JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

DETERMINATION OF SOFOSBUVIR AND VELPATASVIR IN COMBINATION BY DERIVATIVE UV SPECTROPHOTOMETRY

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ABSTRACT

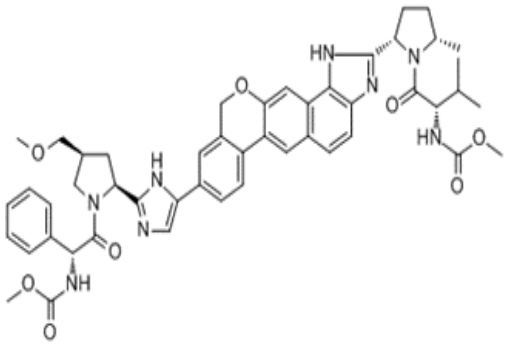
A combination of sofosbuvir and velpatasvir is available in the market for treatment of Hepatitis C viral infections. It has advantage of once daily dose. A new simple derivative spectrophotometric method has been developed and validated for simultaneous estimation of sofosbuvir and velpatasvir. Quantitative estimation was carried out at 260nm and 269 nm for velpatasvir and sofosbuvir respectively, in first derivative mode. The calibration curves were linear over a concentration range of 3-15µg/ml for velpatasvir and 12-60µg/ml for sofosbuvir**.** Method validation was assessed as per the ICH guidelines Q2(R1). The developed method was successfully applied for analysis of these two drugs in a mixture and excellent recovery results were obtained. The method is simple, fast and economic. The method can be applied to the quality control testing of sofosbuvir and velpatasvir in their combination.

Keywords: Sofosbuvir, Velpatasvir, UV derivative spectrophotometry.

INRODUCTION

Sofosbuvir(SOF) and Velpatasvir (VEL)are the direct acting antiviral drugs used in therapy to treat chronic Hepatitis C. Sofosbuviris used in combination therapy with other antiviral medications to treat chronic hepatitis C virus (HCV) infected patients with HCV genotypes 1-6, and to treat HCV and HIV co-infected patients[1]. IUPAC nomenclature is propan-2-yl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl] methoxy-phenoxy phosphoryl]amino]propanoate [2]. 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 Hepatitis C Virus (HCV). IUPAC nomenclature is methyl*N*-[(1*R*)-2-[(2*S*,4*S*)-2-[5-[6-[(2*S*,5*S*)-1-[(2*S*)-2-(methoxycarbonylamino)-3-methyl butanoyl]-5-methylpyrrolidin-2-yl]-21-oxa-5,7diazapentacyclo[11.8.0]henicosa-1(13), 2,4(8), 5,9,11,14(19),15,17-nonaen-17-yl]-1*H*-imidazol-2-yl]-4-(methoxymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl]carbamate.[3] The combination is proven to effectively treat all the 6 HCV genotypes. A combination of these two drugs is available as a tablet in market with a label claim of 100mg velpatasvir plus 400mg sofosbuvir per tablet.

A thorough literature search revealed reported methods of SOF combination with VEL include analytical methods likeLC-MS/MS [4], High-performance +liquid chromatography (HPLC) [5-8], UPLC MS/MS[9],UPLC[10]and HPTLC[11]. Current research was aimed at method development and validation of derivative UV spectrophotometric method for simultaneous estimation of these recently co-formulated antiviral drugs.



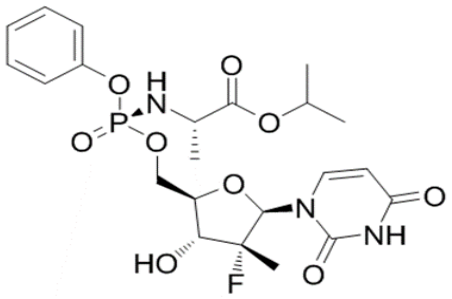


Fig 1.1 Structure of Velpatasvir **Fig 1.2 Structure of Sofosbuvir**

MATERIALS AND METHODS

Calibrated glassware (Class A), weighing balance (Make SHIMADZU, model AY 120), UV spectrometer (Make JASCO, modelV730), sonicator(Make Pharma Solutions), etc.Working standards of velpatasvir and sofosbuvir, distilled water.

Method development

Selection of solvent

HPLC grade methanol was used as a solvent for studies of both the drugs.

Preparation of standard stock solution

A calibrated "class A" volumetric flask of 10 ml was used for preparing the stock solutions of both the drugs. Weighing of individual velpatasvir and sofosbuvir was carried out using a calibrated weighing balance.10mg of velpatasvir was weighed, methanol was added and further proceeded to sonication as the drug was found to dissolve slowly. After sonication the volume was made up to 10 ml. Thus, individual stock solution was prepared. 10mg of sofosbuvir was weighed, methanol was added and further proceeded to make up its volume to 10 ml thus sofosbuvir stock solution was prepared. 1 ml from each of these solutions were pipetted out and diluted with methanol to10 ml (100µg/ml).

Preparation of working solution

Velpatasvir

From the stock solution of velpatasvir, appropriate volume was transferred to 10ml volumetric flasks and the volume was made up with methanol to obtain concentrations in the range of 3-15µg/ml.

Sofosbuvir

From sample solutions of sofosbuvir, appropriate volume was transferred to 10ml volumetric flasks in the similar manner as for velpatasvirto obtain concentrations in the range of 12-60µg/ml.

Determination of λmax

Absorption spectrum was determined in the wavelength range of 200 nm to 400 nm. The spectrum in the first derivative mode was recorded to locate the ZCP (zero crossing point) for each drug such that the other will have appreciable absorbance at that wavelength.

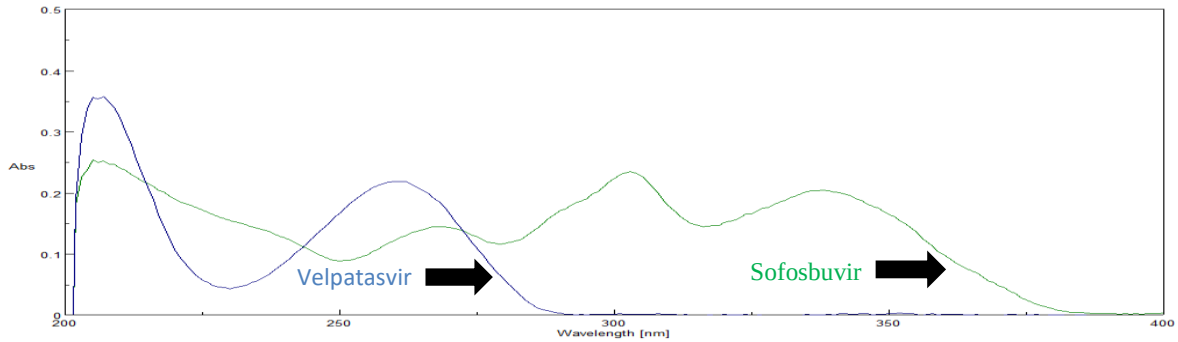


Fig 1.3 Overlay of Velpatasvir and Sofosbuvir

Preparation of calibration curve

Linearity was checked for each drug at the ZCP of the other. Mixtures of these two drugs were prepared and were tested by first derivative method.

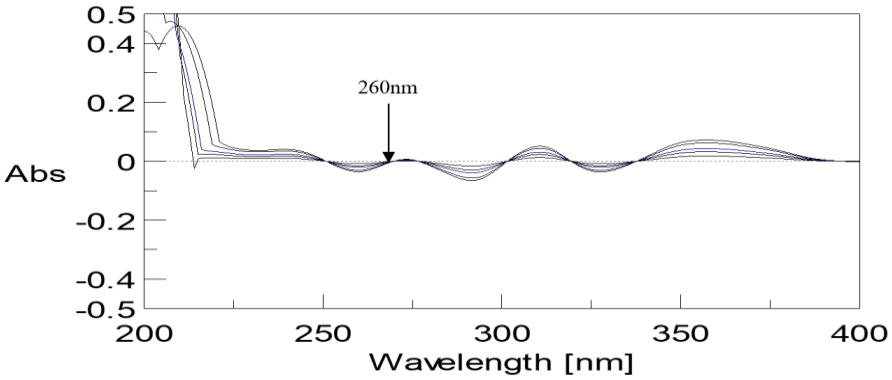


Fig 1.4 Overlain spectra of velpatasvir

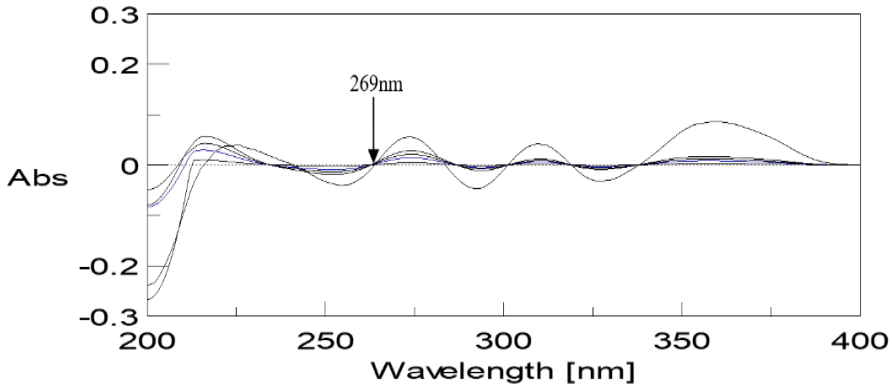


Fig 1.5 Overlain spectra of sofosbuvir

Method validation

Linearity

Linearity was performed at 2 selected wavelengths (i.e., 260nm and 269nm). Mixtures of various concentrations were prepared of Velpatasvir and Sofosbuvir.

Precision

The method precision was studied by intra & inter-day studies for both the drugs. Precision is expressed in terms of % relative standard deviation (%RSD) for a series of measurements.

Robustness

The Robustness for the method was carried out by making small but deliberate changes. The Robustness for the method was performed by a team of three analysts at different wavelength 260 ± 0.5 nm and 269 ± 0.5 nm, effect on absorbance value was noted.

Limit of Detection

Limit of detection is the lowest amount of analyte to be detected in a sample by analytical procedure.

Limit of Quantification

LOQ is the minimum analyte concentration which is quantified with acceptable accuracy and precision.

RESULT AND DISCUSSION

Linearity & range

Selected method was studied for linearity by using linear regression analysis**.**

Table I: Absorbance at 1st order derivative for both the drugs

Sr no	Concentration range (sofosbuvir + velpatasvir)	Absorbance(260nm)	Absorbance(269nm)
1	12+3 µg/ml	-0.01264	0.007370
2	24+6 µg/ml	-0.02625	0.01458
3	36+9 µg/ml	-0.03952	0.02151
4	48+12 µg/ml	-0.05103	0.02765
5	60+15 µg/ml	-0.06702	0.03590

Linearity equations& regression coefficients at both wavelengths, i.e., 260nm & 269nm

$y = -0.1335x+0.077$ $r^2 = 0.9979$ (260nm) for sofosbuvir

$y = 0.0701x+0.0363$ $r^2=0.9983$ (269nm) for velpatasvir

Precision

Absorbance of six replicates of standard mixtures sofosbuvir(12 µg/ml) and velpatasvir (3µg/ml) were noted in first derivative mode. The method was found to be precise. The reproducibility was checked and was expressed in terms of %RSD.

Table II Precision study for velpatasvir(3 µg/ml) & sofosbuvir (12 µg/ml)

Sr.No.	At (260nm)	At (269nm)
1	-0.001511	0.008793
2	-0.00146	0.008845
3	-0.001491	0.008824
4	-0.001485	0.00891568
5	-0.00145	0.009186

6	-0.001501	0.00879
AVG	-0.001483	0.00889228
SD	0.000023639	0.000151019
%RSD	-1.594993341	1.698320163

Limit of Quantification(LOQ) and Limit of Detection(LOD)

Table III

	LOQ	**LOD**
Sofosbuvir	4 µg/ml	1.2 µg/ml
Velpatasvir	1 µg/ml	0.3 µg/ml

Assay of Sofosbuvir and Velpatasvir

The assay procedure was carried out by preparing random mixtures within the specified concentration range.

DISCUSSION

The developed method for the combination by simultaneous estimation by first derivative spectrophotometric technique was found to be economical. This method can be used for routine analysis.

ACKNOWLEDGEMENT

We are grateful to NATCO pharmaceuticals, Hyderabad for providing us the samples of Velpatasvir and Sofosbuvir for our research work. We are also thankful to the Principal and management of AISSMS College of Pharmacy, Pune, Maharashtra for providing us the facilities and the opportunity to carry out research work.

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