm of sodium dihydrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 3.5 with dil. orthophosphoric acid solution. The flow rate was 1.0ml/ min and effluents were monitored at 260 nm. The retention times of Sofosbuvir and Velpatasvir were found to be 2.404 min and 2.986 min, respectively. The linearity for Sofosbuvir and Velpatasvir were in the range of 40-240µg/ml and 10-60 µg/ml respectively. The recoveries of Sofosbuvir and Velpatasvir were found to be 99.64 % and 99.25 % respectively. The proposed method was validated and successfully applied to the estimation of Sofosbuvir and Velpatasvir in pharmaceutical dosage forms.

Keywords: Sofosbuvir, Velpatasvir, Validation, Buffer and ICH Guidelines.

INTRODUCTION

Chemically, Sofosbuvir is propan-2-yl (2S)-2-{{[(2R,3R,4R,5R)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrroimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl}(methoxy)phosphoryl]amino}propanoate. The chemical formula is C27H25FN3O11P. The molecular formula is 529.458 g/mol. Sofosbuvir (tradename Sovaldi) is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients.

Velpatasvir is chemically, (2S)-2-{{[hydroxy(methoxy)methylidene]amino}-1-[25,5S]-2-{{[(2S,4R,5R)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrroimidin-1-yl)-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}]hemicos-1(13),2(4,8),6,9,11,14(19),15,17-nonaen-6-yl]-5-methylpyrrolidin-1-yl}3-methylbutan-1-one. The chemical formula is C49H43N5O3. 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). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients. Velpatasvir acts as a defective substrate for NSSA (Non-Structural Protein 5A), a non-enzymatic viral protein that plays a key role in Hepatitis C Virus replication, assembly, and modulation of host immune responses. Different analytical methods have been reported in the literature for the assay of Sofosbuvir and Velpatasvir in pharmaceuticals and include spectrophotometry, HPLC, UPLC and HPTLC. The present study was to establish a simple, sensitive and
low cost RP-HPLC method for simultaneous estimation of Sofosbuvir and Velpatasvir in bulk as well as in other dosage forms. The developed method was validated as per ICH guidelines.

**MATERIALS AND METHODS**

**Materials**

Sofosbuvir and Velpatasvir were kindly supplied by Natco. Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A tablet VELPANAT (Natco) containing 400mg of Sofosbuvir and 100mg of Velpatasvir were used.

**Instrumentation**

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 μL sample loop. The output signals were monitored and integrated using Empower 2 software.

**Methods**

**Chromatographic conditions**

The elution was isocratic and the mobile phase consisted of a mixture of buffer (accurately weighed 1.414g of sodium dihydrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 3.5 with dil. orthophosphoric acid solution) and acetonitrile (70:30 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A Kromosil C18 (150mm x 4.6 mm, 5µm) was used for determination. The flow rate was 1.0ml/min and the column was operated at ambient temperature (~30°C). The volume of sample injected was 10 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 260 nm.

**Standard Preparation**

Accurately weighed and transferred 40mg of Sofosbuvir and 10 mg of Velpatasvir working Standards into a 100 ml clean dry volumetric flask, add 70 ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 4ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent. The final concentrations of Sofosbuvir and Velpatasvir are 160 µg/ml and 40 µg/ml.

**Sample Preparation**

About 20 tablets were taken and their average weight was calculate. The tablets were crushed to a fine powder and drug equivalent to 40mg and 10mg were transferred to a 100ml volumetric flask, dissolved in diluent. Transfer 4ml from the above solution into 10ml volumetric flask and filtered through 0.45µm membrane filter to get concentration of 160µg/ml and 40µg/ml for Sofosbuvir and Velpatasvir.

**Method Validation**

The developed method was validated as per ICH guidelines 4-6 for its accuracy, linearity, precision, specificity, robustness, limit of detection and limit of quantification by using the following procedures.

**System suitability**

System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated.
2.986 mins and none of the impurities were interfering in its assay. The chromatogram of the drugs is shown in Fig. 1. Calibration curve of Sofosbuvir is shown in Fig. 2 and for Velpatasvir is in Fig. 3. The observed peak area values for respective concentrations are shown in Table 1.

Figure 1: HPLC chromatogram of Sofosbuvir and Velpatasvir in optimized chromatographic conditions

Figure 2: Calibration curve of Sofosbuvir in the range 40 to 240 μg/ml.

Figure 3: Calibration curve of Velpatasvir in the range 10 to 60 μg/ml.

Table 1: Calibration curve of Sofosbuvir and Velpatasvir

| S.No | Sofosbuvir | Velpatasvir |
|------|------------|-------------|
|      | Conc(μg/ml) | Rt(mins) | Area   | Conc(μg/ml) | Rt(mins) | Area   |
| 1    | 40          | 2.405     | 390764 | 10          | 2.987     | 119279 |
| 2    | 80          | 2.406     | 786093 | 20          | 2.982     | 235126 |
| 3    | 120         | 2.402     | 1174300| 30          | 2.978     | 349782 |
| 4    | 160         | 2.401     | 1563383| 40          | 2.964     | 467871 |
| 5    | 200         | 2.407     | 1907925| 50          | 2.992     | 574112 |
| 6    | 240         | 2.409     | 2341934| 60          | 2.995     | 692155 |
The accuracy data, method precision data, intermediate precision data for day and intermediate precision data relating to change of instrument are shown in Table 2, Table 3, Table 4, and Table 5 respectively. Robustness data relating to change in flow rate and robustness data relating to change in mobile phase composition are shown in Table 6 and Table 7 respectively. Results of analysis of laboratory samples are shown in Table 8. Table 9 shows system suitability parameters.

### Table 2: Accuracy data

| S.No | Spiked level | Sofosbuvir | Velpatasvir |
|------|--------------|------------|-------------|
|      |              | Amount added (µg/ml) | Amount present (µg/ml) | Average %Recovery* ± %RSD | Amount added (µg/ml) | Amount present (µg/ml) | Average %Recovery* ± %RSD |
| 1(n=6) | 50% | 40.00 | 40.95 | 98.97 ± 0.43 | 10.00 | 10.04 | 100.07 ± 0.46 |
| 2(n=6) | 100% | 80.00 | 80.03 | 100.34 ± 0.28 | 20.00 | 20.96 | 99.56 ± 0.55 |
| 3(n=6) | 150% | 120.00 | 119.06 | 100.42 ± 0.40 | 30.00 | 29.97 | 99.98 ± 0.59 |

*n=6 (Average of 6 determinations)

### Table 3: Method Precision data of Sofosbuvir and Velpatasvir

| S.No | Conc(µg/ml) | RT(mins) | Area | Conc(µg/ml) | RT(mins) | Area |
|------|-------------|----------|------|-------------|----------|------|
| 1    | 160         | 2.403    | 1562412 | 40          | 2.982    | 272468 |
| 2    | 160         | 2.405    | 1565061 | 40          | 2.989    | 272711 |
| 3    | 160         | 2.405    | 1568363 | 40          | 2.987    | 271649 |
| 4    | 160         | 2.406    | 1566157 | 40          | 2.989    | 270677 |
| 5    | 160         | 2.408    | 1566158 | 40          | 2.991    | 273575 |
| 6    | 160         | 2.401    | 1561519 | 40          | 2.974    | 272713 |
| Mean |              |          | 1564945 |            |          | 273049 |
| Std.dev |            |          | 2560.9  |            |          | 2264.3  |
| %RSD |              |          | 0.2     |            |          | 0.8    |

### Table 4: Intermediate Precision data relating to change of day

| S.No | Conc (µg/ml) | Day-1 | Day-2 | Conc (µg/ml) | Day-1 | Day-2 |
|------|-------------|-------|-------|-------------|-------|-------|
| 1    | 160         | 1566934 | 1569202 | 40          | 275638 | 275929 |
| 2    | 160         | 1567283 | 1560292 | 40          | 272833 | 274633 |
| 3    | 160         | 1569474 | 1563848 | 40          | 279383 | 274182 |
| 4    | 160         | 1565849 | 1564122 | 40          | 271132 | 272901 |
| 5    | 160         | 1563938 | 1565393 | 40          | 270293 | 270322 |
| 6    | 160         | 1562384 | 1563033 | 40          | 274842 | 273932 |
| Mean |              | 1565977 | 1564315 |            | 274020.2 | 273649.8 |
| SD   |              | 2527.214 | 2936.945 |            | 3337.527 | 1905.742 |
| %RSD |              | 0.16 | 0.18 |            | 1.21 | 0.69 |
Table 5: Intermediate Precision data relating to change of instrument

| S.No | Instrument to Instrument | Sofosbuvir | Velpatasvir |
|------|--------------------------|------------|-------------|
|      |                          | Peak area  | Peak area   |
|      |                          | Conc (µg/ml) | Day-1 | Day-2 | Conc (µg/ml) | Day-1 | Day-2 |
| 1    |                          | 160        | 1564847    | 1564283  | 40       | 276282   | 273832  |
| 2    |                          | 160        | 1565838    | 1568822  | 40       | 272837   | 272922  |
| 3    |                          | 160        | 1561934    | 1562838  | 40       | 277927   | 271973  |
| 4    |                          | 160        | 1562931    | 1563848  | 40       | 271983   | 270283  |
| 5    |                          | 160        | 1562482    | 1561344  | 40       | 277282   | 272833  |
| 6    |                          | 160        | 1563013    | 1563939  | 40       | 270484   | 275752  |
| Mean |                          |            | 1563508    | 1564179  |          | 274465.8 | 272932.5 |
| Std.dev |                     |            | 1505.319   | 2512.804 |          | 3094.559 | 1828.069 |
| %RSD |                          |            | 0.09       | 0.16     |          | 1.12     | 0.66     |

Table 6: Robustness data relating to change in flow rate (1.0ml/min)

| S.No | Flow rate (ml/min) | Average Peak Area* | Std.dev | %RSD | Average Peak Area* | Std.dev | %RSD |
|------|-------------------|-------------------|--------|------|-------------------|--------|------|
| 1    | 0.9ml/min         | 1566364           | 1453   | 0.36 | 276606           | 3411   | 0.73 |
| 2    | 1.0ml/min         | 1566108           | 1087   | 0.27 | 278575           | 1400   | 0.30 |
| 3    | 1.1ml/min         | 1566214           | 1233   | 0.30 | 276866           | 2723   | 0.58 |

*n=3 (Average of 3 determinations)

Table 7: Robustness data relating to change in mobile phase composition

| S.No | Mobile phase variation (%) | Average peak area* | Std.dev | %RSD | Average peak area* | Std.dev | %RSD |
|------|---------------------------|--------------------|--------|------|--------------------|--------|------|
| 1    | M.P-1-(BUFFER:ACN::69:31)| 1566072            | 3048   | 0.75 | 277789            | 1720   | 0.37 |
| 2    | M.P-2-(BUFFER:ACN::70:30)| 1566995            | 1237   | 0.30 | 277045           | 1356   | 0.29 |
| 3    | M.P-3-(BUFFER:ACN::71:29)| 1566451            | 1751   | 0.43 | 277058           | 3622   | 0.78 |

*n=3 (Average of 3 determinations)

Table 8: Results of analysis of laboratory samples (Assay)

| S.No | Sample | Label | Amount found | %Purity + RSD* | Amount found | %Purity + RSD* |
|------|--------|-------|--------------|---------------|--------------|---------------|
| 1    | Brand-1 (VELPANAT) | 400mg/100mg | 399.99 | 99.48± 0.30 | 99.96 | 99.25± 0.73 |

*n=3 (Average of 3 determinations)
Table 9: System suitability parameters

| Validation parameter       | Results          |
|---------------------------|------------------|
|                           | Sofosbuvir       | Velpatasvir    |
| Linearity range (µg/ml)   | 40 – 240         | 10 – 60        |
| Regression equation       | \( y = 9676x + 5191 \) | \( y = 6777x + 641 \) |
| Correlation Coefficient(r)| 0.9994           | 0.9999         |
| Accuracy                  | 98.58% to 100.71% | 98.94% to 100.58% |
| Precision (%RSD)          | 0.20             | 0.80           |
| Robustness (%RSD)         |                  |                |
| Flow rate: (1.0ml/min & 1.2ml/min) | NMT 0.36 | NMT 0.73 |
| Mobile phase: Buffer : ACN:MeOH(30:60:10) | NMT 0.75 | NMT 0.78 |
| Interday – (Day 1 & Day 2) | NMT 0.18 | NMT 1.21 |
| Instrument to Instrument  | NMT 0.16 | NMT 1.12 |

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in simultaneous estimation of Sofosbuvir and Velpatasvir in marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of Sofosbuvir and Velpatasvir in bulk and its pharmaceutical dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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