Development and Validation of Q-Absorbance Ratio Spectrophotometric Method for the Simultaneous Estimation of Metformin and Empagliflozin; in Bulk and Formulation.

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ABSTRACT:

The present research work demonstrates an analytical method development for simultaneous estimation of Metformin andEmpagliflozin in combined dosage form using Q-absorbance ratio concept. While method development, two different wavelengths one representingiso-absorptive point (223 nm) and other representing the λ_{max} of Metformin (237 nm) were used. Optimum response was obtained in solvent system that comprisesmethanol and water in ratio of 40:60 v/v.Proposed UV method was found to be linear over the concentration range of 1-10 µg/ml for Metformin and that of 1-20 µg/ml for Empagliflozin.On the basis of recovery studies after standard addition, accuracy of proposed method was found to be in between 99.28 to 100.06 and 99.07 to 99.84% for Metformin and Empagliflozin respectively. Intra-day precision of the method in terms of % relative standard deviation was found to be in between 0.15 to 0.83 and

0.10 to 1.34 for Metformin and Empagliflozin respectively. Inter-day precision range of the method for Metformin and Empagliflozin was found to be in between 0.12 to 0.83 and 0.10 to 1.34 respectively.LODand LOQ of proposed UV method were 0.0368 and 0.1117µg/ml for Metformin and 0.0413 and 0.1252 µg/ml forEmpagliflozin.Proposed UV method was robust and rugged in nature.Proposed methodwas successfully used for the estimation of Metformin and Empagliflozin contents of marketed formulation consisting of APIs and the common excipients.

Keywords: UV- visible spectrometry, Q absorbance ratio, Metformin, Empagliflozin, Validation.

1. INTRODUCTION:

Metformin is the first-line medication for the treatment of type 2 diabetes,chemically it is known as 1, 1-dimethylbiguanide hydrochloride a biguanide derivative which is the most commonly prescribed drug to the patients with type-2 diabetes[1-2]. It brings down the blood glucose levels by decreasing the hepatic glucose production, declining intestinal absorption of glucose and enhancing insulin sensitivity by elevating peripheral glucose utilization and uptake[3-4].

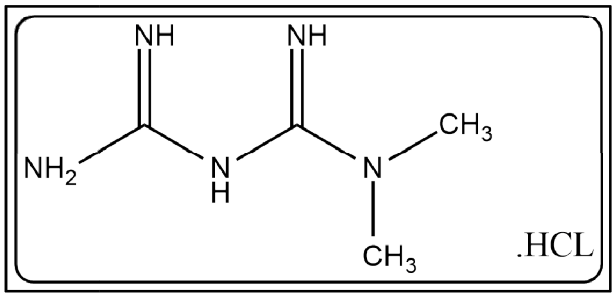

Fig1. Chemical structure of Metformin

Empagliflozin chemically 1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran -3- yl -oxy) benzyl]-benzene which belongs to gliflozin class used in the treatment of type-2 diabetes[5-8]. Empagliflozin is an inhibitor of the sodium glucose co transporter -2 (SGLT-2) thus SGLT-

2reduces blood glucose by blocking glucose reabsorption in the kidney and thereby excreting glucose (i.e., blood sugar) via the urine[9-13].

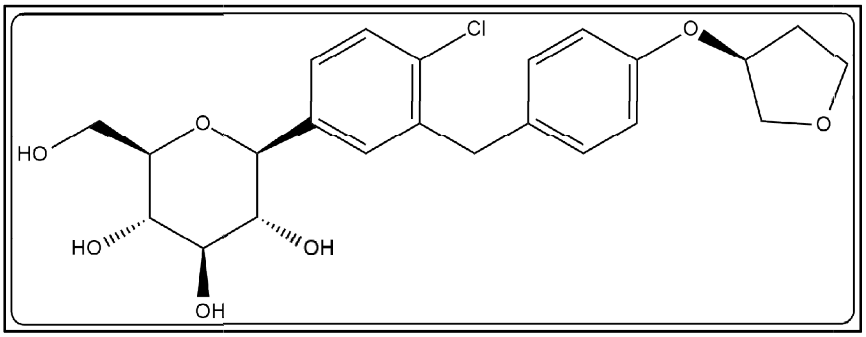

Fig 2. Chemical structure of Empagliflozin

Considering the therapeutic and commercial importance of both drugs it was envisaged that development of simple, economic, accurate, precise yet sensitive UV-visible spectrophotometric method with ability of simultaneous estimation of both Metformin and Empagliflozin will be worth, it would be useful in routine analysis of Metformin and Empagliflozin composition in near future[14-20].

2. EXPERIMENTAL:

Instrumentation:

A double beam UV-visible spectrophotometer (V-530, Jasco)with spectra manager software was used for the method development and validation. Matched quartz cells with 3 cm height and 1 cm path length were used for spectral measurements. Analytical balance (Vibr HT, Essae) was used for the weighing purpose.

Material and Methods:

All chemicals and reagents used for the method development purpose were of analytical or HPLC grade. Pure Metformin and Empagliflozinstandard was purchased from theTCI chemicals (INDIA) pvt.ltd.

Preparation of standard stock solution:

Metformin and Empagliflozinwas weighed separately (1 mg each) and transferred to the 1 ml pre-calibrated volumetric flasks and dissolved in1 mlof methanoland sonicated for 15 min,to achieve a stock solution of 1000 µg/ml (Stock-1). Stock 1 was suitably diluted to achieve solution of 100µg/ml (stock 2).

Determination of maximum wavelength (λ_{max}):

Stock-2 of Metformin and Empagliflozin was diluted suitably so as to obtain solutions of 10µg/ml strength. Resultant Metformin and Empagliflozin solutions were scanned over wavelength range of 800 to 200 nm using medium scanning speed. Obtained spectra were analyzed using Spectra Manager software and the λ_{max} were identified.

Preparation of calibration curve:

Stock 2 of Metformin was diluted suitably so as to achieve seven different calibration standards representing 1, 2, 4, 5, 6, 8 and 10 µg/ml strength whereas Stock 2 of Empagliflozin was diluted to obtain calibration standards with 1,4, 8, 12, 16, 18 and 20 µg/ml strength. From the full spectrum measurement mode (Figure 3 and 4) of stock-2 of Metformin and Empagliflozin, two different wavelengths viz. 223 nm and 237 nm were identified as λ_{max}. The calibration curves representing concentration vs. absorbance were plotted (Figure 3 and Figure 4 respectively).

UV-spectrophotometric method:

Q-Absorption ratio analysis method:

Q-Absorption ratio method comprises use the ratio of absorption at two selected wavelengths (one representingiso-absorptive point and other representingλ_{max} of one of the two components). Proposed method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbance at any two wavelengths is a constant value, independent of concentration and path length. The solutions of 10µg/ml ofmetformin and empagliflozin were scanned in the wavelength range of 400 to 200nm to obtain overlain spectra (fig 5). Two wavelengths, 223nm as iso-absorptive point and 237nm (λmax ofMetformin) were selected for the formation of Q-absorbance ratio equation.

The concentration of the individual components was calculated by using the following equations;

Cx = Qm-Qy/Qx-Qy) ×A1/ax 1

Cy = Qm-Qy/Qy-Qx) ×A1 /ax1

WhereQm = A2 /A1, A 1 is absorbance of sample at iso-absorptive point,

A2 is absorbance of sample at λ_{max} of one of the two components,

Qx = ax2 /ax1,Qy = ay2 /ay1,

ax 1and ax 2 represent absorptivities of Metformin at λ1 and λ2,

ay 1 and ay 2 denote absorptivities of Empagliflozin at λ1 and λ2 respectively;

Cx and Cy be the concentration of Metformin and Empagliflozinrespectively.

3. Validation of UV- visible spectrophotometric methods:

The developed method for simultaneous estimation of Metformin and Empagliflozin was validated as per ICH guidelines. Different parameters like linearity, accuracy, precision, robustness, and ruggedness, limit of detection (LOD) and limit of quantification (LOQ) were evaluated[21-25].

Linearity and Range:

Linearity of the proposed UV method was established using seven different CAL STDs of Metformin and Empagliflozin. CAL STDs of Metformin and Empagliflozinwere analyzed at respective wavelengths of maximum absorbance. Calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis.R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy:

Accuracy may often be expressed as % recovery by the assay of known added amount of analyte. To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%)of its predefined concentration. To the predefined

concentrations, different amounts of Metformin and Empagliflozin were added (standard addition method) and the accuracy was calculated on the basis of percent recovery. For calculating the percent recovery following formula was used.

% RC= (SPS-S/SP) × 100

Where,

SPS = Amount found in the spiked sample

S = Amount found in the sample

SP = Amount added to the sample

% RC = Percent recovery

Precision (Inter-day and Intra-day precision):

The precision of the proposed UV method was established by performing intra- and inter-day UV analysis of predefined samples. The study was performed at three concentration levels (Metformin: 1.5, 5 and 9.5µg/ml andEmpagliflozin: 1.5,12 and 19.5 µg/ml).Samples (n=3) were analyzed at three different time intervals of a day. Study was repeated on three consecutive days. Deviation in the results was calculated in terms of % relative standard deviation (% RSD).

Robustness:

Robustness of the method was assessed by analyzing MQC STDs of Metformin and Empagliflozin 5µg/mland 12µg/ml Resepectivelyat ±1nm of pre-identified wavelength of maximum absorbance for both Metformin and Empagliflozin. The results were calculated in terms of % RSD.

Ruggedness:

Ruggedness of the method was established by analyzing triplicate samples of Metformin and Empagliflozin5µg/ml and 12µg/ml Resepectivelyon two different UV-Visible spectrophotometers viz. V-530, Jasco and BA-UV-2600, Bio age. Results were expressed in terms of % RSD.

Limit of Detection and Quantification:

To determine the limit of detection and quantification (LOD and LOQ), the standard deviations (σ) of response and slope of calibration curve (S) were used. Detection of limit was calculated by (3.3×σ/S) and quantification limit was calculated by (10×σ/S).

4 Application of Method:

Estimation ofMetformin and Empagliflozincontent in pharmaceutical formulation:

The marketedpharmaceutical formulationof Metformin and Empagliflozin (Brand Name Jardiance Met) was analyzedin order to estimate the contents of abovementioned pharmaceutical formulation, 5 mg of formulation was accurately weighed and transferred to calibrated volumetric flask. The contents were dissolved in 5 ml of methanol and obtained solution was filtered through 0.45 µm syringe filter. Filtered solution was suitably diluted and analyzed for Metformin and Empagliflozin content by using proposed UV-Visible spectrophotometric method.

In vitrodrug release studies:

Tablets of empagliflozin and metformin hydrochloride were evaluated for in vitro drug release studies, which were performed using USP Type-I (Basket) dissolution test apparatus. The volume of the dissolution medium was 900mL with a stirring speed of 100 rpm and the temperature was maintained at 37°C±0.5°C. These conditions were kept constant for all dissolution studies. The study was carried out in pH 6.8 phosphate buffer at 10, 15, 20, 30, 45 and 60 min.

5. RESULTS AND DISCUSSION:

Determination of wavelength of maximum absorbance (λ_{max}):

Identification of wavelength having maximum absorbance is prerequisite for quantitative UV analysis. Solution with absorbance value less than 1 were considered to be appropriate for the determination of wavelength having maximum absorbance. Considering the above mentioned point determination of λmax of Metformin and Empagliflozin solution of 10 µg/ml concentration

each were carried out by full scan mode of UV-Visible spectrophotometer. The full scan mode was processed by Jasco UV software and λmax were determined. The λmax was found to be 237nm and 223nm for Metformin and Empagliflozin(Fig. 3 and Fig. 4) respectively. The overlain spectra of both drugs shown in Fig. 5. The two wavelengths were used for the analysis of the drugs were 223 nm (Iso-absorptive point) and 237 nm (λmax of Metformin) at which the calibration curves were prepared for both the drugs.

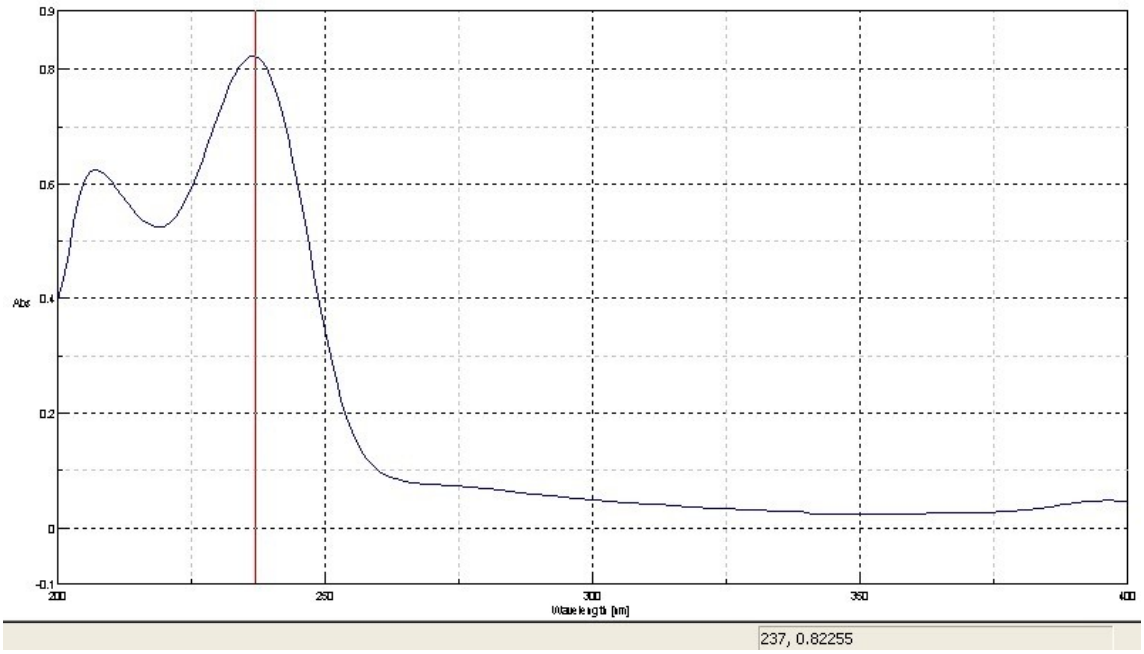

Fig 3.UV-visible spectra of Metformin (237 nm)

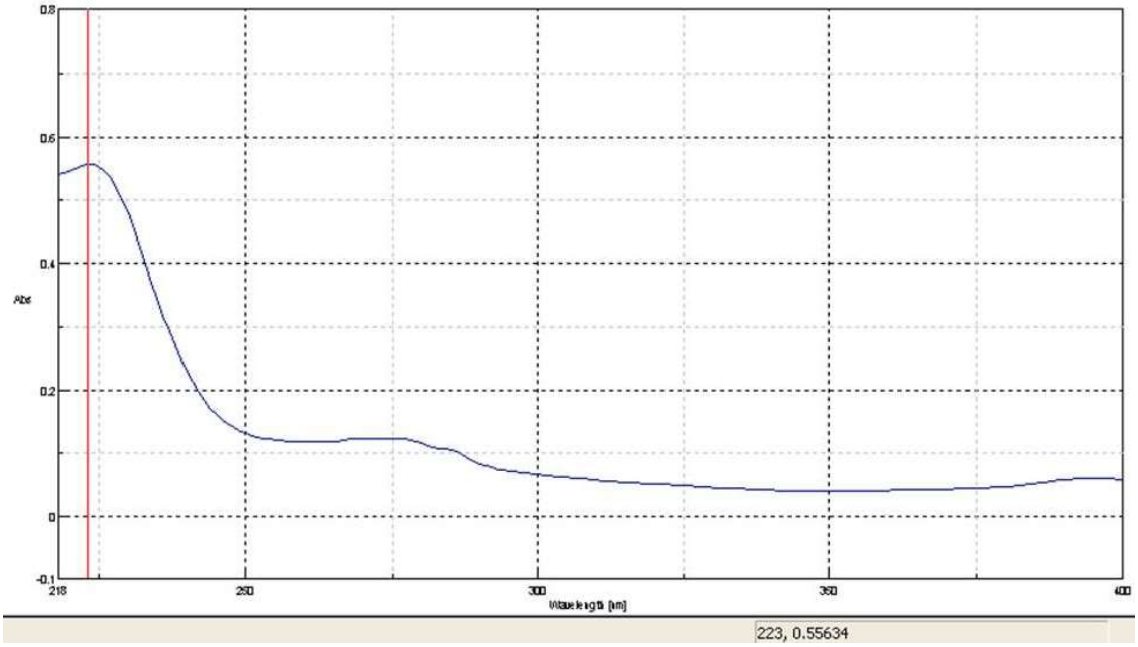

Fig 4.UV-visible spectra of Empagliflozin (223 nm)

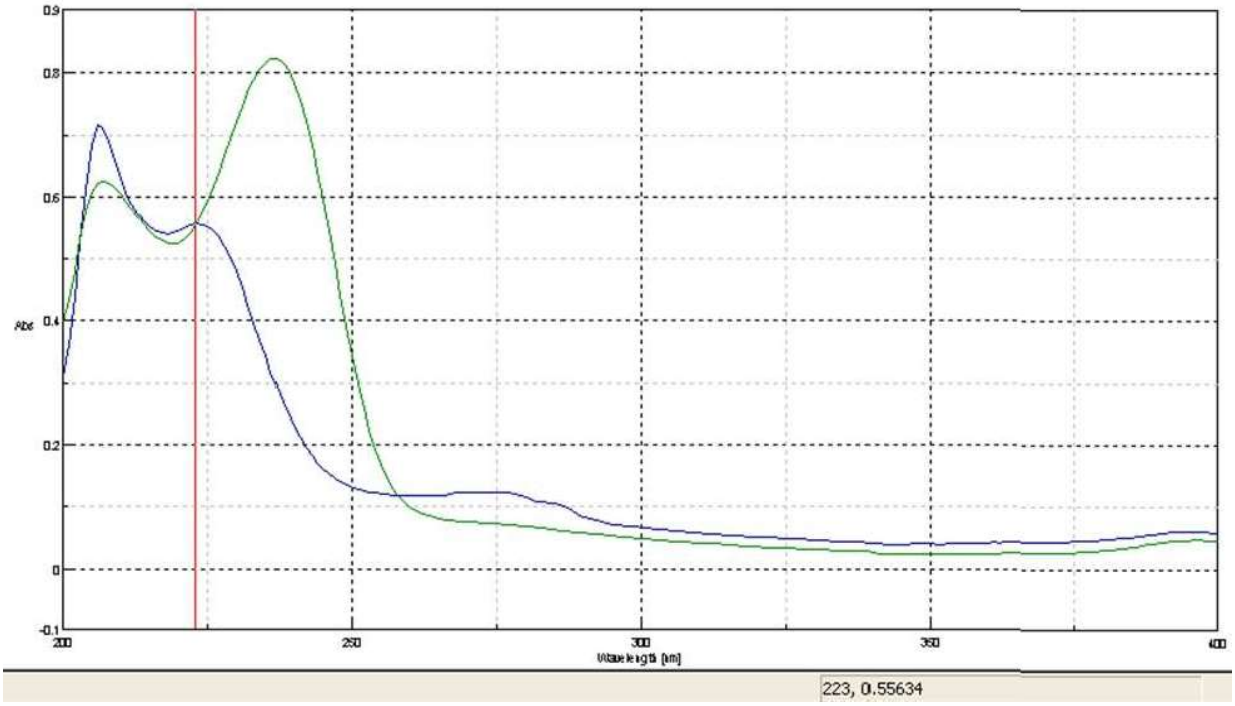

Fig 5.Overlain spectra of Metformin and Empagliflozin

Preparation of Calibration Curve:

(A) Calibration Curve for Metformin:

Calibration curve for Metformin consists of different concentrations of standard solution ranging from 1 -10µg/ml. The solutions were prepared by pipetting out 1,2, 4, 5,6, 8 and 10 µg/ml of the working standard solution of Metformin (100µg/ml) into series of 5 ml volumetric flasks and the volume was adjusted to mark with solvent. the absorbance of the solutions was measured at 223nmand 237nm against solvent ratio of methanol: water (40:60) as a blank.Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity.(Figure 6)

(B) Calibration Curve for Empagliflozin:

Calibration curve for Empagliflozinconsists of different concentrations of standard solution ranging from 1-20µg/ml. The solutions were prepared by pipetting out 1, 4, 8, 12, 16, 18 and 20 µg/ml of theworking standard solution of Empagliflozin(100µg/ml) into series of 5 ml volumetric flasks and the volume was adjusted to mark with solvent ratio.The absorbance of the solutions was measured at 223 nm and 237nm against solvent ratio of methanol: water (40:60) as a blank.Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity. (Figure 7)

6. Method validation: -

Linearity and Range

Linearity and range are the key parameters of analytical method which demonstrates the limit within the intended method to be used for its optimum performance. Considering the importance of linearity and the range, seven points calibration curves of Metformin between the range 1-10 µg/ml and Empagliflozin between the range 1-20µg/ml were plotted. The concentrations and the respective mean absorbance values of Metformin and Empagliflozinare mentioned in (Table 1 & Table 2). Calibration curve were subjected to least square regression analysis yielded an equation; $y = 0.064X + 0.003$ and $y = 0.035X+ 0.003$ with correlation coefficient for Metformin and Empagliflozin in 223nm respectively (Fig. 6 andotherto least square regression analysis yielded an equation; $y = 0.040X + 0.004$ and $y = 0.039X +0.11$ with correlation coefficient for Metformin and Empagliflozinin 237nm respectively (Fig. 7). The linearity study revealed that

the developed UV method was found to be linear adherence to the system of Beers Law over the concentration range of 1 to 10 µg/ml for Metforminand 1 to 20 µg/ml for Empagliflozin.

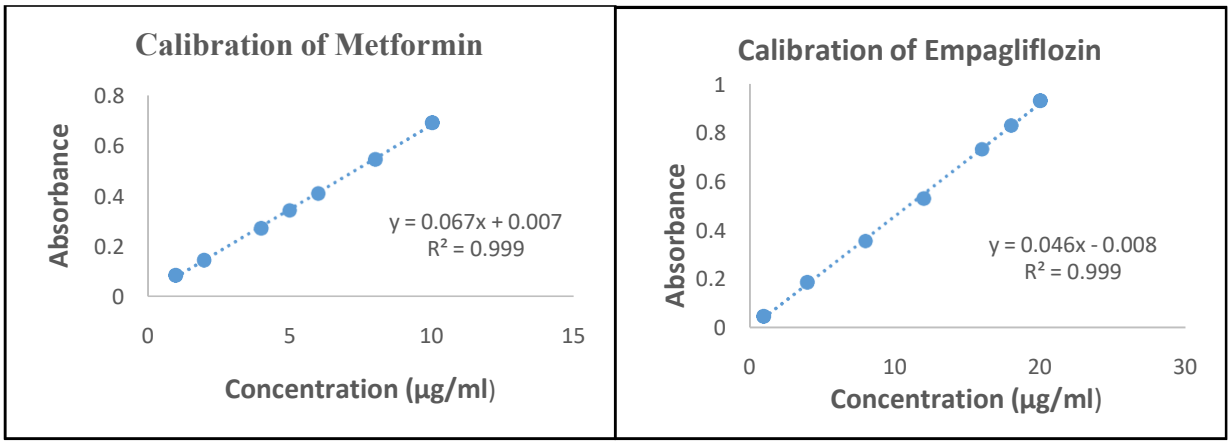

Fig 6. Calibration curve of Metformin and Empagliflozin at 223 nm

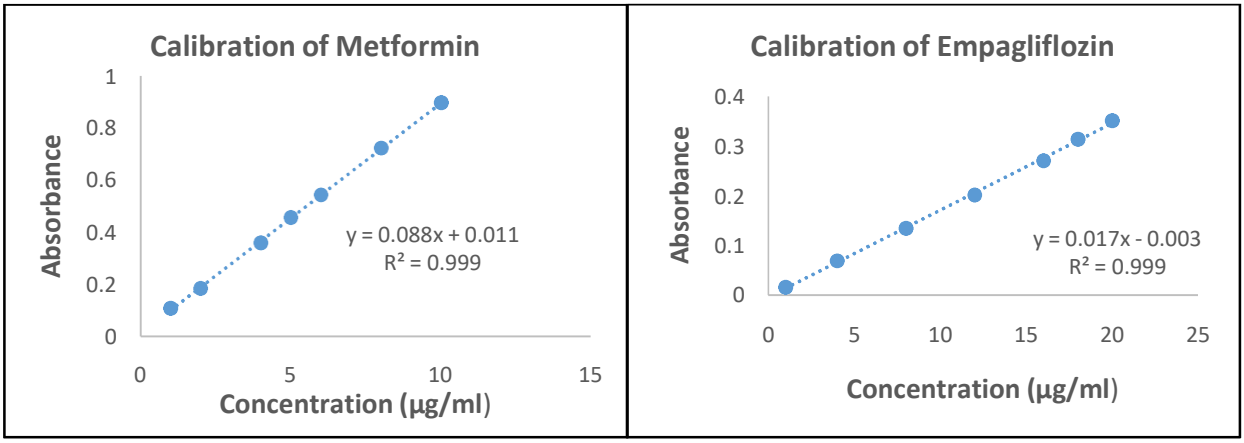

Fig 7. Calibration curve of Metformin and Empagliflozin at 237 nm

Table 1: Calibration data at Iso absorptive Point (223nm)

	METFORMIN		EMPAGLIFLOZIN	
Sr No.	Conc. (µg/ml)	Absorbance	Conc.(µg/ml)	Absorbance

1	1	0.0828± 0.0025	1	0.0452 ± 0.0049
2	2	0.1433 ±0.0034	4	0.1844 ± 0.0026
3	4	0.2689 ± 0.0045	8	0.3534 ± 0.0029
4	5	0.3400 ±0.0023	12	0.5278 ± 0.0046
5	6	0.4068 ± 0.0056	16	0.7290 ± 0.0031
6	8	0.5422 ± 0.0042	18	0.8265 ± 0.0067
7	10	0.6878 ± 0.0076	20	0.9284 ± 0.0035

Table 2: Calibration data at λmax(237nm)

	METFORMIN		**EMPAGLIFLOZIN**	
Sr No.	**Conc. (µg/ml)**	**Absorbance**	**Conc.(µg/ml)**	**Absorbance**
1	1	0.1077 ± 0.0025	1	0.0161 ± 0.0015
2	2	0.1837 ± 0.0048	4	0.0692 ± 0.0052
3	4	0.3582 ± 0.0051	8	0.1345 ± 0.0022
4	5	0.4552 ± 0.0019	12	0.2015 ± 0.0033

5	6	0.5418 ± 0.0063	16	0.2704 ± 0.0027
6	8	0.7213 ± 0.0018	18	0.3132 ± 0.0036
7	10	0.8952 ± 0.0042	20	0.3508 ± 0.0042

Accuracy:

Accuracy is the measure of how close the experimental value is to the true value. The accuracy of an analytical method expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value.sometimes it termed as trueness.Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. Accuracy of UV method for Metformin and Empagliflozin, was established by recovery studies.The results of accuracy studies, determined that thedeveloped UV method is highly accurate as the percent recovery was found to be between 98 to 100% (Table 3).

Table 3: Recovery studies for Metformin and Empagliflozin.

Origin level (µg/ml)	METFORMIN				Origin level (µg/ml)	EMPAGLIFLOZIN			
	Conc. (%)	Amount Added	% Recovery	% RSD		Conc. (%)	Amount Added	% Recovery	% RSD
1.5	80	1.2	99.85 ± 0.29	0.8875	1.5	80	1.2	99.07 ± 0.35	0.7257
5	100	5	99.28 ± 0.18	1.2086	12	100	12	99.84 ± 0.14	0.0397
9.5	120	11.4	100.06 ± 0.32	0.5757	19.5	120	23.4	99.59 ± 0.25	0.3570

Precision:

Precision is the variability among replicate measurements, i.e., how close the values in a series of results are to each other. Precision of the assay was determined by repeatability and intermediate precision, which was studied by comparing the assays on 3 different days. It is expected that an analytical method should generate reproducible outcomes. Precise analytical method leads to accurate results. Considering the importance of reproducible and accurate results, Inter-day, intra-day variations were studied to determine repeatability and intermediate precision of the proposed analytical method. Intermediate precision was determined by analyzing three different levels of Metformin and Empagliflozin concentrations at 1.5, 5, 9.5 and 1.5, 12, 19.5 µg/ml respectively.The results were expressed in terms of mean absorbance values, percent assay and % RSD for the intra-day and inter-day precision study, demonstrated in Table 4-7, respectively for Metformin and Empagliflozin. Percentage RSD values of intra-day precision study were found to be between0.15and 0.83 for Metformin and between 0.10 and 1.34 for Empagliflozin whereas those of inter-day precision study were between 0.12 and0.83 for Metforminand between 0.10 and 1.34 for Empagliflozin. % RSD values were less than 2, demonstrated the precision of developed UV method.

Table 4: Intra-day precision data of UV method for Metformin

Sr. No			Morning			Afternoon			Evening		
	Wavelength (nm)	Conc (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	223	1.5	1.5089	100.04	1.034	1.4923	99.48	0.965	1.5023	100.15	0.507
	237		1.5035	100.234	0.725	1.5055	100.37	0.559	1.4994	99.96	0.557
2	223	5	5.0181	100.363	0.922	5.0003	100.00	0.347	4.9958	99.91	0.623

	237		5.0006	100.014	0.929	5.0029	100.05	0.182	5.0006	100.01	0.149
3	223	9.5	9.5008	100.009	0.512	9.5006	100.06	0.399	9.5059	100.06	0.116
	237		9.4994	99.994	0.166	9.5021	100.02	0.092	9.5017	100.01	0.221

Table 5: Inter-day precision data of UV method for Metformin

Sr. NO	Wavelength (nm)	Conc (µg/ml)	Day 1			Day 2			Day 3		
			Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	223	1.5	1.4984	99.89	0.835	1.5004	100.03	0.540	1.5009	100.06	0.515
	237		1.5028	100.18	0.614	1.5035	100.23	0.469	1.5032	100.21	0.499
2	223	5	5.0047	100.09	0.631	5.0041	100.08	0.607	5.0043	100.08	0.621
	237		5.0014	100.02	0.420	5.0009	100.02	0.409	5.0006	100.01	0.402
3	223	9.5	9.5024	100.02	0.342	9.4961	99.95	0.348	9.5017	100.01	0.344
	237		9.5010	100.01	0.159	9.4992	99.99	0.129	9.5004	100.00	0.145

Table 6: Intra-day precision data of UV method for Empagliflozin

Sr. NO			Morning			Afternoon			Evening		
	Wavelength (nm)	Conc (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	223	1.5	1.5004	100.02	0.7861	1.5000	100	0.8352	1.5039	100.26	0.988
	237		1.5023	100.15	1.2859	1.5011	100.07	1.7418	1.5034	100.23	0.996
	223		12.018	100.15	0.5379	12.003	100.03	0.7459	12.003	100.03	0.702

2	237	12	11.995	99.96	0.5803	12.002	100.01	0.1644	11.993	99.94	0.114
3	223	19.5	19.492	99.96	0.0763	19.495	99.97	0.1539	19.503	100.01	0.099
	237		19.505	100.02	0.1516	19.502	100.01	0.2574	19.502	100.01	0.192

Table 7: Inter-day precision data of UV method for Empagliflozin

Sr. NO	Wavelength (nm)	Conc (µg/ml)	Day 1			Day 2			Day 3		
			Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	223	1.5	1.5014	100.09	0.870	1.5004	100.02	0.718	1.5023	100.15	0.915
	237		1.5023	100.15	1.341	1.5023	100.15	0.982	1.5015	100.10	1.237
2	223	12	12.008	100.07	0.661	12.007	100.05	0.539	12.008	100.07	0.659
	237		11.996	99.97	0.286	11.998	99.98	0.166	11.997	99.98	0.292
3	223	19.5	19.497	99.98	0.109	19.497	99.98	0.108	19.499	99.99	0.100
	237		19.503	100.01	0.200	19.503	100.01	0.176	19.501	100.01	0.161

Robustness

Robustness examine the effect that operational parameters such as temperature, mobile phase composition, detection wavelength etc, have on the analysis results. If the influence of parameter is said to be within a previously specified tolerance, the parameter is said to be within the methods robustness range.Robustness study of proposed UV method was evaluated by using three different solvent. The method was found to be robust as indicated by the % RSD values which are less than 2%. The% RSD values were found to be between0.19 and0.63 for Metforminand between 0.09 and 0.18 forEmpagliflozin, shown in Table 8 for Metformin and Empagliflozin respectively. Percentage RSD values were below 2 depict that the proposed UV method was robust in nature.

Table 8: Robustness study for Metformin and Empagliflozin

Molecule	Conc (µg/ml)	Solvent Ratio (Water: MeOH)	λmax	Absorbance Mean	%RSD
		55:45	223	0.3431	0.4325
			237	0.4538	0.1983

Metformin	5	60:40	223	0.3424	0.4836
			237	0.4531	0.2318
		65:35	223	0.3416	0.6293
			237	0.4526	0.2604
Empagliflozin	12	55:45	223	0.5606	0.1441
			237	0.21220	0.1699
		60:40	223	0.5598	0.1560
			237	0.2115	0.1704
		65:35	223	0.5590	0.1725
			237	0.2106	0.0988

Ruggedness:

Ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different instruments, different elapsed assay times, different assay temperatures, different days etc.Ruggednes analytical methods are free from environmental/external factors impact. The ruggedness of proposed UV method, for Metformin and Empagliflozin solutions were analysed by using two different UV-Visible spectrophotometers. Sample analysis resulted into % RSD values between 0.17 and 0.34 for Metforminand between 0.16 and 0.74 for Empagliflozin. Results showed that the proposed UV method was rugged as % RSD values were less than 2, shown in Table 9.

Table 9: Ruggedness study for Metformin and Empagliflozin

Molecule	**Theoretical Conc (µg/ml)**	**Make of Instrument**	**λ max**	**% RSD**
Metformin	5	V-530, Jasco	223	0.3403
			237	0.1781
		UV-2600, Bio age	223	0.3393
			237	0.1934
Empagliflozin	12	V-530, Jasco	223	0.7353
			237	0.1620
		UV-2600, Bio age	223	0.7027
			237	0.1730

Limit of Quantitation (LOQ) and Limit of Detection (LOD):

Generally, LOQ is the first calibration standard. LOQ represents the lowermost concentration that can be analysed. LOD represents the lowest quantity of substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%). LOD and LOQ of proposed UV method were found to be 0.03686 and 0.11171 µg/ml for Metformin whereas 0.04134 and 0.12527 µg/ml for Empagliflozin, as shown in Table 10 for Metformin and Empagliflozin. Lower LOQ values indicated that the proposed method would be sensitive enough to quantify the Metformin and Empagliflozincontent of samples at its lower level.

Table 10: LOD and LOQ for Metformin and Empagliflozin

Sr. No.	Parameter	Metformin	Empagliflozin
1	LOD	0.03686	0.04134
2	LOQ	0.11171	0.12527

Estimation of Metformin and Empagliflozincontent in pharmaceutical formulation:

The developed UV method was successfully applied for estimation of Metformin and Empagliflozincontent in pharmaceutical formulation. The Metformin and Empagliflozincontent in the pharmaceutical formulation was found to be 100.76 % and 100.81% respectively (Table no 11) by Q-Absorbance method**.**

Table 11: Analysis of content in pharmaceutical formulation

Sr no.	Sample (n=5)	Amount present(µg/ml)	Amount found(µg/ml)	Assay%
1	Metformin	5	5.03	100.76
2	Empagliflozin	12	12.09	100.81

In vitro drug release studies: -

The Marketed formulation Jardiance MetTablets of empagliflozin and metformin hydrochloride were evaluated for in vitro drug release studies, which were performed using USP Type-I (Basket) dissolution test apparatus. The volume of the dissolution medium was 900mL with a stirring speed of 100 rpm and the temperature was maintained at 37°C±0.5°C. These conditions were kept constant for all dissolution studies. The study was carried out in pH 6.8 phosphate buffer at 10, 15, 20, 30, 45 and 60min. 5 mL of sample was withdrawn periodically and replaced with equal volume of fresh dissolution medium. The collected samples were filtered through 0.45µ filter by discarding initial 4mL of solution. Further diluted 2mL of filtrate to 100mL with dissolution medium and analyzed to assess the percent drug dissolved. The percent drug release was obtained is 100.74% & 100.45% for Metformin and Empagliflozin respectively.

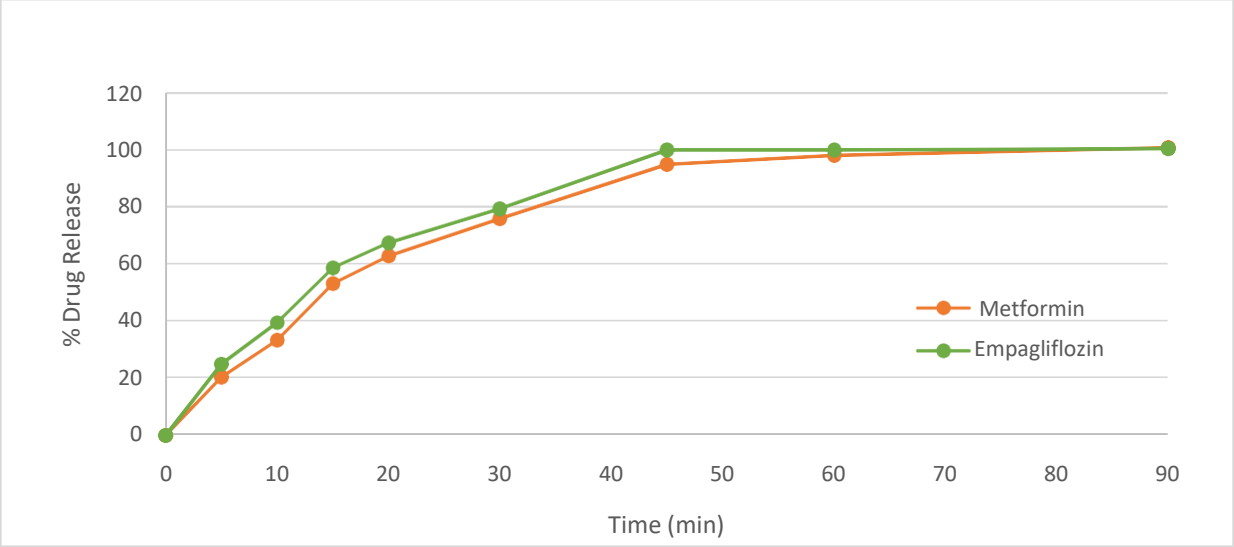

Fig 7.Comparative dissolution profile metforminand empagliflozin tablets.

7. Conclusion:

The simple, precise, accurate, and sensitive UV- visible spectrophotometric method for theQ Absorbance of Metformin and Empagliflozin in a bulk drug and pharmaceutical formulation was

developed and validated. The recovery result confirms the accuracy of method. The proposed method was found to be robust and rugged in nature. Thus,it can be effectively applied for the estimation of Metformin and Empagliflozinin pharmaceutical formulation.

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