METHOD DEVELOPMENT FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN (MET), SAXAGLIPTIN (SXG) AND DAPAGLIFLOZIN (DGF) IN MARKETED FORMULATION USING UV VIS SPECTROPHOTOMETER

ABSTRACT

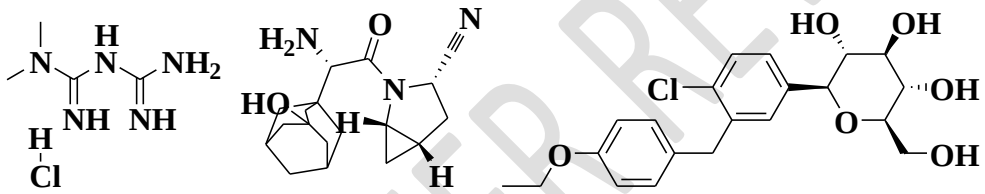
Type 2 diabetes mellitus (T2DM) is a disorder distinguished by resisting insulin effects and/or its reduced secretion leading to high blood sugar levels. It is fast becoming epidemic worldwide and is a major cause of death in the past years. Maintaining a correct blood sugar level is the primary target in the management of T2DM. Developing a single analytical method for estimation of individual drug from a multidrug composition is a very challenging task. A complexation, derivatization, extraction, evaporation and sensitive-free direct a new, simple, precise, accurate, reproducible, and efficient UV spectrophotometric method is developed and validated for the simultaneous estimation of ternary mixture of metformin (MET), saxagliptin (SXG) and dapagliflozin (DGF) in both their bulk form and combined in tablet dosage form recently approved by FDA in 2019 to be used for treatment of Type 2 diabetes mellitus by simultaneous equation method. The solutions of standard and sample were prepared in methanol: water (80:20 v/v). The λmax for MET, SXG, and DGF were 232.0, 212.0 and 272.0nm, respectively. Calibration curves are linear in the concentration ranges 10-50μg/ml for MET, 1-5μg/ml for SXG and 5-25μg/ml for DGF, respectively. Results of analysis of simultaneous equation method were analyzed and validated for various parameters according to ICH guidelines.

Keywords: Diabetes mellitus, Simultaneous equation method, Metformin, Saxagliptin, Dapagliflozin

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a worldwide problem affecting approximately 8% of the adult population, with predictions of more than 400 million cases by 2030 [1]. The prevalence of T2DM implies an urgent need for new treatments and preventative strategies. The disease results from progressive β cell dysfunction in the presence of chronic insulin resistance, leading to a progressive decline in plasma glucose homeostasis. Increased glucagon secretion, gluconeogenesis, renal glucose reabsorption and reduced incretin response are then observed. Maintaining a correct blood sugar level is the primary target in the management of T2DM [2, 3]. The biguanide class consists of metformin, which is used as the first line therapy for T2DM. It acts by reducing glucose production in the liver, and its

decreased absorption in the gastrointestinal tract also increases the insulin sensitivity in the target cell [4]. Saxagliptin are the dipeptidyl peptidase-4 inhibitors, also called gliptins. They increase the levels of incretins in plasma (GLP-1 and GIP), leading to insulin increase and decrease in blood glucose [5, 6]. Dapagliflozin are sodium-glucose cotransporter-2 (SGLT2) inhibitors (Fig. 1). The literature review revealed that several analytical methods have been reported for estimation of metformin, dapagliflozin and saxagliptin individually and in combination with other drugs by UV-Spectrophotometry, RP-HPLC, and HPTLC [7-19]. However, no spectrophotometric method has yet been reported for simultaneous estimation of MET, SXG, and DGF in pharmaceutical dosage forms. These methods mentioned in the literature, especially the chromatographic techniques, are time-consuming, costly, and require expertise. A simple and accurate UV spectrophotometric method developed can be highly useful for the routine analysis of tablet formulations. Hence, an attempt has been made to develop and validate in accordance with ICH guidelines [20].



(A) **(B)** **(C)**

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

MATERIAL & METHOD

Reagents and chemicals

Reference standard of MET, SXG, and DGF was a generous gift from Bioplus life science, Bangalore. Methanol, acetonitrile, HCl was procured from Rankem, RFCL Limited, New Delhi, India. All solvents and reagents were of analytical grade. All the solutions were protected for light and were analyzed on the day of preparations. Triple distilled water was generated in house. QTERNMET® XR Tablet (MET1000mg/ SXG 5mg/ DGF5mg) was purchased from local market of Bhopal, India. Distilled water was obtained by Mili Q apparatus by Millipore (Milliford, USA) for whole experimental work.

Instrument

In UV-spectrophotometric method, Labindia model-3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

Method development

Standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 50ml methanol: water (80:20 v/v) in 100 ml volumetric flask. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 100ml with methanol: water (80:20 v/v) to get a concentration of 1000 µg/ml (Stock-A) for drugs.

Sub stock solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of MET, SXG and DGF and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with methanol: water (80:20 v/v) that gave concentration of 100µg/ml (Stock-B).

Preparation of working standard solution

0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with methanol: water (80:20 v/v). This gave the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml respectively for MET. In same manner 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml and 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml for SXG and DGF respectively also prepared.

Selection of wavelength for linearity

Solutions of 10 µg/ml of MET, 1µg/ml SXG and 5µg/ml DGF were prepared separately. The solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of MET, SXG and DGF was observed at 232.0 nm, 212.0nm and 272.0 nm, respectively. MET showed linearity in the concentration range of 10-50 µg/ml and SXG showed the linearity in the concentration of 1-5 µg/ml and DGF showed linearity 5-25µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration (Figure 2-4).

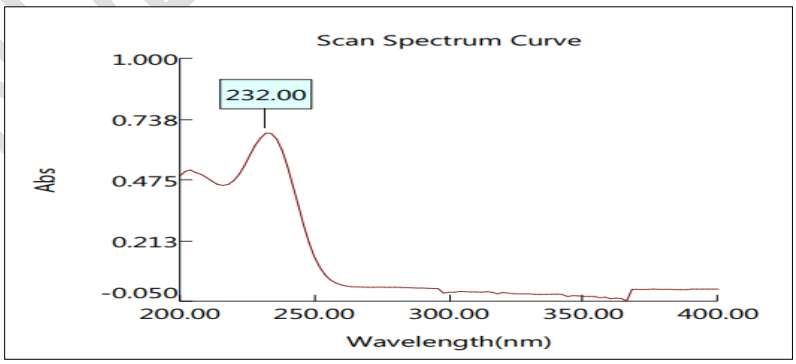


Figure 2: Determination of λ_{max} of MET

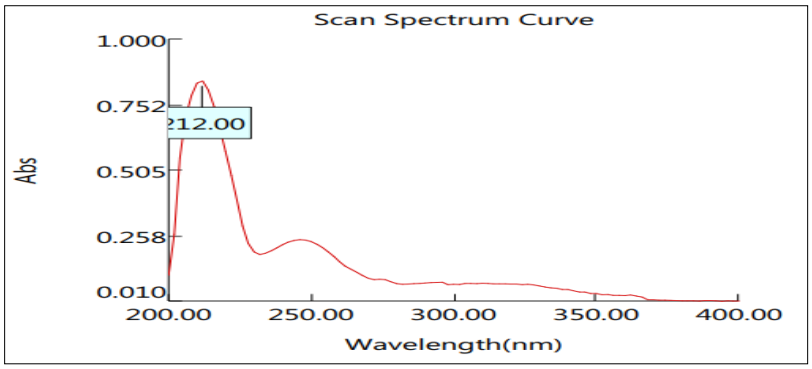


Figure 3: Determination of λ_{max} of SXG

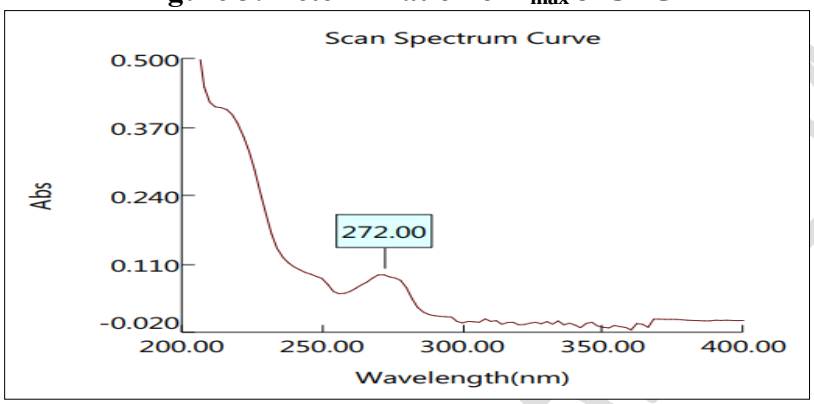


Figure 4: Determination of λ_{max} of DGF

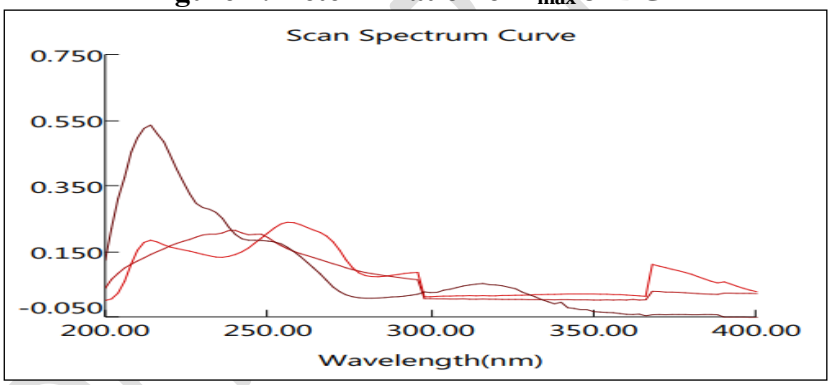


Figure 5: Overlay Spectra of MET, SXG and DGF

Study of Overlay Spectra

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of MET, 10µg/ml of SXG and 10µg/ml of DGF were scanned in the spectrum mode over the range of 200-400 nm against methanol: water (80:20 v/v) as blank and the overlain spectra of the three were recorded. MET showed an absorbance peak at 232.0 nm, whereas SXG at 212.0 nm and DGF at 272.0 nm. The overlain spectra also showed isoabsorptive points at 248.00 nm. Due to difference in absorbance maxima and having no interference with each other so three drug can be simultaneously estimated by simultaneous equation method Figure 5.

Simultaneous equation method (Vierordt's)

Simultaneous equation method is based on the absorption of drugs (X, Y, and Z) at the wavelength maximum of the other. Three wavelengths selected for the method are 232.0 nm, 212.0nm and 272.0nm that are λ_{max} of MET, SXG and DGF respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$CX = \frac{(A1(ay2az3 - az2ay3) - ay1(A2az3 - az2A3) + az1(A2ay3 - ay2A3))}{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - ay2ax3)}$$

$$CY = \frac{(ax1(A2az3 - az2A3) - A1(ax2az3 - az2ax3) + az1(ax2A3 - A2ax3))}{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - ay2ax3)}$$

$$CZ = \frac{(ax1(ay2A3 - A2ay3) - ay1(ax2A3 - A2ax3) + A1(ax2ay3 - ay2ax3))}{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - ay2ax3)}$$

Where, A_1, A_2 and A_3 are absorbance's of mixture at 232.0 nm, 212.0nm and 272.0nm respectively, ax1, ax2 and ax3 are the absorptive of MET at 232.0, 212.0 and 272.0 nm respectively, ay1, ay2 and ay3 are the absorptive of SXG at 212, 232 and 272 nm respectively, az1, az2 and az3 are the absorptive of DGF at 272, 212 and 232nm respectively.

Methods validation

Validation of the method was carried out in accordance with the International Conference on Harmonization Q2B guidelines 2005 [20].

Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in table 1.

Accuracy

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. Result of recovery study shown in table 2.

Precision

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. Result of precision shown in table 3.

Analysis of Tablets Formulation

Twenty tablets were taken and determine the average weight, tablets ground to a fine powder; amount equal to 10mg of MET (0.1mg SXG and 0.1mg DGF) was taken in 10 ml volumetric flask. Then 5ml of methanol: water (80:20 v/v) was added and the flask was sonicated for about 10 min to solubilize the drug present in capsule powder and the volume was made up to the mark with methanol: water (80:20 v/v). After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with methanol: water (80:20 v/v) to get the final concentrations of all three drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times.

Table 1: Results of linearity of MET, SXG and DGF

PARAMETER	MET	SXG	DGF
Concentration (µg/ml)	10-50	1-5	5-25
Correlation Coefficient (r^2)*	0.998	0.999	0.999
Slope (m)*	0.030	0.121	0.017
Intercept (c)*	0.013	0.000	0.001

*Value of three replicate

Table 2: Results of recovery study

% Level	% MEAN±SD*		
	MET	SXG	DGF
80 %	98.65±1.284	98.83±0.521	98.65±0.769
100 %	99.41±0.434	97.84±1.042	98.85±0.664
120 %	99.24±0.601	98.54±0.740	99.13±0.465

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

Table 3: Results of precision

Parameter	% MEAN±SD*		
	MET	SXG	DGF
Repeatability	99.012±0.213	96.101±0.067	98.521±0.124
Intermediate precision			
Day to day precision	99.441±0.124	96.707±0.060	99.001±0.090
Analyst-to-Analyst	99.702±0.079	97.578±0.057	98.977±0.076
Reproducibility	99.637± 0.086	96.168±0.087	99.412±0.060

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

Table 4: Assay of tablets formulation

	% Conc. Found		
	MET	SXG	DGF
Replicate 1	99.79	99.5	99.48
Replicate 2	99.65	99.27	99.49
Average	99.72	99.38	99.48

S. D.	0.099	0.163	0.007
% RSD	0.099	0.164	0.007

The proposed method was validated for precision, accuracy, specificity, linearity and range, robustness and ruggedness. Validation of the proposed method was carried out in accordance with the International Conference on Harmonization [20] guidelines. The linearity of the calibration plots was confirmed by the high value of the correlation coefficients (r 2 = 0.999 for MET, 0.998 for SXG, and 0.999 for DGF). Recovery was in the range of 97.84-99.41%; the values of standard deviation and % RSD were found to be less than 2 showing the high accuracy of the method. Robustness and ruggedness were also carried out and percentage RSD was found to be less than 2.0%. The assay of MET, SXG, and DGF was found to be 99.72%, 99.38%, and 99.48%. Thus, the method provides a simple, convenient, rapid and accurate way to determine MET, SXG, and DGF simultaneously.

CONCLUSION

The Vierordt's method has been successfully applied for simultaneous determination of MET, SXG, and DGF in combined sample solution, and they were found to be accurate, simple, rapid, and precise. Once the equations were constructed, analysis required only measuring the absorbance values of the sample solution at the selected wavelengths followed by few simple calculations. The proposed method was completely validated showing satisfactory data for all the method validation parameters tested. Simultaneous equation method comparably noted to be very efficient in every aspect. Unlike HPLC, by using simultaneous equation method (UV) the data's can be generated applying simple calculations. So these methods can be easily and conveniently adopted for routine quality control analysis of these cited drugs.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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