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Research Article

# Development And Validation of New RP-HPLC Method For the Simultaneous Estimation of Sofosbuvir And Velpatasvir In Combined Pharmaceutical Dosage Form

**P. Seetharamaiah*[1], Nagaraju Pappula[1], JVLN. Seshagiri Rao[2] and D. Gowrisankar[2]**

[1]*Hindu College of Pharmacy, Amaravathi Road, Guntur – 522002, A.P., India*
[2]*University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P., India*

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## ABSTRACT

A simple, rapid, accurate, precise, specific and sensitive reverse phase-HPLC method has been developed and validated for the simultaneous estimation of Sofosbuvir and Velpatasvir in bulk and pharmaceutical dosage form. The chromatographic separation was performed on the Discovery C18 column (250mm×4.6mm, 5µm particle size), using a mobile phase of OPA (0.1%): Acetonitrile = 50:50 (v/v), at a flow rate of 1.0 ml/min in an ambient temperature of 25˚C with the detection wavelength of 260nm. The retention times of Sofosbuvir and Velpatasvir were 2.47 min and 3.32 min respectively. The linearity was performed in the concentration range of 100-600 µg/mL, 25-150 µg/mL each of Sofosbuvir and Velpatasvir with a correlation coefficient of 0.998 and 0.999 for Sofosbuvir and Velpatasvir respectively. The proposed method has been validated for specificity, linearity, range, accuracy, precision and robustness were within the acceptance limit according to ICH guidelines and the developed method can be employed for routine quality control analysis in the bulk and combined pharmaceutical dosage form.

## INTRODUCTION

Sofosbuvir [1-2] 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). Treatment options for chronic Hepatitis C have advanced significantly since 2011, with the development of direct acting antivirals such as sofosbuvir. As a pro-drug nucleotide analogue, sofosbuvir is metabolized into its active form as the antiviral agent 2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate (also known as GS-461203), which acts as a defective substrate for NS5B (non-structural protein 5B). NS5B, an RNA-dependent RNA polymerase, is essential for

***Corresponding Author:** P. Seetharamaiah
**Address**: *Hindu College of Pharmacy, Amaravathi Road, Guntur – 522002, A.P., India*
**Email** : pappulanagaraju@gmail.com
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the transcription of Hepatitis C viral RNA and for its high replicative rate and genetic diversity. Sofosbuvir and other direct acting antivirals are therefore very potent options for the treatment of Hepatitis C, as they exhibit a high barrier to the development of resistance. Sofusbuvir (**Fig.-1)** has a molecular mass of 529.45 g/mol, molecular formula of $C_{22}H_{29}FN_3O_9P$ and has IUPAC name is Propan-2-(2S)-2-{[(S)-{[(2R,3R,4R,5R)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy}(phenoxy) phosphoryl]amino}propanoate.

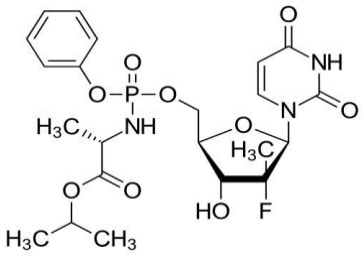

**Figure 1: Chemical structure of Sofosbuvir**

Velpatasvir [3] 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 acts as a defective substrate for NS5A (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. Treatment options for chronic Hepatitis C have advanced significantly since 2011, with the development of Direct Acting Antivirals such as velpatasvir. Notably, velpatasvir has a significantly higher barrier to resistance than the first generation NS5A inhibitors, such as ledipasvir and daclatasvir, making it a highly potent and reliable alternative for treatment of chronic Hepatitis-C. Velpatasvir **(Fig.-2)** has a molecular mass of 883.01 g/mol, molecular formula of $C_{49}H_{54}N_8O_8$ and has IUPAC name is (2S)-2-{[hydroxy(methoxy)methylidene]amino}-1-[(2S,5S)-2-(17-{2-[(2S,4S)-1-[(2R)-2-{[hydroxyl(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-methylpyrrolidin-1-yl]-3-methylbutan-1-one.

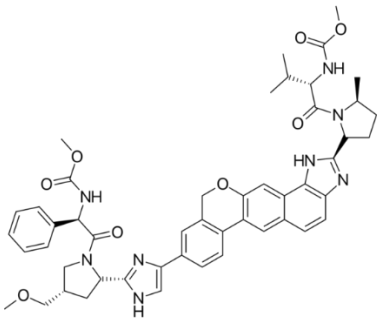

**Figure 2: Chemical structure of Velpatasvir**

## LITERATURE:

Some analytical methods have been reported for the determination of sofusbuvir [4-10] in pharmaceutical dosage forms and biological samples. But only few analytical methods using chromatography [11-26] have been reported for the simultaneous determination of sofusbuvir and velpatasvir in combined dosage forms. The present study concentrates on simultaneous RP-HPLC method and its validation in a novel fixed dose combination tablet of sofusbuvir and velpatasvir following the ICH directions.

## EXPERIMENTAL

### INSTRUMENTATION

HPLC system (Waters) with binary HPLC pump (model number 2695), PDA detector (model number 2998) and degasser with 10 µL injection loop were used. The chromatographic data was processed by Waters Empower2 software. Electronic balance ELB 300 and Digisun pH meter were used.

### MATERIALS

The reference samples of sofosbuvir and velpatasvir (API) were obtained from M/s. Sun pharma limited, Ahmadabad, India. The branded formulation (tablets) (Velpanat tablets containing Sofosbuvir and Velpatasvir) manufactured by M/s. Natcopharma limited, Hyderabad was procured from the local market. HPLC grade methanol, acetonitrile and analytical grade orthophosphoric acid were obtained from M/s. Rankem Chemicals Ltd, Mumbai, India. Milli-Q water dispensed through a 0.22 µ filter of the Milli-Q water purification system (Millipore, Merck KGaA, Darmstadt, Germany) was used throughout the study.

**Chromatographic Conditions**

The chromatographic separation and analysis of selected drug combination were worked out on a Discovery C18 column (250mm×4.6mm, 5µm particle size). The mobile phase used is a mixture of OPA (0.1%): Acetonitrile = 50:50 (v/v), at a flow rate of 1.0 ml/min. Mobile phase filtration using 0.45 µm membrane filter was done and 10 min sonication was performed. The column temperature is 25±2ºC while analyzing. The analyte elution was monitored using a photodiode array detector set at 260 nm. 10 µL is the injection volume.

**Preparation of stock standard and tablet sample**

About 200 mg of sofosbuvir and 50 mg of velpatasvir were accurately weighed and transferred into a 50 mL clean dry volumetric flask containing 30 mL of the diluent. The solution was sonicated for 10 min and then volume was made up to the mark with a further quantity of the diluent to get a concentration of 4000 µg/mL of sofosbuvir and 1000 µg/mL velpatasvir (Stock solution). A mixed working standard solution was further prepared by diluting the above stock solution to obtain a concentration of 400 µg/mL of sofosbuvir and 100 µg/mL of velpatasvir**.**

Twenty tablets of the commercial sample of 'Velpanat' were weighed and finely powdered. An accurately weighed portion of powdered sample equivalent to 200 mg of sofosbuvir and 50 mg of velpatasvir was transferred into a 50 mL volumetric flask containing 30 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drugs and the volume made up with a further quantity of the diluent. Then, this mixture was filtered through a 0.45 µ membrane filter. Further, 1 mL of the above filtrate was pipetted into a 10 mL volumetric flask and the volume was made up with the diluent.

**Method Optimization**

Parameters of chromatography such as time of retention, peak tailing, theoretical plates count, and resolution were determined to optimize the method. For that, trials are carried out - different mobile phase ratios and different stationary phase types, with temperature difference, values of pH and flow rate. On this basis method is employed to separate sofosbuvir and velpatasvir from themselves and also from stress degradants, discovery C18 (250 × 4.6 mm, 5 µm) column having temperature 25±2ºC was selected which gave good symmetric and sharp peaks. Based on less analysis time, peak response, peak symmetry and column efficiency, and mobile phase used is a mixture of OPA (0.1%): Acetonitrile = 50:50 (v/v), at a flow rate of 1.0 ml/min using photodiode array detector, a wavelength of 260 nm was selected as detection wavelength. The chromatographic parameters optimized exhibit a good peak shape, resolution and a good number of theoretical plates. The typical chromatogram of velpatasvir and sofosbuvir by the developed method is presented in **Figure-3**.

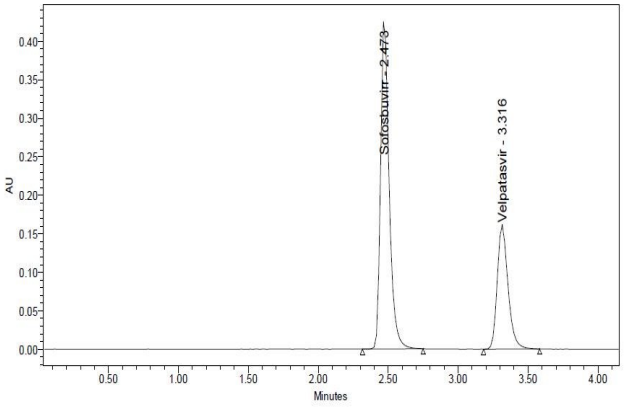


**Figure 3: Chromatogram of standard solution of sofosbuvir and velpatasvir**

## METHOD VALIDATION

Validation was performed by referring to ICH guidelines. HPLC Suitability, linearity, selectivity, accuracy, sensitivity, precision, specificity and robustness were determined.

## SYSTEM SUITABILITY

Study of System suitability established by six consecutive injections with the same working standard solutions. The number of theoretical plates was found to be > 2000, USP tailing was < 2 and USP resolution is above 2. The values for the system suitability parameters of the method, as presented in Table- 1, are within acceptance limits.

**Table-1: System Suitability of sofosbuvir and velpatasvir**

| S. No. | Sofosbuvir | | | Velpatasvir | | |
|---|---|---|---|---|---|---|
| | Area | USP Plate Count | USP tailing | Area | USP Plate Count | USP tailing |
| 1 | 4205215 | 6915 | 1.37 | 1728204 | 8432 | 1.34 |
| 2 | 4199444 | 6687 | 1.41 | 1698342 | 8432 | 1.34 |
| 3 | 4189975 | 6687 | 1.41 | 1674356 | 8166 | 1.32 |
| 4 | 4205289 | 6742 | 1.39 | 1719675 | 8152 | 1.30 |
| 5 | 4213456 | 7358 | 1.33 | 1728202 | 8642 | 1.30 |
| 6 | 4225677 | 6839 | 1.41 | 1719436 | 8403 | 1.31 |
| Mean | 4206509 | | | 1711369 | | |
| Std. Dev. | 12182.9 | | | 21168.3 | | |
| % RSD | **0.29** | | | **1.24** | | |

## SPECIFICITY:

Specificity is the ability to determine unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the method was performed by injecting blank solution, placebo solution and working standard solutions of sofosbuvir and velpatasvir separately. No interfering peaks were observed at the retention times of sofosbuvir and velpatasvir. Hence, the method is specific for the simultaneous determination of sofosbuvir and velpatasvir.

## LINEARITY AND SENSITIVITY

Linearity was done by preparing standard solutions of sofosbuvir and velpatasvir at five concentration levels. The linearity of detector response for sofosbuvir and velpatasvir was verified by prepared solutions in concentration range 100-600 µg/mL and 25-150 µg/mL respectively. Peak area of each sample against respective concentration of analytes was found to be linear. Correlation coefficient of sofosbuvir and velpatasvir are 0.998 and 0.999. Linearity results were presented in Table-2. This method's sensitivity was represented as limits of quantification (LOQ) and detection (LOD). Determining detection and quantitation limits is based on the signal-to-noise ratio with 3:1 & 10:1. Determined LOD values and LOQ values for sofosbuvir and velpatasvir were shown in Table-2.

**Table-2: Regression Analysis and Sensitivity of Sofosbuvir and Velpatasvir**

| Parameter | Sofosbuvir | Velpatasvir |
|---|---|---|
| Linearity (µg/mL) | 100-600 µg/mL | 25-150 µg/mL |
| Equation of regression (A= SC + I) | y = 10172x+5976.5 | y = 16839x+24692 |
| Slope (S) | 10172 | 16839 |
| Intercept on Y-axis (I) | 5976.5 | 24692 |
| Regression coefficient (R2 ) | 0.998 | 0.999 |
| LOD (µg/mL) | 0.44 µg/mL | 0.33 µg/mL |
| LOQ (µg/mL) | 1.32 µg/mL | 1.01 µg/mL |

**A = Peak area of analyte; I = analytes' concentration (µg/mL)**

## PRECISION AND ACCURACY

For precision studies, the same standard solutions of sofosbuvir and velpatasvir were injected 6 times into the HPLC system on the same day. The percentage RSD values calculated for peak areas of sofosbuvir and velpatasvir were less than 0.6 % (Table-3) indicating the precise assay with the developed HPLC method. For accuracy, the percentage recovery was calculated for both active ingredients. The results (Table-6) are acceptable with good percent recovery.

**Table 3: Results of repeatability of sofusbuvir and velpatasvir**

| S. No. | Sofosbuvir | | | Velpatasvir | | |
|---|---|---|---|---|---|---|
| | Area | USP Plate Count | USP Tailing | Area | USP Plate Count | USP Tailing |
| 1 | 4136808 | 6815 | 1.36 | 1676927 | 8433 | 1.34 |
| 2 | 4166672 | 6787 | 1.42 | 1674769 | 8432 | 1.33 |
| 3 | 4166672 | 6683 | 1.41 | 1659724 | 8165 | 1.35 |
| 4 | 4146929 | 6755 | 1.39 | 1665289 | 8154 | 1.31 |
| 5 | 4136794 | 7359 | 1.34 | 1681692 | 8644 | 1.30 |
| 6 | 4171736 | 6837 | 1.43 | 1669206 | 8405 | 1.32 |
| MEAN | 4154269 | | | 1671268 | | |
| SD | 15980.6 | | | 8077.2 | | |
| % RSD | **0.4** | | | **0.5** | | |

### Intermediate Precision:

Six replicate injections of the same dilution were analyzed on two different days for verifying the variation in the precision. The % RSD of the results for sofosbuvir and velpatasvir were found to be 0.2 and 0.3 respectively, which are within acceptable limit of ≤2. Hence, the method is reproducible on different days. This indicates that the method is precise. The results are shown in the Table 4 & 5.

**Table 4: Results of Intermediate precision of sofosbuvir**

| S. No. | Average area (n=6) | USP Plate Count | USP Tailing |
|---|---|---|---|
| Day 1 | 4221403 | 6784 | 1.25 |
| Day 2 | 4221401 | 6696 | 1.36 |
| Overall average | 4221402 | | |
| SD | 10005.1 | | |
| % RSD | **0.2** | | |

**Table 5: Results of Intermediate precision of velpatasvir**

| S. No. | Average area (n=6) | USP Plate Count | USP Tailing |
|---|---|---|---|
| Day 1 | 1726580 | 8554 | 1.32 |
| Day 2 | 1726582 | 8495 | 1.28 |
| Overall average | 1726581 | | |
| SD | 5707.9 | | |
| % RSD | **0.3** | | |

**Recovery Test**

Further evaluation of the accuracy of the method was carried through recovery test. Recovery test was determined by means of the standard addition method. The recovery experiments were performed by adding sofosbuvir and velpatasvir standards at three concentration levels to the placebo for three times. The recovery test results are summarized in Table 6. Hence, the obtained results indicated that the developed HPLC method was accurate enough for simultaneous quantitative evaluation of sofosbuvir and velpatasvir. There was no interference noticed from the common excipients of the tablet.

**Table 6: Results of recovery experiments of sofosbuvir and velpatasvir**

| Preanalysed amount (µg/ml) | | Spiked amount (µg/ml) | | % Recovered | |
|---|---|---|---|---|---|
| **Sofosbuvir** | **Velpatasvir** | **Sofosbuvir** | **Velpatasvir** | **Sofosbuvir** | **Velpatasvir** |
| 200 | 50 | 100 | 25 | 99.13 | 99.06 |
| 200 | 50 | 100 | 25 | 99.58 | 99.50 |
| 200 | 50 | 100 | 25 | 99.10 | 100.36 |
| 200 | 50 | 200 | 50 | 99.04 | 100.57 |
| 200 | 50 | 200 | 50 | 99.02 | 100.53 |
| 200 | 50 | 200 | 50 | 99.10 | 100.16 |
| 200 | 50 | 300 | 75 | 99.77 | 101.25 |
| 200 | 50 | 300 | 75 | 99.25 | 100.43 |
| 200 | 50 | 300 | 75 | 99.91 | 100.23 |
| | | | MEAN | 99.32 | 100.25 |
| | | | SD | 0.304 | 0.569 |
| | | | %RSD | **0.34** | **0.63** |

**Robustness**

Under the slightly varied chromatographic conditions (mobile phase's flow rate ± 0.1 mL/min & temperature in the column ± 2ºC), sofosbuvir and velpatasvir peaks were well separated and there was no significant change in the system suitability parameters (Table-7 and Table-8), which illustrated the robustness of the method.

**Table 7: Robustness study for sofosbuvir**

| Condition | Mean area | % assay | % difference |
|---|---|---|---|
| Optimized | 4213158 | 99.65 | ----- |
| Flow rate at 0.9 mL/min | 4210341 | 99.04 | 0.61 |
| Flow rate at 1.1 mL/min | 4213265 | 99.79 | 0.14 |
| Mobile phase: | | | |
| • Buffer-acetonitrile (55:45) | 4214210 | 100.11 | 0.46 |
| • Buffer-Acetonitrile (65:35) | 4214231 | 100.15 | 0.50 |
| Column Temperature: | | | |
| • at 25°C | 4210335 | 99.03 | 0.62 |
| • at 35°C | 4212844 | 99.59 | 0.06 |

**Table 8: Robustness study for Velpatasvir**

| Condition | Mean area | % assay | % difference |
|---|---|---|---|
| Optimized | 1722424 | 99.81 | ---- |
| Flow rate at 0.9 mL/min | 1723566 | 100.03 | 0.22 |
| Flow rate at 1.1 mL/min | 1722856 | 99.97 | 0.16 |
| Mobile phase: | | | |
| Buffer-acetonitrile (55:45) | 1722652 | 99.65 | 0.16 |
| Buffer-Acetonitrile (65:35) | 1722243 | 99.23 | 0.58 |
| Column Temperature: | | | |
| at 25°C | 1718839 | 99.02 | 0.79 |
| at 35°C | 1723646 | 100.05 | 0.24 |

**Stability of formulation solution:**

The sample solutions were analyzed at 0 h (comparison sample) and after 24 h (stability sample) by keeping at ambient room temperature. Stability was determined by determining %RSD for the formulation sample solutions. The sample solution injected after 24 h by keeping at room temperature (30°C) did not show any appreciable change. The deviation in the assay was not more than 2 and the results are shown in Table 9.

**Table 9: Stability data of sofosbuvir and velpatasvir**

| Drug | %Assay at 0 h* | %Assay at 24 h* | Deviation |
|---|---|---|---|
| **Sofosbuvir** | 99.56 | 99.01 | 0.55 |
| **Velpatasvir** | 100.05 | 99.85 | 0.20 |

*n=6 for each parameter

## DISCUSSION ON THE RESULTS

A Discovery C18 (4.6 x 250 mm; 5 µm) was selected as the stationary phase for separation of both drugs and detection was carried out at 260 nm. Initially, reverse phase liquid chromatography separation was attempted using various ratios of methanol and water and acetonitrile and water as the mobile phases, in which both the drugs were not eluted properly, and the resolution was also poor. Further trials were also performed to optimize the organic content of mobile phase phosphate buffer. The retention times were found to about 2.473 and 3.316 min for sofosbuvir and velpatasvir respectively.

## CONCLUSION

In the present study, a new simple, precise and accurate HPLC method was developed and validated for the simultaneous estimation of sofosbuvir and velpatasvir in tablet dosage form. In this method, a Discovery C18 (4.6 x 250 mm; 5 µm) was selected as the stationary phase. A 50:50 v/v mixture of phosphate buffer and acetonitrile was used as the mobile phase at a flow rate of 1.0 mL/min. Under the optimized conditions, the retention times obtained for sofosbuvir and velpatasvir were 2.473 and 3.316 min respectively. This HPLC method was validated as per the ICH guidelines. In this method, the number of theoretical plates is above 2000, tailing factor is less than 2 and RSD of peak area is less than 2, which indicates that the optimized method met the limits of system suitability parameters. The linearity ranges obtained for the drugs are sofosbuvir and velpatasvir showed in 25-150 µg/mL and 100-600 µg/mL respectively. The percent mean recovery values of sofosbuvir and velpatasvir were found to be 99.32 and 100.25% respectively and it showed that the proposed method is accurate. RSD values of repeatability and intermediate precision were ≤2 and hence the method is precise. The lowest values of LOD and LOQ as obtained by the proposed HPLC method

indicate that the method is sensitive. The solution stability studies of method indicate that sofosbuvir and velpatasvir were stable up to 24 h. Chromatographic conditions were deliberately changed slightly to check the robustness of the method and the results showed the reliability of the method as no appreciable changes in the results were observed.

The proposed method has some benefit over the earlier reported methods. Both the drugs were eluted with a simple mobile phase with good resolution. The retention times of the drugs obtained in this method were short. The short run time shows the speed of analysis which enables more number of samples to be analyzed per unit time. The proposed RP-HPLC method is precise and accurate and can be used for routine quality control analysis for simultaneous determination of Sofosbuvir and Velpatasvir in their tablet dosage forms.

## REFERENCES


1. Ghayathri VR, Vinodhini C, Gayatri S, Chitra K, Drug profile of sofosbuvir - a nucleotide analog inhibitor of the hepatitis C virus polymerase, World Journal of Pharmacy And Pharmaceutical Sciences 2014;3:1411-1416.
2. HarmeetKaur Bhatia, Harmanjit Singh, NipunjotGrewal, NavreetKaurNatt, Sofosbuvir: A novel treatment option for chronic hepatitis C infection, Journal of Pharmacology and Pharmacotherapeutics 2014; 5:278-284.
3. Brieva T, Rivero A, Rivero-Juarez A, Pharmacokinetic drug evaluation of velpatasvir plus sofosbuvir for the treatment of hepatitis Cvirus infection, Expert opinion on drug Metabolism and Toxicology 2017;13:483-490.
4. Lalitha KV, Raveendra Reddy J, Devanna N, Stability indicating RP-HPLC method development and validation for estimation of Sofosbuvir in pharmaceutical dosage form, The Pharmaceutical Innovation Journal 2018;7: 656-662.
5. Annapurna MM, Ravi Teja G, Chaitanya SM, New Stability Indicating Ultrafast Liquid Chromatographic Method for the Determination of Sofosbuvir in Tablets, Asian Journal of Pharmaceutics 2018;12:151-158.
6. Shaik John Saida, Muniappan M, Manikanta Kumar A, Muralidaran K, Ramulu Y, VenkatRao S, Estimation of Sofosbuvir with Validated Ultra High Performance Liquid chromatographic (UHPLC) Method in its bulk and Formulations, Der Pharmacia Sinica 2017;8:10-15.
7. Swathi P, RajeswarDutt K, V. Rao KKN, Alagar Raja M, RP-HPLC Method Development and Validation for Estimation of Sofosbuvir in Pure and Tablet Dosage Form,Asian Journal of Pharmaceutical Technology 2017;7:153-156.
8. Prashant M. Khedkar, Moreshwar P. Mahajan, Sanjay D. Sawant, Development and Validation of UV Spectrophotometric Method for the Estimation of Sofosbuvir (SFS) in Bulk and Tablet Formulation, International Journal of Pharma Research & Review 2017;6:1-4.
9. Nebsen M, Eman S. Elzanfaly, Stability-Indicating Method and LC–MS-MS Characterization of Forced Degradation Products of Sofosbuvir, Journal of Chromatographic Science 2016; 54:1631–1640.
10. Shahram Miraghaei, Bahareh Mohammadi et.al, Development and validation of a new HPLC-DAD method for quantification of sofosbuvir in human serum and its comparison with LC–MS/MS technique: Application to a bioequivalence study 2017; 1063:118-122.
11. Rani JS, A new RP-HPLC method development and validation for simultaneous

estimation of sofosbuvir and velpatasvir in pharmaceutical dosage form. International Journal of Engineering Technology Science and Research 2017; 4:145-152.

12. Kalpana N, Kumar JS, Ramachandran D, Analytical method development and validation for the simultaneous estimation of sofosbuvir and velpatasvir drug product by reverse phase high performance liquid chromatography method. Asian Journal of Pharmaceutical and Clinical Research 2018; 11:164-168.
13. Mamatha J, Devanna N, Simultaneous RP-HPLC method development and it's validation for estimation of sofosbuvir and velpatasvirin their combined dosage form. Rasayan Journal of Chemistry 2018; 11:392-400.
14. Vanaja B, Vageesh NM, Kistayya C, Urukundu V, RP-HPLC method development and validation for simultaneous estimation of sofosbuvir and velpatasvirin pure and pharmaceutical dosage form. Innovative International Journal of Medical and Pharmaceutical Sciences 2018; 3:45-48.
15. Jyothi U, Umadevi B, Analytical method development and validation for the simultaneous estimation of sofosbuvir and velpatasvirdrug product by RP-HPLC method. Indo American Journal of Pharmaceutical Research 2017; 7:402-409.
16. Kumaraswamy G, Pranay K, Rajkumar M, Lalitha R, Novel stabilty indicating RP-HPLC method simultaneous determination of sofosbuvir and velpatasvirin bulk and combined tablet dosage forms. Innovative International Journal of Medical and Pharmaceutical Sciences 2017;32:45-48.
17. Sarath N, Seshagirirao JVLN, A stability indicating RP-HPLC method for simultaneous estimation of velpatasvir and sofosbuvir in combined tablet dosage forms, World journal of pharmacy and pharmaceutical sciences 2017;6:1596-1611.
18. Madan Mohan Reddy M, GowriSankar D, SeshagiriRao JVLN, A Novel metho development and validation of RP-Hplc method and stress degradation study of determination of sofosbuvir and velpatasvir in bulk and combined tablet dosage form, European Journal of Biomedical and Pharmaceutical sciences 2018;5:490-501.
19. Sandya Rani J, Devanna N, Development and validation of RP-Hplc Method for the simultaneous estimation of sofosbuvir, velpatasvir and voxilaprevir in bulk and tablet dosage forms, Rasayan Journal of Chemistry 2018;11: 452-459.
20. Sridevi M, Siva Rao T, Gangu Naidu CH, Development and validation of liquid chromatographic method for simultaneous determination of sofosbuvir, velpatasvir and voxilaprevir in fixed tablet dosage form, European Journal of Biomedical and Pharmaceutical sciences 2018;5: 351-360.
21. Nagaraju T, Vardhan SVM, Ravi Kumar D, Ramachandran D, A New RP-HPLC Method for the Simultaneous Assay of sofosbuvir and ledipasvir in Combined Dosage Form, International Journal of Chemical Technology and Research 2017;10:761-768.
22. Mastanamma SK, Chandini SK, Reehana SK, Saidulu P, Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of Sofosbuvir and Ledipasvir in bulk and their combined dosage form, Future Journal of Pharmaceutical Sciences 2017;1-8.
23. Sreenivasarao B, Varaprasadreddy M, Simultaneous analysis of ledipasvir and sofosbuvir in bulk and tablet dosage form by stability indicating high performance liquid chromatographic method, Global journal for research analysis 2017;6:505-509.

24. Mohamed El-Kassem M Hassouna, Maha Mohammed Abdelrahman, Mahmoud Abdelfatah Mohamed, Assay and Dissolution Methods Development and Validation for Simultaneous Determination of Sofosbuvir and Ledipasvir by RP-HPLC Method in Tablet Dosage Forms, Journal of Forensic Sciences & Criminal Investigation 2017;1:1-11.
25. Abdallah OM, Abdel-Megied AM, Gouda AS, Development a validated highly sensitive LC-MS/MS method for simultaneous quantification of Ledipasvir, sofosbuvir and its major metabolite GS-331007 in human plasma: Application to a human pharmacokinetic study, Journal of Pharmaceutical Biomedical Analysis 2017;143:305-310.
26. Vamsi Reddy P, AshaRanjani V, Chandra Sekhar R, ShyamSundar M, RP-HPLC Method Development and Validation for Simultaneous Estimation of Ledipasvir and Sofosbuvir in Bulk and Tablets, International Journal of Chemistry and Pharmaceutical Sciences 2017;5:148–153.

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