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Simultaneous Chromatographic Analysis of Simeprevir and Sofosbuvir in Combined Formulation

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ABSTRACT

New RP-HPLC method have been developed for simultaneous analysis of simeprevir and sofosbuvir in pharmaceutical formulation and applied to stability studies of drugs. The analytes were quickly eluted using a phosphate buffer at pH 3.50 and acetonitrile in a 25:75 %v/v ratio on a Hypersil BDS C18 column measuring 150 x 4.6 mm with a particle size of 5 µm. The detection was facilitated using a PDA detector at a wavelength of 253 nm. The analytes went through chromatography at a steady flow rate of 1 mL/min. Simeprevir and sofosbuvir were eluted at 2.1 min. and 5.3 min., respectively, demonstrating good resolution. The method was validated in accordance with ICH guidelines. The linearity range of simeprevir was determined to be 25 - 75 µg/mL, while for sofosbuvir, it was found to be 10 - 30 µg/mL. The % RSD values (< 2) in precision studies indicate the method's reproducibility. The percentage recoveries were 100.1% for simeprevir and 99.36% for sofosbuvir, both of which were found to be within the acceptable limits. The validated method was effectively utilized for the assay of formulation and stability studies of drugs under various stress conditions..

Keywords: *Simeprevir, sofosbuvir, RP-HPLC, method development, validation, stability studies*

1. INTRODUCTION

Simeprevir is a “non-structural protein 3 and 4A (NS3/NS4A) inhibitor of the hepatitis C virus (HCV) protease complex. It's a second-generation protease inhibitor that's taken orally once a day with food. It is used to treat HCV genotype 1 infection in patients with compensated liver disease. Simeprevir chemical structure is (1R,4R,6S,7Z,15R,17R)-N-cyclopropylsulfonyl-17-[7-methoxy-8-methyl-2-(4-propan-2-yl-1,3-thiazol-2-yl)quinolin-4-yl]oxy-13-methyl-2,14-dioxo-3,13-diazatricyclo[13.3.0.04,6]octadec-7-ene-4-carboxamide and shown in Figure 1 [1].

Sofosbuvir is a nucleotide prodrug with potential HCV inhibiting activity that prevents viral multiplication by inhibiting the NS5B polymerase. Sofosbuvir chemical structure is “propan-2-yl (2S)- 2-[[[(2R,3R,4R,5R)- 5-(2,4-dioxopyrimidin-1-yl) -4-fluoro- 3-hydroxy- 4-methyloxolan- 2-yl] methoxy-phenoxyphosphoryl]amino]propanoate and shown in Figure 2 [2].

The USFDA has approved the grouping of simeprevir (Olysio) and sofosbuvir (Sovaldi) are antiviral drugs to treat chronic genotypes of HCV [3-4]. A drug combination widely used and both are oral medications used to treat hepatitis C. They are effective against hepatitis C genotypes 1, 2, 3, 4, 5, and 6 [5].

After literature we found limited analytical methods such as spectrophotometric, spectroflurimetric and chromatographic methods for estimating simeprevir and sofosbuvir alone and in combination with other medicines have been disclosed. There is a need of sensitive and rapid analytical method for quality control of these drugs. Hence the present investigation was done to optimize the chromatographic conditions for concurrent analysis of simeprevir and sofosbuvir in bulk and pharmaceutical formulation.

CHEMICALS AND REAGENTS

The reference standards of simeprevir and sofosbuvir were procured from Hetero Laboratories, Hyderabad, India. A local pharmacy supplied marketed formulations (Olysio and Sovaldi) with label claims of 150 mg of simeprevir and 400 mg of sofosbuvir per pill. The mobile phase solvents were supplied from Merck in Mumbai, India. Premixed mobile phase was used as diluent throughout the study.

2. METHODOLOGY

Instrumentation

The Alliance Waters HPLC (2695) with a PDA detector and connected to Empower 2 software. Hypersil BDS C18 (150 x 4.6 mm, 5 µm) column at 30 ºC, auto sample injector (10 µL) was used for the analysis of drugs. Digital weighing balance (Satorius, BSA224SCW), Digital pH meter (Lab India, AD102U), Ultra sonicator (Spincotech, RK 106), Hot air oven (bio-techniques, India) were utilized for this work [6-8].

Chromatographic conditions

Chromatographic separation was achieved by Hypersil BDS C18 (150 x 4.6 mm, 5 µm) column at a 30 °C constant column oven temperature with isocratic elution mode. The mobile phase at a flow rate of 1.0 mL/min is composed of acetonitrile and phosphate buffer at pH 3.5 in a ratio of 75:25 % v/v. The mobile phase was degassed and filtered prior to use. The injection volume was 10 µL, and the PDA detector operated at a wavelength of 253 nm [9].

Preparation of standard solution

Divided 50 mg sofosbuvir and simeprevir into 50 mL volumetric flasks, dissolved, and filled with mobile phase. To achieve a concentration of 20 µg/mL simeprevir and 50 µg/mL sofosbuvir, dilute 2.0 mL and 5.0 mL standards in a 100 mL volumetric flask with mobile phase [10].

Preparation of sample solution

Each 20 of Olysio and Sovaldi tablets were measured and powdered. 150 mg simeprevir and 400 mg sofosbuvir tablet powder was carefully weighed into a 250 mL volumetric flask. Add 150 mL of mobile phase to the flask, sonicate for 10 minutes, and fill to the mark. It underwent 0.45 µm filtering. Before analysis, 3 mL of this solution was combined with mobile phase in a 100 mL volumetric flask [11-14].

Method validation

The presented method's system applicability, precision, specificity, linearity, accuracy, LOD, LOQ, and robustness have all been verified according to ICH Q2 (R1) requirements [15-16].

System suitability

The system suitability test was used to monitor the efficiency of the improved technique. It was done six times using a freshly produced working standard solution injected into HPLC under optimum conditions. Analyte peak retention duration, resolution, theoretical plate number, peak size, and tailing factor were examined chromatographically [6-8].

Precision

The percentage of RSD within one day and day to day of the suggested method fluctuation was calculated by measuring the analyte quantity in the standard solution from six replicate preparations. Results have been tabulated [6-8].

Specificity

The method's specificity was determined by comparing the chromatograms of blank (mobile phase) and placebo solutions to the chromatogram of the test solution (analytes in mobile phase). The placebo solution contains all of the excipients usually employed in the production of tablet dosage forms [6-8].

Linearity

The linearity test was carried out for 5 concentrations ranging from 50% to 150 percent of the test concentration. Linearity curves for methods with sample concentration to peak area were drawn, and the y-intercept and slope of the curve were calculated [6-8].

Accuracy

The method's accuracy was measured by the recovery of a standard spiked to the sample's target concentration at 50, 100, and 150 %. The medicine recovery percentage was calculated from the chromatogram peak area [6-8].

LOD and LOQ

The basis of the formula suggested in ICH Q2 guidelines. LOD = 3.3σ/s and LOQ = 10σ/s [6-8].

Robustness

Intentionally altering the chromatographic conditions and monitoring capability of the % RSD of retention times [6-8].

Assay of tablets

Several tagged analytes in tablet dose form were analyzed simultaneously using the suggested approach. Two commercially available dosage forms, Olysio and Sovaldi, as well as an in-house tablet formulation were subjected to the assay. Before diluting with mobile phase to achieve an appropriate concentration within the linearity range, the drugs were first extracted into the mobile phase using an ultrasonicator. The solution was then made in the same way as described above (the sample preparation stage). Using the improved approach, triplicate solutions were analyzed to find the percentage of drug content in each analyte. The peak areas were then calculated [13-14].

Forced degradation studies

Forced degradation studies on drugs were steered as per ICH guidelines. Stress studies were employed on simeprevir and sofosbuvir by exposing a stock solution of 1.6 mg/mL sofosbuvir and 0.6 mg/mL simeprevir with 3 mL of 0.5 N HCl (acid hydrolysis), 3 mL of 0.5 N NaOH (base hydrolysis), 3 mL of 5 % H_2O_2 (oxidative degradation), 60 °C (thermal stress) and UV 254 nm (photo stress) for 24 h. These solutions were diluted with mobile phase and the chromatograms were obtained to test the sample's stability after it was introduced into the system [13-14].

3. RESULTS AND DISCUSSION

Optimization of chromatographic parameters

In order to achieve HPLC separation of sofosbuvir and simeprevir, initial trials were carried out with the goal of determining appropriate and optimal chromatographic conditions. Different chromatographic parameters, such as mobile phase and their proportions, different columns were carefully studied.

The best separation of the analytes under isocratic conditions, probable combinations of buffers with variable pH values and organic solvents were tested on different columns and the method was finalized; chromatogram was illustrated in fig. 1.

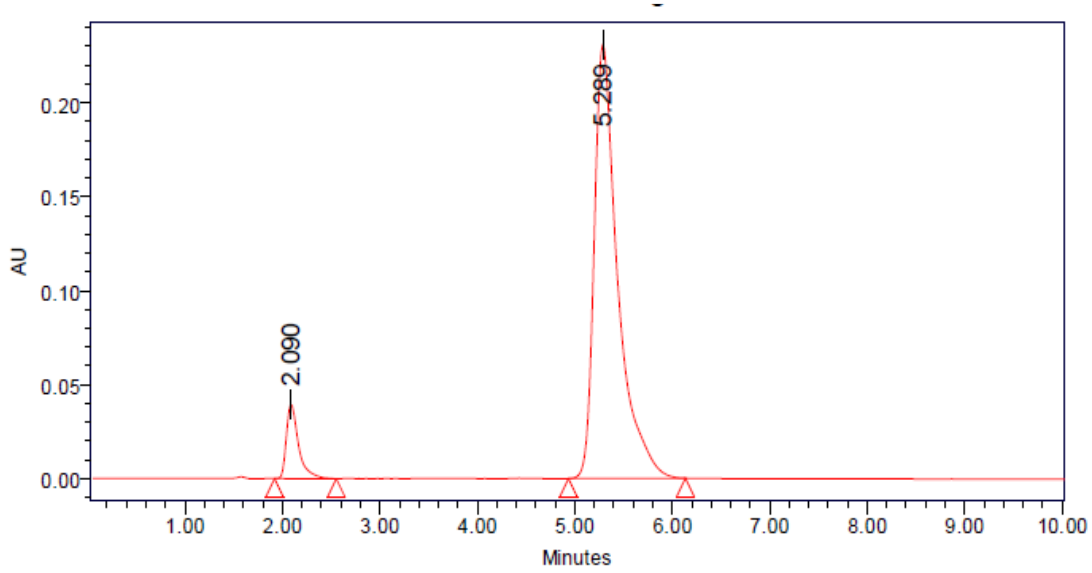

Fig. 1: HPLC peaks of simeprevir and sofosbuvir under optimized conditions

Method validation

System suitability

Plate count, tailing, retention, and resolution are indications of system suitability criteria. Data was collected from five duplicate injections of standard drug solution during the testing. System suitability parameters for the proposed method were publicized in Table 1 and found that all of the parameters were within the acceptable range [17-19].

Table 1: Results of system suitability test

S. No.	Simeprevir			Sofosbuvir			
	Rt (min.)		USP Plate Count	Rt (min.)	Tailing factor	USP Plate Count	Resolution
1	2.089	1.32	5836	5.338	1.44	8986	6.8
2	2.091	1.44	5705	5.312	1.45	8797	6.3
3	2.075	1.36	5864	5.299	1.46	8418	6.2
4	2.095	1.38	5764	5.314	1.43	8876	6.3
5	2.078	1.41	5891	5.346	1.39	8688	6.5
6	2.087	1.35	5756	5.334	1.41	8745	6.2
Specification	-	<1.5	>2000	-	<1.5	>2000	>2.0

Linearity

The method showed linearity in the range of 10 - 30 µg/mL for sofosbuvir and 25-75 µg/mL for simeprevir. The linear regression equation for sofosbuvir was Y = 339866 X -70161, with a correlation coefficient of 0.999, and for simeprevir it was Y = 9410 X + 1280, with a correlation coefficient of 0.999.

Precision

Six duplicate injections of mixed standard solution with concentrations of 20 µg/mL and 50 µg/mL of sofosbuvir and simeprevir, respectively, were used to test the accuracy of the established method in terms of repeatability. Determined the RSD (%) values of the findings corresponding to the peak area and retention time and the data obtained for intra-day and inter-day precision studies were given in the Table 2. In terms of retention times and peak areas, the intra-day and inter-day chromatograms are identical. No significant difference (between intra-day and inter-day precision) is observed. The % RSD values for the intra-day and inter-day studies were both less than 2.0, indicating that the suggested approach is repeatable.

Accuracy

After spiking with additional standard drug at three different levels (50%, 100 %, 150 %) to the sample solution containing 20 µg/mL and 50 µg/mL of sofosbuvir and simeprevir, respectively, the proposed method was used for extraction and subsequent estimation of simeprevir and sofosbuvir from pharmaceutical formulation. Three chromatograms of each spiked concentrations are recorded for comparison and found that the retention times remained same, but peak areas proportionally changed. The findings were tabulated in Table 2. The % recovery was in between 98.0-102.0 % and %RSD was shown to be below 2.0 %, suggesting accuracy of proposed method.

Specificity

Retention times of simeprevir and sofosbuvir were 2.09 and 5.33 min respectively. In this method, there were no interference peaks in the blank and placebo during the retention times of these drugs. However, the formulation and standard chromatograms gave characteristic peaks for simeprevir and sofosbuvir. Thus, the specificity of proposed analytical method was ensured.

Sensitivity

The ICH Q2 recommendations algorithms LOD values were found to be 1.05 µg/mL and 2.36 µg/mL for sofosbuvir and simeprevir respectively.

The LOQ value was found to be 3.54 µg/mL and 8.39 µg/mL sofosbuvir and simeprevir respectively. These values indicate the adequate sensitivity of proposed method.

Robustness

To test the method's adaptability, deliberate changes in optimum process parameters such as column temperature, flow rate, and buffer pH were made, and the results are shown in Table 2. For all variables, the percent RSD was less than 2, indicating that the approach was reliable.

Table 2: Results of method validation

Parameter	Sofosbuvir			Simeprevir		
Precision						
Repeatability	Peak area	SD	RSD (%)	Peak area	SD	RSD (%)
Intra-day	6759742	47955	0.71	470842	1750	0.37
Inter-day	6800127	57491	0.85	470351	2028	0.43
Accuracy						
Level of recovery	Spiked amount (µg/ml)	Recovery %	RSD (%)	Spiked amount (µg/ml)	Recovery %	RSD (%)
50%	10	99.3	0.28	25	100.1	0.36
100%	20	99.6	0.32	50	100.7	0.32
150%	30	99.3	0.33	75	100.0	0.41
Robustness						
Factor	% RSD of Retention time			% RSD of Retention time		
Flow rate (0.9 mL/min)	0.23			0.24		
Flow rate (1.1 mL/min)	0.29			0.15		
Temperature (28 ^{0}C)	0.13			0.14		
Temperature (32 ^{0}C)	0.11			0.15		
Buffer (pH 3.3)	0.22			0.20		
Buffer (pH 3.7)	0.24			0.25		

RSD- Relative standard deviation (n=3); SD- standard deviation

Assay of tablet formulation

An assay of currently available tablet dosage forms (Olysio® and Sovaldi®) was used to assess the proposed method and in-house tablets containing 150 mg of simeprevir and/or 400 mg of sofosbuvir, given in Table 3. Sample and standard chromatograms showed identical analytical peaks at their retention times and indicated that selected drugs are clearly separated and showed no interfering peaks, due to excipients (Fig. 2). Hence, the proposed method is selective for determination of title analytes. The assay findings were compared to the labelled quantities of the related substances and the % assay of simeprevir and sofosbuvir was found to be in the range of 99-100%.

Table 3: Assay Results of sofosbuvir and simeprevir in tablets

Formulation	Drug	Label claim (mg)	Amount found (mg)	% Assay
In-house tablets	Sofosbuvir	400	399.1	99.75
	Simeprevir	150	149.32	99.55

Olysio®	Simeprevir	150	149.78	99.85
Sovaldi®	Sofosbuvir	400	399.6	99.90

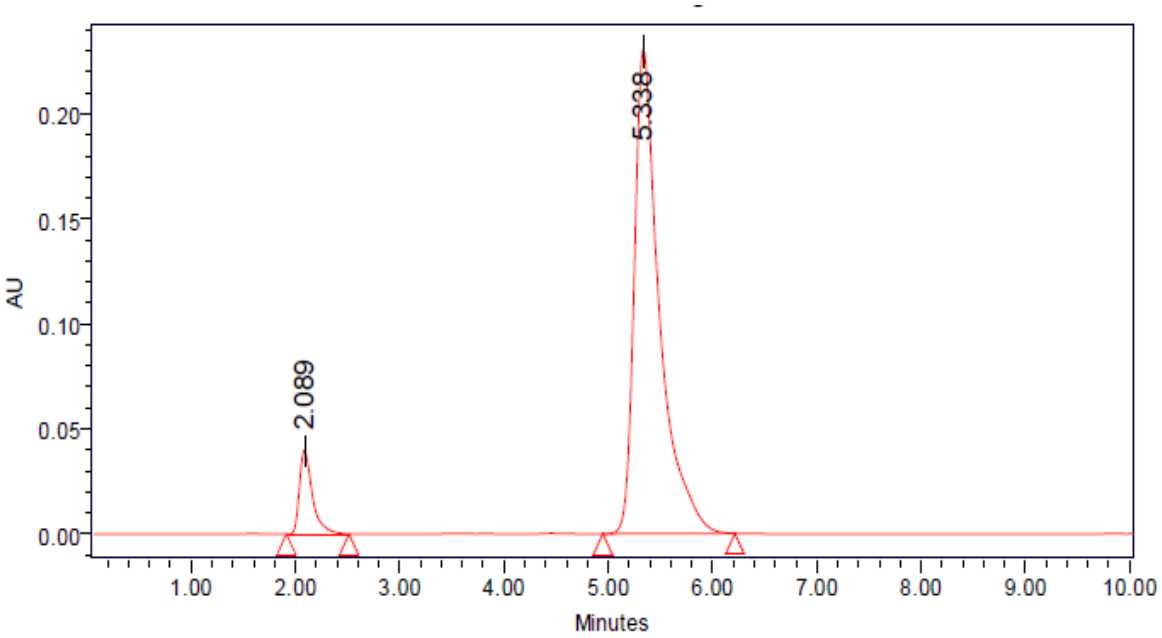

Fig. 2: Chromatogram of formulation (tablets) sample

Forced degradation studies

To prove the method's stability-indicating capability, forced degradation tests were carried out. The developed RP-HPLC method was applied to study the behaviour of drugs, under various stress conditions such as acidic, alkaline, oxidative, photolytic and thermal. Summary of degradation studies is reported in Table 4. The projected method was found highly specific for determination of simeprevir and sofosbuvir, without interference of degradants and the current method is appropriate to resolve the degradation products. It can then be used to analyse target drug samples from both accelerated and long-term stability studies. The chromatograms of samples (subjected to various stress conditions) revealed well-separated analyte and degradation product peaks, with no amendment in analyte peak retention times. Significant degradation of both drugs is observed in acid, base and oxidative conditions.

Table 4: Stability study results of sofosbuvir and simeprevir

Stress condition	Condition per 1 day	Sofosbuvir		Simeprevir	
		% Assay	% Degradation	% Assay	% Degradation
Control	-	99.5	0	99.41	0.15
Acid Hydrolysis	0.5N HCl	89.9	9.85	80.44	18.12
Basic Hydrolysis	0.5N NaOH	94.2	4.32	85.13	13.63
Thermal Degradation	60°C	99.1	0.22	99.24	0.11
Photo Degradation	UV-254nm	98.2	0.89	99.09	0.52
Oxidative Degradation	5% H_2O_2	95.7	4.11	90.22	8.22

4. DISCUSSION

Using C18 column (150 x 4.6 mm, 5 µm) and a flow rate of 0.8 mL/min were used with a basic mobile phase consisting of methanol and water at a ratio of 50:50 % v/v. The 10-minute duration did not reveal any peaks. The water and methanol ratios in a 70:30:45:55 v/v respectively. Below 10 min., the only peaks that were noticed were at 9.6 min. and 7.2 min., respectively. Accordingly, the organic phase was switched to acetonitrile using a C18 column (150 x 4.6 mm, 5 µm) with a flow rate of 1.2 mL/min and a column temperature of 30 °C. This was done to improve the peak shape and retain late eluting peaks earlier. Eluted at 1.96 and 2.26 minutes, respectively, both peaks show lesser resolution. I used orthophosphoric acid to bring the pH of the water down to 4.0 then switched to a Hypersil BDS C18 column (150 x 4.6 mm, 5 µm). A large peak was produced, and the chromatogram revealed empty peaks. The analytes showed satisfactory reactivity and resolution of around 10 when the pH of the phosphate buffer was slightly changed to 3.5 using orthophosphoric acid in a 75:25 v/v organic to buffer ratio carried out at a flow rate of 1.0 mL/min. In accordance with ICH Q2 (R1) standards, all validation criteria, including system suitability studies, accuracy, linearity, precision, and robustness, are adhered to. There is no matrix interference with the proposed RP-HPLC technique, making it suitable for testing API, dosage forms, and stability samples.

CONCLUSION

The contemporary work entails the successful chromatographic method development for analysis of fixed-dose combinational antiviral drugs. The present work has applications in real-time use in the pharmaceutical industry, because of the use of aqueous media and well defined and separated retention times. The present report facilitates the use of RP-HPLC methods for accomplishing enhanced performance such as robustness, ruggedness and allowing the scope for continual improvement during analysis of two drugs. Further, the developed analytical methods will have applications in quality control testing of studied drugs. The projected method was applied to study the stability behavior of drugs under various stress conditions and permitted the analysis of drugs, in spiked human plasma. RP-HPLC method was not reported till to date for this combination and has been the first report.

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CONFLICTS OF INTERESTS

Declared none

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