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Development and quantification of UPLC method for the simultaneous estimation of voxilaprevir, sofosbuvir, and velpatasvir in formulations

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ABSTRACT

Aim

The aim of the present research work was to develop a sensitive, rapid and accurate, RP-UPLC method for the simultaneous estimation of voxilaprevir (VXR), sofosbuvir (SFR), and velpatasvir (VLR) in formulations.

Materials and methods

The chromatographic separation of mixture of VXR, SFR, and VLR was attained in isocratic method utilizing a mobile phase of 0.01N potassium dihydrogen orthophosphate (pH 4.8) and methanol in the proportion of 50:50% v/v utilizing a CHS C18 column which has dimensions of 100×2.1mm,2.0 µmparticlesizeandtheflowrateof1.0mL/min.Thedetectionsystemwasmonitoredat260nm wavelength maximum with 1.0 mL injection volume.

Results

The retaining time for VXR, SFR, and VLR was achieved at 1.468 min, 0.606 min, and 0.848 min, respectively.

Conclusion

The developed method was highly sensitive, rapid, precise, and accurate than the earlier reported methods. The total run time was decreased to 3.0 min; hence, the technique was more precise and economical. The projected method can be utilized for routine analysis in quality control department in pharmaceutical companies.

Keywords:RP-UPLC, Sofosbuvir, Velpatasvir, Voxilaprevir.

INTRODUCTION

Voxilaprevir (VXR), sofosbuvir (SFR), and velpatasvir (VLR) drugs were combined in a single dosage form (film coated tablet) in the brand name of Vosevi for the treatment of hepatitis-C. These three drugs will acts against hepatitis-C virus (HCV) in three different mechanisms.[1,2] VXR produce its antiviral activity by binding reversibly and inhibiting the non-structural protein (NS) 3/4A serine protease of HCV. Subsequently viral reproduction of HCV genetic material and translation into a single polypeptide, NS3, and its activating cofactor NS4A are responsible for splitting genetic components into the following non-structural and structural proteins essential for gathering into mature virus: NS3, NS4A, NS4B, NS5A, and NS5B[2]. By inhibiting viral protease NS3/4A, VXR therefore prevents viral replication and function. VXR chemically designated as (1R, 18R, 20R, 24S, 27S, 28S)- N-[(1R,2R)-2-(Difluoromethyl)-1-{[(1-methylcyclopropyl) sulfonyl] carbamoyl} cyclopropyl]-28-ethyl-13,13-

difluoro- 7-methoxy -24-(2-methyl-2- propanyl)-22, 25-dioxo-2, 21-dioxa-4, 11, 23, 26 tetraazapentacyclodioxanonacosa -3(12), 4, 6, 8, 10-pentaene- 27-carboxamide with molecular weight of 868.94 g/ mole[3-5] [Fig.1].

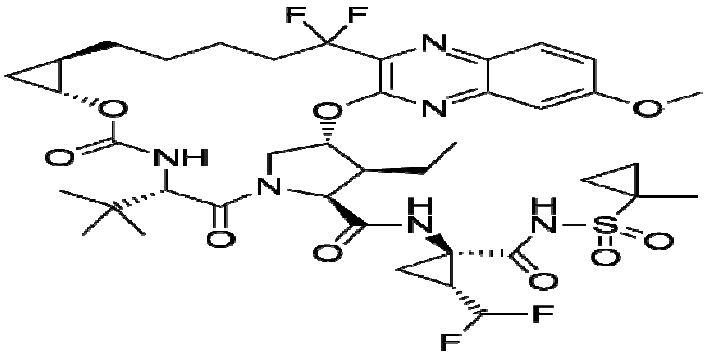


Fig.1 Structure of Voxilaprevir

SFR chemically designated as isopropyl(2S)-2-[(2R,3R,4R, 5R)-5-(2,4- dioxopyrimidin-1-yl)-4-Fluoro –hydroxy-4- methyl -tetrahydrofuran-2-yl] methoxy –phenoxy- phosphoryl] amino] propionate with molecular weight of 529.4 g/mole [Fig.2].SFRpreventsHCVNS-5BRNA-dependentRNA polymeraseandactsasachainterminator.Specificallyitalso inhibits HCV viral replication by binding to the two Mg^{+2}ions present in HCV NS5B polymerases.[2,6,7].

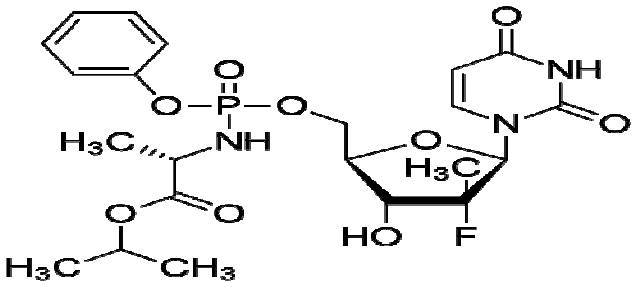


Fig.2 Structure of Sofosbuvir

VLR is a selective NS-5A inhibitor which binds to domain-I of NS-5A comprising amino acids 33–2021. This NS-5A inhibiting component competes with RNA for binding at this site. Inhibition of NS-5A is also known toinduce redistribution of the protein to lipid droplets. The exact role of NS-5A in RNA replication is not yet understood although it is known to be an important constituent. VLR is chemically designated as Methyl{(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)- 1-{(2R)-2-[(methyl carbonyl) amino]-2-phenyl acetyl]- (methoxy methyl)-2-pyrrolidinyl]-1H- imidazol-4-yl}-1,- 11-dihydroisochromeno[4', 3:'6,7]naphtha[1, 2-d] imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbonate with molecular weight of 883.02 g/ mole[8-10] [Fig.3].

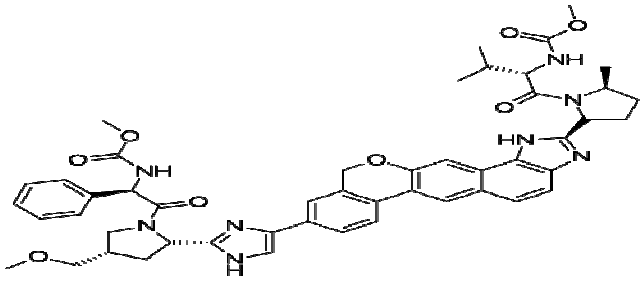


Fig.3 Structure of Velpatasvir

The literature review discloses that a very few spectroscopic,[7] LC-MS/MS,[8] and high performance liquid chromatographic techniques[9-13] have been reported for estimation of VXR, SFR, and VLR. Based on the reported HPLC methods, there is a need to develop a rapid, sensitive reversed-phase-UPLC method for simultaneous estimation of VXR, SFR, and VLR in bulk and formulations.

MATERIALS AND METHODS

Chemicals and reagents

The standard components of VXR, SFR, and VLR were provided as a gift sample from spectrum Pharma Research Solutions, Hyderabad. VOSEVI tablets labelled to contain VXR 100 mg, SFR 400 mg, and VLR 100 mg wereprocured fromthelocalmarket.HPLCgradeacetonitrileandmethanol were obtained from A.B enterprises, Mumbai, India. Orthophosphoric acid was bought from Ranchem,Mumbai, India. HPLC grade water was processed by utilizing Milli-Q Millipore water purification system used during the method development.

Instrumentation (UPLC)

Chromatographic measurements were made on a Waters alliance (Waters Corporation, Milford, MA, USA) 2695 model which consisted of two (AD/LC/32) solvent delivery modules, a PDA (AD/LC/77) detector, quaternary gradient pumps, in built an auto injector valve with a 20 µL loop. The system was controlled through a system controller (SCL-10A) and a personal computer using Empower 2 software installed on it. The mobile phase was degassed using a Shimadzu sonicator (Model: Power sonic 405). Absorbance spectra were recorded using Shimadzu UV-Visible spectrophotometer (Model: UV-1800) employing quartz cell of 1.0 cm of path length and weighing was done with Shimadzu balance (Model: AUX220).

Preparation of buffer

Accurately weighed 1.36 g of potassium dihydrogen orthophosphate in a 1000 mL of volumetric flask add about 900 mL of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1 mL of triethylamine then PH adjusted to 4.8 with dilute ortho phosphoric acidsolution.

Preparation of stock and standard solution

Accuratelyweighedandtransferred10mgofSFRand 2.5mg of VLR and 2.5 mg of VXR working standards into a 25 mL clean and dry volumetric flask, add 3/4th volume of diluent (water:acetonitrile[50:50v/v]),sonicatedfor5minandmade uptofinalvolumewithdiluent.1.0mLfromtheabovestock solution was taken into a 10 mL volumetric flask and made upto10mLtoget40µg/mLofSFR,10µg/mLofVLR,and 10 µg/mL ofVXR.

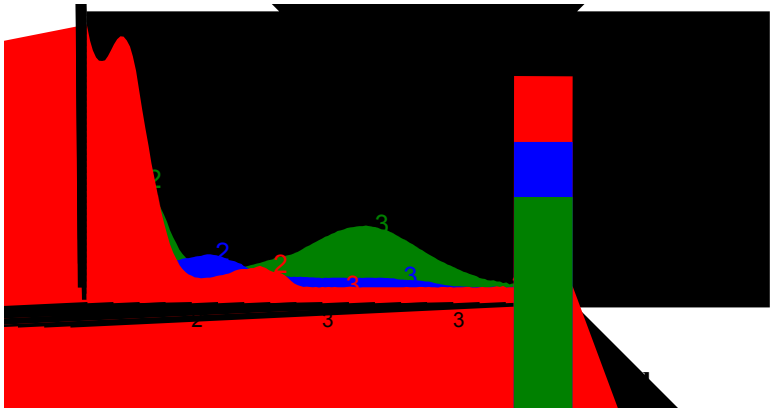
Preparation of sample solution

Five tablets were weighed and calculated the average weight of tablets and then the weight equivalent to one tablet was transferred into a 100 mL volumetric flask containing 50 mL of diluent and sonicated for 25.0 min. Further, the volume made up with diluent and subjected for filtration. From the filtrate, 1.0 mL solution was pipetted out into a 10.0 mL volumetric flask and made up to 10.0 mL with diluent [9-14].

RESULT

In the present work, we selected UPLC to reduce the total run time. Method development was executedwith different columns and mobile phases. The sample solution of VXR (10 µg/mL), VLR (10 µg/mL) and SFR (10 µg/mL) of UV overlay graph showed that drugs absorb at 260 nm (Fig.4).

So, the wavelength selected for the estimation of VXR, SFR and VLR were at 260 nm. With different mobile phase compositions and stationary phases three different trials were executed and fourth trail was optimized. In all the three trials: merged peaks were observed in trail -1 (Fig.5), peak shape was poor and tailing in the trail -2 (Fig.6) and base line was poor in the trial -3 (Fig.7). Optimized chromatographic peaks were shown in Fig.8.



Finally, the method was optimized with mobile phase of 0.01N potassium dehydrogenase orthophosphate (pH 4.8) and methanol in the proportion of 50:50% v/v utilizing a CHS C18 column which has dimensions of 100 × 2.1 mm, 2.0 µm particle size, and the flow rate of 1.0 mL/min. It is the ability of a method to unequivocally evaluate the analyte components in the presence of other components such as impurities, degrades, and excipients expected to be present. This parameter was estimated by injecting and evaluatingtheblank,placebo,standardandsamplesolutions, and chromatograms, respectively.[14] Chromatograms of blank, placebo, and sample solutions are shown no peaks at the retaining time of VXR, SFR, and VLR peaks. The chromatograms of VXR, SFR, and VLR of standard, blank, formulation, and placebo are represented in Fig.8.

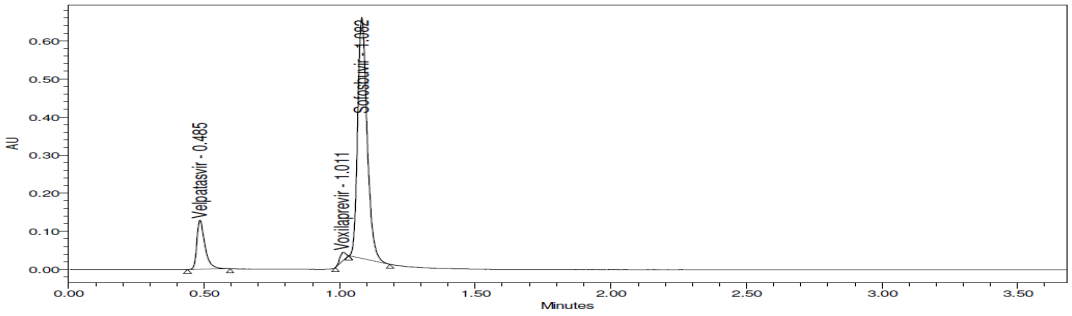


Fig.5 Representative Chromatogram of trial-1

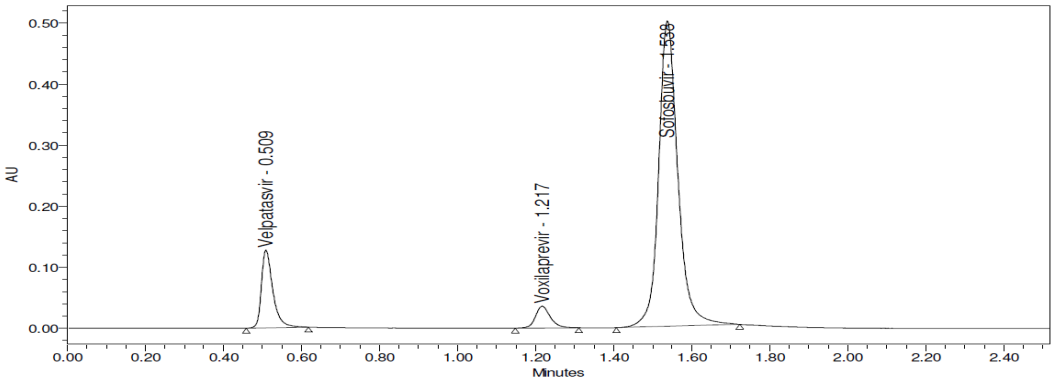


Fig.6 Representative Chromatogram of trial-2

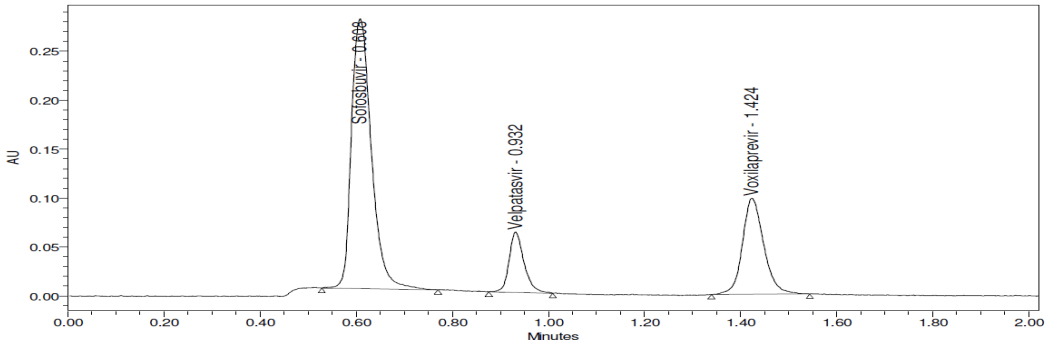


Fig.7 Representative Chromatogram of trial-3

Table.1. The major trials details

S.No	Trials	Column	Mobile Phase	Observation
01	**Trial -1**	Hypersil C18 (100 x 3 mm, 2.1 µm)	Methanol: 0.1% OPA (1:1)	Merged peaks were observed
02	**Trial -2**	Kinetex C18 (100 x 3 mm, 2.1 µm)	Buffer: ACN (30:70 v/v) (Buffer: 0.01N Potassium dihydrogen ortho phosphate and pH 4.8 adjusted with OPA)	Peak shape was poor
03	**Trial -3**	Acquity BEH C18 (100 x 3 mm, 1.7µm)	Methanol:Buffer (70:30 v/v) (Buffer: 0.01N Potassium dihydrogen ortho phosphate and pH 4.8 adjusted with OPA)	Base line was poor

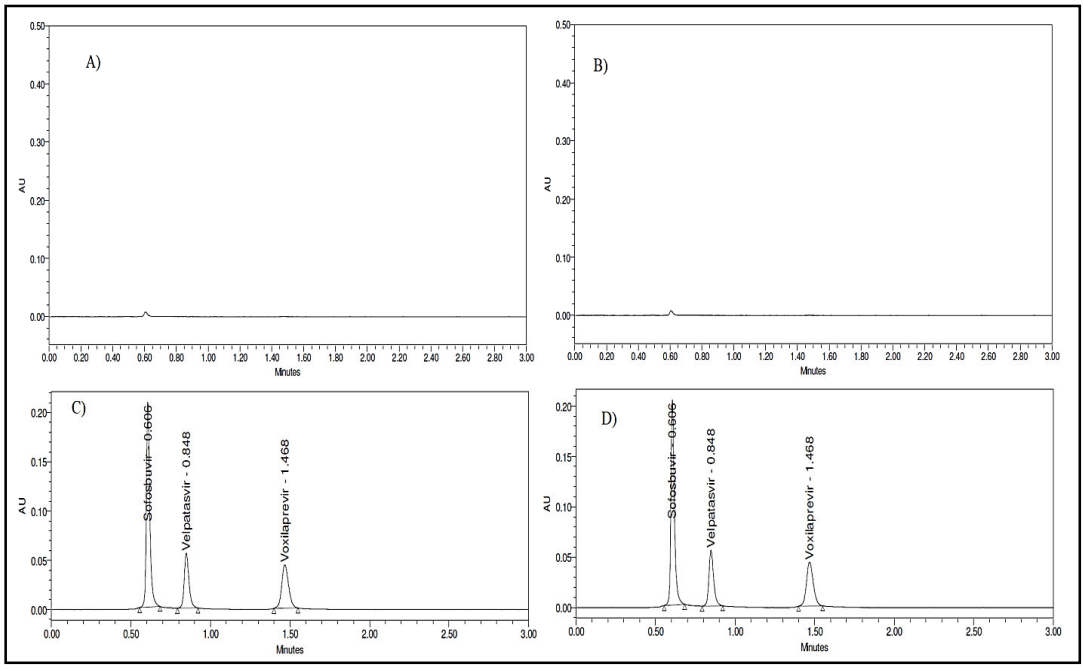


Fig.8 Chromatograms of A) blank, B) Placebo, C) Standard and D) Formulation

Optimized chromatographic conditions

After systematic trials with different mobile phase compositionsandotherparametersinvolvedinthetechnique, the following chromatographic conditions wereemployed:

- Mobile phase: 0.01N KH_2PO_4: Methanol

(50:50v/v)

- Flow rate: 1.0mL/min
- Column: CHS C18 100 × 2.1 mm 2.0 m.
- Detector wave length: 260nm
- Column temperature:30°C
- Injection volume: 1.00 mL
- Run time: 3.0 min
- Diluent: Water:Acetonitrile (50:50v/v).

Assay of marketed formulation

Themarketed formulation of VOSEVI (tablet) was evaluated by infusing 1.5 µl of reference and analyte solutions six times into the chromatographic system and the resulting chromatograms of analytes were documented. The quantity of anaytes existed in the marketed formulation was estimated by equating the peak area of reference and analyte. The % assay of SFR, VLR and VXR were found to be 99.0–101.0%.

CONCLUSION

A sensitive, rapid, and accurate, RP-UPLC method for the simultaneous estimation of VXR, SFR, and VLR in formulations was developed. Retention timeandtotalruntimesofanalyteswеredecreased.Retention times for VXR, SFR, and VLR were achieved at 1.468 min, 0.606 min, and 0.848 min, respectively. Mean assay value ofVXR,SFR,andVLRwasfound to be 99.90%, 99.87%, and 99.91%, respectively. Hence, the developed method was rapid and economical that can be applicable in routine analysis of these drugs in quality control department of pharmaceutical companies.

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