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Research Article

A Novel, Validated UV Spectrophotometric Method For The Simultaneous Estimation Of Metformin Hydrochloride And Empagliflozin In Fixed-Dose Combinations

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ABSTRACT

A validated UV spectrophotometric approach has been developed for the concurrent quantification of Metformin HCl and Empagliflozin in both pure powder form and two-component dosage forms using simultaneous equation method. The methodology relies on determining the absorbance of both Metformin HCl and Empagliflozin, in the concentration range of 2-10µg/ml and 0.1-5µg/ml, respectively, at their respective λmax of 234 nm and 224 nm. The correlation coefficient of linearity for Metformin HCl and Empagliflozin in the range 2-10µg/ml and 0.1-5µg/ml was 0.999, 0.998, and 0.9998, 0.9998, respectively. The findings have been statistically validated in accordance with ICH criteria.

INTRODUCTION

Spectroscopy is a scientific discipline that explores how matter interacts with electromagnetic radiation.[1] UV-Vis spectroscopy, a specific analytical technique, measures the absorption or transmission of UV or visible light wavelengths by a sample compared to a reference or blank. This property, influenced by the sample's composition, can provide insight into its components and their concentrations.[2] In therapeutics, combination drug products have been crucial for a long time. When correctly formulated, fixed-combination medications can increase convenience, reduce costs, and sometimes enhance safety and efficacy. However, analyzing samples with multiple components presents a significant challenge in contemporary research. Multicomponent analysis

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has recently gained attention as a fascinating focus for analytical chemists.[3] The majority of relevant analytes have additional molecules absorbing in the same spectral area with them in their dose forms, therefore traditional UV spectral tests cannot be utilized to determine their concentration. UV spectrophotometric methods are mostly employed in multicomponent analysis, which minimizes the laborious process of separating interferents and permits the measurement of a greater number of analytes, hence cutting down on the time and expense of analysis. [3] Metformin hydrochloride (MFN) a biguanide antidiabetic is chemically identified as 1,1-dimethyl biguanide hydrochloride. It is a medication used mostly for people who are overweight and is used to treat type 2 diabetes. When some insulin is present, they have an anti-diabetic impact rather than increasing insulin secretion. Possible mechanisms of action include delayed GI tract glucose absorption, enhanced insulin sensitivity, intracellular glucose release, and hepatic gluconeogenesis suppression. [4–7] Empagliflozin (EMPA) is 1-chloro-4-(β-D-glucopyranose-1-yl)-2-(4-(tetrahyrofuran-3-yloxybenzyl) benzene). It is an inhibitor of sodium-glucose co-transporter 2 (SGLT-2) that is mostly present in the proximal tubules of the kidney's nephronic components.[8] Empagliflozin inhibits the kidney's glomerular filtrate's ability to reabsorb glucose, lowering blood glucose levels in individuals with type 2 diabetes.[9] In the kidney, glucose is reabsorbed from the glomerular filtrate by SGLT-2 co-transporters. Because SGLT2 inhibition has a glucuretic action, which lowers the renal threshold for glucose and inhibits renal absorption, more glucose, or blood sugar, is excreted in the urine. [10-11]

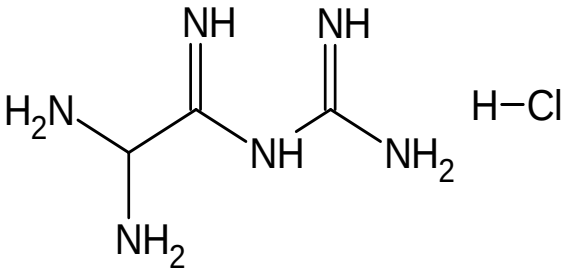

Figure 1: Structure of Metformin HCl

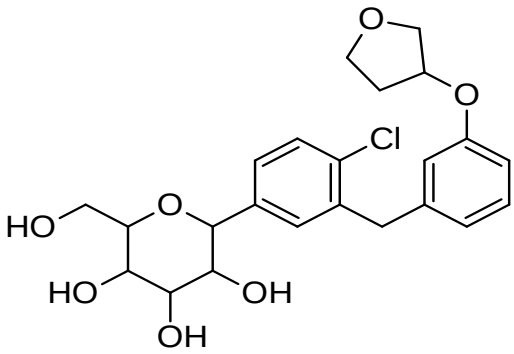

Figure 2: Structure of Empagliflozin

Literature review reveals that there are numerous approaches for estimating the dosages of Metformin HCl and Empagliflozin both alone and in combination with other medications. [12–15] This method was developed with the aim of replacing organic, toxic solvents like methanol, ethanol used in the above studies with eco-friendly, cost-effective, and readily available solvents such as water and IPA. By employing the simultaneous equation method, an effort was made to establish and validate a simple, precise, and accurate UV technique for the simultaneous estimation of Empagliflozin and Metformin HCl in their combined dosage form. Method validation followed ICH norms.[16]

MATERIALS

Analytical grade reagents and chemicals were utilized for the study. Received gift samples of MFN and EMPA from Synokem Pharmaceuticals Ltd. JARDIANCE MET, the mixed dosage form, was bought. Methanol HPLC grade purchased from Merck Life Sciences Pvt, Ltd.

EQUIPMENT

The Hitachi UH 5300 double beam UV-vis spectrophotometer and matching quartz cells with

a 1 cm path length were utilized. The material was weighed using a Shimadzu electronic analytical balance (ATX224). GT SONIC Professional Ultrasonic cleaner was used for sonication of solutions.

METHODOLOGY

Preparation of Standard Stock Solution and calibration curve

MFN and EMPA were individually dissolved in 50 ml standard flask with IPA-Water mixture (50:50) to yield a concentration of 1 mg/ml (Solution A) for each component. The standard stock solution of EMPA and MFN was further diluted by taking 1ml of solution and diluted with IPA-Water mixture to 10 ml (100 µg/ml Solution B). Accurately pipetted out 5ml and made up to 50ml to obtain 10 µg/ml (Solution C). Precisely pipette 2, 4, 6, 8, and 10 ml of solution C into 10 ml standard flasks for MFN calibration. The volume was then adjusted with an IPA-Water mixture to the appropriate level. Precisely pipette out 0.1, 1, 2, 3, 4, and 5 ml of solution B into a 10 ml standard flask for EMPA calibration. The volume was then adjusted using an IPA-Water mixture.

Determination of Wavelength of Maximum Absorption

Using IPA-water mixture as blank, the 10 µg/ml solution of EMPA and MFN was exposed to a UV spectrophotometric scanner (200-350 nm) to ascertain the λmax of EMPA and MFN. Two distinct peaks were visible in the scanning spectra of the 10 µg/ml solutions of MFN and EMPA at 224 and 234 nm, respectively. Additionally, the overlay spectra of MFN and EMPA were captured. Figure 5.

Determination of Absorbance

The absorbance of the further diluted solutions of solution C , corresponding to Empagliflozin (0.1-5µg/ml) and Metformin (2-10µg/ml), was measured at wavelengths of 234 nm and 224 nm, respectively.

Analysis of Tablet Formulation

20 tablets of JARDIANCE MET (12.5/500 mg EMPA and MFN) were weighed, triturated, and powder equivalent to 50 mg of MFN and 1.25 mg of EMPA was put into a 10 ml volumetric flask along with 5 ml of IPA-Water mixture. The flask was then sonicated for 10 minutes. After being adjusted to the proper volume, the mixture was filtered using Whatman filter paper. 1 ml of the resulting solution was diluted with IPA- Water mixture to 10 ml, making the solution of concentration 500 µg/ml and 12.5 µg/ml of MFN and EMPA respectively. Again 0.1 ml of the resultant solution was added to a 10 ml volumetric flask that had been diluted with an IPA-Water mixture to generate a concentration of 5 µg/ml and 0.125 µg/ml of MFN and EMPA respectively.

Simultaneous Equation Method (Vierodt's Method):

A multi-component system consisting of two components X and Y, each of which absorbs at the λmax of the other, λ1 (234 nm) being the wavelength of maximum absorbance of X (MFN) and λ2 (224 nm) being the wavelength of maximum absorbance of Y (EMPA).

Cx and Cy be the concentration of X and Y respectively in the diluted sample. Two equations are constructed based up on the fact that at λ1 and λ2 the absorbance of the mixture is the sum of the individual absorbances of X and Y.

At λ1

A1= ax1bcx+ay1bcy……(1)

At λ2

A2= ax2bcx+ay2bcy…….(2)

For measurements in 1cm cell, b= 1, therefore,

Rearrange equation (2)

CY= A2-ax2cx/ay2

Substituting for Cy in equation (1) and rearranging gives

Cx = (A2ay1-A1ay2) / (ax2ay1 ax1ay2)…….(3)

Cy = (A1ax2-A2ay2) / (ax2ay1 ax1ay2)……(4)

The absorptivities of X at λ1 and λ2 = ax1 and ax2 respectively.

The absorptivities of Y at λ1 and λ2, = ay1 and ay2 respectively.

The absorbance of the diluted sample at λ1 and λ2 = A1 and A2 respectively.

Cx and Cy be the concentrations of X and Y respectively in the diluted sample.[17]

METHOD VALIDATION

Linearity (Calibration curve)

Calibration curves were constructed by plotting absorbance vs. concentrations of MFN and EMPA, at their respective λmax, and regression equations were computed. The calibration curves were graphed over five distinct concentrations for MFN ranging from 2 to 10 µg/mL and six distinct values for EMPA ranging from 0.1 to 5 µg/mL. (Fig.6,7 and Fig.8,9).

Accuracy

The usual addition method was utilized to calculate the recoveries of MFN and EMPA in order to assess the accuracy of the procedure. Tablet dosage form sample solutions were supplemented with known concentrations of MFN and EMPA (80%, 100%, and 120%). MFN and EMPA quantities were approximated. (Table 3) displays the results. The results demonstrate how accurate the procedure is.

Precision

The degree of repeatability or the analytical method's repeatability under typical operating conditions is measured by precision. Two levels of precision were taken into consideration: repeatability and intermediate precision.

Repeatability

Six results at 100% test concentration, a mixture of 5 µg/mL MFN and 0.125 µg/mL EMPA were used to assess the method's repeatability. The repeatability of the suggested approaches is demonstrated by the RSD values, which were found to be less than 2% (Table 3).

Intermediate precision

Using six determinations of the mixture of 5 µg/mL MFN and 0.125 µg/mL EMPA, the intermediate precision was examined. Over the course of three days, the stock solution was simultaneously made and examined. At 234 and 224 nm, the absorbance of the resultant solution was measured. Variations in the data over three days were analyzed, and statistical validation was done (Table 3).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

$$\mathbf{LOD = 3.3^* (\sigma/S)}$$

$$\mathbf{LOQ = 10^* (\sigma/S)}$$

where, σ = Standard deviation

S= Slope of Calibration curve

RESULTS

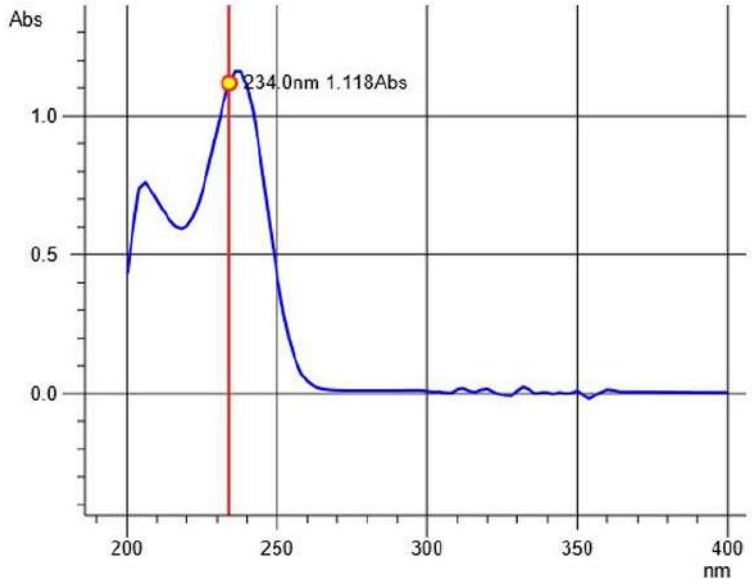

Figure: 3 UV spectra of Metformin HCl

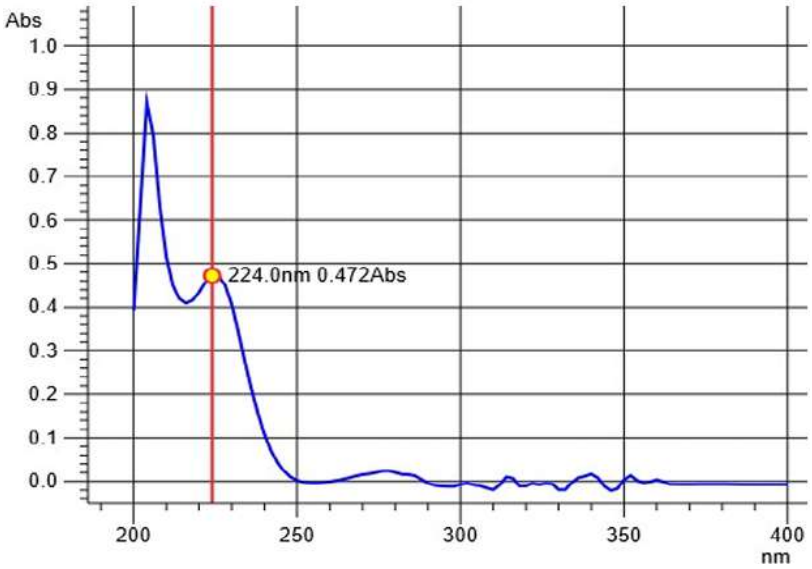

Figure: 4 UV spectra of Empagliflozin

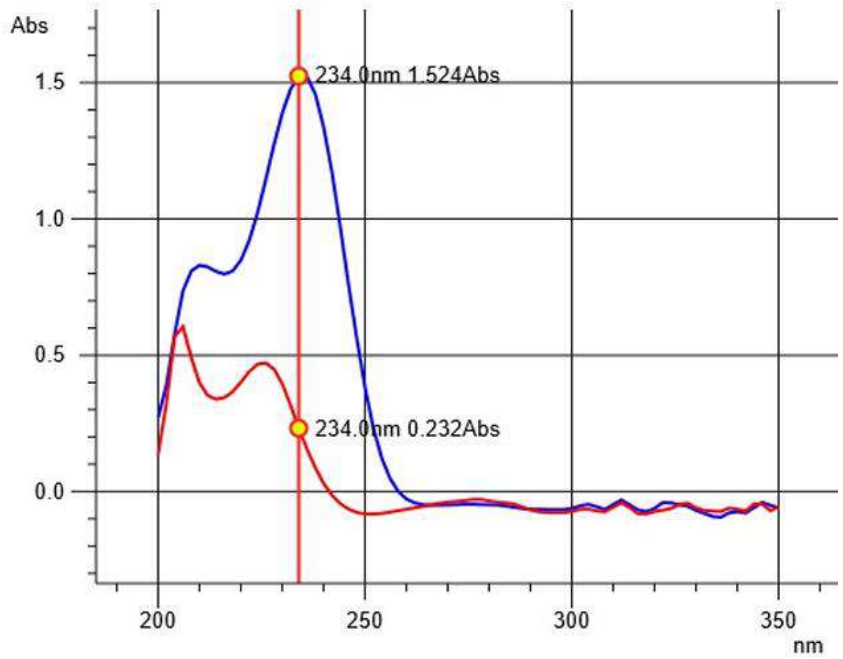

Figure: 5 Overlay spectra of MFN and EMPA

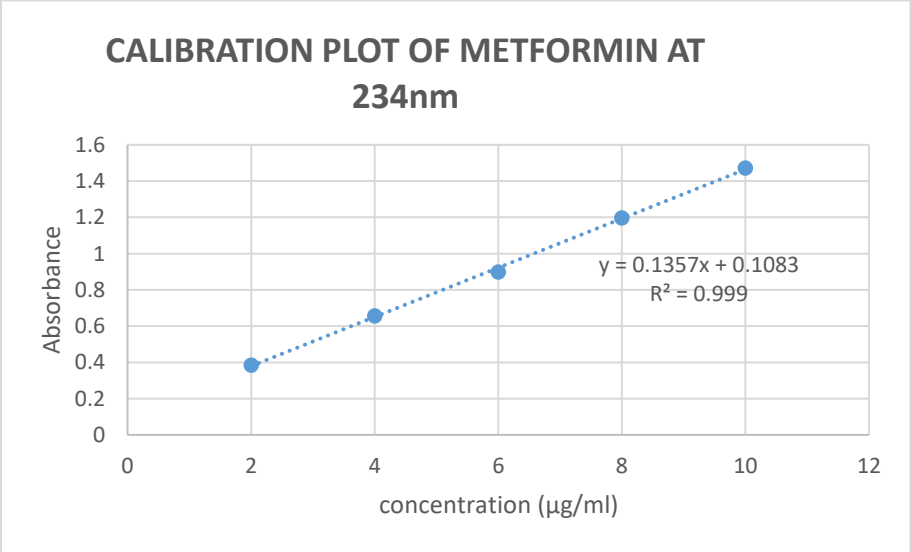

Figure: 6 Calibration plot of MFN at 234nm.

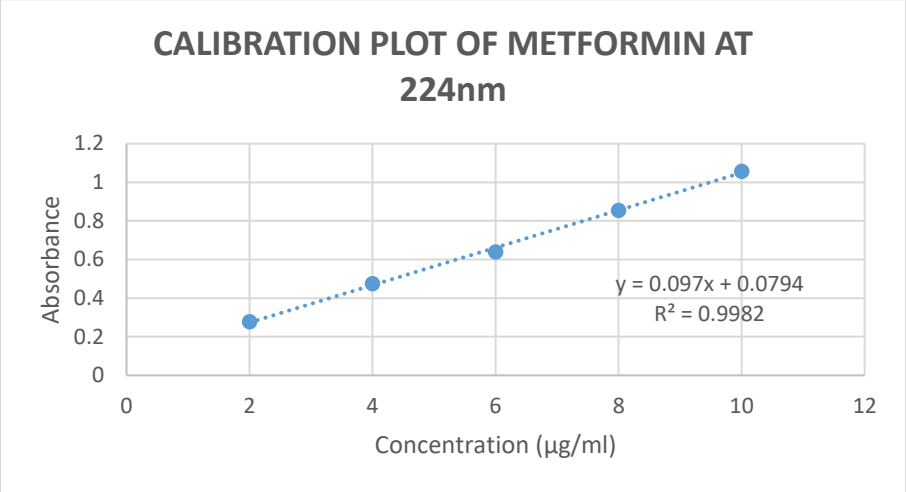

Figure: 7 Calibration plot MFN at 224nm

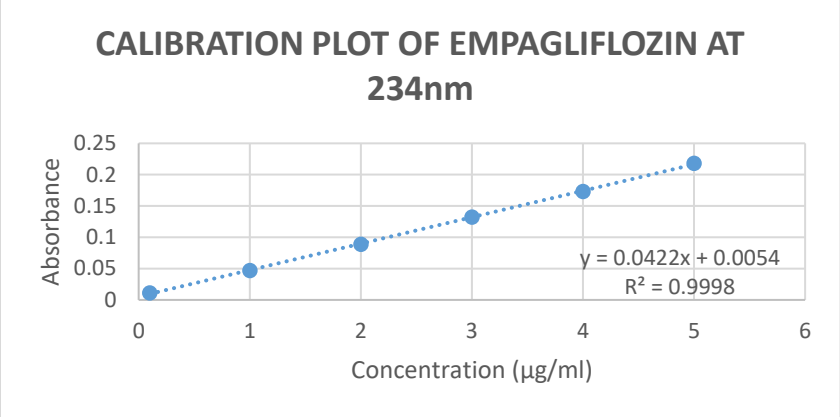

Figure: 8 Calibration plot of EMPA at 234nm

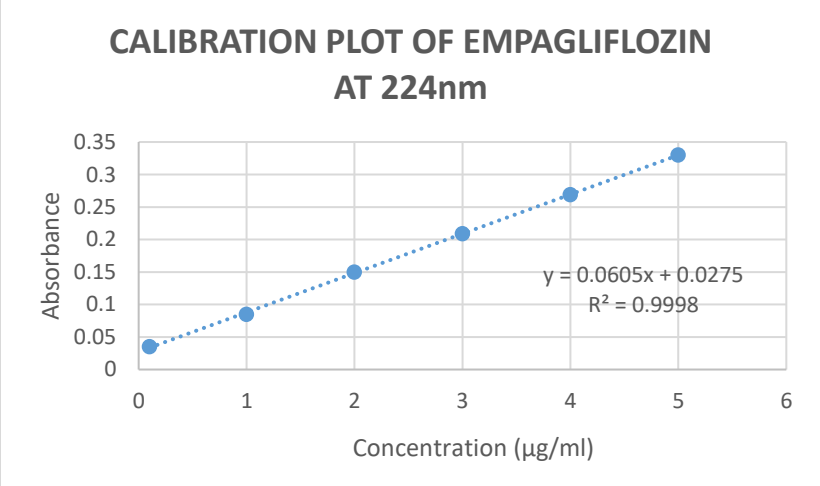

Figure: 9 Calibration plot of EMPA at 224nm

Absorptivity of Metformin HCl and Empagliflozin

Table 1: Absorptivity of drugs

Sr. No	Drug	Absorptivity	
		234nm	224nm
1	Metformin	0.1604	0.1152
2	Empagliflozin	0.0546	0.1172

Table 2: Overview of key results

Parameters	Simultaneous equation method			
	MFN		EMPA	
	234nm	224nm	234nm	224nm
Linearity / range	2-10µg/ml	2-10 µg/ml	0.1-5 µg/ml	0.1-5 µg/ml
Correlation coefficient	0.999	0.9998	0.9998	0.9998
Intercept	0.1083	0.0794	0.0054	0.0275
Slope	0.1357	0.097	0.0422	0.0605

Table 3: Summary of validation criteria

Parameter		MFN		EMPA	
Accuracy %		98.62-100.57		92.50-93.34	
Precision	Repeatability	0.4224		1.9303	
	Intermediate	0.7277		1.9721	
LOD		0.3298 (234nm)	0.4406 (224nm)	0.0448 (234nm)	0.08261 (224nm)
LOQ		0.9996 (234nm)	1.3354 (224nm)	0.1360 (234nm)	0.2503 (224nm)

Table 4: Tablet analysis report

Formulation	Label claim	Amount found (mg)	% Label claim
Tablet (JARDIANCE MET)	MFN 500 mg	485.5 mg	97.07%
	MPA 12.5 mg	11.80 mg	94.47%

CONCLUSION

The technique proved effective in estimating the MFN and EMPA content of tablets that contained 12.5 mg of EMPA and 500 mg of MFN. It was discovered that the procedure was highly sensitive, precise, accurate, reproducible, and specific with no interference from excipients by carefully considering the validation parameters. The method could be viewed as economical and eco-friendly because it was developed utilizing easily available, reasonably priced and relatively lesser toxic solvents for drug analysis. The validation of the procedures adhered to the recommendations made by the ICH guidelines. Therefore, the suggested techniques can be used for regular quality control of MFN and EMPA in formulations with combination doses.

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