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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF EMPAGLIFLOZIN AND METFORMIN HYDRO-CHLORIDE IN COMBINED DOSAGE FORM

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Keywords:

Empagliflozin, Metformin hydrochloride, Isobestic point, Simultaneous equation method, Absorption ratio method, Combined dosage form

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ABSTRACT: Two simple spectrophotometric methods have been developed for the simultaneous estimation of Empagliflozin and Metformin hydrochloride from the tablet dosage form. Method-I simultaneous equation method involves the measurement of absorbances at two wavelengths 224 nm (λ_{max} of Empagliflozin) and 233nm (λ_{max} of Metformin hydrochloride) using Methanol and Water as diluent. Method – II Absorption ratio method involves the measurement of absorbances at two wavelengths 233 nm (λ_{max} of Metformin Hydrochloride) and 266 nm (λ_{max} of Isobestic point). The linearity lies between 0.1-25 µg/ml for Empagliflozin and 0.5-25 µg/ml Metformin hydrochloride in both methods. The accuracy and precision of the methods were determined and validated statistically. The two methods exhibited good reproducibility and recovery with a relative standard deviation of <2%. Both the methods were found to be rapid, specific, precise, accurate, and reproducible and can be successfully applied for the routine analysis of Empagliflozin and Metformin hydrochloride in a combined dosage form.

INTRODUCTION: Empagliflozin is chemically (2S, 3R, 4R, 5S, 6R) -2- [4-chloro- 3- [[4-[(3S) -oxolan- 3- yl] oxyphenyl] methyl] phenyl] - 6- (hydroxymethyl) oxane- 3, 4, 5- triol. The chemical structure of Empagliflozin is shown in **Fig. 1** [1]. Empagliflozin is a sodium-glucose co- transporter-2 (SGLT-2) inhibitor, which is found almost exclusively in the proximal tubules of nephronic components in kidney [2]. Empagliflozin lowers the blood glucose level in people with type 2 diabetes by blocking the reabsorption of glucose from glomerular filtrate in the kidney.

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SGLT-2 co-transporters are responsible for the reabsorption of glucose from the glomerular filtrate in the kidney [3, 4]. The glucuretic effect resulting from SGLT2 inhibition reduces renal absorption and lowers the renal threshold for glucose, therefore promoting excretion of excess glucose (*i.e.*, blood sugar) in the urine. Additionally, it contributes to reduced hyperglycemia and also assists in weight loss and blood pressure reduction [5, 6].

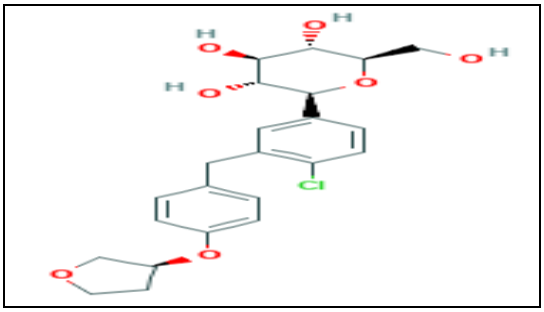

FIG. 1: STRUCTURE OF EMPAGLIFLOZIN

Metformin hydrochloride is chemically N, N-Dimethylbigunide hydrochloride. The chemical structure of Metformin is shown in **Fig. 2**. Metformin hydrochloride is a biguanide anti-hyperglycemic agent, used for treating non-insulin-dependent diabetes mellitus (NIDDM) [7].

Biguanide lowers blood glucose without causing over hypoglycemia and increases the glucose uptake and utilization in the skeletal muscle thereby reducing insulin resistance and reduce hepatic glucose production (gluconeogenesis). It improves glycemic control by decreasing hepatic glucose production, as well as decreasing glucose absorption and increasing insulin-mediated glucose uptake by peripheral tissue [8]. These effects are mediated by the initial activation of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole-body energy balance, and the metabolism of glucose and fats [9, 10].

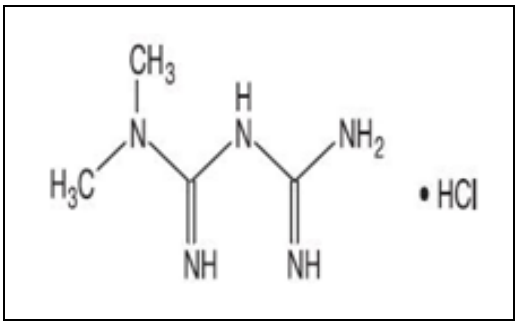

FIG. 2: STRUCTURE OF METFORMIN HYDROCHLORIDE

Only a few analytical methods have been reported for UV method development and validation of Empagliflozin and Metformin hydrochloride in Dosage form [11-15]. A literature survey shows that there are many methods for the estimation of Empagliflozin and Metformin separately and in combination with other drugs. Therefore, an attempt was made for method development and validation of a simple, precise and accurate, UV method for the simultaneous determination of Empagliflozin and Metformin hydrochloride drugs in their combined dosage using Simultaneous equation method and Absorption ratio method.

MATERIALS AND METHODS:

Apparatus and Equipment: An Elico Double Beam SL 210 UV- VIS Spectrophotometer was used for all absorbance measurements with 10 mm path length quartz cuvettes.

Reagents and Chemicals: Active Pharmaceutical ingredients Metformin Hydrochloride and Empagliflozin were obtained as a gift sample from Dr. Reddy's Laboratories. The Pharmaceutical dosage form (Jardiance met 12.50 Empagliflozin and 500 Metformin hydrochloride) was purchased from a local pharmacy. Methanol used in the analysis was of Analytical grade and Distilled water used was freshly prepared by distillation.

Methodology:

Preparation of Standard Stock Solution (1000 µg/ml): Standard stock solutions of Empagliflozin and Metformin hydrochloride were prepared separately by adding 10 mg of the drug to methanol taken in 10 ml volumetric flask and volume were made up to the mark with methanol. The resulting solution contains 1 mg/ml of the drug. The standard stock solution of Empagliflozin and Metformin hydrochloride was further diluted by taking 1 ml in a 10 ml volumetric flask and made up to the mark using distilled water (100 µg/ml). The stock solution of Empagliflozin and Metformin hydrochloride was further diluted with distilled water to obtain a concentration of 10 µg/ml.

Determination of Wavelength of Maximum Absorption: The 10 µg/ml solution of Empagliflozin and Metformin hydrochloride was subjected to a UV spectrophotometric scanning (200-400 nm) to determine the λ_{max} of Empagliflozin and Metformin hydrochloride using water as blank. The scanning spectra of 10 µg/ml solution of Empagliflozin and Metformin hydrochloride showed clear peaks at 224 nm and 233 nm respectively. The overlay spectra of Empagliflozin and Metformin hydrochloride were also recorded. From the overlay spectra, iso-absorptive point of Empagliflozin and Metformin hydrochloride was calculated **Fig. 3, 4, 5**.

Analysis of Tablet Formulation: 10 tablets of JARDIANCE MET (12.5/500 mg Empagliflozin and Metformin hydrochloride) were weighed and triturated using mortar and pestle and powder equivalent to 500 mg of Metformin hydrochloride was taken. A quantity equivalent to 500 mg of Metformin Hydrochloride and 12.5 mg of Empagliflozin was transferred into a 100 ml volumetric flask containing 50 ml of methanol and sonicated for 10 min. The final volume was made

up to the mark and filtered through Filter Paper HM2 Qualitative Circles (12.5 cm). 1 ml of the resulting solution was diluted with distilled water to 50 ml making the solution of 100 µg/ml. 2 ml of the resulting solution was again transferred to 10 ml volumetric flask diluted with distilled water and volume was adjusted up to mark. The present concentration is 0.5/20 µg/ml (Empagliflozin / Metformin). The absorbance was taken at 224nm, 233 nm and 266 nm against a distilled water blank. The concentration of Empagliflozin and Metformin Hydrochloride was calculated by using the below equation.

The assay was performed using two methods, I- Simultaneous equation method & II- Absorption ratio method. Data represented in **Tables 2, 3** and **4**.

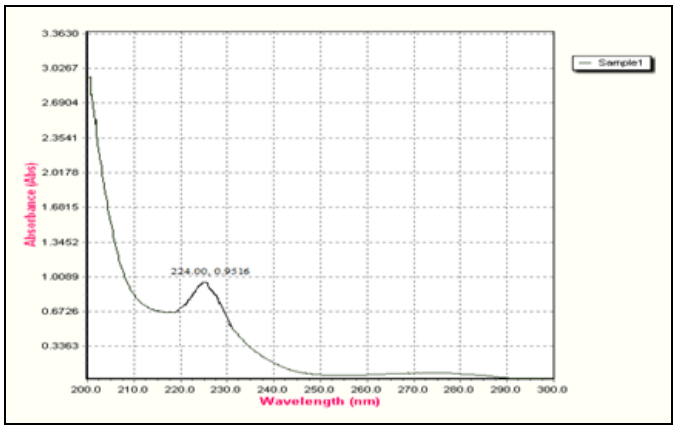

FIG. 3: UV SPECTRA OF EMPAGLIFLOZIN

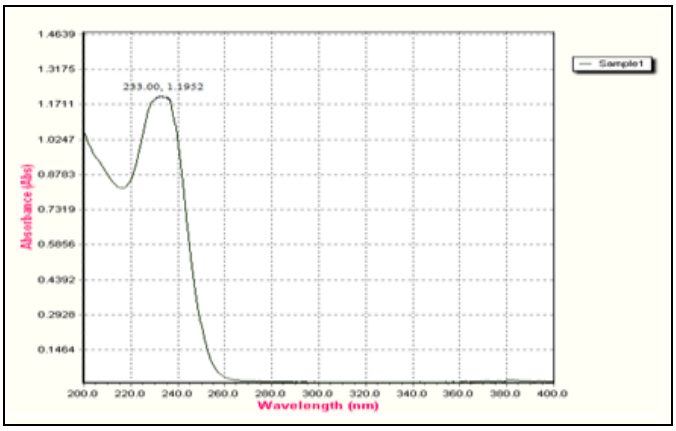

FIG. 4: UV SPECTRA OF METFORMIN HYDROCHLORIDE

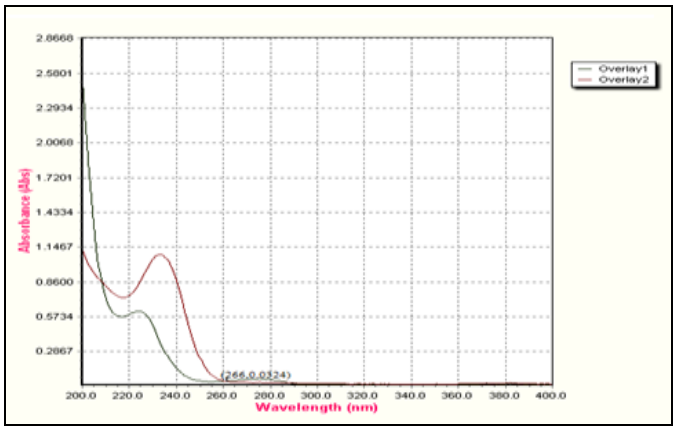

FIG. 5: OVERLAY UV SPECTRA OF EMPA AND MET

Method I: Simultaneous Equation Method (Vierodt's Method): A multi-component system consisting of two components X and Y, each of which absorbs at the λ_{max} of the other, λ_1 (233 nm) being the wavelength of maximum absorbance of X (Metformin HCl) and λ_2 (224 nm) being the wavelength of maximum absorbance of Y (Empagliflozin).

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

$$Cx = (A_2ay_1 - A_1ay_2) / (ax_2ay_1 - ax_1ay_2)$$

$$Cy = (A_1ax_2 - A_2ay_2) / (ax_2ay_1 - ax_1ay_2)$$

The absorptivities of X at λ_1 and λ_2 = ax_1 and ax_2 respectively.

The absorptivities of Y at λ_1 and λ_2, = ay_1 and ay_2 respectively.

The absorbance of the diluted sample at λ_1 and λ_2 = A_1 and A_2 respectively.

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

Determination of Absorptivity Value: The absorptivity value of Empagliflozin and Metformin hydrochloride from each solution was calculated using the following formula and the results are presented in **Table 1**.

Absorptivity = Absorbance (gm/100ml) / Concentration

Method II - Q-Absorption Ratio Method: This method is a modification of the method of the simultaneous equation. According to this method, the ratio of absorbance at any two wavelengths for a substance, which obeys Beer's law, is a constant value independent of concentration and path length. This constant is termed as 'Hufner's Quotient or Q-value. This method involves the measurement of absorbance at two wavelengths, one being the λmax of one of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), called as Iso- absorptive point.

C_x and C_y= concentration of X and Y respectively.

λ_1 and λ_2= 266 nm & 233nm.

A= absorbance of sample at iso-absorptive point.

a_1 and a_2 = Absorptivity of x and y at iso-absroptive wavelength.

Qm = (Absorbance of sample solution at λ max of one of the components (λ2)) / (Absorbance of sample solution at iso-absorptive wavelength)

Qx = (Absorptivity of X at λ max of one of the components (λ2)) / (Absorptivity of X at iso-absorptive wavelength)

Qy = (Absorptivity of Y at λ max of one of the components (λ2)) / (Absorptivity of Y at iso-absorptive wavelength)

The concentration of each component can be calculated as:

$$Cx = (Qm-Qy) / (Qx-Qy) \times A/\alpha 1$$

$$Cy = (Qm-Qx) / (Qy-Qx) \times A/ \alpha 2$$

Method Validation: [16]

Linearity: Linearity is the ability of the method to elicit test results that are proportional to the concentration of the analyte in the sample.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under prescribed conditions.

- **Repeatability/Intraday or Assay Precision:** Precision under the same operating conditions over a short interval of time. It was carried out by measuring 6 different samples of the same concentration at different intervals of time.
- **Intermediate Precision:** It expresses precision within – laboratory variations: different days, different analysts, different equipment, *etc*. It was carried out by different analysts.

Accuracy: It is the closeness of test results obtained by the method to the true value. It was determined by percent recovery of the standard API to the blank. The average recovery of the analyte of 50%, 100%, 150% solution was calculated. Prepare 1, 2, 3 µg/ml of Empagliflozin and Metformin Hydrochloride solution in three sets from the standard stock solution. Spike the standard Empagliflozin and Metformin Hydrochloride in the concentration of 50%, 100% and 120% solutions to the sample. The tablet formulation (sample) is prepared in a lab according to the label claim.

Ruggedness: Is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories or different analysts. It was performed by 2 different analysts.

Robustness: It is the capacity of the method to remain unaffected by small but deliberate variations in method parameters. From the standard stock solution, 10µg/ml was taken. The analysis was performed by changing the wavelength of Empagliflozin (222 nm, 226 nm), Metformin Hydrochloride (231 nm, 235 nm) and Isobestic point (264 nm, 268 nm).

RESULTS AND DISCUSSION: The method developed in the present study provides a convenient, precise and accurate way for the simultaneous analysis of Empagliflozin and Metformin hydrochloride using II methods. The absorption maxima of Empagliflozin, Metformin hydrochloride was found to be 224 nm and 233 nm respectively. The isoabsroptive point was found to be 266 nm. The assay of Metformin hydrochloride and Empagliflozin was found to be 19.79 µg/ml and 0.49 µg/ml respectively by method I. For method II the assay value for Metformin hydrochloride and Empagliflozin are 19.76 µg/ml and 0.49 µg/ml. The results are shown in **Table 1, 2, 3, 4**.

Linearity was studied by diluting a standard stock solution of Empagliflozin and Metformin Hydrochloride to 0.1 to 20 µg/ml and 0.5 to 20 µg/ml concentrations respectively. Calibration curves with concentration versus absorbance were plotted at their respective wavelengths *i.e.*, 224 nm and 233 nm. The standard curves for Empagliflozin and Metformin Hydrochloride were linear and exhibited good correlation coefficient (R^2 = 0.999 & 0.9995) respectively are shown in **Fig. 6, 7** respectively and data is presented in **Table 5, 6**. The % RSD was calculated for both the methods at Interday and intraday precision, and the result was found to be <2and results are shown in **Table 7, 8**. Accuracy was calculated for the method I and is shown in **Table 9**. Ruggedness % RSD results were found to be < 2 and are shown in **Table 10, 11**. Robustness was found to be within limits and results are shown in **Table 12, 13**.

TABLE 1: SIMULTANEOUS EQUATION VALUES

Drug	Absorbance maxima (λ_1-233nm)	Absorbance maxima (λ_2- 224nm)
Formulation (Jardiance- Met)	2.4020 (A_1)	1.8196 (A_2)
Absorbance of Metformin Hydrochloride	1.1952	0.8950
Absorbance of Empagliflozin	0.7213	0.9516
Absorptivity of Metformin Hydrochloride	0.11952 (a_{x1})	0.08950 (a_{x2})
Absorptivity of Empagliflozin	0.07213 (a_{y1})	0.09516 (a_{y2})

C_x = 1.8196 × 0.07213 – 2.4020 × 0.09516 / 0.08952 × 0.07213 – 0.11952 × 0.09516 = 19.79 µg/ml

C_y = 2.4020 × 0.08952 – 1.8196 × 0.11952 / 0.08952 × 0.07213 – 0.11952 × 0.09516 = 0.49 µg/ml

Simultaneous Equation Method:

TABLE 2: ASSAY FOR METHOD-I

Amount present (12.50 mg) Empagliflozin	Amount present (%label claim)	Amount present (500 mg) Metformin HCl	Amount present (% label claim)
12.25	98	494	98.80
12.25	98	494	98.80
12.29	98.32	494.25	98.85
12.30	98.40	494.75	98.95
12.49	99.92	494.5	98.90
% RSD	0.757274		0.065944

TABLE 3: ABSORPTION RATIO METHOD VALUES

Drug	λ_2 (233nm)	Λ_1 (266nm)
Jardiance- Met	2.3980 (A_1)	0.0324 (A_2)
Absorbance of Metformin Hydrochloride	1.1952	0.0129
Absorbance of Empagliflozin	0.7213	0.1386
Absorptivity of Metformin Hydrochloride	0.11952	0.00129 (a_1)
Absorptivity of Empagliflozin	0.07213	0.01386 (a_2)

Qm = (2.3980) / (0.0324) = 74.01

Qx = (0.11952) / 0.00129 = 92.65

Qy = 0.07213 / (0.01386) = 5.20

Cx = (74.01-5.20) / (92.56-5.20) × (0.0324) / (0.00129) = 19.76 µg/ml

Cy = (74.01-92.65) / (5.20-92.65) × (0.0324) / (0.01386) = 0.49µg/ml

TABLE 4: ASSAY FOR METHOD- II

Amount Found Empagliflozin (12.50 mg)	Amount present (% Label claim)	Amount found Metformin (500 mg)	Amount present (% Label claim)
12.25	98	494	98.80
11.99	95	494	98.80
12.44	99.52	494.25	98.85
12.25	98	494.75	98.95
12.50	100	494.50	98.90
% RSD	1.257274		0.065944

Linearity:

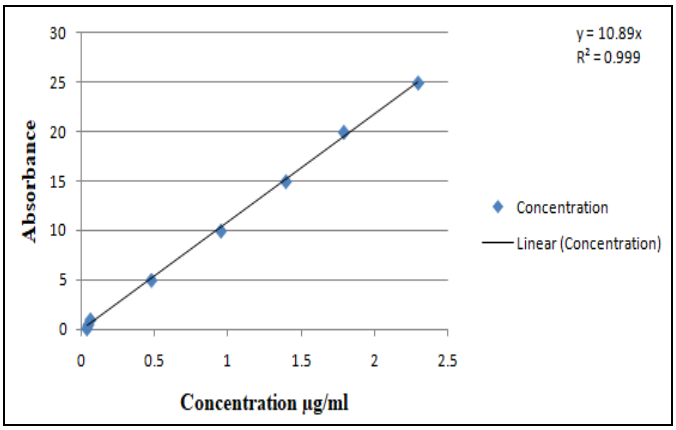

FIG. 6: CALIBRATION CURVE OF EMPAGLIFLOZIN (0.1-20 µg/ml)

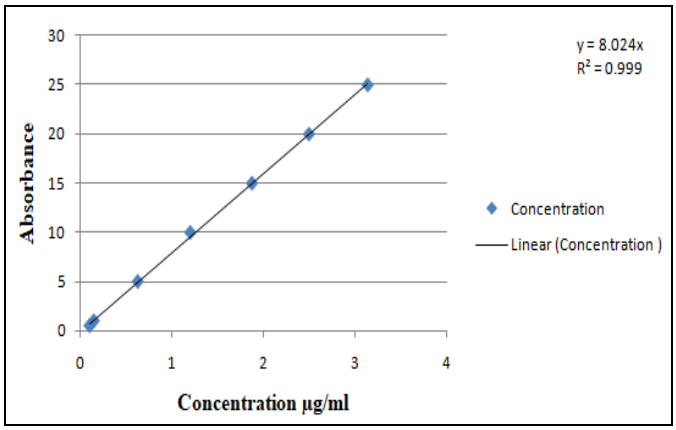

FIG. 7: CALIBRATION CURVE OF METFORMIN HYDROCHLORIDE (0.5-20 µg/ml)

TABLE 5: STANDARD CURVE DATA OF EMPAGLIFLOZIN

Concentration (µg/ml)	Absorbance
0.1	0.0356
0.5	0.0459
1	0.0599
5	0.4765
10	0.9516
15	1.3956
20	1.7922
25	2.2993

TABLE 6: STANDARD CURVE DATA OF METFORMIN HYDROCHLORIDE

Concentration (µg/ml)	Absorbance
0.5	0.0956
1	0.1412
5	0.6213
10	1.1952
15	1.8695
20	2.4921
25	3.1334

Precision:

TABLE 7: PRECISION STUDY DATA FOR METHOD- I

INTERDAY PRECISION		INTRADAY PRECISION	
Empagliflozin (%) @224nm	Metformin Hydrochloride (%) @233nm	Empagliflozin (%) @224nm	Metformin Hydrochloride (%) @233nm
98	98.8	87.16	99.03
101.17	98.84	88.13	99.01
98.25	98.85	89.97	98.44
99.72	98.98	89.04	98.17
99.11	98.93	89.84	98.79
98	98.8	89.84	99.06
%RSD 1.260396	0.073959	1.280763	0.372405

TABLE 8: PRECISION STUDY DATA FOR METHOD- II

INTERDAY PRECISION		INTRADAY PRECISION	
Metformin Hydrochloride (%) @233nm	Isobestic Point (%) @266nm	Metformin Hydrochloride (%) @233nm	Isobestic Point (%) @266nm
98.96	95.13	98.4	91.02
98.9	93.52	98.61	90.27
98.84	93.77	98.38	91.11
99.19	92.17	99.19	92.17
98.89	92.13	98.2	90.36
98.36	93.91	98.27	89.67
% RSD 0.275819	1.22224	0.367443	0.956173

Accuracy:

TABLE 9: ACCURACY STUDY DATA (SIMULTANEOUS EQUATION METHOD)

Drug	Sample + Standard	Percentage	%Recovery	% Mean Recovery	%RSD
MET	20+1	80	99.42-100%	99.62333%	0.32%
EMPA	0.5+1		97.92-98.10%	97.91667%	0.18%
MET	20+2	100	099.42-99.53%	99.47333%	0.05%
EMPA	0.5+2		99.25-99.57%	99.39333%	0.16%
MET	20+3	120	99.8-100%	99.94%	0.05%
EMPA	0.5+3		100-100.05%	100.05%	0.04%

Ruggedness:

TABLE 10: RUGGEDNESS STUDY DATA BY DIFFERENT ANALYST FOR METHOD 1

@233nm				@224nm	
Metformin	Analyst 1	Analyst 2	Empagliflozin	Analyst 1	Analyst 2
	99.01	99.02		96.20	93.52
	98.92	98.61		96.67	92.98
	98.91	99.02		98.67	92.42
	98.96	98.93		97.16	91.34
	98.86	99.03		97.40	91.08
	98.95	98.95		98.63	93.02
% RSD	0.05144	0.162325		1.040194	1.065018

TABLE 11: RUGGEDNESS FOR METHOD-II

@233nm				@266nm	
Metformin	Analyst 1	Analyst 2	Isobestic Point	Analyst 1	Analyst 2
	98.83	98.20		98.12	90.36
	98.80	98.83		98	92.36
	98.76	98.90		99.84	93.52
	98.81	98.84		96.76	93.77
	98.84	99.19		96.67	92.17
	98.68	98.89		96.23	92.13
% RSD	0.059944	0.330212		1.366569	1.318514

Robustness:

TABLE 12: ROBUSTNESS STUDY DATA AT DIFFERENT WAVELENGTH FOR METHOD- I

Empagliflozin	222nm	226nm	Metformin	231nm	235nm
	100	96.61		99.30	98.35
	96	98.30		99.45	98.28
	96.73	97.34		99.42	98.35
	96.73	99.40		99.39	98.29
	100	97.10		99.33	98.38
	100	98.30		99.34	98.26
% RSD	1.977443	1.039016		0.058129	0.048743

TABLE 13: ROBUSTNESS STUDY DATA AT DIFFERENT WAVELENGTH FOR METHOD- II

Metformin	231nm	235nm	Empagliflozin	264nm	268nm
	99.30	98.43		106.14	88.00
	99.20	98.48		109.74	87.90
	99.34	98.26		104.89	88.70
	99.27	98.42		106.58	86.00
	99.19	98.26		109.76	87.25
	99.23	98.30		108.18	89.98
% RSD	0.059349	0.098029		1.863423	1.523225

CONCLUSION: Based on the result obtained new, simple, rapid; precise UV spectrophotometric method was developed for the simultaneous estimation of Empagliflozin and Metformin hydrochloride. Hence, this method can be applied for the estimation of Empagliflozin and Metformin hydrochloride in drug testing laboratories and pharmaceutical industries.

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CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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