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Research Article

STABILITY INDICATING DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR RP-HPLC METHOD

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ABSTRACT:

Sofosbuvir and Velpatasvir were developed with Std Discovery 250 x 4.6 mm, 5m. Buffer-containing MP OPA :MeCN in the 55:45 ratio was poured across the column at 1 ml/min. This procedure employed 0.1% Perchloric acid buffer. The temperature was 30°C. Optimised wavelength was 230 nm. Sofosbuvir and Velpatasvir had 2.146 and 2.770 min retention times. Sofosbuvir and Velpatasvir had 0.4 and 0.5 RSD. %Recovery was 100.09% for Sofosbuvir and 100.62% for Velpatasvir. Sofosbuvir and Velpatasvir regression equations yielded LOD, LOQ values of 0.24, 0.73, and 0.15, 0.45. The regression equations for Sofosbuvir and Velpatasvir are y = 91520.x + 1773.9 and y = 179637x + 22360, respectively. Reduced retention and run time for better method development.

Key Words: Sofosbuvir and Velpatasvir, RP – HPLC.

INTRODUCTION

Hepatitis C is a viral illness characterised by acute inflammation of the liver. Chronic hepatitis C can result in severe hepatic injury. Infectious hepatitis C virus (HCV) is transmitted by direct contact with blood containing the virus. For the majority of individuals with the persistent, known as chronic, hepatitis C infection, the preferred therapy is with newer antiviral drugs. Chronic hepatitis C may typically be effectively treated with these medications.[1]

Antiviral medications are pharmaceuticals specifically authorised by the Food and Drug Administration (FDA) to treat or manage viral infections. They selectively engage certain phases within the viral life cycle. While an ideal antiviral medication should possess efficacy against both actively replicating and latent viruses, the majority of the currently known antiviral medicines only demonstrate effectiveness against replicating viruses.[2]

sofosbuvir and Velpatasvir are a combination of drugs used for treatment of Hepatitis C. These drugs work by reducing the amount of hepatitis C virus in your body, which helps your immune system fight the infection and may help your liver recover. Chronic hepatitis C infection can cause serious liver problems such as scarring (cirrhosis), or liver cancer.[3] The combined therapy regimen of sofosbuvir and velpatasvir shown great efficacy in HCV patients with genotypes 1–6, including those with prior treatment experience and cirrhosis. With the exception of genotype 3, the addition of ribavirin did not result in a substantial enhancement of SVR12 rates. Additionally, further research should examine the impact of adding ribavirin into this treatment plan in individuals with HCV genotype 3.[4]

Background

Sofosbuvir: It is a direct-acting antiviral agent used to treat specific hepatitis C virus (HCV) infections in combination with other antiviral agents. It is chemically known as propan-2-yl (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. Sofosbuvir is recommended for the treatment of adult

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patients with chronic hepatitis C virus (HCV) genotypes 1, 2, 3, 4, 5, or 6 infection; when used in combination with Velpatasvir as the combination product Epclusa; or in combination with Ribavirin if associated with decompensated cirrhosis.[5]

Velpatasvir: It 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). It is known as methyl N-[(1R)-2- [(2S,4S)-2-(5-{6-[(2S,5S)-1-[(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl]-5-methylpyrrolidin-2-yl]-21-oxa-5,7-diazapentacyclo[11.8.0.0^{3,11}.0^{4,8}.0^{14,19}]henicosa-1,3(11), 4(8), 6,9,12,14,16,18-nonaen-17-yl}-1H-imidazol-2-yl)-4-(methoxymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl] carbamate. [6]

The Sofosbuvir and Velpatasvir is a single tablet, once a day regimen that combines two pan-genotypic, high potency and high genetic barrier antiviral molecules, providing >95% of SVR across all GTs with favourable safety and tolerability across a broad patient population even for decompensated cirrhotic subjects.[7]

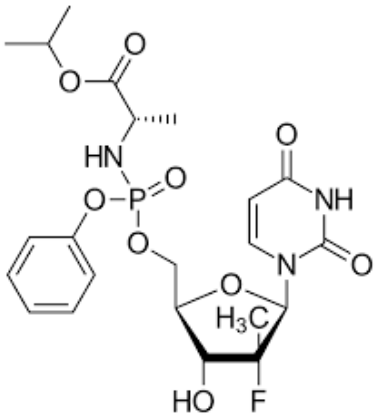

Figure 1 : structure of Sofosbuvir

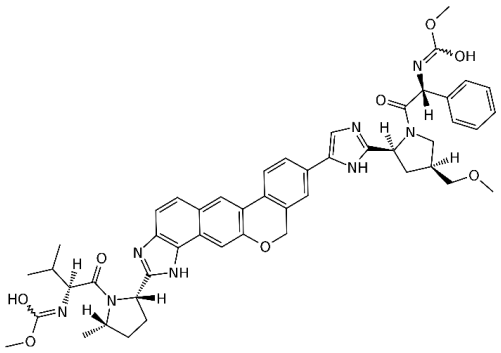

Figure 2: Structure of Velpatasvir

An extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Sofosbuvir, Velpatasvir, and their medicinal dose form using RP-HPLC.[8- 14] must be validated and developed as per ICH guidelines

Materials and Methods

Spectrum pharma Research Solution provide with Sofosbuvir and Velpatasvir pure drugs (API) gift samples and Combination Sofosbuvir and Velpatasvir tablets (Velpanat) received from local market. The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective:

The primary objective of this study is to provide a highly exact, accurate, sensitive, specific, consistent, and efficient analytical method for the simultaneous quantification of Sofosbuvir and Velpatasvir in their pure form and throughout tablet formation.

Table 1: Chromatographic Conditions:

Mobile phase	OPA : Acetonitrile (55:45)
Flow rate	1 ml/min
Column	Discovery C18 (4.6 x 150mm, 5µm
Detector wave length	260 nm
Column temperature	26°C
Injection volume	10µL
Run time	5.0 min

Preparation

Std Stock Sol Prep: Weighed 40mg Of Sofosbuvir And 10mg Of Velpatasvir Into 50ml Flasks, Added 3/4th Diluents, Then Set For Sonication For About 15 Min. Later Makeup The Flask With Diluent And Label It As Standard Stock Solution. (800 µg/Ml Sofosbuvir, 200µg/Ml Velpatasvir)

The Sample Stock Sol, 5 Tablets Average Weight Was Found. Then, One Tablet Was Put Into A 100ml Vf, 50 Ml Of Diluents Were Added, And The Mixture Kept For Sonication For 25 Min. The Vol Was Then Makeup With The Diluent And Filtration Through Hplc Filters (2000µg/Ml Of Sofosbuvir And 1000µg/Ml Of Velpatasvir).

100% Solution Or Working Sample Sol, 0.2 Ml Of The Filtered 100% Sol Was Added To 10 Ml Vf And Saturated With Diluent. (Sofosbuvir At 100µg/Ml And Velpatasvir At 50µg/Ml)

Standrad Working Sols (100% Sol) From Each Stock Sol 1 Ml Sol Was Pipetted Out In 10ml Vf And Makeup With Diluent. (50µg/Ml Sofosbuvir, 25µg/Ml Velpatasvir)

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Sofosbuvir (80ppm) and Velpatasvir (20ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should be not more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Table 2: System suitability results

S no	**Sofosbuvir**			**Velpatasvir**		
Inj	**RT (min)**	**USP Plate Count**	**Tailing**	**RT (min)**	**USP Plate Count**	**Tailing**
1	2.141	6364	1.25	2.765	7494	1.28
2	2.146	6312	1.11	2.767	7944	1.27
3	2.146	6305	1.15	2.770	7138	1.28
4	2.147	6713	0.73	2.771	7862	1.28
5	2.148	6846	0.73	2.780	7801	1.30
6	2.153	6912	1.18	2.782	7542	1.28

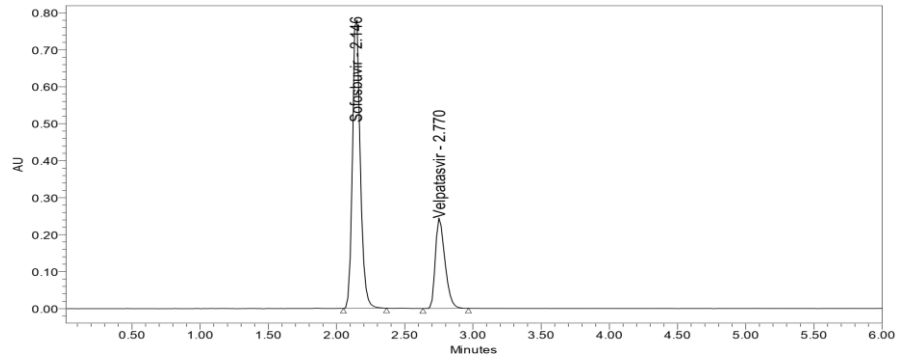

Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area
Velpatasvir	2.146	3113131
Sofosbuvir	2.770	1123010

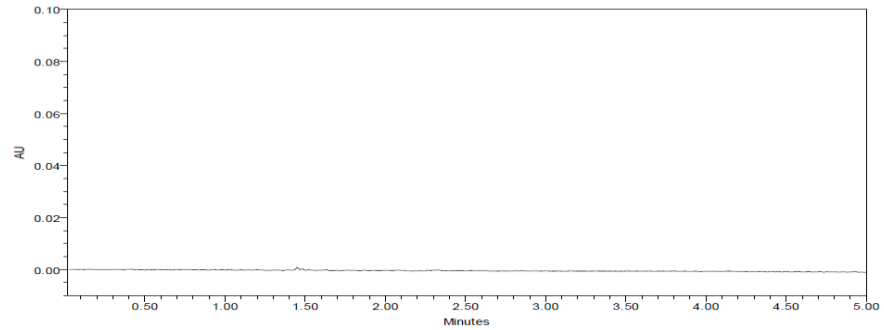

Figure 4: Blank

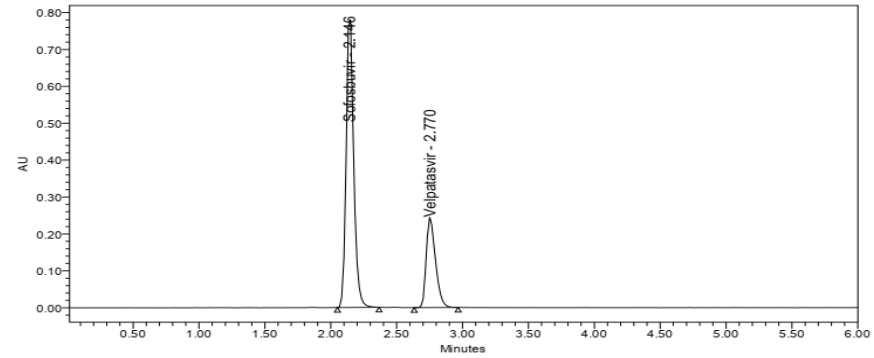

Figure 5: Specificity of Sofosbuvir and Velpatasvir

Linearity:

Calibration data is given in table 4 and regression data in table 4 and calibration curve in figure 4, 5

Table 4: Calibration data of Sofosbuvir and Velpatasvir

Sofosbuvir		Velpatasvir	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
20	1850045	5	915951
40	3604691	10	1827650
60	5513555	15	2699696
80	7424145	20	3635646
100	9155092	25	4466056
120	10960701	30	5435996

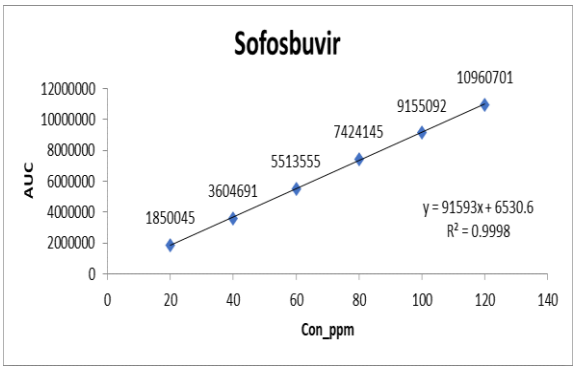

Figure 6 Calibration curve of Sofosbuvir

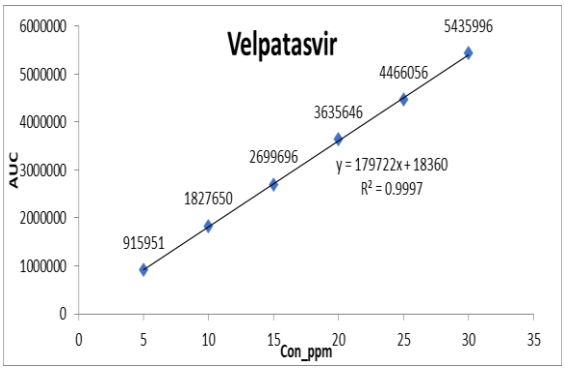

Figure 7 Calibration curve of Velpatasvir

Table 5: regression data

Parameter	Sofosbuvir	Velpatasvir
Conc range (µg/mL)	20-120	5-30
Regression Equation	y = 91593x + 6530.6	y = 179722x + 18360
Co-relation	0.999	0.999

Accuracy:

Recovery data shown in table 6

Table 6: recovery data of Sofosbuvir and Velpatasvir

	Sofosbuvir			Velpatasvir		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	40	40.24	100.59	10	10.10	101.04
	40	40.28	100.71	10	10.10	100.97
	40	40.09	100.24	10	10.00	99.97
100%	80	80.03	100.03	20	20.17	100.84
	80	79.74	99.67	20	20.18	100.90
	80	79.74	99.68	20	20.16	100.79
150%	120	120.53	100.44	30	30.18	100.59
	120	119.04	99.20	30	30.22	100.73
	120	120.25	100.20	30	30.01	100.04
% recovery	101.09			100.65		

System precision was performed and the data was shown in table 7

Table 7: System precision of Sofosbuvir and Velpatasvir

S. No	Area of Sofosbuvir	Area of Velpatasvir
1.	7313676	3665437
2.	7376363	3657466
3.	7384364	3627364
4.	7384364	3694363
5.	7304736	3628466
6.	7393746	3604746
Mean	**7359542**	**3646307**
S.D	**39477.9**	**32262.4**
%RSD	**0.5**	**0.9**

The % RSD for the peak areas of Sofosbuvir and Velpatasvir obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Sofosbuvir and Velpatasvir and shown in table 8.

Table 8: method Precision

S. No	Area of Sofosbuvir	Area of Velpatasvir
1.	7383746	3623547
2.	7383633	3653646
3.	7304363	3627364
4.	7376264	3638265
5.	7386364	3623747
6.	7376364	3623747
Mean	**7368456**	**3631719**
S.D	**31674.1**	**12136.7**
%RSD	**0.4**	**0.3**

From the above results, the % RSD of method precision study was within the limit for Sofosbuvir and Velpatasvir.

Robustness: Robustness conditions like Flow minus (1.1ml/min), Flow plus (1.3ml/min), mobile phase minus (50B:50A), mobile phase plus (65B:40A), temperature minus (21°C) and temperature plus(31°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 9: Robustness data for Sofosbuvir and Velpatasvir

Condition	%RSD of Sofosbuvir	%RSD of Velpatasvir
Flow rate (-) 0.7ml/min	0.5	0.7
Flow rate (+) 0.9ml/min	0.9	0.8
Mobile phase (-) 50B:50A	0.4	0.8
Mobile phase (+) 60B:40A	0.4	0.3
Temperature (-) 27°C	0.5	0.7
Temperature (+) 33°C	0.5	0.6

Sensitivity:

Table 10: sensitivity of Sofosbuvir and Velpatasvir.

Molecule	LOD	LOQ
Sofosbuvir	0.24	0.73
Velpatasvir	0.04	0.13

Force Degradation Studies: table 11 shows degradation conditions and table 12 shows the obtained degraded data and purity plot chromatogram in figure 8,9.

Table 11: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxdation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

Table 12: degradation data

Type of degradation	Sofosbuvir			Velpatasvir		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	7073646	95.86	4.14	3448464	95.88	4.12
Base	6947547	96.12	3.88	3417666	95.73	4.27
Peroxide	7275466	95.09	4.91	3594646	96.37	3.63
Thermal	7338464	97.96	2.04	3628464	97.18	2.82
Uv	7337444	97.50	2.50	3638466	98.46	1.54
Water	7352746	99.57	0.43	3617363	99.46	0.54

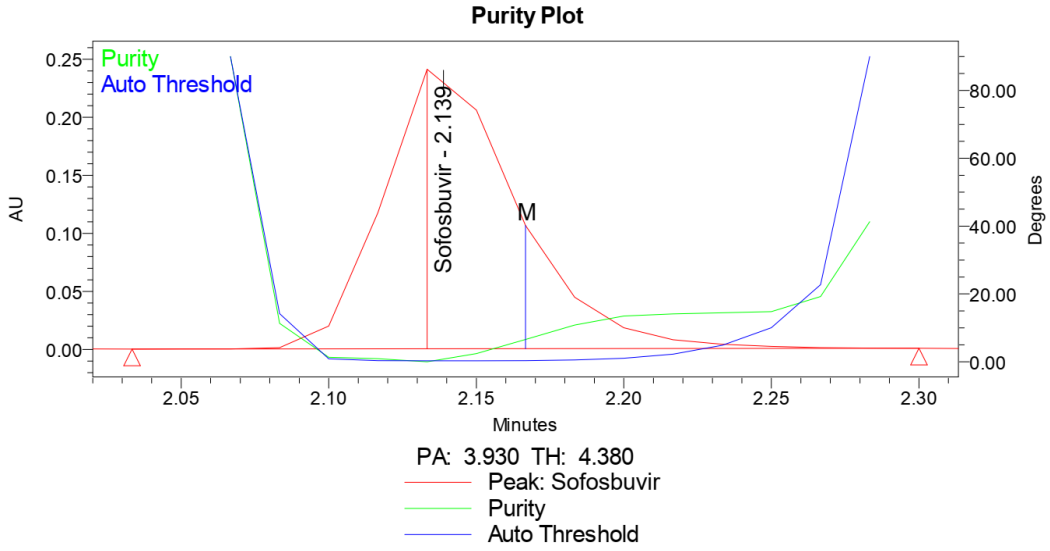

Figure 8: Purity plots for Acid Condition for Sofosbuvir

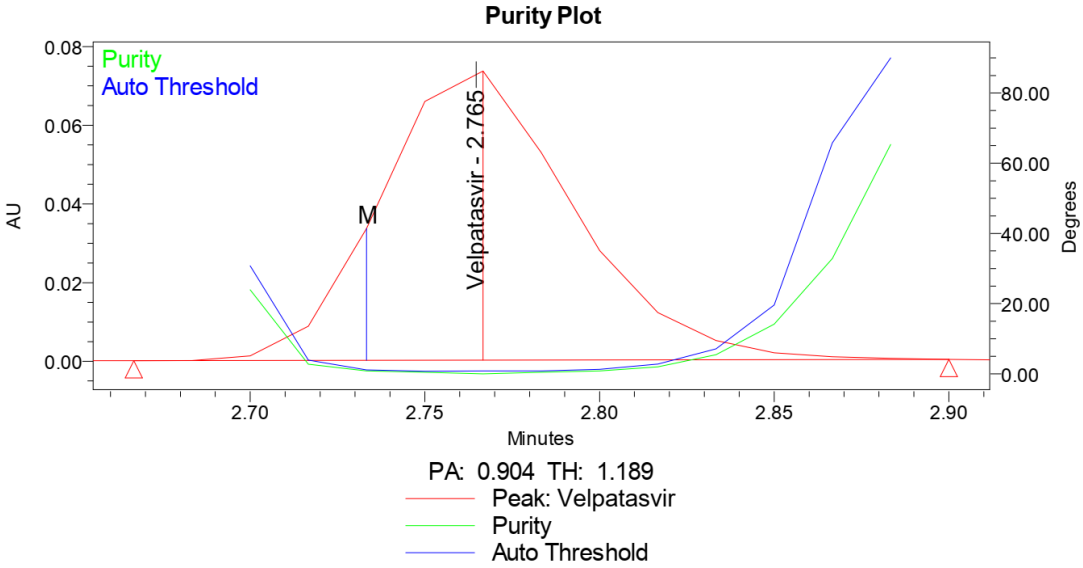

Figure 9: Purity plots for Acid Condition for Velpatasvir.

Assay: Velpanat Tablet, bearing the label claim Sofosbuvir 400mg, Velpatasvir 100mg. Assay was performed with the above formulation. Average % Assay for Sofosbuvir and Velpatasvir obtained was 99.71% and 99.73% respectively.

Table 11: assay data

	Sofosbuvir			Velpatasvir		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	7313676	7383746	100.13	3665437	3623547	99.18
2	7376363	7383633	100.13	3657466	3653646	100.00
3	7384364	7304363	99.05	3627364	3627364	99.28
4	7384364	7376264	100.03	3694363	3638265	99.58
5	7304736	7386364	100.16	3628466	3623747	99.18
6	7393746	7376364	100.03	3604746	3623747	99.18
Avg	**7359542**	**7368456**	**99.92**	**3646307**	**3631719**	**99.40**
Stdev	**39477.9**	**31674.1**	**0.43**	**32262.4**	**12136.7**	**0.3**
%RSD	**0.5**	**0.4**	**0.43**	**0.9**	**0.3**	**0.3**

Assay was calculated by the formula:

		AT	WS	1	100	10	P	FV		
	% Assay = --------X--------X-------X------- X -------X --------X ----------								X 100	
		AS	100	10	1	1	100	L.C		
AT		Average Peak area of sample in test solution								
AS		Mean peak area of sample in standard solution								
WS		Weight of drug working standard taken in mg								
P		Assay of drug working standard in % on dried basis								
L.C		Label Claim								

Figure 10. Assay formula

Conclusion:

The tests revealed that the novel suggested method for simultaneous estimation of Sofosbuvir and Velpatasvir is easy, precise, and accurate. Its high resolution, shorter retention duration, and separation of degradants contribute to its effectiveness. The proposed approach is cost-effective and suitable for standardised assessments in the pharmaceutical industry.

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