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A Study of New Method Development, Validation and Forced Degradation for Simultaneous Analysis of Dapagliflozin and Saxagliptin in Pharmaceutical Dosage Form by HPLC Method

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ABSTRACT

A simple, precise and stability-indicating Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for simultaneous quantification of Dapagliflozin (DGFZ) and Saxagliptin (SGPT) in combined dosage form. The developed method has been validated with respect to precision, linearity, accuracy, robustness, ruggedness, sensitivity, solution stability. The method has been developed with ammonium dihydrogen phosphate buffer (pH 6.8) and methanol in a ratio of 65:35 v/v as mobile phase at a flow rate of 1.5 ml/min over Intersil ODS C18 column (250 mm × 4.6 mm × 5 µ). The UV detection wavelength was fixed at 280 nm. The column temperature being maintained at ambient temperature. The method shown good linearity with correlation coefficient values of 0.9992 and 0.999 for DGFZ and SGPT. The percent recoveries of two drugs found within the limits of (98.00-102.0%). The Limit of Quantification (LOQ) concentrations of DGFZ and SGPT are 0.312 µg/ml and 0.156 µg/ml respectively. The Limit of Detection (LOD) concentrations of DGFZ and SGPT are 0.156 µg/ml and 0.078 µg/ml respectively. According to International Conference on Harmonization (ICH) guidelines Forced degradation study was validated.

Keywords: RP-HPLC, Stability-indicating, Dapagliflozin, Saxagliptin, Sample stability, Forced degradation

INTRODUCTION

Dapagliflozin (DGFZ) is a sodium-glucose cotransporter-2 inhibitor and it works by decreasing the amount of sugar the body absorbs and increasing the amount of sugar that leaves the body in the urine (Figures 1 and 2) [1]. Saxagliptin (SGPT) is used for the treatment of type 2 diabetes (Figure 2) [2]. SGPT is a dipeptidyl peptidase-4 inhibitor. It works by increasing the amount of insulin released by human body and by decreasing the amount of sugar made by human body [3].

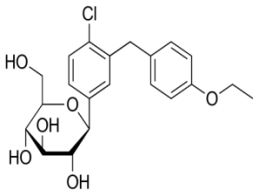


Figure 1: Structure of dapagliflozin

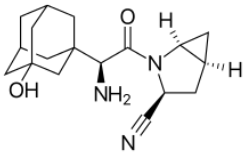


Figure 2: Structure of saxagliptin

Jani BR et al. [4], developed UV spectroscopic method for simultaneous estimation of DGFZ and Metformin Hydrochloride in synthetic mixture. Mohammad Yunoos et al. [5], developed a stability indicating High Performance Liquid Chromatography (HPLC) method for simultaneous determination of metformin HCl and DGFZ in bulk drug and tablet dosage form. This method was developed with hypersil BDS C18, 250 mm column at 240 nm with 0.1% orthophosphoric acid, acetonitrile as mobile phase in the ratio of 50:50% (v/v) at a flow rate of 1 ml/min. Jani BR et al. [6], reported a UV method for simultaneous estimation of DGFZ and metformin hydrochloride in a synthetic mixture. Shyamala et al. [7], developed a simultaneous method for quantification of DGFZ and metformin HCl in tablet dosage form. In this method the mobile phase was phosphate buffer: methanol: acetonitrile in the ratio of 50:30:20 at pH 6.5. Flow rate was 1 ml/min, at 240 nm UV detector wavelength. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for DGFZ were found to be 3.650 µg/ml and 3.649 µg/ml respectively. This method was found to be good as the percentage recovery of Metformin HCL and DGFZ were found to be 100.67 and 99.54 respectively. Afshan Urooj et al. [8], reported a HPLC method for simultaneous estimation of DGFZ and Metformin in bulk and in synthetic mixture. The method was developed with Photo Diode Array (PDA) detector with acetonitrile and water (75:25% v/v) as mobile phase. The percentage recoveries of the methods are in between 99.3-99.6%. LOD and LOQ were found to be 5.0 µg/ml and 15.2 µg/ml for metformin and 3.7 µg/ml and 11.4 µg/ml for DGFZ. Manasa Sanagapati et al. [9], reported a HPLC method for determination of DGFZ with PDA detector at 245 nm using acetonitrile and ortho phosphoric acid (55:45) as mobile phase. The linearity of this method was 25-150 µg/ml. Rambabu et al. [10], reported a HPLC method for the estimation of DGFZ in bulk and tablet formulation. This method was reported with PDA detector at 210 nm. The linearity of the method was 25-150 µg/ml and recovery was in the range of 98.95-101.72%.

In the literature survey more methods are reported for simultaneous estimation of SGPT and metformin. Mohammad Yunoos et al. [11], reported RP-HPLC method for simultaneous determination of metformin hydrochloride and SGPT in bulk and combined tablet dosage form. This method was developed with Hypersil ODS C18 column, KH_2PO_4 buffer, acetonitrile and methanol in the ratio of 25:50:25 (% v/v/v) as the mobile phase at a flow rate of 1.0 ml/min. In this method accuracy reported in the ranges of 99.62-99.93% and 99.66-99.80% for metformin hydrochloride and SGPT respectively. R. Pravin cumar et al. [12], developed RP-HPLC method for simultaneous estimation of metformin and SGPT in tablets. The methods was developed with C18 column using phosphate buffer, acetonitrile and methanol in the ratio 75:15:10 as the mobile phase at pH 5.0. The detector wave length is 225 nm. Nyola Narendra et al. [13], developed a HPLC method with 0.02 M potassium dihydrogen phosphate, Acetonitrile and methanol in the ratio of 50:25:25 (v/v/v) at pH 4.3. The linearity concentrations range for SGPT 10-50 µg/ml and for metformin 5-25 µg/ml. The recoveries of SGPT and metformin were 100.48 and 101.1% respectively. S.M. Mhaske et al. [14], developed as spectrophotometric method. P.B.N. Prasad et al. [15], developed RP-HPLC method with C18 column, 0.05 M KH_2PO_4 buffer, methanol and acetonitrile in the ratio of 60:20:20 (% v/v)as a mobile phase at a flow rate of 0.6 ml/min at UV detection at 220 nm wavelength. The LOD and LOQ of metformin were found to be 0.112 µg/ml and 0.373 µg/ml, respectively.

Objective

The present study is concerned with the development and validation of SGPT and DGFZ in formulation by high performance liquid chromatography.

MATERIALS AND METHODS

Apparatus and chemicals

The method has been developed and validated with Peak LC P7000 HPLC (Isocratic) system with 20 µl rheodyne injector and UV/Visual detectorUV7000 and PEAK chromatographic version 1.06. The SSBV and VPSV were scanned with UV-Visible spectrophotometer (Tech comp-UV 2301, make Japan) with Hitachi software. DGFZ purchased from Hikal Ltd, Mumbai and SGPT was obtained from Jubilant Life Sciences Ltd., Amroha. HPLC grade solvents, water, acetonitrile and methanol were procured from Merck, Mumbai. Method was developed with Intersil ODS C18 column (250 mm × 4.6 mm × 5 µ) at 280 nm.

Standard stock solution preparation

Chromatographic conditions

Optimization of chromatographic conditions: Drugs solubility was examined and mobile phase was fixed after trial with different ratios of mobile phase combinations. The detection wavelength was optimized in the double beam spectrophotometer, by scanning sample in the range of 200-400 nm. From the overlaid spectrum of DGFZ and SGPT UV absorption wavelength of 280 nm was selected for the simultaneous quantification of DGFZ and SGPT in HPLC method. The overlay scanning spectra showed in Figures 3 and 4. The finalized HPLC conditions are showed in Table 1.

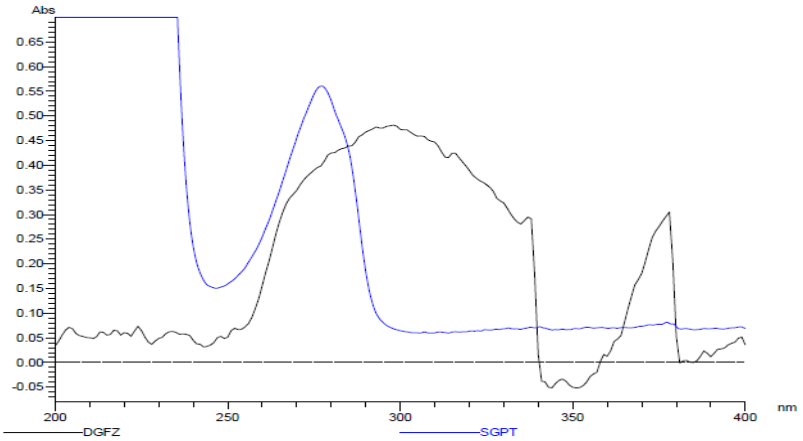
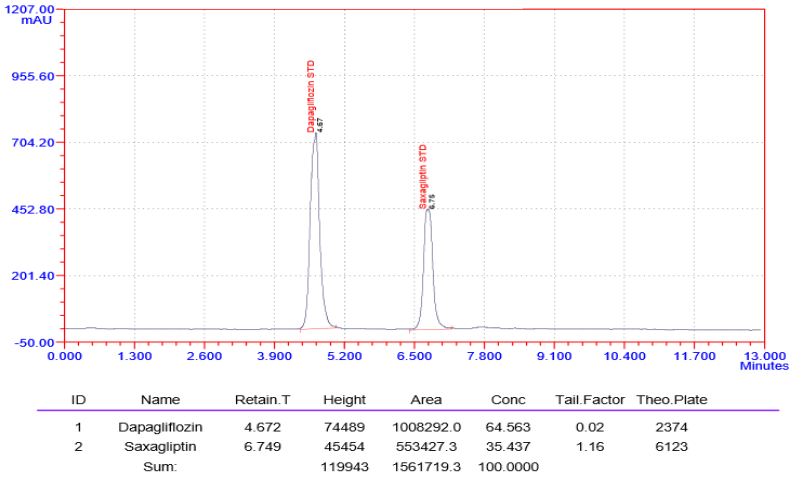


Figure 3: UV scanning overlay spectrum of DGFZ and SGPT

Table 1: Chromatographic conditions of developed method

S. No.	Parameter	Condition
1	Mobile phase	$NH_4H_2PO_4$ buffer: Methanol 65%:35%, v/v
2	pH	6.8
3	Column	Intersil ODS C18 column (250 mm × 4.6 mm × 5 µ)
4	Flow rate	1.5 ml/min
5	UV detector wavelength	280 nm
6	Run time	13 min
7	Sample volume	20 µl



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Dapagliflozin	4.672	74489	1008292.0	64.563	0.02	2374
2	Saxagliptin	6.749	45454	553427.3	35.437	1.16	6123
	Sum:		119943	1561719.3	100.0000		

Figure 4: Standard chromatogram of DGFZ and SGPT

Validation

Linearity test

The linearity was checked over the concentration ranges of about 2.5-40.0 µg/ml and 1.25-20.0 µg/ml for DGFZ and SGPT. The total runtime time was 13 min. The calibration curves were linear in the studied range and equations of the regression analysis were obtained. The slope, intercept and the correlation coefficient were determined (Figures 5, 6 and Table 2).

Table 2: Linearity results of developed method

S. No.	Percentage of concentration	DGFZ		SGPT	
		Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
1	25%	2.5	670011	1.25	434396
2	50%	5.0	808182	2.5	488309
3	100%	10	1008292	5	553427
4	200%	20	1374293	10	678730
5	400%	40	2106062	20	937664
6	r^2	0.9992		0.9990	
7	Slope	37678.69		26255.1	
8	Intercept	609348.4		415025	

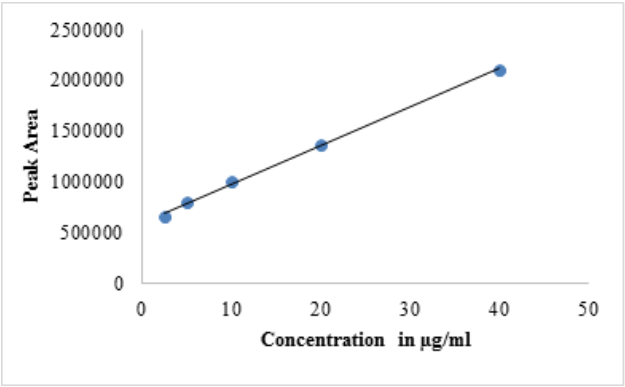


Figure 5: Linearity graph of DGFZ

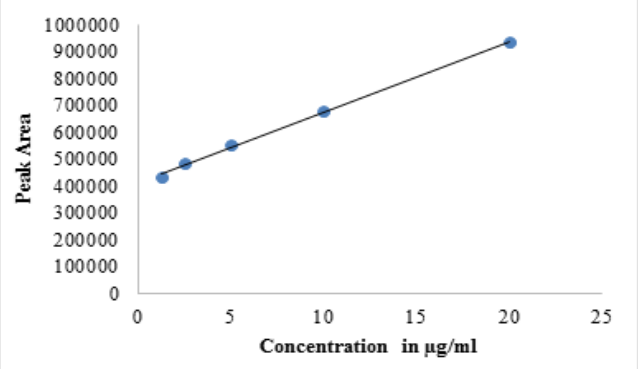


Figure 6: Linearity graph of SGPT

Precision

Intra-day precision was investigated by replicate applications and measurements of peak area of DGFZ and SGPT for six times on the same day under similar conditions. Inter-day precision was obtained from %RSD values obtained by repeating the assay six times on two different days. The %RSD was calculated (Tables 3 and 4) which was within the acceptable limit i.e., less than 2.0.

Table 3: Intraday results of developed method

S. No.	Peak Area obtained for	
	DGFZ at 10 µg/ml	SGPT at 5.0 µg/ml
Injection-1	995731	553501
Injection-2	998322	551839
Injection-3	1008369	557830
Injection-4	1010634	557359
Injection-5	1014100	560927
Injection-6	1012090	556493
% of RSD	0.759	0.583

Table 4: Interday results of developed method

S. No.	Peak Area obtained for	
	DGFZ at 10 µg/ml	SGPT at 5.0 µg/ml
Injection-1	991325	554675
Injection-2	1020672	556445
Injection-3	996804	562139
Injection-4	992875	552141
Injection-5	1004969	553451
Injection-6	1019076	564890
% of RSD	1.29	0.91

Ruggedness

Ruggedness of the method was validated with different analyst with different system. Ruggedness of the method is 0.88 % for DGFZ and 1.74% for SGPT between the two sets of data. The data indicates the ruggedness of method is good (Table 5).

Table 5: Ruggedness results of developed method

S. No.	Peak Area obtained for	
	DGFZ at 10 µg/ml	SGPT at 5.0 µg/ml
Injection-1	992651	542577
Injection-2	999359	563535
Injection-3	998286	550007
Injection-4	1014129	550240
Injection-5	1000895	564304
Injection-6	1013941	565792
% of RSD	0.88	1.74

Robustness

Robustness is an indication of reliability of the analytical method during normal usage Table 6. The Mobile phase ratio was changed ±15%, the buffer solution pH was changed ± 0.2, and detector wavelength was changed ± 3 nm.

Recovery

Accuracy of the method was determined by recovery studies. Recovery test was conducted at the levels of 50%, 100%, 200%. The recovery studies were carried out three times and the percentage recovery was calculated for both drugs and shown in the Table 7.

Table 6: Robustness results of developed method

Chromatographic condition	Concentration of DGFZ (µg/ml)	Peak area	% of change	Concentration of SGPT (µg/ml)	Peak area	% of change
At normal conditions	10.0	1008292	0.0	5.0	553427	0.0
Mobile phase	10.0	991325	1.68	5.0	554675	0.22
Mobile phase	10.0	1020672	1.22	5.0	556445	0.54
pH 6.82	10.0	996804	1.13	5.0	562139	1.57
pH 6.78	10.0	992875	1.52	5.0	552141	0.23
Detector wavelength: 238 nm	10.0	1004969	0.32	5.0	553451	0.004
Detector wavelength: 232 nm	10.0	1019076	1.06	5.0	564890	2.07
% of RSD		1.29		% of RSD	0.91	

Table 7: Recovery results of developed method

Concentration level	DGFZ	True area	% of recovery	SGPT	True area	% of recovery
50%	805409	808182	99.65	487280	488309	99.78
100%	1017155	1008292	100.87	553849	553427	100.07
200%	1377263	1374293	100.21	681714	678730	100.43
	Average recovery		100.24	Average recovery		100.09

Sensitivity

The sensitivity was determined by signal to noise ratio. The standard solution was serially diluted and injections were made to obtain chromatogram. The results were showed in Table 8.

Table 8: LOQ and LOD results of developed method

S. No.	Test	DGFZ	SGPT
1	LOQ	0.3125 µg/ml	0.15625 µg/ml
2	LOD	0.15625 µg/ml	0.078125 µg/ml

Solution stability

Solution stability [16-18] period for standard DGFZ and SGPT has been determined by keeping the solution 36 h at room temperature. After 6, 12, 18, 24, 30 and 36 h the solutions have been analyzed under same conditions Table 9. All insignificant changes have been observed for the chromatographic responses for the solution analyzed, relative to freshly prepared standard. Up to 24 h the percentage of change is found to be less than 2%.

Table 9: Solution stability results of developed method

S. No.	Time period (h)	Concentration of DGFZ (µg/ml)	Peak area	% of change	Concentration of SGPT (µg/ml)	Peak Area	% of change
1	0	10.0	1008292	0.0	5.0	553427	0.0
2	6	10.0	1003272	0.49	5.0	555445	0.36
3	12	10.0	1009021	0.07	5.0	551090	0.42
4	18	10.0	1004354	0.39	5.0	558484	0.91
5	24	10.0	1009904	0.15	5.0	558001	0.82
6	30	10.0	984790	2.33	5.0	540919	2.26
7	36	10.0	900067	10.73	5.0	546997	1.16

Forced degradation study

The degradation behavior [19-22] of DGFZ and SGPT under various stress conditions has been investigated by developed HPLC Method.

Oxidation: To 1 ml of stock solution of DGFZ and SGPT, 1 ml of 20% hydrogen peroxide has been added. The solution was kept for 30 min. For degradation study, the resultant solution was diluted to obtain standard concentration and 10 µl solution has been injected into the system. The chromatograms were recorded to assess the stability of sample. The degradation chromate gram is shown in Figure 7.

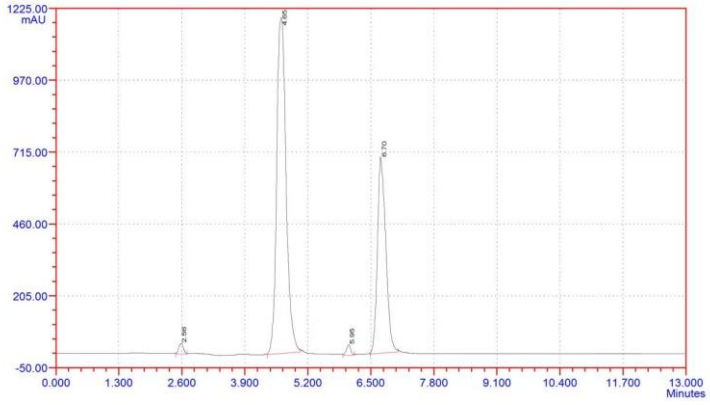


Figure 7: Oxidative degradation chromatogram

Acid degradation: To 1 ml of stock solution of DGFZ and SGPT, 1 ml of 2 N hydrochloric acid is added and refluxed for 30 min. The resultant solution is diluted to obtain standard concentration and 10 µl solution injected into the system. The chromatograms have been recorded to assess the stability of sample. The degradation chromate gram is shown in Figure 8.

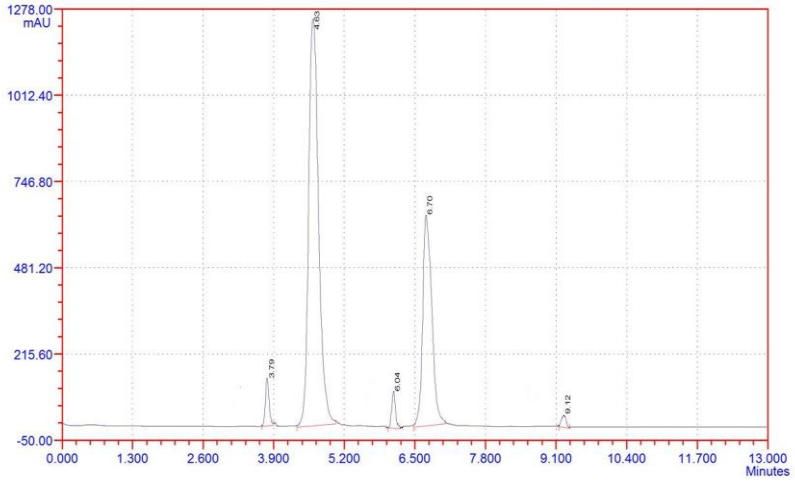


Figure 8: Acid degradation chromatogram

Alkali degradation studies: To 1 ml of stock solution of DGFZ and SGPT, 1 ml of 2 N NaOH is added and refluxed for 30 min. The resultant solution was diluted to obtain standard concentration and 10 µl solution injected into the system. The chromatograms have been recorded to assess the stability of sample. The degradation chromate gram is shown in Figure 9.

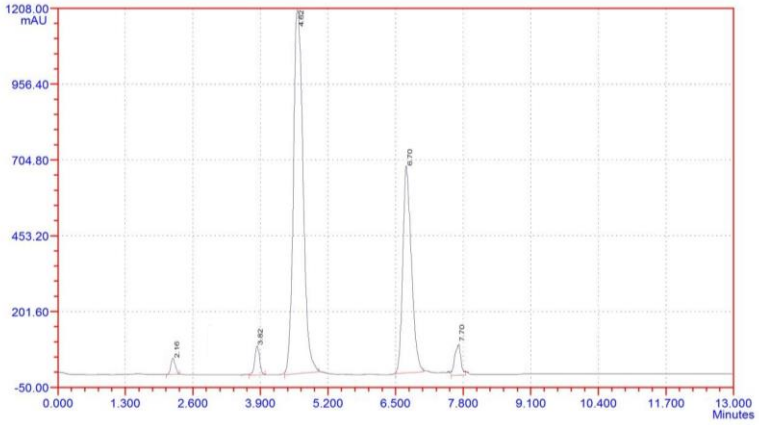


Figure 9: Alkali degradation chromatogram

Thermal/dry heat degradation studies: The standard drug solution has been placed in oven at 1050℃ for 6 h to study dry heat degradation. The final solution was diluted to obtain standard concentration and 10 µl solution injected into the system. The chromatograms have been recorded to assess the stability of sample. The degradation chromatogram gram is shown in Figure 10.

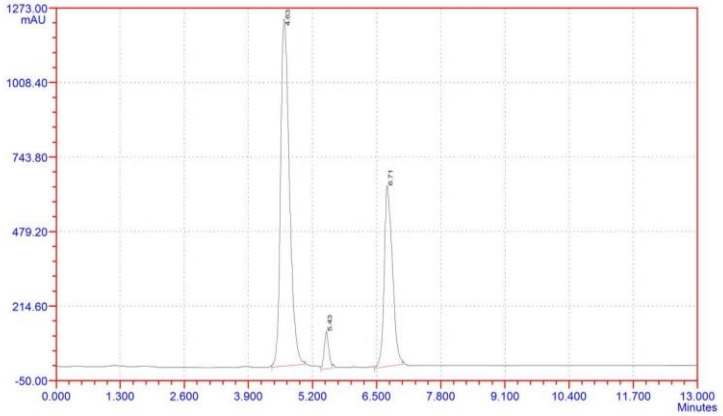


Figure 10: Thermal degradation chromatogram

Photo stability studies: The photochemical stability of the drug has also been studied by exposing the 100 µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt h/m^2 in photo stability chamber. The final solution was diluted to obtain standard concentration and 10 µl solution injected into the system. The chromatograms were recorded to assess the stability of sample. The degradation chromate gram was showed in Figure 11.

Table 10: Degradation study results of developed method

Chromatographic condition	Concentration of DGFZ (µg/ml)	Peak area	% of degradation	Concentration of SGPT (µg/ml)	Peak area	% of degradation
No degradation	10.0	1008292	0.0	5.0	553427	0.0
Oxidative degradation	10.0	911893	9.56	5.0	516932	6.59
Acid degradation	10.0	986525	2.15	5.0	509110	8.0
Alkali degradation studies	10.0	946549	6.12	5.0	503120	9.09
Thermal/dry heat degradation studies	10.0	918717	8.88	5.0	531924	3.88
Photo stability studies	10.0	958504	4.93	5.0	538534	2.69

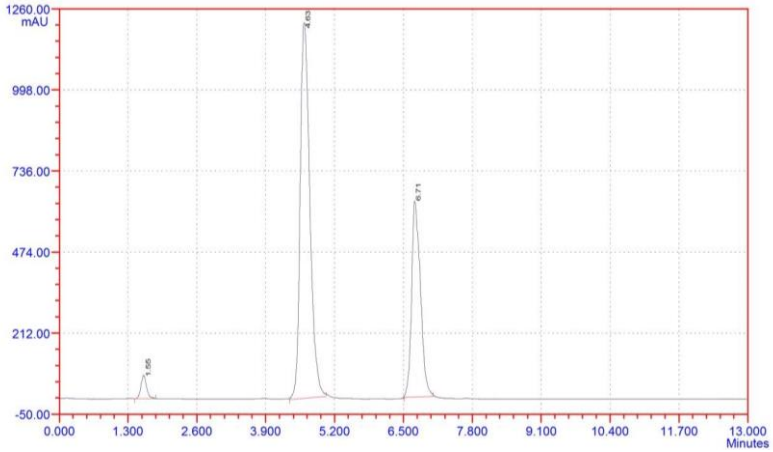
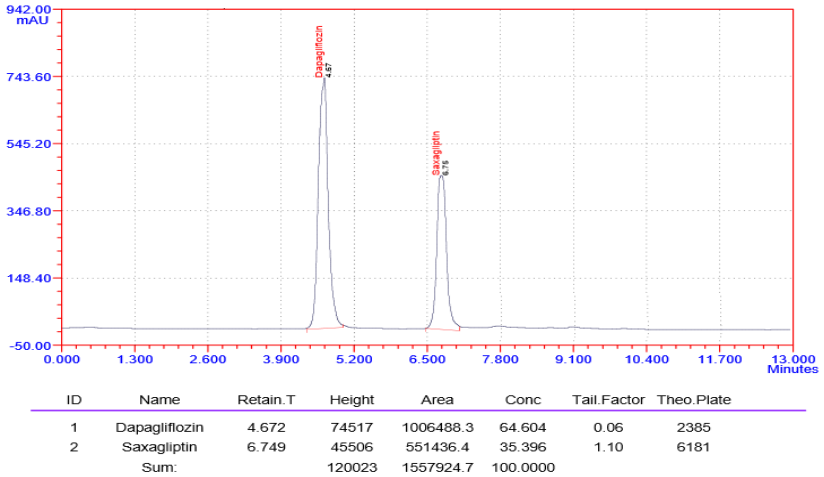


Figure 11: Photo stability degradation chromatogram

Formulation analysis

Table 11: Formulation analysis results

Formulation	Drug	Label claim	Sample concentration	Standard area	Formulation area	Amount of drug found	% of accuracy
Qtern	DGFZ	10 mg	10 µg/ml	1008292	1006488	9.982 µg/ml	99.82%
	SGPT	5 mg	5.0 µg/ml	553427	551436	4.982 µg/ml	99.64%



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Dapagliflozin	4.672	74517	1006488.3	64.604	0.06	2385
2	Saxagliptin	6.749	45506	551436.4	35.396	1.10	6181
	Sum:		120023	1557924.7	100.0000		

Figure 12: Formulation chromatogram of DGFZ and SGPT

RESULTS AND DISCUSSION

A new RP- HPLC method has developed for simultaneous estimation of DGFZ and SGPT in a formulation. It is shown that the method is precise, accurate, reproducible, linear, selective and specific providing the reliability of the method. The method is validated over the concentration range of 2.5-40.0 µg/ml for DGFZ and 1.25-20.0 µg/ml for SGPT. A linearity result is shown in Table 2. The mean percent recovery of DGFZ is 100.24%, SGPT is 100.09%. Recovery results are shown in Table 7. The intra and inter-day precision, ruggedness and robustness has been conducted at standard concentration, the percentage of RSD value for all tests is less than 2%. The LOQ concentration of DGFZ is 0.3125 µg/ml and SGPT is 0.15625 µg/ml. The LOD concentration of DGFZ was 0.15625 µg/ml and SGPT was 0.078125 µg/ml. The drug solution stability test has been performed at 0, 6, 12, 18, 24 and 30 h. Up to 30 h the solution is stable and error is below 3%. The forced degradation study has been conducted at standard concentration. Results are shown in Table 10. The formulation analysis results were showed in Table 11 and Figure 12.

CONCLUSION

In the literature, there is no HPLC method for simultaneous estimation of DGFZ and SGPT. The analytical method developed is simple and has good accuracy and reproducibility. It can be used for the estimation of DGFZ and SGPT in bulk drug and in a formulation. The method was validated for linearity, accuracy, precision, ruggedness, robustness LOD, LOQ and recovery. The separation method developed produce acceptable values of recovery.

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