Egypt. J. Anal.Chem, Vol. 28, PP. 22-27 (2019)

Rapid High-Performance Liquid Chromatographic Method for Determination of Simeprevir In Pharmaceutical Dosage Form

Ahmed A. Youssef, Yousry M Issa & Kareem M. Nabil

[1]Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt

[2]Pharmagene Specialized Analytical Services, Giza, Egypt

Summary: Rapid, simple and selective HPLC method was developed and validated for determination of simeprevir (SIM) in pharmaceutical capsules dosage form. Chromatographic separation was achieved by using RP-C18 column as stationary phase and acetonitrile (ACN): potassium phosphate, 70:30 (v/v), as mobile phase in a flow rate 1.5 mL/min. The effluent was detected using fluorescence detector. The method showed a linear range 10-2000µg/mL with excellent correlation coefficient> 0.999. Parameters of validation were studied to ensure obtaining accurate and precise data. The developed method was applied successfully to the analysis and determination of SIM in OLYSIO® (150 mg/capsule) as pharmaceutical dosage form.

Introduction

Simeprevir (SIM) with IUPAC name 2R,3aR,10Z,11aS,12aR,14aR)-N-(Cyclopropylsulfonyl)-2-{[2-(4-isopropyl-1,3-thiazol-2-yl)-7-methoxy-8-methyl-4-quinolinyl]oxy}-5-methyl-4,14-dioxo-2,3,3a,4,5,6,7,8,9,11a,12,13,14,14atetradecahydrocyclopenta[c]cyclopropa[g][1,6] diazacyclotetra-decine-12a(1H)-carboxamide was recently discovered as antiviral agent for treatment of hepatitis C, genotype 1 HCV.[1,2] Due to high number of infected people around the world by hepatitis C so it's important to develop appropriate analytical method for determination of such drug in pharmaceutical dosage form. The published papers for determination of SIM, which is few by the way, involve using LC/MS/MS,[3] which is costly technique. The present work involves determination of SIM using new, rapid and selective HPLC-Fluorescence method with unique detection and successfully applied for analysis of pharmaceutical dosage form.

Experimental

Chemicals and reagents

Simeprevir (SIM) (Fig. 1) was purchased from Alsachim, Strasbourg, France. Sodium dihydrogen phosphate, dipotassium hydrogen phosphate and potassium dihydrogen phosphate was purchased from TechnoPharm Chem (India). Potassium hydroxide was purchased from Sigma Aldrish (Spain), 85% phosphoric acid was purchased from SD fine (India), N,N-Dimethylformamide was purchased from BDH laboratory suppliers (England) and HPLC grade ACN was purchased from Scharlab S.L. (Spain).

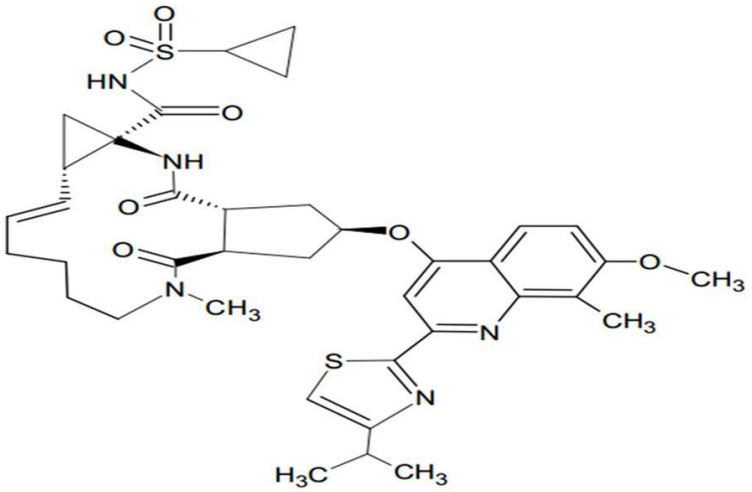

Fig. (1). Structure of simeprevir

Instrumentation and equipment

The analysis was carried out on Agilent HPLC Systems using HPLC 1200 series with autosampler for injection, pump, and degasser for delivering and degassing mobile phase into the system and 1100 series HPLC fluorescence detector for detection. The chromatographic separation was performed on Xbridge (150 x 4.6 mm, 5 µm particle size) and Atlantis (150 x 4.6 mm, 5 µm particle size) as stationary phase. Ultrasonication Barnstead aquawave (USA) used for degassing solutions. GHP membrane filter PALL corporation with 0.45 µm particle size (USA).

Chromatographic conditions

Different mobile phases were used such as 0.02 mol/L sodium or potassium dihydrogen phosphate and dipotassium hydrogen phosphate, as the aqueous constituent in the mobile phase while ACN was used as the organic constituent. The pH of the mobile phase was adjusted to 6.5 using 85% phosphoric acid and 1.0 mol/L potassium hydroxide. Two flow rates of the mobile phase have been tested, 1.0 and 1.5 mL/min, on Xbridge (150 x 4.6 mm, 5 µm particle size) as stationary phase at 30˚C. The injection volume was 1.0 µL. The fluorescence detection was used at 337 and 420 for excitation and emission respectively, which found that those wavelengths gave most suitable signal for a suitable sensitivity and selectivity.

Preparation of mobile phase buffer

A phosphate buffer solution, 0.02 mol/L, of pH 6.5 was prepared by weighing accurately 2.72 g of potassium dihydrogen phosphate followed by dissolving in deionized water. The pH was adjusted by using 1.0 M potassium hydroxide and then the total volume was completed to the mark in 1.0 liter volumetric flask.

Preparation of standard solutions

A stock standard solution of SIM was prepared in HPLC grade ACN to have a final concentration 750 µg/mL and then stored at -20˚C. Working solution of concentration 75 µg/mL was prepared by successive dilution using the same solvent mixture of the mobile phase. All SIM working solutions for intra-day and inter-day studies were prepared by further dilution using 50% ACN.

Application for pharmaceutical formulation

Transfer 5 capsules of OLYSIO® (150 mg SIM/capsule as label claim), to a 1.0 liter volumetric flask then 30 mL deionized water was added and shake till disintegration. Add 100 mL N,N- Dimethylformamide and sonicate for 15 min then add 600 mL ACN and sonicate for 45 min. The total volume was completed to the mark with ACN to have a concentration of 750 µg/mL. This solution was diluted to have a concentration of 75 µg/mL SIM and then injected six times to HPLC after filtration through a 0.45 µm GHP membrane filter. Standard solution of 75 µg/mL was injected against the previous solution.

Results and discussion

Optimization of chromatographic conditions

Very simple isocratic chromatographic separation method was developed for determination of SIM. ACN containing different types of buffers (pH 6.5) were tested, such as sodium phosphate, dipotassium hydrogen phosphate and potassium dihydrogen orthophosphate,to ensure obtaining a short run time and good peak symmetry. The results indicated that 0.02mole/L of Potassium dihydrogen orthophosphate is the optimum buffer with mobile phase composition, buffer:ACN, 30:70 (v/v), with respect to peak shape and symmetry.

Two different stationary phases have been investigated, Atlantis (150 x 4.6 mm, 5 µm particle size) and Xbridge (150 x 4.6 mm, 5 µm particle size). Although the results showed comparable retention time for both types, Xbridge had more acceptable peak shape at 3.7 min .

Detection of SIM was carried out by scanning the excitation and emission wavelengths over the range 200-500 nm using florescence detector. The results showed that 337 and 448 nm is the most acceptable excitation and emission wavelengths as indicated by the low background emission and non-interfering peak SIM peak.[(4)]

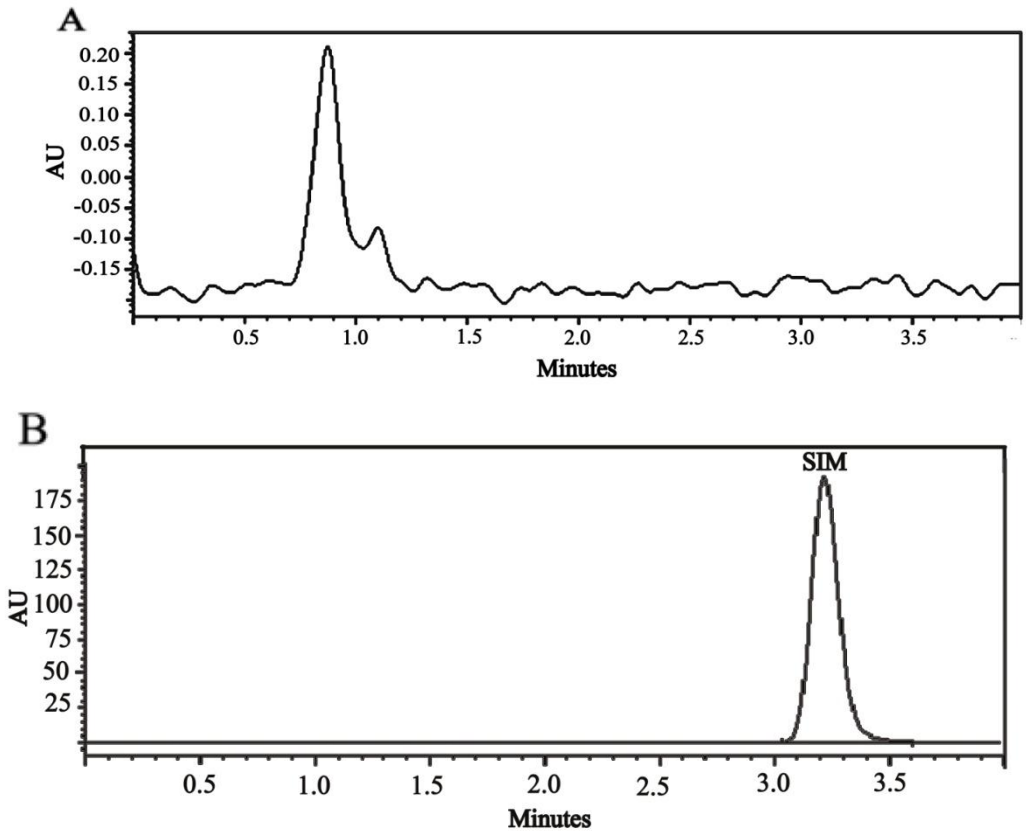

Fig. (2). SIM free (A) and spiked samples at 60 ng/mL (B).

Method validation

Validation of the present method was based on FDA guidelines.[5] The following parameters have been studied:

The linearity of the developed method was investigated over the concentration range 10-2000µg/mL. The calibration curves resulting from direct injection of the mobile phase containing different concentrations of standard SIM into HPLC system were subjected to regression analysis. The regression equation was Y=20.62X-20.76, indicated high linearity of the calibration graphs as showed by the correlation coefficient values(> 0.999).

The selectivity of the method was studied by injection of drug free capsule excipients solution.[6] There were no interfering peaks from injection of the extraction solution of the capsule excipients at the retention time of SIM. The method was selective for SIM (Fig. 2).

Reproducibility was studied by injection of 75 µg/mL of SIM six times using the optimized conditions. The results showed that RSD was 0.22 % which ensure high precision of the present method.

Standard addition method was used for solutions preparation at level of 80, 100 and 120% level of the injected test concentration,[7] 75 µg/mL. Each concentration was prepared and injected three times at the same day and at three successive days, for intra- and Inter-day studies respectively. The results show that the accuracy are within the range 102.84-104.57% with RSD

0.29-0.61% and 104.24-105.44% with RSD 0.77-1.45%, for intra-and inter-day, respectively (Table 1).

Table 1 Intra-day and Inter-day data for determination of SIM

Nominal concentration (µg/mL)	Intra-day				Inter-day			
	Found[a] (µg/mL)				Found[b] (µg/mL)			
	Mean[a]	SD	Accuracy (%)	RSD (%)	Mean[b]	SD	Accuracy (%)	RSD (%)
60	61.09	5.68	102.84	0.45	61.92	18.4	104.24	1.45
75	77.64	4.65	104.57	0.29	78.29	12.42	105.44	0.77
90	91.83	11.52	103.06	0.61	92.73	30.28	104.08	1.58

a) Mean of 3 determinations during day, Intraday.

b) Mean of 3 determinations during three days, Inter-day.

The sensitivity of the present method was studied by evaluating the limit of detection (LOD) and the lower limit of quantification (LLOQ) [8] for the standard SIM. The results showed that, the LOD and LLOD were 3.0, 10.0 µg/mL, respectively, which indicates the high sensitivity of the developed method (Table 2).

Table 2 Analytical parameters for quantitation of SIM.

Parameter	Characteristic
Linearity range (µg/mL)	10-2000
Slope	20.62
Intercept	-20.76
Correlation coefficient	1
LOD (µg/mL)	3
LOQ (µg/mL)	10

Application for pharmaceutical formulation

The present method was applied for determination of SIM in OLYSIO® as capsule formulation. The claimed concentration of SIM was 150 mg/Capsule. The results of the present method showed that the average concentration of SIM is 149.22 mg/capsule with recovery and RSD values 99.48% and 0.93, respectively (Table 3).

Table 3 Assay of SIM in OLYSIO® capsules.

Label Claim(mg)	150
Amount found (mg)±SD	149.22±1.39
% label claim	99.48
% RSD (n=6)	0.93

Conclusion

The developed method allows determination of SIM in pharmaceutical dosage form by unique, selective and sensitive detection by HPLC-Fluorescence. This study was validated successfully and able to show linearity in the range 10-2000µg/mL (with low LLOQ). Also can be applied for routine analysis due to short run time.

Acknowledgments

Authors greatly thank Dr. Mohamed Rashed, CEO of Pharmagene (CRO), Giza, Egypt for providing such help, assistance and support during the research.

References

1. https://en.wikipedia.org/wiki/Simeprevir, accessed on 2017-5-30.
2. https://www.olysio.com/chronic-hepatitis-c-treatment/what-is-olysio, accessed on 2017-5-30.
3. A. Ariaudoa, F. Favataa, A. De Nicolòa, M. Simielea, L. Pagliettia, L. Boglionea, C. S. Cardellino , C. Carcieri , G. Di Perri and A. D'Avolio . J. Pharm. Biomed. Anal., **125**, 369–375 (2016).
4. I. Khana, Z. Iqbal, A. Khanb, M. Hassana, F. Nasira, A. Razadand, L. Ahmad, A. Khan and M. Akhlaq Mughal . J Chromatogr B., **1033**, 261–270 (2016).
5. FDA US. Guidance for Industry. Method Validation. Rockville, MD,USA: US Department of Health and Human Services, US FDA/Center for Drug Evaluation and Research (2001).
6. A. Acheampong, W. Gyasi, G. Darko, J. Apau, and S. Addai-Arhin. Springer plus, **5**, 625-632 (2016).
7. A. Sruthi, P. Tejaswi, N. Thanuja, D. Sudheer Kumar and P. VivekSagar. J. pharmacy research 6, 140-144 (2013).
8. A. M. Atta, S. Mostafa, I. Salama and M. S. Gomaa. . IJPCBS, **6**, 48-61 (2016).