UPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND LEDIPASVIR IN TABLET DOSAGE FORM

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ABSTRACT

A simple, precise, specific and stability-indicating UPLC (Ultra Performance Liquid Chromatography) method was developed and validated for the simultaneous estimation of anti-viral drugs Simeprevir and Sofosbuvir in combined dosage form. The method was developed using SD C18 column (100 mm×2.1 mm, 1.8μm) with isocratic elution. 0.1% ortho phosphoric acid buffer and acetonitrile (60:40 v/v) were used as mobile phase with 0.2 ml/min flow rate at ambient temperature. The detection wavelength was fixed at 220 nm. The run time was within 2 min. The method was validated in terms of linearity, accuracy and reproducibility. Calibration plots were linear over the range of 37.5-225 μg/ml for Simeprevir and 100-600 μg/ml for Sofosbuvir. The Recovery was in the range of 98-102% with the relative standard deviation of less than 2% for both drugs. The mean recoveries were found to be 99.41% and 99.91% for Simeprevir and Sofosbuvir respectively. The relative standard deviation (RSD) was found to be < 2.0% for both drugs. The limit of detection and the limit of quantification for the Simeprevir were found to be 0.51 and 1.55 μg/ml respectively and for Sofosbuvir 0.50 and 1.52 μg/ml respectively. The proposed method was validated and successfully applied to the estimation of Simeprevir and Sofosbuvir in tablet dosage form.

KEYWORDS: Simeprevir, Sofosbuvir, UPLC, Validation, Stability.

INTRODUCTION

UPLC is an emerging area of analytical separation science which retains the practicality and principles of HPLC while increasing the overall interlaced attributes of speed, sensitivity and resolution. Speed and peak capacity can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC[1-2] by using fine particles. UPLC takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates[3-5] for increased speed, sensitivity and superior resolution. In this article we explored the potential of UPLC to improve the analysis of the samples that are encountered during pharmaceutical development and manufacturing. Particular emphasis has been placed on determining whether UPLC can reduce analysis times without compromising the quantity and quality of the analytical data generated compared to HPLC.

Simeprevir is a protease inhibitor for HCV NS3/4A protease, which is required for replication of the virus. Simeprevir is indicated in patient with hepatitis C virus[6] (HCV) genotype 1 for the treatment of chronic hepatitis

as a combination therapy, which includes peg interferon alfa and ribavirin.

Sofosbuvir acts against HCV and is categorized as a direct-acting antiviral agent. Sofosbuvir is nucleotide analog inhibitor, which specifically inhibits HCV NS5B polymerase. Sofosbuvir prevents HCV viral replication by binding to the two Mg^{2+} ions present in HCV NS5B polymerase's GDD active site motif. Sofosbuvir is used in combination therapy to treat chronic hepatitis C virus (HCV) infected patients with HCV genoptype 1,2,3, or 4, and to treat HCV and HIV co-infected patients.[7-9] The combination therapy includes either ribavirin alone or ribavirin and peg-interferon alfa.

Combination therapy of Simeprevir and Sofosbuvir tablets are available as Evotaz formulation. Simultaneous estimation of both drugs is highly desirable as this would allow more efficient generation of clinical data and could be more cost-effective than separate assays. To the best of our knowledge, there are few available on RP-UPLC method for Simeprevir and Sofosbuvir in combined tablet dosage form with short run time. [10-12] It is, therefore, felt necessary to develop a new method for

simultaneous determination of both the drugs with shorter run time. We intend to opt for a faster chromatographic technique UPLC, for the said study.

The chemical structures of both drugs were shown in Fig.1.
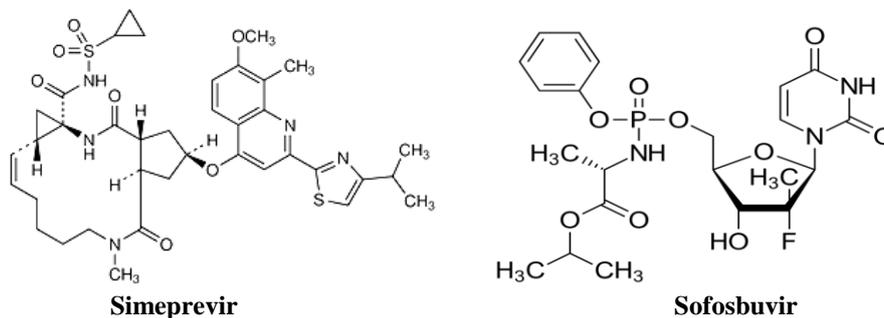
Simeprevir   **Sofosbuvir**
Fig. 1: Chemical Structures of Simeprevir and Sofosbuvir.

MATERIALS AND METHODS

Chemicals and Reagents
Pure standard samples of Simeprevir and Sofosbuvir were obtained as gifted samples from Ranchem Pharmaceuticals Ltd. and its marketed formulation in the brand name of Evotaz [Label claim containing Simeprevir 150mg and Sofosbuvir 75 mg] were procured from local pharmacy. Water (UPLC-Grade), Acetonitrile (UPLC-Grade; Rankem) and Methanol (UPLC-Grade Rankem), ortho phosphate buffer, Ortho-phosphoric acid (Rankem). All dilutions were performed in standard class-A, volumetric glassware.

Chromatographic conditions
The analysis of the drug was carried out on a Waters Acquity UPLC 2695 system (Milford, MA, USA) equipped with a binary solvent (loop capacity 10 µl), column manager composed of column oven, pre column heater, (100 mm×2.1 mm) column with 1.8 µ particle size and a photo diode array detector. Data acquisition, data handling and instrumentation control were performed by Empower software version 2.0. SD C_{18} column was used to optimize the method.

Buffer preparation
0.1% OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with UPLC grade water.

Mobile phase preparation
Prepare a filtered and degassed mixture of Buffer (pH - 2.0), 0.1% OPA: Acetonitrile (60:40%).

Diluent preparation
Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Sample preparation
5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered

by UPLC filters (4000µg/ml of Sofosbuvir and 1500µg/ml of Simeprevir).

RESULTS AND DISCUSSION

Method development
Initial trials were carried by the author in developing the proposed RP-UPLC method.

A variety of mobile phases were investigated in the development of a stability-indicating UPLC method for the analysis of Simeprevir and Sofosbuvir. A mixture of 0.1% v/v Acetonitrile (pH 2.0 adjusted with o-Phosphoric acid) (60:40, v/v) was found to be the most suitable mobile phase for ideal separation of Simeprevir and Sofosbuvir. The solvent mixture was filtered through a 0.22 µ PVDF filter and sonicated before use. It was pumped through the column at a flow rate of 0.2 ml/min. The column was maintained at an ambient temperature. The detection of the drug was monitored at 220 nm. The run time was set at 2 min. Under these optimized chromatographic conditions the retention time obtained for the drugs Simeprevir and Sofosbuvir was 0.969min and 1.289min respectively. A typical chromatogram showing the separation of the drugs is as shown in Fig.2.
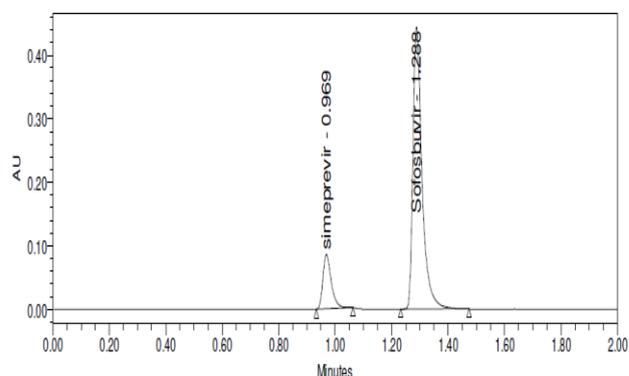
Fig. 2: RP-UPLC optimized chromatogram of Simeprevir and Sofosbuvir.

Method validation
Validation of developed and optimized method
The validation of developed method was done as per ICH guidelines which include System suitability (retention time, peak area), Precision (system precision, method precision), Accuracy, Linearity, Robustness (flow rate, wavelength, mobile phase ratio, column temperature, p^H) and Solution stability at 25ºC.

Specificity
The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method was evaluated by checking peak purity, USP tailing, plate count and resolution of Simeprevir and Sofosbuvir during study. The interference of blank and placebo with the elution of the present cited drugs solutions of diluents and placebo were injected into the chromatographic system with the mentioned

chromatographic conditions and their respective chromatograms were recorded. The peak purity of both the drugs was found satisfactory under different conditions.

Linearity
The linearity of the proposed method was accessed by calculating slope, intercept and correlation coefficient $[r^2]$ of standard curve. Simeprevir and Sofosbuvir showed a linearity range in between 37.5-225 and 100-600μg/ml respectively and the slope and intercept of the calibration plot of Simeprevir and Sofosbuvir were 1256x+1270 and 2437x+6748 with correlation coefficients obtained was 0.999 for the two drugs. The linearity results of both the drugs were shown in Tab.1 and Linearity curves of Simeprevir and Sofosbuvir were depicted in Fig.3.

Tab.1: Linearity results for Simeprevir and Sofosbuvir

Simeprevir		Sofosbuvir	
Conc.(μg/ml)	Peak area	Conc.(μg/ml)	Peak area
0	0	0	0
37.5	47315	100	246219
75	96424	200	495741
112.5	148322	300	757878
150	186699	400	976346
187.5	235682	500	1233634
225	284235	600	1456804
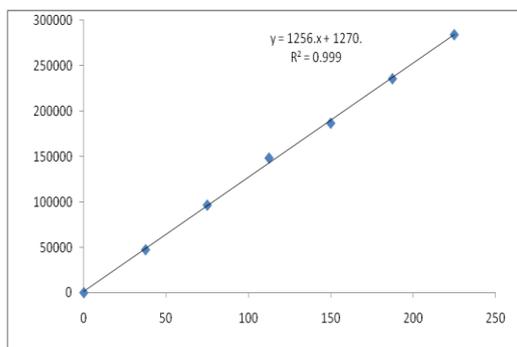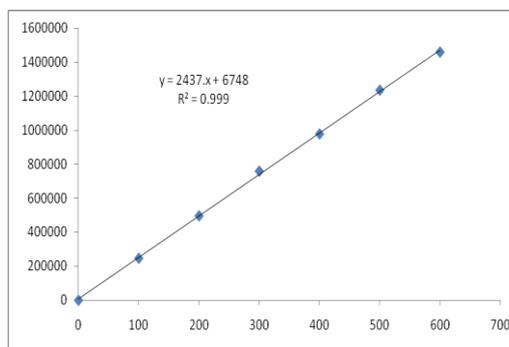
A	B

Fig. 3: Linear calibration plot for Simeprevir (A) and Sofosbuvir (B).

Sensitivity
The limit of detection (LOD) and limit of quantification (LOQ) were established at signal-to noise ratio of 3:1 and 10:1 respectively. The LOD of Simeprevir and Sofosbuvir was found to be 0.51μg /ml & 0.50μg /ml respectively. The LOQ of Simeprevir and Sofosbuvir was found to be 1.55μg /ml & 1.52μg/ ml respectively.

Precision
The precision of Simeprevir and Sofosbuvir by proposed RP-UPLC method was ascertained by replicate analysis of homogeneous samples of Tablet powder. The precision of the proposed method was checked by carrying out six independent assays of test samples. Mean, SD and an RSD (%) value of six assays was

calculated. Intermediate precision was carried out by analyzing the samples on a different day on another instrument. System precision and method precision both were under the permissible limit i.e. 1% and 2% respectively. The results were given in Tab.2 and the low % RSD values of within a day for sofosbuvir and ledipasvir revealed that the proposed method is highly precise.

Table 2: System precision table of Simeprevir and Sofosbuvir.

S. No.	Area of Simeprevir	Area of Sofosbuvir
1.	179599	968062
2.	180152	976143
3.	181370	968003
4.	182764	982584
5.	183603	986159
6.	181550	980516
Mean	181506	976911
S.D	1514.6	7601.9
%RSD	0.8	0.8

Accuracy
To assess the accuracy both the samples were studied in three different concentrations of 50%, 100% & 150% and recovery was found within the acceptance criteria i.e. 95-105% as shown in Tab. 3. % Recovery was obtained as 99.41% and 99.91% for Simeprevir and Sofosbuvir respectively. It is revealing that the developed RP-UPLC method was found to be accurate.

Table 3: Results of Accuracy for Simeprevir and Sofosbuvir.

S. No.	Accuracy level	Injection	Simeprevir % Recovery	Sofosbuvir % Recovery
1.	50%	1	99.18	98.94
		2	100.40	100.58
		3	98.80	99.43
2.	100%	1	98.28	100.44
		2	99.03	99.66
		3	100.08	100.58
3.	150%	1	99.15	100.66
		2	99.74	98.47
		3	100.07	100.44

Robustness
The robustness of the developed method was evaluated by altering few experimental conditions and evaluating the resolution between two adjacent peaks of Simeprevir and Sofosbuvir. The data results were shown in Tab.4.

Table 4: Robustness data for Simeprevir and Sofosbuvir.

S. No.	Condition	%RSD of Simeprevir	%RSD of Sofosbuvir
1	Flow rate (-) 1.1ml/min	0.8	0.9
2	Flow rate (+) 1.3ml/min	0.8	0.6
3	Mobile phase (-) 35B:65A	0.9	1.0
4	Mobile phase (+) 45B:55A	0.9	0.9
5	Temperature (-) 25°C	0.4	0.4
6	Temperature (+) 35°C	0.6	0.5

Formulation assay
The validated method was applied on commercially available Evotaz tablets. The results of the assay undertaken yielded 99.70% and 100.79% of the label claim for Simeprevir and Sofosbuvir. Results of the assay indicated that the method is quite selective for the analysis of Evotaz without interference from the excipients used to formulate and produce these tablets.

Stability studies
A stability-indicating assay method should accurately measure the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities. . It is also mentioned that forced decomposition studies (stress testing) at temperatures in 10 °C increments above the accelerated temperatures, extremes of pH, under oxidative and photolytic conditions should be carried out on the drug substance and drug product so as to establish the inherent stability characteristics and degradation pathways to support the suitability of the proposed analytical procedures. In this article author performed different types of degradation studies and results shown in Tab.5 and shown in Fig.4.

Table 5: Degradation studies data.

Type of degradation	Simeprevir			Sofosbuvir		
	Area	**% recovered**	**% degraded**	**Area**	**% recovered**	**% degraded**
Acid	173151	95.19	4.81	930592	95.39	4.61
Base	177073	97.35	2.65	950353	97.42	2.58
Peroxide	178758	98.27	1.73	958733	98.28	1.72
Thermal	180931	99.47	0.53	969822	99.41	0.59
UV	180886	99.44	0.56	970139	99.45	0.55
Water	180945	99.47	0.53	967073	99.13	0.87
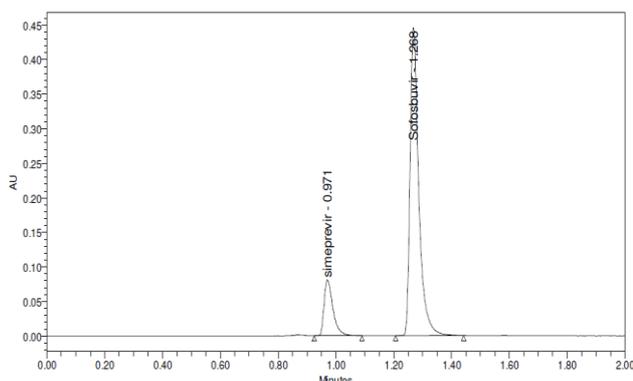
Fig. 4: Acid degradation chromatogram.

CONCLUSION

It was concluded that the proposed new RP-UPLC method developed for the quantitative determination of Simeprevir and Sofosbuvir in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phase was simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine estimation of Simeprevir and Sofosbuvir depending upon the availability of chemicals and nature of other ingredients present in the sample. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. Application of this method for estimation of Simeprevir and Sofosbuvir from tablet dosage form and stressed samples showed that neither the degradation products nor the excipients interfered in the estimation of drug. Hence, this method was specific, stability-indicating and can be successfully used for the estimation of drug in bulk and pharmaceutical dosage form.

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