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Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF METFORMIN, SAXAGLIPTIN, AND DAPAGLIFLOZIN

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ABSTRACT

Objective: A new combination of Metformin, Saxagliptin, and Dapagliflozinfixed-dose combination of antidiabetic medication is being used as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes.

Methods: Chromatography was carried out using Kromasil C18 250×4.6, 5.0 mm column with a flow rate of 1.0 ml/min. The mobile phase comprised of pH 4.8 0.01 N Potassium dihydrogen phosphate buffer and Acetonitrile in the ratio of 65:35 is used. The developed method has been validated for various parameters such as precision, accuracy, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), and solution stability. The stability-indicating capability of the method was established by forced degradation studies under stress conditions such as acid, base, peroxide, UV, thermal, and humidity.

Results: The retention times of Metformin, Saxagliptin, and Dapagliflozin were found to be 2.294, 2.869, and 3.887 min, respectively. The method was specific and linear (R^2>0.999) for MET, SXG, and DGF. The LOD and LOQ were found to be 13.85 µg/ml and 41.95 µg/ml for MET, 0.06 µg/ml and 0.19 µg/ml for SXG and 0.19 µg/ml and 0.57 µg/ml for DGF, respectively. The mean % recovery obtained was found to be 100.21% for MET, 99.83% for SXG, and 99.97% for DGF, respectively.

Conclusion: Hence, the chromatographic method developed was said to be rapid, simple, specific, sensitive, precise, accurate, robust, and reliable that can be effectively applied for routine analysis in research institutions, quality control departments in industries.

Keywords: Method development, Validation, Metformin, Saxagliptin, Dapagliflozin.

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INTRODUCTION

Metformin, Saxagliptin, and Dapagliflozin is an antidiabetic medication used as an adjunct to diet and exercise to improve glycemic control in adults with Type 2 diabetes [1]. It is taken orally. The most common side effects include nose and throat infections, hypoglycemia and effects on the gut such as nausea, vomiting, diarrhea, abdominal pain, and loss of appetite. The fixed-dose combination was approved for medical use in the United States in May 2019 and in the European Union in November 2019 [2].

Metformin, a biguanide anti-hyperglycemic agent is the first-line medication used in the treatment of Type 2 diabetes, especially for the people who are obese, and it is also being used in the treatment of polycystic ovary syndrome [3]. Metformin (Mary Rebecca Y, 2021) is generally preferred for gestational diabetes over insulin [4]. The working of Metformin includes decreasing of glucose production by the liver, thereby increasing the insulin sensitivity of body tissues by exerting an anorexiant effect, finally leads to reduced intake of calories. Saxagliptin, is an oral hypoglycemic belonging to the class of the dipeptidyl peptidase-4 inhibitors [5]. Saxagliptin is used as monotherapy or in combination with other drugs for the treatment of Type 2 diabetes (Jamwal, 2020). The mechanism of action includes increasing the amount of insulin produced by the body immediately after meals when blood sugar is high. Saxagliptin is not used as the drug of choice to treat Type 1 diabetes or diabetic ketoacidosis. Dapagliflozin is used to treat Type 2 diabetes and also Type 1 diabetes with certain restrictions [6]. It can also be used to treat adults suffering with heart failure with reduced ejection fraction and ultimately to reduce the risk of cardiovascular death and hospitalization for heart failure. The chemical structures of Metformin, Saxagliptin and Dapagliflozin were depicted in Figs. 1-3 respectively.

Extensive literature review was conducted and identified that, till now, no RP-HPLC method was reported for the three drug combination Metformin, Saxagliptin, and Dapaglifloxin. Few other analytical, spectrophotometric, and HPTLC densitometric methods [7-14] have been reported for the estimation of Metformin, Saxagliptin, and Dapaglifloxin individually and or along with drug combinations in pharmaceutical preparations.

The aim of this study is to develop and validate a new simple, accurate and economic stability-indicating HPLC method with less runtime, which would be able to separate and quantify the combination of MET, SXG, and DGF in a single run. The developed method was validated as per ICH guidelines [15,16] and can be applied lucratively to quality control purposes.

METHODS

Equipment

HPLC analysis was carried out on Waters Alliance-HPLC system equipped with 2695-separation module connected to 2996-photo diode array detector, and the data were acquired by Empower® version 2.The other equipment used were Mettler Toledo ME 204 weighing balance, Magnetic stirrer, Eutech 700 pH meter, Double distillation apparatus. Ultrasonic bath (Labman Scientific Instruments Pvt. Ltd. Chennai, India) was used for sonication of the samples. Hot air oven was used to carry out thermal degradation studies. UV crosslinker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 and 300 nm was used for photodegradation studies.

Chemicals and reagents

Metformin, Saxagliptin, and Dapaglifloxin working standards were obtained as gift samples by Spectrum Pharma Limited, Hyderabad. HPLC grade solvents acetonitrile, water, and methanol were used.

Analytical grade chemicals sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, Orthophosphoric acid, Triethylamine, and potassium dihydrogen phosphate were purchased from E. Merck Limited, Mumbai, India.

Chromatographic conditions

HPLC analysis was carried out on Waters Alliance-HPLC system equipped with 2695-separation module connected to 2996-photo diode array detector, and the data were acquired by Empower® version 2. Separation was achieved using Kromasil C18 250×4.6, 5.0 mm as a column with mobile phase 0.01 N Potassium dihydrogen phosphate buffer (pH 4.8) and Acetonitrile in the ratio 65:35. The samples were estimated using 10 µL injection volume, flow rate was maintained at 1.0 ml/min with runtime of 7 min, and the temperature was maintained at 30°C throughout the analysis. Detection of the drugs was attained using PDA detector at 260 nm wavelength.

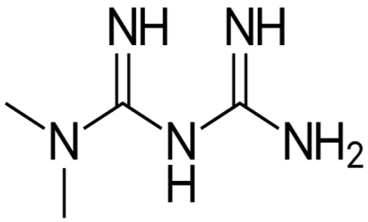

Fig. 1: Structure of metformin

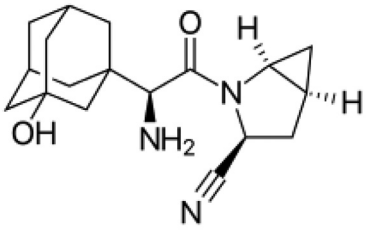

Fig. 2: Structure of saxagliptin

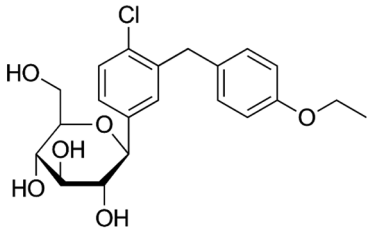

Fig. 3: Structure of dapagliflozin

Preparation of working standard solution

Accurately weighed and transferred 500 mg of Metformin, 2.5 mg of Saxagliptin, and 5mg of Dapagliflozin working Standards into a 50 ml clean dry volumetric flask. To that 10 ml of diluent was added and sonicated for 10 min to dissolve. The final volume was made with the diluent and filtered through 0.45 µ nylon filter to obtain a final concentration of 10,000 µg/ml Metformin, and 50 µg/ml Saxagliptin and 100 µg/ml of Dapagliflozin. From the above stock solution, 1 ml was pipetted out into a 10 ml volumetric flask and then made up to the final volume with diluent. The final concentration obtained for Metformin, Saxagliptin, and Dapagliflozinwere 1000 µg/ml, 5 µg/ml, and 10 µg/ml, respectively.

Preparation of sample solution

Ten tablets were weighed accurately, and average weight was calculated. The tablets were then crushed, and a portion of powder equivalent to the weight of one tablet was weighed and transferred to a 100 ml volumetric flask. Approximately, 50 ml of diluent was added and sonicated for 15 min with intermittent shaking. Now 20 ml of diluent was added and again sonicated for around 25 min. The contents were then restored to room temperature and diluted to final volume with diluent to obtain a concentration of 10,000 µg/ml Metformin, and 50 µg/ml Saxagliptin and 100 µg/ml of Dapagliflozin. This was considered as the stock solution.

The stock solution was filtered through 0.45 µ nylon filter and 0.5 ml of the filtered solution was transferred to a 10 ml volumetric flask and the final volume was made with diluent. The final concentration of the solution obtained consisted of 1000 µg/ml, 5 µg/ml, and 10 µg/ml of Metformin, Saxagliptin, and Dapagliflozinrespectively.

Method validation

The developed RP-HPLC method was validated according to ICH guidelines Q2 (R1) in order to determine the validation parameters such as system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness, and robustness.

System suitability

System suitability parameters were estimated to validate the performance of the system. 10 µL of standard solution was injected 5 times into the system and the chromatograms were recorded. System suitability parameters were determined, and all the parameters were found to be within the specified limits.

Specificity

The specificity of the developed analytical method was determined by injecting the 100 µg/mL concentration solutions each of blank, placebo, 1000 µg/ml, 5 µg/ml, and 10 µg/ml concentration solutions of Metformin, Saxagliptin, and Dapagliflozin respectively.

Precision

Repeatability/method precision was evaluated by injecting the six replicates of MET, SXG, and DGF with the same concentration

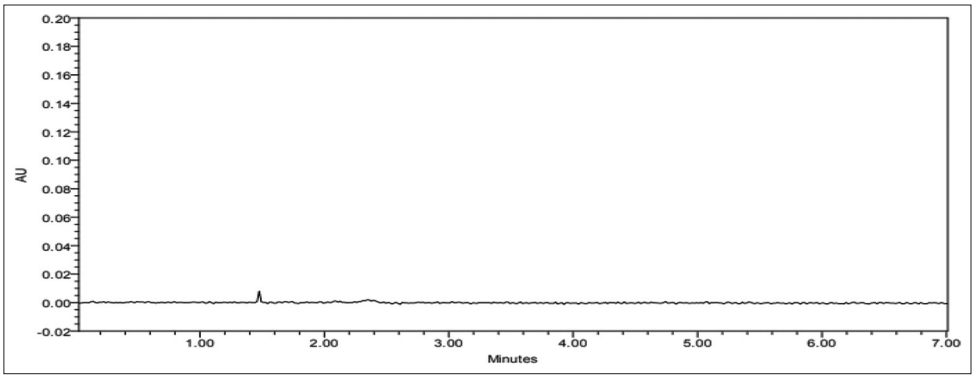

Fig. 4: Chromatogram of blank

(100 µg/mL) and % assay, % RSD was determined. Reproducibility/ Ruggedness/Intermediate precision was performed using different analysts, on different system and on the other day in the same laboratory.

Accuracy

Accuracy was calculated by performing recovery study using the spiking method. It was carried out by spiking certain known amounts (50%, 100%, and 150%) of the working standard solution to the pre-analyzed sample. To determine the accuracy of the proposed method, the solutions were prepared in triplicates, injected into the system, and the chromatograms were recorded.

Linearity

The linearity of the proposed method was determined by injecting the standard solutions prepared of different concentrations of MET, SXG, and DGF. 6 working standard solutions of different concentrations between 250 and 1000 µg/ml for MET, 1.25–7.5 µg/ml for SXG, and 2.5–15 µg/ml for DGF were prepared, injected into the system and the chromatograms were recorded. The results were evaluated by least-squares regression analysis, and the calibration equation and correlation coefficient were calculated.

LOD and LOQ

LOD and LOQ of the present method was established using calibration curve method. Solutions of MET, SXG, and DGF, which were prepared in triplicates in the range of linearity, were injected into the system.

Robustness

To determine the robustness of the developed method, a few of the experimental conditions were calculatingly changed, and the system suitability parameters of MET, SXG, and DGF peaks were estimated. The flow rate was altered by ±0.1 mL/min, the column temperature was altered by ±5°C. The composition of organic phase of the mobile phase was changed ±5% from the original composition maintaining the aqueous phase composition as constant.

Solution stability

About 10 µl of standard solution was injected 6 times to determine the solution stability at 0 h (initial) and at 24 h (final). The chromatograms were recorded, and the system suitability parameters were evaluated.

Forced degradation studies

To determine the stability-indicating parameter of the developed method, stress studies were performed on working standard solutions of concentrations 1000 µg/ml, 5 µg/ml, and 10 µg/ml of MET, SXG, and DGF, respectively.Degradation was attempted by exposing to the stress conditions 1.2 million lux hours followed by 200 Watt hours (photolytic stress), heat (exposed at 105°C for 6 h), acid (2 N HCl for 2 h at 60°C), alkaline (2 N NaOH for 2 h at 60°C), oxidation (20% peroxide for 30 min at 60°C), water (refluxed for 12 h at 60°C), and humidity (exposed to 85% RH for 72 h). The prepared solutions were injected into the system, chromatograms were recorded, and the stability of the method was evaluated.

Table 1: System suitability data

Parameter	MET	SXG	DGF	Acceptance criteria
USP Plate count*	3349	4325	6390	NLT 3000
%RSD	0.5	1.6	0.7	NMT 2.0
Peak Tailing*	1.5	1.4	1.3	NMT 2.0
Resolution*	-	3.4	5.3	>1.5

MET: Metformin, SXG: Saxagliptin, DGF: Dapagliflozin

Table 2: Precision data

S. No.	Peak areas	% Assay	Peak areas	% Assay	Peak areas	% Assay
	MET		SXG		DGF	
1	3475028	99.55	297118	101.06	380281	100.78
2	3508798	100.52	293187	99.73	371354	98.41
3	3487685	99.91	296191	100.75	381394	101.07
4	3527095	101.04	288614	98.17	372796	98.80
5	3529660	101.12	297796	101.29	383928	101.75
6	3526124	101.01	294716	100.25	375346	99.47
Mean	3509065	100.53	294604	100.21	377517	100.05
SD	23047.5	0.660	3374.3	1.15	5074.6	1.345
% RSD	0.7	0.7	1.1	1.1	1.3	1.3

Table 3: Accuracy data

Drug name	Conc. (%)	Amount spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Statistical parameters
MET	50	500	501.57	100.32	Mean %: 100.21
	100	1000	999.58	99.96	SD: 0.82
	150	1500	1506.34	100.42	%RSD: 0.8
SXG	50	2.5	2.48	99.63	Mean %: 99.83
	100	5	5.02	100.29	SD: 1.09
	150	7.5	7.47	99.58	%RSD: 1.09
DGF	50	5	4.99	99.65	Mean %: 99.97
	100	10	10.09	100.97	SD: 1.28
	150	15	14.89	99.41	%RSD: 1.2

Table 4: Linearity data

S. No.	MET		SXG		DGF	
	Concentration (µg/ml)	Peak area*	Concentration (µg/ml)	Peak area*	Concentration (µg/ml)	Peak area*
1	250	883603	1.25	77266	2.5	94018
2	500	1762818	2.5	155740	5	188384
3	750	2698635	3.75	225616	7.5	289643
4	1000	3455468	5	291166	10	378713
5	1250	4292326	6.25	365239	12.5	464490
6	1500	5233693	7.5	444306	15	557330
	Regression equation y=3439.5x+44843 R^2=0.9992		Regression equation y=57811x+6964.2 R^2=0.9992		Regression equation y=36959x+5367.7 R^2=0.9994	

*Average peak area of 3 replicate injections for each concentration

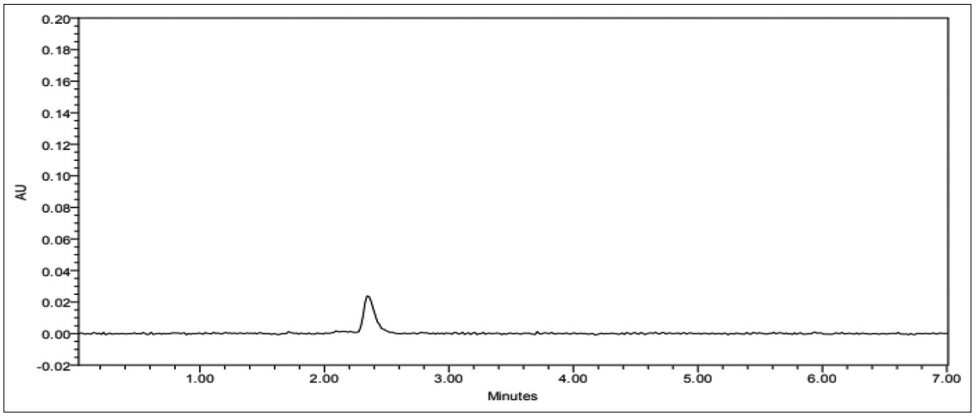

Fig. 5: Chromatogram of placebo

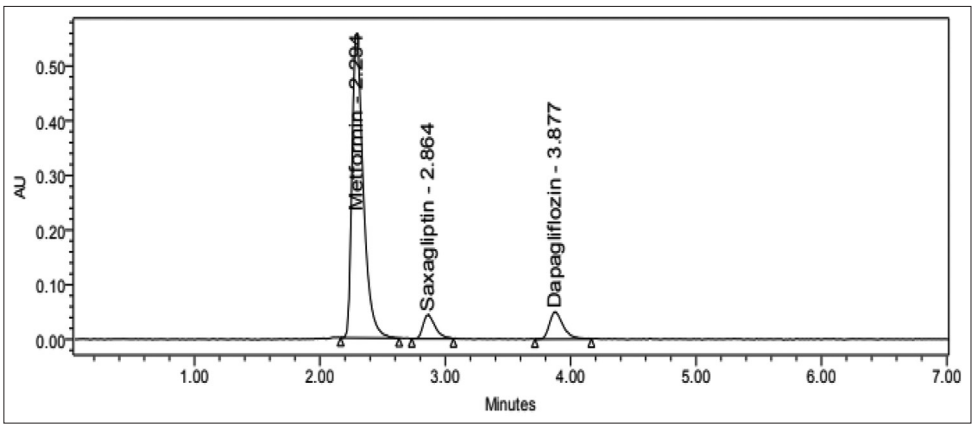

Fig. 6: Chromatogram of mixture of Metformin, Saxagliptin, and Dapagliflozin

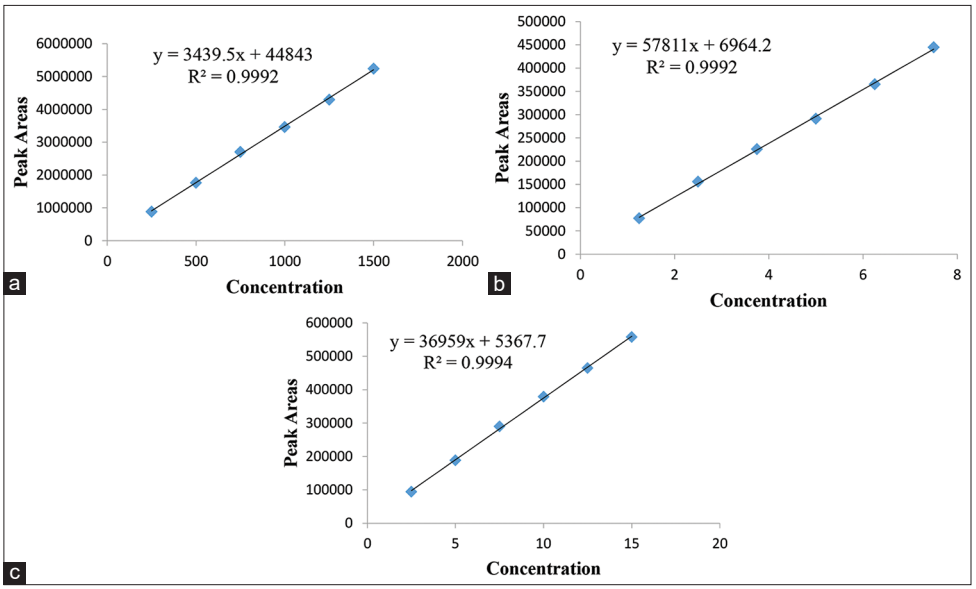

Fig. 7:(a) Standard curve of Metformin. (b) Standard curve of Saxagliptin. (c) Standard curve of Dapagliflozin

RESULTS AND DISCUSSION

System suitability

The column efficiency was identified from the theoretical plate count which was found to be more than 3000 and tailing factor which was found to be between 0.80 to 2.0, and the %RSD obtained was <2.0%. The results of system suitability parameters are summarized in Table 1. and were found to be satisfactory.

Specificity

The results of specificity indicate the non-interference of the co-eluting peaks at the retention times of MET, SXG, and DGF which can be used to determine the purity of the analyte peak and indicates that there is no interference with the excipients used in the formulation. The obtained chromatograms were summarized in Figs. 4-6. Figs. 4 and 5 confirm that the blank and placebo peaks were not interfering at the retention time of MET, SXG, and DGF.

Table 5: LOD and LOQ data

Drug name	LOD (µg/ml)	LOQ (µg/ml)
MET	13.85	41.95
SXG	0.06	0.19
DGF	0.19	0.57

Table 6: Robustness data

Parameter	System suitability parameters				
	RT (min)	Plate count	Peak tailing	Resolution	% RSD
Optimized method					
MET	2.294	3349	1.5	-	0.5
SXG	2.869	4325	1.4	3.4	1.6
DGF	3.887	6390	1.3	5.3	0.7
Flow rate (1.1 ml/min)					
MET	2.165	3161	1.4	-	0.8
SXG	2.671	4006	1.2	3.3	1.6
DGF	3.562	5649	1.2	5.1	1.5
Flow rate (0.9 ml/min)					
MET	2.596	3256	1.4	-	0.8
SXG	3.247	4147	1.3	3.3	1.4
DGF	4.393	6179	1.2	5.3	0.8
Organic phase (60:40)					
MET	2.153	3257	1.4	-	0.8
SXG	2.824	4036	1.3	2.7	1.4
DGF	3.757	5576	1.2	4.5	1.2
Organic phase (70:30)					
MET	2.405	3182	1.4	-	0.9
SXG	3.155	3895	1.3	3.9	1.4
DGF	4.453	5380	1.2	5.7	1.2
Temperature (35°C)					
MET	2.396	3251	1.3	-	0.9
SXG	2.934	3853	1.2	2.9	1.3
DGF	3.898	5117	1.2	4.6	1.2
Temperature (25°C)					
MET	2.411	3165	1.3	-	0.4
SXG	3.231	3652	1.2	4.1	1.4
DGF	4.634	5183	1.2	5.9	0.9

Table 7: Solution stability at 0 h (initial)

Parameter	MET	SXG	DGF	Acceptance criteria
USP Plate count*	3349	4325	6390	NLT 3000
%RSD	0.5	1.6	0.7	NMT 2.0
Peak Tailing*	1.5	1.4	1.3	NMT 2.0
Resolution*	-	3.4	5.3	>1.5

Table 8: Solution stability at 24 h (final)

Parameter	MET	SXG	DGF	Acceptance criteria
USP Plate count*	3428	4424	6431	NLT 3000
%RSD	1.5	1.4	1.6	NMT 2.0
Peak Tailing*	1.4	1.3	1.3	NMT 2.0
Resolution*	-	3.4	5.4	>1.5

*Average of 6 replicate injections

Precision

% Assay for MET, SXG, and DGF obtained was found to be in the range of 98–102%, and the % RSD for MET, SXG, and DGF were found to be within 2%. The results, which were summarized in Table 2, confirm that the developed method was precise, rugged, and reproducible.

Accuracy

The % recovery for MET, SXG, and DGFwere within the range of 98–102%. The % RSD for MET, SXG, and DGFwere found to be within 2%. From the results in Table 3, it was evident that the developed method was accurate.

Table 9: Forced degradation studies at different stress conditions

Stress condition	% Degradation	Purity Angle	Purity Threshold
Metformin			
Acid	5.75	0.293	0.363
Base	4.07	0.308	0.351
Peroxide	3.46	0.367	0.581
Thermal	2.45	0.275	0.384
Photo Stability	1.90	0.288	0.642
Water	1.18	0.371	0.410
Saxagliptin			
Acid	5.98	0.562	0.681
Base	4.02	0.277	0.578
Peroxide	3.12	2.167	2.581
Thermal	1.84	1.908	2.746
Photo Stability	1.22	2.066	7.783
Water	0.80	2.008	2.433
Dapagliflozin			
Acid	5.67	0.662	0.725
Base	4.10	0.767	1.012
Peroxide	3.66	0.791	1.064
Thermal	2.07	0.719	1.202
Photo Stability	1.69	0.789	3.120
Water	0.74	0.731	0.957

Linearity

Linearity was evaluated by analyzing different concentrations of the standard solutions. R^2 value was found to be greater than 0.999 for MET, SXG, and DGF. From the results which were provided in Table 4, confirms good linearity among the various concentrations of MET, SXG, and DGF. The standard curves of MET, SXG, and DGFwere shown in Fig. 7a-c, respectively.

LOD and LOQ

The LOD and LOQ of MET, SXG, and DGF were calculated from calibration curve method using following equations (ICH, Q2 (R1)) using standard deviation and the slope of calibration curve. The results were tabulated in Table 5.

Robustness

From the results presented in Table 6, it was evident that the system suitability parameters of MET, SXG, and DGF remained unaffected by deliberate changes. These results confirm that the present developed method was robust.

Solution stability

Results from Tables 7 and 8 indicate that the system suitability parameters at 0 h (initial) and at 24 h (final) are within the acceptable limits according to ICH guidelines which indicates that the standard solution was stable till 24 h.

Forced degradation studies

To determine the purity of MET, SXG, and DGF, samples were subjected to intended degradation along with blank and placebo which were analyzed with the optimized HPLC conditions. In all the degradation conditions, the peaks of MET, SXG, and DGF were well resolved which indicates that the sample was not degraded. The peak purity of MET, SXG, and DGF was determined based on purity angle and purity threshold. The results summarized in Table 9 confirm that the present developed method can be considered to "stability-indicating."

CONCLUSION

A simple, precise, accurate, linear, and robust RP-HPLC method has been developed for the simultaneous determination of Metformin, Saxagliptin, and Dapagliflozin in active pharmaceutical ingredients. The proposed method was validated in accordance with ICH guidelines by evaluating various parameters. The method was found to be specific

where there is no interference of the peaks of blank, placebo, and the excipients used in the formulation. The results from the degradation studies where the purity angle is less than purity threshold indicates the purity of MET, SXG, and DGF peaks and the method was found to be stability-indicating. Thus, the stability-indicating RP-HPLC method developed for the estimation of MET, SXG, and DGF can be implemented in the routine analysis in various pharmaceutical industries.

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AUTHOR'S CONTRIBUTION

Haritha Pavani Kondeti has involved in the design of the study, sampling, and experimental analysis. Gowri Sankar Dannana has been contributed in data analysis and data interpretation. Both the authors drafted the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest in publishing this manuscript.

AUTHOR'S FUNDING

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