Development and Validation of Spectrophotometric Method for Assay Determination and In Vitro Dissolution Studies of Sofosbuvir Tablets

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Summary: In vitro dissolution of sofosbuvir 400 mg tablets dosage form was performed, using USP dissolution apparatus type-II (paddle type), at 75rpm ± 4 %, and 900mL ± 1%, 0.05 M phosphate buffer pH 6.8 ± 0.05 equilibrated at 37.0 ± 0.5ºC as dissolution medium. Percentage of dissolved sofosbuvir as a function of time was determined using the straight line equation and linear regression using zero order and first order ANOVA based kinetics model. Comparative dissolution studies on two different generic brands A and B was performed comparing the drug release profile with innovator brand Sovaldi 400 mg tablets. The comparison of dissolution profiles was evaluated using model independent approach. The values of similarity factor f_2 were (4 and 3) and the difference factor f_1 were (64 and 50) for both generic products A and B respectively. A simple and precise spectrophotometric method was developed for estimation of sofosbuvir in dissolution medium based on spectrophotometric detection at wavelength 262 nm. The specific absorbance (A = 1%) of sofosbuvir was 178.5 ± 4% and Beer's law was obeyed in the concentration ranges 4µg mL^{-1} to 48µg mL^{-1}. The method was validated appropriately for accuracy, precision, linearity, and specificity, according the guidelines of United State Pharmacopoeia and International Conference on Harmonization. The calibration curve was linear with correlation coefficient (r > 0.9999) and there was no spectral interference from excipients present in the tablets dosage form. This method is precise, rapid and specific for determination of sofosbuvir in tablets dosage form and successfully applied for assay determination and in vitro dissolution studies.

Keywords: Sofosbuvir, GS-7977, Spectrophotometric, In vitro Dissolution.

Introduction

Hepatitis C Virus (HCV) infections is a substantial global health problem and its prevalence is estimated 3 % (170 to 200 million) of the world population [1-3]. HCV is the leading cause of serious liver diseases, like fibrosis, cirrhosis, hepatocellular carcinoma and liver-transplantation. It is estimated that 350,000 to 500,000 patients dying each year from liver disease associated with HCV infections [4-6]. Before the recent development of direct acting antiviral (DAA) drugs in 2011, the HCV regimens for all major six genotypes and sub types were limited to different combination therapy of interferon and pegylated alfa interferon with rebivirin [7-8]. The efficacy and tolerability of interferon based therapy was not ideal due to unfavorable safety profile with high level of toxicities, adverse reactions and suboptimal sustained virologic response (SVR) rate [9-10]. The development of different DAA drugs for HCV opened a new area to achieve a high SVR rate, simplify and shorten the treatment duration and completely eliminate the interferon based regimens for all genotypes [11]. The regimens with first generation antiviral NS3 protease e.g., Telaprevir, Boceprevir, Simeprevir, Faldaprevir, and Asunaprevir drugs improved the SVR rate against different HCV genotypes but could not eliminate the use of interferon completely [12-15]. The recent development of novel NS5A polymerase e.g., Ledipasvir, Daclatasvir and NS5B nucleotides e.g., Sofosbuvir (SOF) achieved the goal of safe regimen and high tolerability with improved SVR rate. The combination of SOF with other DAAs eliminated the need of interferon and Ribavirin completely [16-17].

SOF formally GS-7977 (Sovaldi 400 mg Tablets) is a novel HCV nucleotide analog (NS5B polymerase inhibitor). It was first developed by Gilead Sciences (GS) USA and approved by US Food and Drug Administration (FDA) for the treatment of chronic hepatitis C (CHC) in 2013 [18-21]. SOF is chemically (*S*)-isopropyl-2-((*S*)-(((2*R*, 3R, 4R, 5R)-5-(2, 4-dioxo-3, 4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyl tetra hydrofuran-2-yl) methoxy)-(phenoxy) phosphoryl amino) propionate having molecular formula ($C_{22}H_{29}FN_3O_9P$) and structure formula as shown in (Fig. 1). SOF is white to off-white non-hygroscopic crystalline solid exhibits pH-independent high solubility in aqueous medium across a pH ranges from 1.2 - 7.7. It has low apparent intestinal permeability, and belongs to the class III of Biopharmaceutics Classification System (BCS) [22-23].

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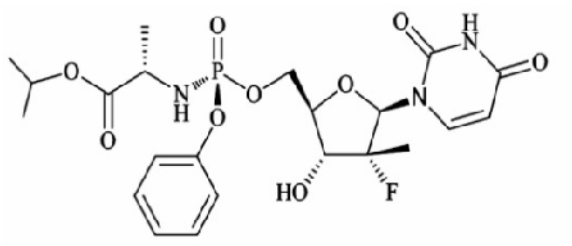

Fig. 1: (Sofosbuvir GS-7977).

The bioavailability of a drug from oral dosage form depends on its release, dissolution and permeability through the gastrointestinal tract. Considering the pH dependent solubility of SOF, the gastrointestinal pH will influence on release of drug substance and due to low apparent intestinal permeability the bio-availability of the product will be affected. To check the release and solubilization of the active drug substance from the drug product, under physiological conditions the In vitro dissolution testing is important. The dissolution test is also important to assess development of new formulation, consistency in developed formulation and ensuring the product quality and performance after certain changes in formulation [24-28]. To ensure the in vitro dissolution studies of a product according to the requirement of FDA [29], it is important to have an accurate and simple analytical method to quantify the release of drug substance from tablets dosage form.

Various analytical methods for quantitative determination of SOF and therapeutic monitoring have been reported in literature e.g., UPLC-ESI-MS/MS [30-33], SPE-LC [34], LC-MS/MS [35-37], and LC-ESI-QTOF–MS/MS [38]. Analysis of fixed dose combination, bulk drug and dosage form of SOF are also reported in literature *i.e*., HPLC-UV [39-40]. However these methods are specific for therapeutic monitoring, degradation studies and simultaneous determination of fixed-dose combination. To the best of our knowledge no specific spectrophotometric method is available for assay determination and in vitro dissolution studies of SOF tablets dosage form. Considering the therapeutic importance and dissolution behavior of SOF, a simple, precise and specific spectrophotometric method is desired. In the present study, a UV spectrophotometric method was developed to quantify the content of SOF in tablets dosage form *i.e.* assay determination and in dissolution studies. The suitability of the method was confirmed by a validation processes according to the guidelines of United State Pharmacopoeia (USP) [41] and International Conference on Harmonization (ICH) [42]. The developed method is based on spectrophotometric detection of SOF at wavelength 262 nm using double beam spectrophotometer and quartz cells of 1 cm path length. The proposed method is rapid, accurate, and precise and can be used for routine evaluation of SOF tablets assay content and in vitro dissolution studies.

Experimental

Materials and Chemicals

The reference standard of SOF, generic brands of tablets and excipients for placebo e.g., microcrystalline cellulose (USP), magnesium stearate British Pharmacopeia (BP), croscarmellose sodium (BP), polyvinyl alcohol (USP), colloidal anhydrous silica European Pharmacopeia (EP), titanium dioxide (USP), macrogol (EP), purified talc (BP), and mannitol (BP) was provided by Genome Pharmaceuticals (Pvt) Ltd Pakistan. Innovator product Sovaldi-400 mg tablets (Galid Science, Inc. USA) was purchased from local Pharmacy. Potassium dihydrogen orthophosphate (KH_2PO_4) analytical, sodium hydroxide (NaOH) analytical, and methanol was purchased from Sigma-Aldrich (Germany).

Instrumentation

USP tablet dissolution apparatus type-II (Galvano Scientific Pak) and Shimadzu UV-1601 double beam spectrophotometer with 1-cm quartz cell (Shimadzu Japan) was used for dissolution studies and analysis. Shimadzu electronic balance AW220 (Shimadzu Japan), Ultrasonic bath SONOREX (Bandelin Germany), Millipore vacuum filtration assembly and Milli-Q water distillation system (Millipore USA) were also used in the present studies.

Solution preparation

Dissolution medium

Potassium dihydrogen orthophosphate 0.05 M solution was prepared by dissolving accurately 40.8 gm in about 5.0 L purified water using 6.0 L volumetric flask, the pH of this solution was adjusted using sodium hydroxide dilute solution and diluted to 6.0 L using purified water.

SOF reference and sample stock solution

SOF reference stock solution 0.5 mg mL^{-1} were prepared by dissolving equivalent to 50 mg SOF reference material in methanol using 100 mL

volumetric flask and diluted to volume with dissolution medium. Sample solution of tablet dosage form was prepared by dissolution of 10 tablets of each brand in 1L dilution medium and filtered through 0.45µm nylon filter. The above solutions were stored at 2–8◦C and further diluted for working solutions of required concentration in method development and validation.

Sample solution in dissolutions studies were prepared on the same way using 10 mL sample collected from dissolution bowl at specified intervals ± 2%. These solutions were diluted to the required concentration using dissolution medium and filtered through 0.45µm nylon filter.

Method Development and Optimization

Due to capability for rapid analysis and good repeatability, spectrophotometric methods have widespread usage in the drug analysis. The conjugated group attached at belt K and benzene ring at belt B and E1 on molecular structure of SOF **(**Fig. 1) theoretically support for the absorption in UV region and better option for development of spectrophotometric method. To check the solubility and molar absorptivity of SOF in different medium, a series of SOF reference solutions in different medium were scanned in UV range from 200 nm to 400 nm. The maximum absorbance of SOF solution in all medium were found at wavelength 261 ± 2 nm and the specific absorption was calculated from the ratio of absorbance and concentration. To optimize the sensitivity of SOF in dissolution medium at 262 nm, a serial concentration of SOF reference solution in dissolution medium e.g.10, 20, 30, and 40 µg mL^{-1} were analyzed. The stability of absorbance value in dissolution medium was crossed checked against the absorbance value of freshly papered solution and the relation of absorbance with concentration was determined according to beer's law. Due to sensitive, stable and repeatable absorbance values of SOF at 262 nm, wavelength 262 nm was selected for quantitative determination. To ensure the spectral interference of tablet matrix (placebo) and dissolution medium, the absorbance values of all excipients were studied from 200 to 400 nm. The analytical method was further validated for recovery, precision, specificity and linearity according to the guidelines of USP and ICH.

In vitro dissolution and drug release studies

Using the FDA specified parameters for SOF tablets dissolution [29], drug release studies of tablets dosage form was performed. USP dissolution apparatus type-II (paddle type), at 75rpm ± 4 %, and 900ml ± 1%, 0.05 M phosphate buffer pH 6.8 ± 0.05 as dissolution medium was selected for dissolution studies. The medium was equilibrated at 37.0 ± 0.5ºC and tablets Samples were added to each dissolution bowl with time gap of 2 min to manage sample collection at predetermined time intervals e.g., 5, 10, 15, 20, and 30 min. 10mL samples were withdrawn using bent SS cannula from halfway between the top of the media and top of the paddle, not less than 1cm away from the wall of bowl. The sampled volume was replaced immediately in dissolution bowel with an equal volume of the dissolution medium (maintained at 37.0 ± 0.5ºC). The sample aliquots were filtered through 0.45µm nylon filter and diluted to the required concentration using dissolution medium. The absorbance values for each solution were determined at 262 nm using spectrophotometer and the content of SOF released was calculated against the standard reading of SOF solution in same medium. The percentage of SOF dissolved at each collection time was calculated on the base of labeled amount of SOF (considered 100%). To obtain the in vitro dissolution profile of the product, the cumulative percentage of SOF released was plotted against time. The dissolution efficiency (DE) of profiles was evaluated using the trapezoidal rule from the area under curve at time t1. Comparative dissolution studies on two different generic brands A and B were performed and the drug release profiles were compared with innovator brand Sovaldi 400 mg tablets. The comparison of dissolution profiles was evaluated according to the requirement of immediate release pharmaceutical dosage form using model independent and model dependent approach. The value of difference factor (f_1) was calculated using formula ($f_1 = \{[\sum_{t=1}^{n}|R_t-T_t|][\sum_{t=1}^{n} R_t]\}x100$) and similarity factor (f_2) was calculated using formula ($f_2 = 50 \times \log \{[1+(1/n)\sum t_{=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\}$). Where n is the number of time points, R_t is the % dissolution value of the reference at time t, and T_t is the % dissolution value of the test at time t. The value of f_1 was evaluated against limits ≤ 15 (0 to 15) and f_2 against ≥ 50 (50 to 100). The correlation coefficient (R2) was evaluated for linear (time x % dissolution of SOF) and first order (time x log % dissolution) kinetics modeless.

Results and Discussion

Method development and optimization

The absorption spectrum of SOF **(**Fig. 2a and 2b) showed that SOF solution in different medium have UV absorption peak at 262 ± 2 nm and it is suitable for quantification of drug substance in drug product. The sensitivity of SOF at 262 nm was optimized using serial concentration of SOF reference solution *i.e*., 10, 20, 30, and 40 µg mL^{-1} in

different medium *i.e*. methanol, 0.1 M hydrochloric acid solution, acetate buffer pH 4.5, simulated intestinal fluid and phosphate buffer pH 6.8. The specific absorbance of SOF in each medium was calculated as shown in Table-1. Based on suitable absorbance of SOF at 262 nm, the analytical method was further validated by evaluation of different performance parameters *i.e*., recovery, precision, specificity and linearity according to USP and ICH guidelines.

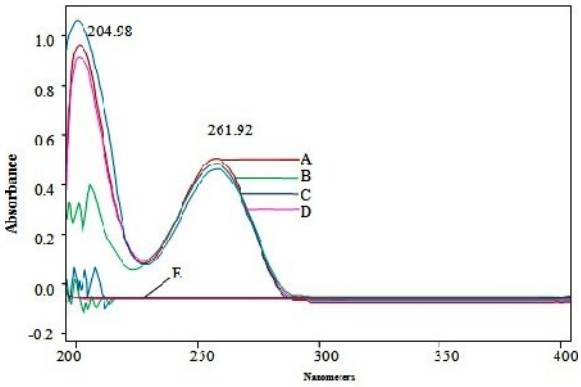

Fig. 2: (a) Absorption spectrum of SOF in different medium
A) methanol, B) 0.1 M HCl, C) acetate buffer, D) simulated gastric fluid and E) Medium (Blank / Placebo).

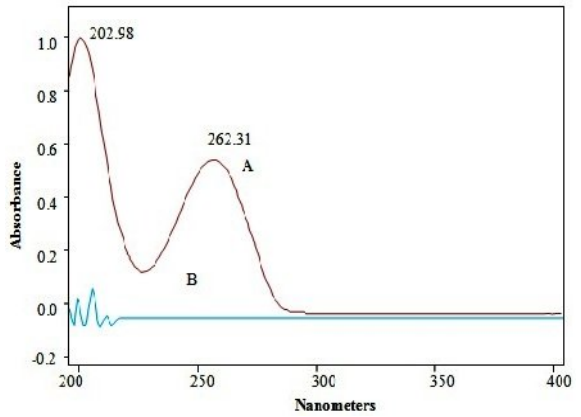

Fig. 2: (b) Absorption spectrum A SOF in Phosphate Buffer and B Medium (Blank / Placebo).

Table-1: Absorptivity and λ max of SOF in different medium

Medium	Observed λ max	Specific absorption (A = 1%)
Methanol	261 nm	192.5 ± 6.3
0.1 M HCl	262 nm	178.9 ± 5.4
Acetate buffer pH 4.5	261 nm	165.3 ± 6.8
Simulated intestinal fluid	262 nm	153.5 ± 9.3
Phosphate buffer pH 6.8	262 nm	176.5 ± 5.8

Method validation

Accuracy and Recovery

For the study of accuracy and recovery, six concentrations levels containing known amount of SOF (8, 16, 24, 32, 40 and 48 µg mL^{-1}) in dissolution medium were analyzed each replicate (n=3). The values of recovery, relative error (RE %) and relative standard deviation (RSD %) were evaluated against acceptable limits ± 2%. Results (Table-2) showed that recovery of SOF at all concentration levels was 100 ± 2% and the values of RE % and RSD % were less than ± 2 %. All the results of recovery studies were within acceptable limits and the method was accurate and repeatable.

Table 2: Results of accuracy and recovery

Conc. of SOF Analyzed µg mL^{-1}	Recovery	%RE	RSD
8	98.97 ± 1.07	1.03	1.08
16	99.44 ± 1.41	0.56	1.42
24	100.53 ± 0.89	-0.53	0.89
32	99.2 ± 0.54	0.8	0.54
40	99.48 ± 0.17	0.52	0.17
48	99.95 ± 0.42	0.05	0.42

Repeatability and Precision

Precision and repeatability of the analytical method was assured by analyzing replicate (n=6) reference solution in dissolution medium at two concentration level 20 % and 120%, having known amount of SOF 8 and 48 µg mL^{-1}. The RSD % of absorbance values was evaluated for acceptance limits of 2 %. The results (Table-3) showed that the values of RSD% for replicates (n=6) of each concentration level was less than 2.0%. The results for precision and intermediate precision studies showed that the given method is precise and repeatable within the acceptable limits.

Robustness

Robustness of the method was assured by applying small changes (± 2 % of the given value) in the pH of dissolution medium and the detection wavelength on spectrophotometer. Replicates (n=6) of solutions containing known amount of SOF (8 and 48 µg mL^{-1}) were analyzed after applying small changes (± 2%) in the given value of pH of dissolution medium and wavelength. The value of RSD for absorbance was evaluated against acceptance limits of 2%. The results (Table-4.0) showed that there is no affect on the absorbance value and recovery of SOF. The results showed that the method is robust and suitable for quantification of SOF in dissolution medium.

Table-3: Results of precision and intermediate precision.

Sample Conc / Days	20 % (8 µg mL^{-1})		120 %(48 µg mL^{-1})	
	Absorbance	Recovery %	A	Recovery %
Day 1	0.14 ± 0.002	100.09 ± 1.32	0.85 ± 0.003	100.02 ± 0.32
RSD	1.429 %	1.32 %	0.353 %	0.32 %
Day 2	0.142 ± 0.002	99.5 ± 1.33	0.85 ± 0.002	100.21 ±0.26
RSD	1.408 %	1.34 %	0.235 %	0.26 %
Day 3	0.14 ± 0.001	99.62 ± 0.97	0.85 ± 0.006	100.23 ± 0.68
RSD	0.714 %	0.97 %	0.706 %	0.68 %

Table 4: Results of robustness studies

SOF Conc. (µg mL^{-1})	Absorbance	RSD %	Recovery %	RSD %
20 %	0.14 ± 0.002	1.429	99.98 ± 1.25	1.25
120 %	0.84 ± 0.003	0.357	100.21 ± 0.39	0.39

Linearity

Seven concentrations levels covering the analysis range from 20 to 140 % having known concentration SOF (4, 8, 16, 24, 32, 40 and 48 µg mL^{-1}), replicates (n=3) were analyzed. The mean absorbance values were plotted on Y axis against concentration on X axis. Based on linear regression equation (A = Slope C x Y intercept) and graphical presentation the relation of concentration and response was evaluated. LOD and LOQ were calculated using expression (3.3 $^{\delta}$ /slope) and (10^{δ}/slope) respectively. The statistical data derived from linearity studies (Table-5) showed that the method is linear and the value for correlation coefficient is 0.9999, LOD = 0.94 µg mL^{-1} and LOQ = 2.84 µg mL^{-1}. The given method is linear and Beer's law obeyed over a concentration ranges from 4µg mL^{-1} to 48µg mL^{-1}.

Table-5: Statistical data derived from calibration curve.

Correlation R	0.9999
Slope	0.0176
Intercept	0.0012
SD	0.005
LOD (µg mL^{-1})	0.94
LOQ (µg mL^{-1})	2.84

Specificity and placebo interference

To check the interference of excipients present in tablet dosage form, solution of placebo was prepared in phosphate buffer pH 6.8 using all excipients of tablets except adding the active ingredient. This solution was analyzed on UV range from 200 nm to 400 nm. There was no significant absorbance of dissolution medium and all the excipients present in dosage form in the given range from 220 nm to 400 nm (Fig 2b). The results showed that the given method is specific for SOF and suitable for assay quantification and in vitro dissolution studies of tablets dosage form.

Stability of solutions

Stability of SOF in dissolution medium was assessed at three concentration levels 120, 60 and 20 % containing known amount of SOF (48, 24 and 8 µg mL^{-1}). These solutions were exposed to different conditions *i.e.*, room temperature (15-25ºC) for 7 days, and cool temperature (02- 08ºC) for 15 days. Results were evaluated by analyzing replicates (n = 3) of each solution comparing the absorbance values with the values for freshly prepared solution in the same medium. The results (Table-6) showed that there was no change in the absorbance values of SOF in dissolution medium and the solution were stable for 7 days stored at room temperature and for 15 days stored at cool temperature in refrigerator.

Application of method and In vitro dissolution studie

The method was successfully applied for quantification of SOF in assay determination of SOF tablets dosage form (Table-7). SOF was recovered 100 ± 2 % for (n=3) composite samples of 10 tablets of each brand separately. In vitro dissolution studies were performed on SOF tablets two generic products and innovator product. The dissolution profile for each brand was developed and compared with innovator brand. The results of dissolution studies (Table-7) showed that the value of RSD% was less than 5 % at initial point and less than 2% for other intervals. The value of similarity factor f_2 was (4 and 3) and the difference factor f_1 was (64 and 50) for dissolution profiles of both generic products A and B respectively. The graphical presentation (Fig. 3) showed that the dissolution profiles of generic brands A and B are similar to that of innovator brand and the value of correlation coefficient (r) using linear kinetics for brand A was (0.9945) and brand B was (0.9766). The results of dissolution and drug release of all the brands meets the USP criteria (Q=80%) in 15 and 20 min.

Table-6: Results of stability studies of solutions

Concentration µg mL^{-1}Analyzed	SOF Concentration Recovered %			
	(15-25ºC)7 days		(02- 08ºC)15 days	
	% Recovery	% RSD	% Recovery	% RSD
8	99.15 ±1.16	1.17	99.55 ± 1.22	1.23
24	99.86 ± 1.2	1.2	99.76 ± 0.83	0.83
48	99.76 ± 0.91	0.91	99.52 ± 0.83	0.83

Table-7: Results of comparative dissolution studies.

Time (Min)	Innovator brand			Generic Brand A			Generic Brand B		
	% Dissolution		% Mean ± SD	% Dissolution		% Mean ± SD	% Dissolution		% Mean ± SD
	Min	Max		Min	Max		Min	Max	
5	73.37	79.60	76.46 ± 2.10	66.43	69.69	68.48 ± 1.78	73.37	79.60	67.54 ± 1.23
10	95.46	97.45	96.48 ± 0.65	85.27	90.23	88.43 ± 1.79	95.46	97.45	92.21 ± 1.23
15	97.87	100	98.84 ± 0.77	94.62	98.44	96.6 ± 1.43	97.87	100.00	97.57 ± 1.40
20	99.43	101.13	100.14 ± 0.62	98.44	101.13	99.67 ± 0.97	99.43	101.13	99.84 ± 1.07
30	95.751	102.83	98.77 ± 2.64	97.31	98.87	98.16 ± 0.51	95.751	102.83	99.55 ± 0.89

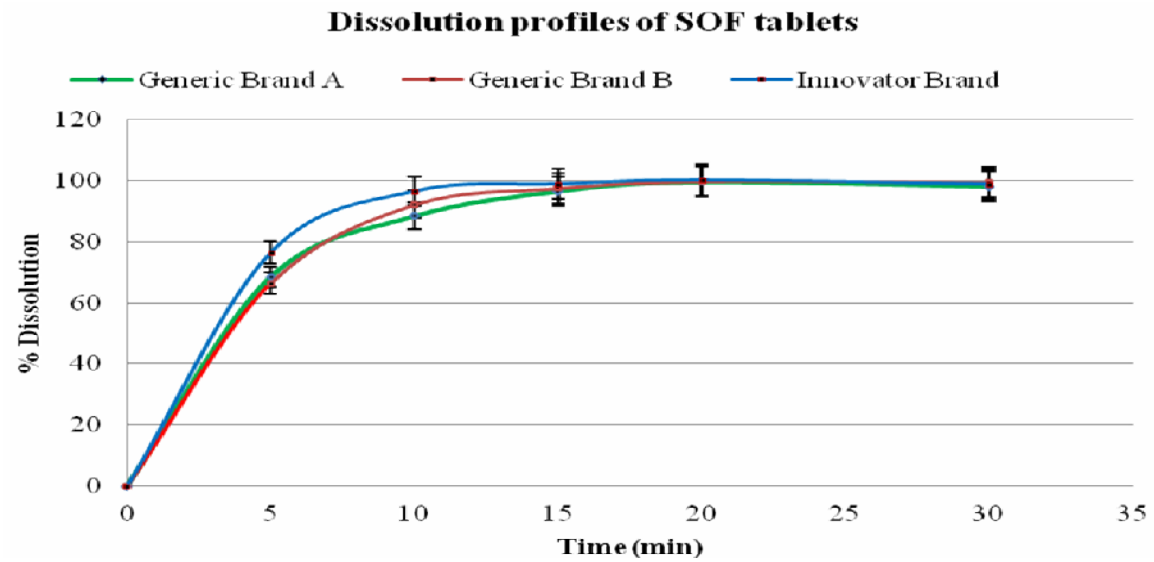

Fig. 3: Comparative dissolution profile of SOF brands.

Conclusions

SOF is the novel and selective (NS-5B) HCV polymerase inhibitor and has low apparent intestinal permeability and high solubility at pH ranges from 1.2 - 7.7. Due to its vital clinical role for the treatment of Hepatitis C, it is important to determine the delivery of correct and specified dose to the patient at physiological conditions.

To ensure the assay and in vitro dissolution of tablets dosage form, a spectrophotometric method was developed and successfully validated according to the requirements of USP and ICH guidelines. All the results of the tests carried out for the validation of the method were in complete agreement with the required limits and criteria. The validated method was successfully applied to in vitro dissolution studies of SOF tablets dosage form. Comparative dissolution studies of two generic brands versus innovator brand were performed using same validated method. Based on our results it was concluded that the method was accurate, precise and specific for quantification of SOF in tablet dosage form and in vitro dissolution studies.

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