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# Simultaneous Spectrophotometric Estimation of Metformin, Saxagliptin and Dapagliflozin in Marketed Formulations

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## ABSTRACT

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and/or decreased production, resulting in elevated blood sugar levels. It is rapidly spreading around the world and has become a leading cause of mortality in recent years. The basic goal of T2DM treatment is to maintain a healthy blood sugar level. It is a difficult undertaking to develop a single analytical method for estimating individual drugs from a multidrug combination. A novel, simple, precise, accurate, repeatable, and effective UV spectrophotometric approach is developed and validated for the simultaneous quantification of a ternary combination of metformin (MET), saxagliptin (SXG), and dapagliflozin (DAG) (DGF). The FDA recently authorised bulk forms and mixed in tablet dosage forms for use in the treatment of Type 2 diabetes mellitus using the simultaneous equation approach. Methanol: water (80:20 v/v) was used to make the standard and sample solutions. MET, SXG, and DGF had maximum values of 232.0, 212.0, and 272.0nm, respectively. The calibration curves for MET, SXG, and DGF are linear in the concentration ranges of 10-50g/mL, 1-5g/mL, and 5-25g/mL, respectively. According to ICH rules, the results of the simultaneous equation technique analysis were examined and validated for various parameters.

*Keywords: Diabetes mellitus; simultaneous equation method; metformin; saxagliptin; dapagliflozin.*

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## 1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a global disease that affects around 8% of the adult population, with more than 400 million cases predicted by 2030 [1]. The high incidence of T2DM necessitates the development of innovative therapies and prevention techniques. The condition develops as a result of increasing cell malfunction in the setting of chronic insulin resistance, resulting in a gradual loss of plasma glucose homeostasis. The result is increased glucagon secretion, gluconeogenesis, renal glucose reabsorption, and a decreased incretin response. The major goal of T2DM management is to maintain a healthy blood sugar level [2,3]. Metformin, a member of the biguanide family, is used as a first-line treatment for T2DM. It works by decreasing glucose synthesis in the liver and decreasing glucose absorption in the gastrointestinal tract, as well as improving insulin sensitivity in the target cell [4]. The dipeptidyl peptidase-4 inhibitors, also known as gliptins, include saxagliptin. They raise the levels of incretins (GLP-1 and GIP) in the blood, resulting in a rise in insulin and a drop in blood glucose [5,6]. The sodium-glucose cotransporter-2 (SGLT2) inhibitors dapagliflozin (Fig. 1). Several analytical techniques for estimating metformin, dapagliflozin, and saxagliptin separately and in combination with other medicines have been published in the literature [7-19], including UV-Spectrophotometry, RP-HPLC, and HPTLC. However, no spectrophotometric approach for simultaneous measurement of MET, SXG, and DGF in pharmaceutical dose forms has yet been disclosed. 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].

## 2. MATERIALS AND METHODS

### 2.1 Reagents and Chemicals

Bioplus life science, Bangalore, generously donated the MET, SXG, and DGF reference standards. Rankem, RFCL Limited, New Delhi, India provided the methanol, acetonitrile, and HCl. Analytical grade solvents and reagents were used throughout the experiment. All of the solutions were light-protected and evaluated on the day of the preparations. In-house triple distilled water was produced. The QTERNMET® XR Tablet (MET1000mg/ SXG 5mg/ DGF5mg) was acquired in Bhopal, India's local market. For the whole experiment, Millipore's Mili Q equipment (Milliford, USA) was used to get distilled water.

### 2.2 Instrument

In the UV-spectrophotometric approach, Labindia model-3000+ series quartz cells with a wavelength precision of 1 nm were utilised.

### 2.3 Method Development

#### 2.3.1 Standard stock solution (Stock-A)

Dissolving 100 mg of each medication in 50 mL methanol: water (80:20 v/v) in a 100 mL volumetric flask yielded standard stock solutions. To achieve a concentration of 1000 g/ml (Stock-A) for medicines, the flask was sonicated for around 10 minutes to solubilize the drug and the volume was brought up to the mark 100ml with methanol: water (80:20 v/v).

#### 2.3.2 Sub stock solution (Stock-B)

Aliquots of 2.5 ml were extracted using a pipette from standard stock solution A of MET, SXG, and DGF and deposited into 25 ml volumetric flasks individually and diluted to a concentration of 100 g/ml with methanol: water (80:20 v/v) (Stock-B).

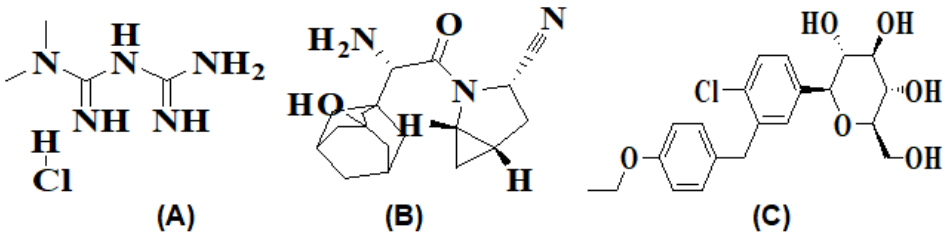


**Fig. 1. Chemical structure of (A) Metformin (B) Saxagliptin (C) Dapagliflozin**

### 2.3.3 Preparation of working standard solution

0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml of sub stock solution (Stock-B) were taken individually and volume was increased up to 10 ml with methanol: water (80:20 v/v) in a 10 ml volumetric flask. For MET, this resulted in solutions of 10g/ml, 20g/ml, 30g/ml, 40g/ml, and 50g/ml, respectively. 1g/ml, 2g/ml, 3g/ml, 4g/ml, 5g/ml, 10g/ml, 15g/ml, 20g/ml, and 25g/ml for SXG and DGF, respectively, were made in the same way.

For linearity, wavelength selection is important.

Separate solutions of 10 mg/ml MET, 1 mg/ml SXG, and 5 mg/ml DGF were produced. From 200 nm to 400 nm, the solutions were examined in spectrum mode. At 232.0 nm, 212.0 nm, and 272.0 nm, respectively, the highest absorbance of MET, SXG, and DGF was detected. 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 (Figs 2-4).

## 2.4 Study of Overlay Spectra

The working standard solution is made from the concentration of the standard stock solution. The overlain spectra of 10g/ml of MET, 10g/ml of SXG, and 10g/ml of DGF were scanned in the spectrum mode over the range of 200-400 nm against methanol: water (80:20 v/v) as a blank and recorded. The absorbance peak for MET was 232.0 nm, while SXG was 212.0 nm and DGF was 272.0 nm. At 248.00 nm, the overlay spectra revealed isoabsorptive spots. Because of the differences in absorbance maxima and the fact that they do not interfere with one other, three drugs may be evaluated concurrently using the simultaneous equation approach (Fig. 5).

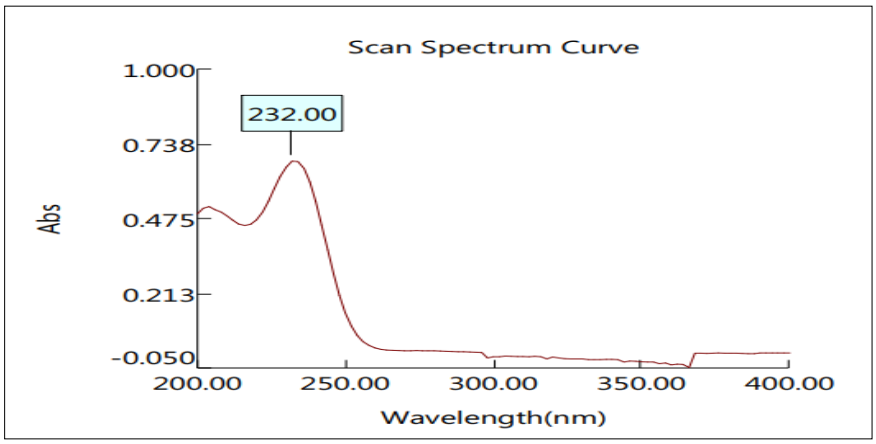


**Fig. 2. Determination of $\lambda_{max}$ of Metformin**

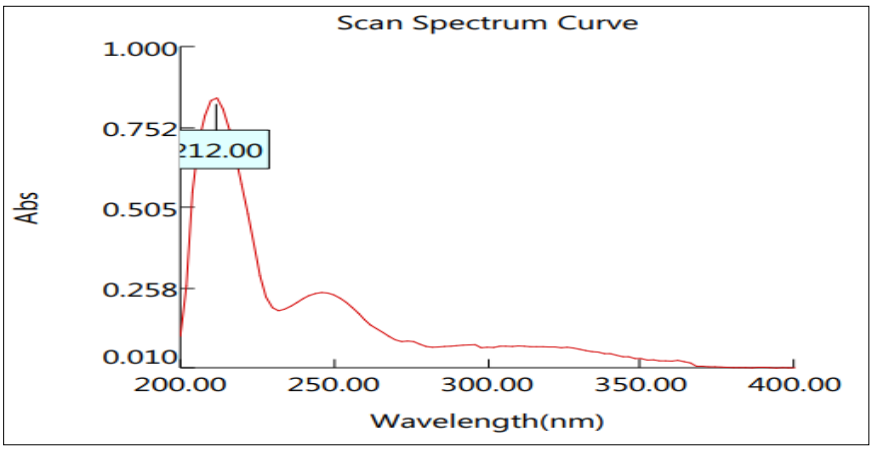


**Fig. 3. Determination of $\lambda_{max}$ of Sexagliptin**

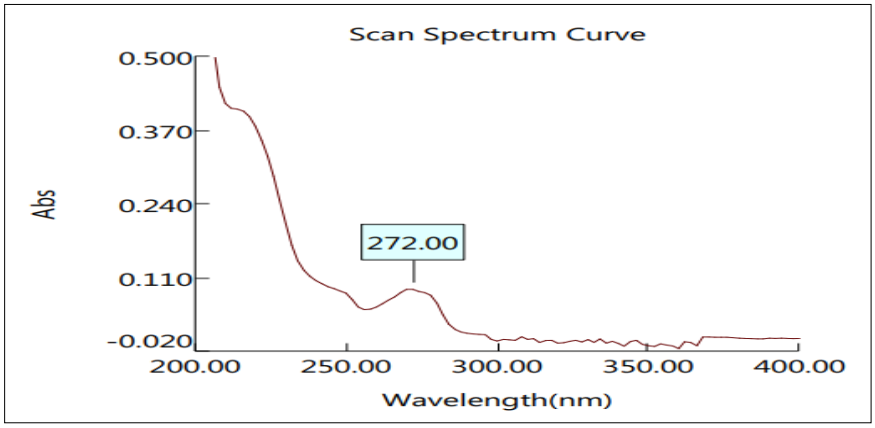


**Fig. 4. Determination of $\lambda_{max}$ of Dapagliflozin**

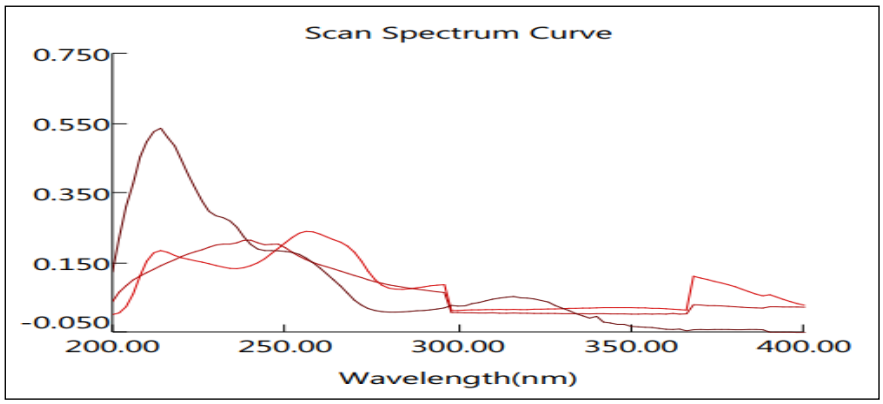


**Fig. 5. Overlay Spectra of MET, SXG and DGF**

### 2.4.1 Simultaneous equation method (Vierordt's)

The simultaneous equation technique is based on drug absorption at the wavelength maximum of the other (X, Y, and Z). The method uses three wavelengths: 232.0 nm, 212.0 nm, and 272.0 nm, which are the maximum of MET, SXG, and DGF, respectively. The absorbances were measured at the chosen wavelengths, and the absorptivities (A1 percent, 1cm) for both medications were calculated as the average of five separate measurements. The following equations were used to calculate the concentrations in the sample.

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

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

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

Where A1, A2 and A3 are the absorbances of the mixture at 232.0 nm, 212.0 nm, and 272.0 nm, 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 n.

### 2.4.2 Validation of methods

The approach was validated using the Q2B recommendations from the International Conference on Harmonization in 2005 [20].

## 2.5 Linearity

The linearity of the analytical technique was tested to see if it could produce test findings proportionate to the analyte concentration in the sample within a specific 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.

## 2.6 Accuracy

Recovery studies were used to evaluate the validity and reliability of the suggested approaches. At three replicates and three concentrations, the recovery of additional standards was found to be (80%, 100%, and 120%). When the percent value is close to 100, SD and percent RSD are less than 2, it indicates that the method is accurate. Table 2 shows the results of the recovery study.

## 2.7 Precision

The repeatability and intermediate precision of the medication were used to determine precision. The accuracy under the same operating conditions over a short period of time is shown by the repeatability result. Within-laboratory variance on various days and analyst to analyst variation by different analysts are both reflected in the intermediate precision research. When the SD and percent RSD are both less than 2, the approach is said to be precise. Table 3 shows the accuracy result.

## 2.8 Analysis of Tablets Formulation

Twenty tablets were collected to estimate the average weight, and the tablets were crushed into a fine powder; 10 mg of MET (0.1 mg SXG and 0.1 mg DGF) was placed in a 10 ml volumetric flask. The flask was then sonicated for around 10 minutes to solubilize the medication present in the capsule powder, after which the volume was brought up to the mark with methanol: water (80:20 v/v). Filtration was performed after sonication using Whatman filter paper No. 41. Filtrate was collected and diluted with a mixture of methanol and water (80:20 v/v) to bring the final concentrations of all three medicines into the acceptable range. The concentrations were determined by observing the absorbances of final dilutions at different wavelengths. The procedure was repeated for five times.

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 follows the standards of the International Conference on Harmonization [20]. The strong correlation coefficients (r2 = 0.999 for MET, 0.998 for SXG, and 0.999 for DGF) verified the linearity of the calibration plots. The recovery rate was 97.84-99.41 percent, while the standard deviation and percent RSD values were both less than two, indicating the method's high accuracy. Robustness and ruggedness tests were also performed, with a percentage RSD of less than 2.0 percent. The results of the MET, SXG, and DGF assays were 99.72 percent, 99.38 percent, and 99.48 percent, respectively. As a result, the approach gives a quick, easy, and accurate way to estimate MET, SXG, and DGF all at once.

**Table 1. Results of linearity of MET, SXG and DGF**

| Parameter | MET | SXG | DGF |
|---|---|---|---|
| Concentration (µg/ml) | 10-50 | 1-5 | 5-25 |
| Cor. Coeff ($r^2$)* | 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 |

## 3. CONCLUSION

Vierordt's approach has been successfully used to determine MET, SXG, and DGF in a mixed sample solution, and it has been proven to be accurate, easy, quick, and exact. After constructing the equations, all that was left to do was measure the absorbance values of the sample solution at the designated wavelengths and do a few basic calculations. The suggested technique was fully validated, with good results for all of the method validation parameters that were examined. Simultaneous equation approach is known to be extremely efficient in every way. Unlike HPLC, data may be obtained via simple calculations utilising the simultaneous equation technique (UV). As a result, these methodologies may be readily and quickly used for routine quality control examination of the medications mentioned.

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## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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