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Method development of metformin, saxagliptin and dapagliflozin in marketed formulation by HPLC

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***Abstract*---**The fixed dose combination of Metformin, Saxagliptin and Dapagliflozin is a recently approved antidiabetic medication. The aim of this study was to develop a simple, rapid, sensitive, and validated isocratic reversed phase-high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Metformin, Saxagliptin and Dapagliflozin in bulk and formulation as per ICH Guidelines. The developed method was found simple, sensitive and economical for the simultaneous estimation of MET, SXG and DGF in their tablet formulation. The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. The robustness of developed method was checked by changing in the deliberate variation in solvent.

***Keywords*---**metformin, saxagliptin and dapagliflozin, simultaneous estimation, HPLC validation.

Introduction

The prevalence of type 2 diabetes mellitus (T2DM), which affects 8% of the population worldwide, is expected to rise to 400 million cases by 2030 [1]. The prevalence of T2DM suggests a pressing need for innovative therapies and prevention measures. Plasma glucose homeostasis gradually deteriorates as a result of the disease's growing cell dysfunction in the presence of chronic insulin resistance. The following effects include increased glucagon secretion, gluconeogenesis, diminished incretin response, and renal glucose reabsorption. The main goal of treating T2DM is to maintain a healthy blood sugar level [2, 3]. Metformin, a member of the biguanide class, is prescribed as the initial treatment for type 2 diabetes. It works by decreasing the liver's ability to produce glucose, and the target cell's sensitivity to insulin is further increased by its decreased intestine absorption [4]. The gliptins, commonly known as dipeptidyl peptidase-4 inhibitors, include saxagliptin. They raise plasma levels of incretins (GLP-1 and GIP), which causes insulin to rise and blood glucose to fall [5, 6]. Inhibitors of sodium-glucose cotransporter-2 (SGLT2) include dapagliflozin (Fig. 1). Following a review of the literature, it was discovered that numerous analytical techniques, including UHPLC, RP-HPLC have been described for the quantification of metformin, dapagliflozin, and saxagliptin alone and in combination with other medications [7–30]. Not any simple cost effective method has been developed for the estimation MET, SXG and DGF, Hence, an attempt has been made to develop and validate in accordance with ICH guidelines [20].

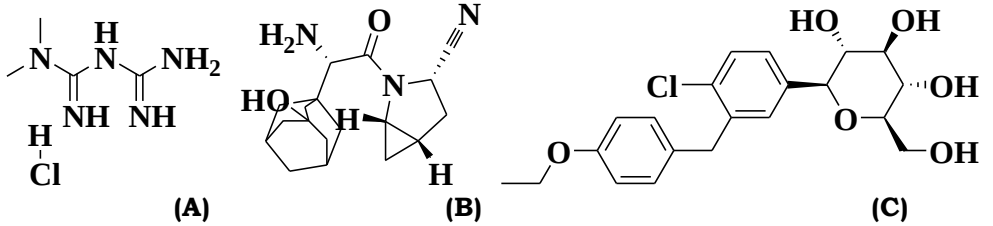


Figure 1 Chemical structure of (A) Metformin (B) Saxagliptin (C) Dapagliflozin

Material and Methods

Instrument

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

Drug and Chemicals

MET, SXG and DGF were obtained as pure sample from Pharmaceutical Company, as gift samples along with their analytical reports. HPLC grade methanol and acetonitrile was obtained from Merck (India) limited. Potassium Dihydrogen orthophosphate and Ortho- phosphoric acid (GR grade) was obtained from S.D. Fine Chemicals Ltd, Mumbai, India. All other chemical used were of

analytical grade. Triple distilled water was used for whole experiment was generated in house.

Methods
Selection of Mobile Phase

Initially to estimate MET, SXG and DGF in fix dosage form number of mobile phase in different ratio were tried. The mobile phase found to be most suited for analysis was 20mM KH2PO4: Acetonitrile (pH 3.5 with OPA) in the ratio of 30:70v/v, taking into account system suitability parameters such as RT, Tailing factor, No. of theoretical plates, and HETP. To remove particulate debris, the mobile phase was filtered through 0.45m filter paper and then degassed using sonication. For the analysis, a flow rate of 1.0 ml/min was used.

Preparation of standard Stock solution

Accurately weighed 10 mg of MET, SXG and DGF was transferred into 10 ml volumetric flasks separately and dissolved in 5 ml of methanol and sonicate for 10 min., then volume was made up to 10 ml with Acetonitrile. Concentration of MET, SXG and DGF in methanol was 1000µg/ml. (stock- A).

Preparation of Sub Stock Solution

1 ml of solution was taken from stock-A of MET, SXG and DGF and transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent (Acetonitrile) to give concentration of 100µg/ml (Stock-B).

Preparation of Different Solution

0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with (Acetonitrile). This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml for drug. In same manner 0.1µg/ml, 0.2µg/ml, 0.3µg/ml, 0.4µg/ml, 0.5µg/ml of SXG and DGF also prepared.

Linearity and Calibration Graph

A series of dilutions ranging from 5-25 g/ml for MET, 5-25 g/ml for SXG, and 0.1-0.5 g/ml for DGF were created to establish the linearity of the analytical method. All of the solutions were filtered and injected through a 0.2m membrane filter, and chromatograms were obtained at 275 nm three times. Between the mean peak area and the respective concentration, a calibration graph was drawn, and a regression equation was established.

Validation of HPLC Method development
Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were used to assess the accuracy of the suggested approaches at three different levels: 80 percent, 100 percent, and 120 percent. The recovery investigations were carried out by mixing a specified amount of MET, SXG, and DGF standard solution with pre-analysed tablet powder. The solutions were then re-analyzed using the provided methodologies. To determine the recovery of the added drug sample, the entire analysis method was repeated. This recovery analysis was carried out three times for each of the five concentration levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to day was performed by analyzing 5 different concentration of the drug for three days in a week.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of tablets formulation

Amount corresponding to 100 mg of MET (0.25 mg SXG and 0.5 mg DGF) was taken in a 10 ml volumetric flask, which was weighed and ground to a fine powder. The flask was then sonicated for around 10 minutes to solubilize the medication included in the tablet powder, after which the volume was made up to the mark with methanol. Filtration was carried out after sonication using a 0.45 membrane filter. Filtrate was collected and diluted with methanol until the final concentrations of both medicines were within the acceptable range. The concentrations were acquired using the calibration curve method and the mean area of final dilutions was observed. The process was carried out five times more.

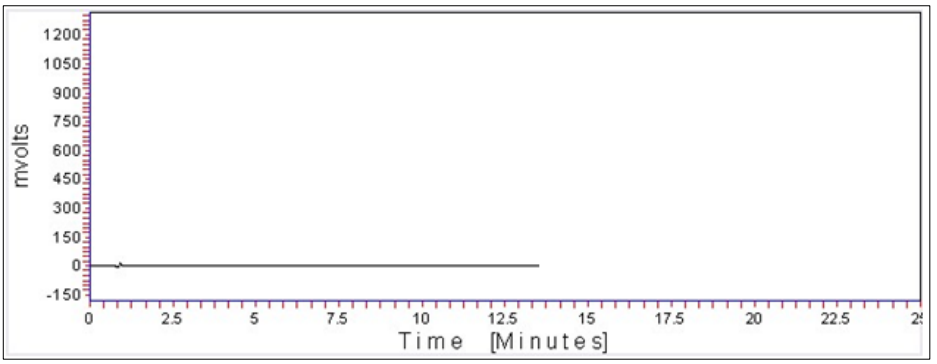
Results and Discussion

This work was majorly emphasized to establish new RP-HPLC method for the simultaneous quantification of MET, SXG and DGF in their recently approved fixed dosage combinations. After multiple systematictrials, the optimum chromatographic condition having well-resolved peaks with better peak shape was achieved by using an isocratic mobile phase The RP-HPLC method was developed for estimation of Metformin (MET), Saxagliptin (SXG) and Dapagliflozin (DGF) in combined formulation by isocratically using 20mM

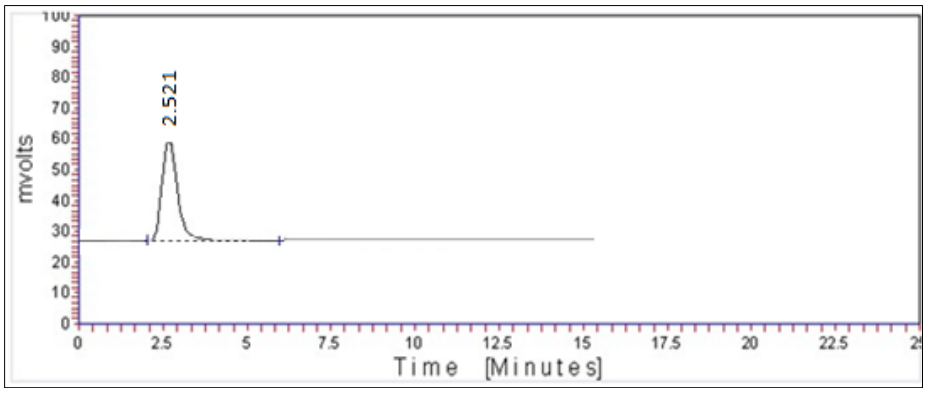
KH2PO4: Acetonitrile (pH 3.5 with OPA) in the ratio of 30:70v/v as mobile phase, Prontosil C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 275mm. Then developed method was validated by using various parameters.

Method validation System suitability test System suitability was enumerated by performing six independent injections of working standard solutions of the drugs. Parameters including % relative standard deviation (RSD), the number of theoretical plates, retention time, and tailing factors were calculated. The mean values for Tailing Factor were found to be 1.127±0.018%, 1.142±0.021% and 1.145±0.026 for MET, SXG and DGF respectively. The specificity of the method was evaluated by performing an analysis of the standard solution and blank solution for the presence of possible interferences Figure 1. The HPLC chromatogram for the drug and blank showed no interfering peaks. Five point calibration curves were obtained in a concentration range of 5µg/ml-25µg/ml for MET, 0.1µg/ml-0.5µg/ml for SXG and 0.1µg/ml-0.5µg/ml for DGF. Peak area and concentration data were subjected to least square regression analysis and the response of the drugs was found to be linear in the investigated concentration ranges. The linear regression equations were y = 51.23x+2.699 for MET, y = 1418x+0.056 for SXG and y = 1202x+2.328 respectively. The coefficient of determination (R^2) values was 0.999, 0.999 and 0.999 for MET, SXG and DGF respectively. The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method.

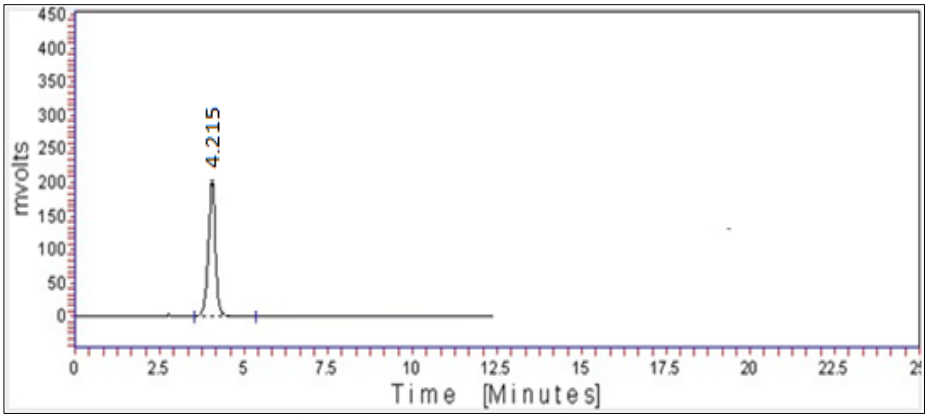
Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. The robustness of developed method was checked by changing in the deliberate variation in solvent. The results of the analysis of synthetic mixture were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drugs.



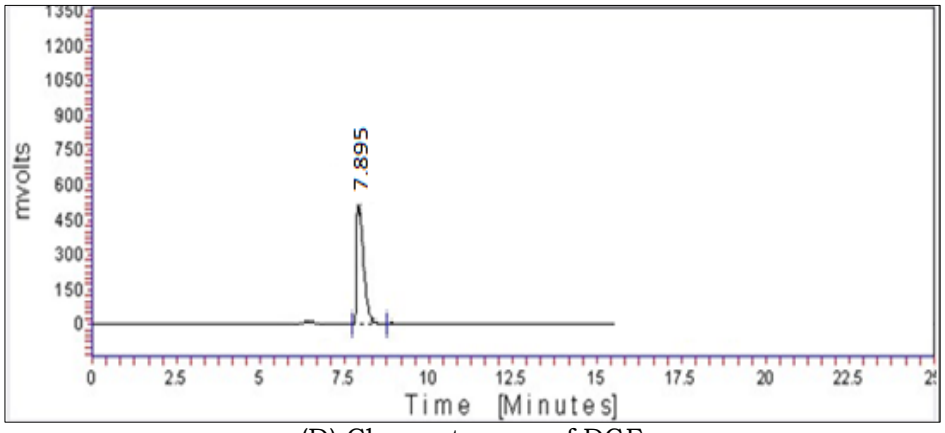
(A) Chromatogram of Blank



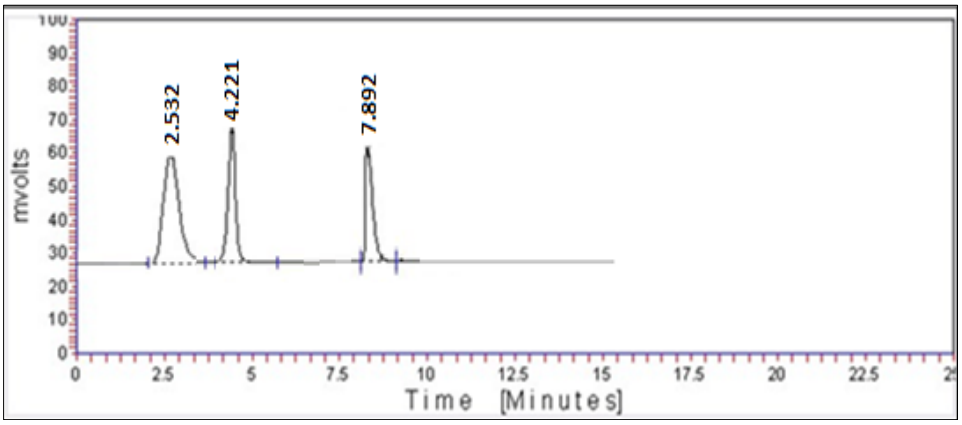
(B) Chromatogram of MET



(C) Chromatogram of SXG



(D) Chromatogram of DGF



(E) Chromatogram of MET, SXG, DGF

Figure 1: (A) Chromatogram of Blank, (B) Chromatogram of MET, (C) Chromatogram of SXG, (D) Chromatogram of DGF, (E) Chromatogram of MET, SXG, DGF

Table 1: Results of linearity of Metformin (MET), Saxagliptin (SXG) and Dapagliflozin (DGF)

PARAMETER	MET	SXG	DGF
Concentration (µg/ml)	5-25	0.1-0.5	0.1-0.5
Correlation Coefficient (r^2)*	0.999	0.999	0.999
Slope (m)*	51.23	1418	1202
Intercept (c)*	2.699	0.056	2.328

*Value of three replicate

Table 2: Results of recovery study

% Level	% MEAN±SD*		
	MET	SXG	DGF
80%	98.86±0.452	96.21±0.519	95.96±1.425
100%	98.97±0.729	96.24±0.944	95.11±1685
120%	98.79±0.579	97.10±1.524	97.06±1.566

***** Value of three replicate and five concentrations.

Table 3: Results of precision

Parameter	% MEAN±SD*		
	MET	SXG	DGF
Repeatability	99.395±0.070	97.089±0.009	97.567±0.009
Intermediate precision			
Day to day precision	99.548±0.056	95.100±0.007	95.967±0.007
Analyst-to-Analyst	99.497±0.054	95.033±0.006	97.350±0.010
Reproducibility	99.535±0.079	96.617±0.009	96.378±0.008

***** Value of five replicate and five concentrations

Table 4: LOD and LOQ of MET, SXG and DGF

Name	LOD (µg/ml)	LOQ (µg/ml)
MET	0.50	1.50
SXG	0.012	0.035
DGF	0.018	0.050

Table 5: Analysis of tablets formulation of MET, SXG and DGF

Conc. Present (µg/ml)			Replicate-1					
			Conc. Found (µg/ml)			% Conc. Found		
MET	SXG	DGF	MET	SXG	DGF	MET	SXG	DGF
5	0.1	0.1	4.99	0.1	0.1	99.80	100.00	100.00
10	0.2	0.2	9.95	0.19	0.19	99.50	95.00	95.00
15	0.3	0.3	14.88	0.29	0.29	99.20	96.67	96.67
20	0.4	0.4	19.95	0.38	0.39	99.75	95.00	97.50
25	0.5	0.5	24.85	0.49	0.48	99.40	98.00	96.00
					MEAN	99.53	96.93	97.03
					SD	0.249	1.127	1.894
					% RSD	0.250	1.194	1.952

Table 6: Analysis of tablets formulation of MET, SXG and DGF

Conc. Present (µg/ml)			Replicate-2					
			Conc. Found (µg/ml)			% Conc. Found		
MET	SXG	DGF	MET	SXG	DGF	MET	SXG	DGF
5	0.1	0.1	4.98	0.1	0.1	99.60	100.00	100.00
10	0.2	0.2	9.99	0.19	0.18	99.90	95.00	90.00
15	0.3	0.3	14.95	0.29	0.30	99.67	96.67	100.00
20	0.4	0.4	19.96	0.39	0.39	99.80	97.50	97.50
25	0.5	0.5	24.85	0.49	0.49	99.40	98.00	98.00
					MEAN	99.67	97.43	97.10
					SD	0.192	1.832	4.129
					% RSD	0.193	1.880	4.252

Conclusion

The developed method was found simple, sensitive and economical for the simultaneous estimation of MET, SXG and DGF in their tablet formulation. Validation of developed methods was performed according to ICH guidelines. The standard deviation, % RSD for the methods are low, reflecting a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The advantage of method was found being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed methods can be successfully applied for determination and dissolution testing of selected drugs in commercial formulation.

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