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RESEARCH ARTICLE

New Validated UV Spectrophotometric Method for Simultaneous Marketed Brand Gibtulio Met (12.5/500mg) Fixed Dose Combination Tablets

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ABSTRACT

A validated UV spectrophotometric method has been developed for the simultaneous quantification of Gibtulio Met (12.5/500mg) Fixed Dose Combination dosage forms using the simultaneous equation method. This approach is based on measuring the absorbance of Metformin HCl and Empagliflozin within the concentration ranges of 2-10 μg/ml and 0.1-5 μg/ml, respectively, at their respective λ max values of 235 nm and 225 nm. The linearity correlation coefficients for Metformin HCl and Empagliflozin in these concentration ranges were 0.999, and 0.9998 respectively. The results were statistically validated in compliance with ICH guidelines.

Keywords: Metformin; Empagliflozin; ICH guidelines

INTRODUCTION

Spectroscopy examines how matter interacts with electromagnetic radiation. UV-Vis spectroscopy, a widely used analytical method, measures the absorption or transmission of UV and visible light by a sample, offering insights into its composition and concentration. Fixed-dose combination medications, like Metformin HCl and Empagliflozin, are essential in treating conditions such as type 2 diabetes. However, analyzing multi-component formulations poses significant challenges due to overlapping spectral properties. Traditional UV methods often fail to distinguish individual components. To overcome this, UV spectrophotometric techniques are commonly used for multi component analysis, simplifying the process, and reducing time and costs.

Metformin HCl is a Biguanides Antidiabetic that lowers blood glucose levels by improving insulin sensitivity, slowing glucose absorption in the gastrointestinal tract, and reducing hepatic glucose production. Empagliflozin, and SGLT-2 inhibitor, reduces glucose reabsorption in the kidneys, effectively lowering blood sugar in diabetic patients. While many methods exist to estimate these drugs individually or in combination, this study aims to replace harmful solvents like Methanol and ethanol with eco-friendly alternatives, such as water and Methanol, for simultaneous estimation. The developed method is validated in accordance with ICH guidelines to ensure precision, accuracy, and simplicity using the simultaneous equation method.

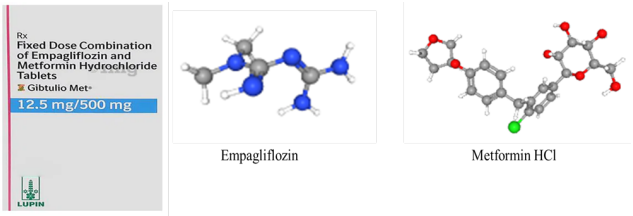

Fig. 1: Structure of Metformin and Empagliflozin of Brand of Gibtulio Met

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EXPERIMENT

Chemicals and Reagents

Analytical-grade reagents and chemicals were used for the study. The samples were obtained from a local distributor representing Lupin Pharma, specifically the brand Gibtulio Met in mixed dosage form. Methanol (HPLC grade) was sourced from Merck Life Sciences Pvt. Ltd.

Equipment

A Shimadzu UV-1900i double-beam UV-Vis spectrophotometer with 1 cm path length quartz cells was used for the analysis. The materials were weighed using a Shimadzu electronic analytical balance, and a Professional Ultrasonic Cleaner was employed for sonication of the prepared solutions.

Preparation of Standard Stock Solution and Calibration Curve

Gibtulio Met was dissolved in a 50 ml standard flask using a Methanol-Water mixture (50:50) to achieve a concentration of 1 mg/ml (Solution A) for each component. The standard stock solution was further diluted by taking 1 ml of Solution A and diluting it with the Methanol-Water mixture to 10 ml, yielding a concentration of 100 μg/ml (Solution B). From this, 5 ml was accurately pipette and diluted to 50 ml to obtain a 10 μg/ml solution (Solution C). For the calibration curve, 2, 4, 6, 8, and 10 ml of Solution C were precisely transferred into 10 ml standard flasks, and the volume was adjusted to the appropriate level using the Methanol-Water mixture.

Determination of Wavelength of Maximum Absorption

A 10 μg/ml solution of Gibtulio Met was prepared, with a Methanol-Water mixture used as the blank. This solution was subjected to UV spectrophotometric scanning across the wavelength range of 200-400 nm to determine the λ_{max} of Gibtulio Met. The scanning spectra revealed two distinct peaks at 225 nm and 235 nm, respectively, indicating the absorption maxima. Additionally, the overlay spectra of the 10 μg/ml solution were recorded for further analysis, as shown in Figure 2 this data provides critical insight into the compound's UV absorbance characteristics for accurate quantification.

Determination of Absorbance

The absorbance of the further diluted solutions of Solution C, corresponding to Gibtulio Met concentrations ranging from 2 to 10μg/ml, was measured at wavelengths of 225 nm and 235 nm, respectively shown in Figure 2.

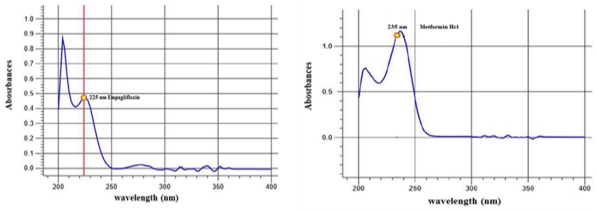

Fig. 2: UV spectra of Empagliflozin and Metformin HCl

Analysis of Tablet Formulation

Twenty tablets of Gibtulio Met (12.5/500 mg Empagliflozin and Metformin HCl) were weighed and triturated. A portion of the powder, equivalent to 1.25 mg of Empagliflozin and 50 mg of Metformin HCl, was transferred into a 10 ml volumetric flask with 5 ml of a Methanol-Water mixture. The flask was sonicated for 10 minutes, and after adjusting to the appropriate volume, the mixture was filtered using Whatman filter paper. 1 ml of the filtrate was diluted to 10 ml with the Methanol-Water mixture, yielding a solution with concentrations of 500μg/ml Metformin HCl and 12.5μg/ml Empagliflozin. Subsequently, 0.1 ml of this solution was further diluted in a 10 ml volumetric flask with the Methanol-Water mixture to achieve concentrations of 5μg/ml Metformin HCl and 0.125μg/ml Empagliflozin.

Simultaneous Equation 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 (Metformin HCl) and λ2 (225 nm) being the wavelength of maximum absorbance of Y (Empagliflozin). 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

$$A1 = ax1bcx + ay1bcy \quad (1)$$

At λ2

$$A2 = ax2bcx + ay2bcy \quad (2)$$

For measurements in 1cm cell, b= 1, therefore, Rearrange Equation (2)

CY= A2-ax2cx/ay2

Cx = (A2ay1-A1ay2) / (ax2ay1ax1ay2) (3)

Cy = (A1ax2-A2ay2) / (ax2ay1ax1ay2) (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 $\lambda 1$ and $\lambda 2$ = A1 and A2 respectively. Cx and Cy be the concentrations of X and Y respectively in the diluted sample.

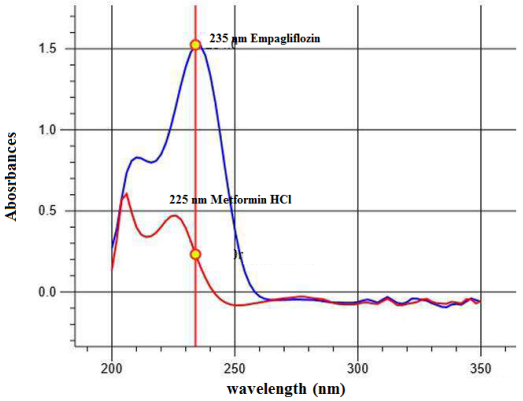

Fig. 3: Overlay spectra of Gibtulio Met

Method Validation

Linearity (Calibration curve): Calibration curves were constructed by plotting absorbance vs. concentrations of MFN and EMPA, at their respective λ_{max}, 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 5μg/mL (Figure 4).

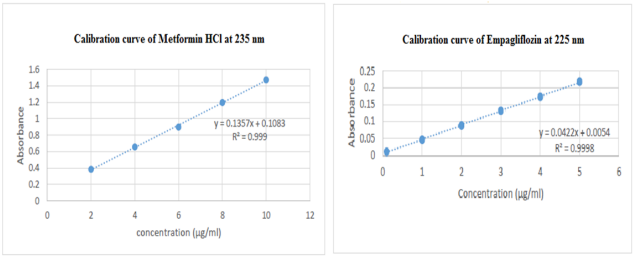

Fig. 4: Calibration curve of Metformin HCl (MFN) and Empagliflozin (EMPA)

Table 1: Simultaneous equation method for Metformin HCl (MFN) and Empagliflozin (EMPA)

Parameters	Simultaneous equation method	
	MFN	EMPA
	225 nm	235 nm
Linearity / range	2-10μg/ml	0.1-5μg/ml
Correlation coefficient	0.999	0.9998
Intercept	0.1083	0.0054
Slope	0.1357	0.0422

N = 6

Absorptivity of Metformin HCl and Empagliflozin:

Table 2: Absorptivity of drugs

Sr. No.	Drug	Absorptivity
1.	MFN (235 nm)	0.1652
2.	EMPA (225 nm)	0.0536

N=6

- **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 5μg/mL MFN and 0.125μg/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 5μg/mL MFN and 0.125μg/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):**

1. LOD = 3.3* (σ/S)
2. LOQ = 10* (σ/S)

Where, σ = Standard deviation, S= Slope of Calibration curve.

Table 3: Result of validation parameters

Parameter		MFN	EMPA
Accuracy %		98.99 %	94.50 %
Precision	Repeatability	0.4131	1.9358
	Intermediate	0.8231	1.9823
LOD		0.3654	0.4365
LOQ		0.9982	0.1365

N= 6

Table 4: Tablet analysis report

Formulation	Label Claim	Amount founds	% Label Claim
Tablet of Gibtulio Met	MFN (500 mg)	498 mg	99.60%
	EMPA (12.5 mg)	12.05	99.69 %

N = 6

RESULT AND DISCUSSION

The developed method demonstrated exceptional efficacy in quantifying Gibtulio Met content in combination tablets containing 12.5 mg of Empagliflozin (EMPA) and 500 mg of Metformin (MFN). Through comprehensive analysis of validation parameters, the method was found to be highly sensitive, precise, accurate, and reproducible, exhibiting specificity with no interference from tablet excipients.

One of the noteworthy aspects of this method is its eco-friendly and cost-effective nature. By employing readily available and relatively non-toxic solvents, the procedure offers a sustainable approach to drug analysis. The method aligns with green chemistry principles, minimizing environmental impact while maintaining analytical efficiency.

Validation followed the rigorous standards set forth by ICH guidelines, ensuring that the process meets global benchmarks for pharmaceutical analysis. As a result, the proposed method is highly suitable for routine quality control of combination dose formulations containing MFN and EMPA. Its reliability and eco-conscious design make it a viable choice for both laboratory and industrial applications in the pharmaceutical sector.

CONCLUSION

The developed technique demonstrated exceptional efficiency in quantifying Gibtulio Met content in tablets containing 12.5 mg of EMPA and 500 mg of MFN. By meticulously addressing key validation parameters, the method proved to be highly sensitive, precise, accurate, reproducible, and specific, with no interference from excipients. Moreover, the use of easily accessible, cost-effective, and less toxic solvents enhanced the eco-friendliness and economic viability of the procedure. The validation adhered strictly to ICH guidelines, confirming its robustness. Consequently, this innovative method is highly suitable for routine quality control of MFN and EMPA in combination dosage forms, ensuring both reliability and sustainability.

Conflict of interest

Authors don't have any conflict of interest.

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