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Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs and combined dosage forms

N.Padmaja[1], Mulagiri Sharath Babu[2] and G. Veerabhadram*[2]

[1]*Faculty of Pharmacy, University College of Technology, Osmaina University, Hyderabad, India*
[2]*Department of Chemistry, University College of Science, Osmaina University, Hyderabad, India*

ABSTRACT

Two simple, precise and economical UV spectrophotometric methods have been developed for the simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk and pharmaceutical dosage forms. Method A is simultaneous equation method (Vierodt's Method), which is based on measurement of absorption at 272nm and 234nm i.e. λmax of Empagliflozin and Metformin hydrochloride respectively. Method B is Absorbance ratio (Qanalysis method) which is based on measurement of absorption at wavelength of 254nm and 226nm i.e. iso absorptive point of Empagliflozin and Metformin hydrochloride and λmax of Empagliflozin respectively. Linearity was observed in the concentration range of 5-25μg/ml for Empagliflozin and 2-12μg/ml for Metformin hydrochloride. The accuracy of methods was assessed by recovery studies and was found to be within range of 98.99-101.12% for both Empagliflozin and Metformin hydrochloride. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory.

INTRODUCTION

Empagliflozin

Empagliflozin chemically,(1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran-3-yl-oxy) benzyl]-benzene (Fig.1) is an orally administered selective sodium glucose cotransporter-2 (SGLT-2) inhibitor, which lowers blood glucose in people with type 2 diabetes by blocking the reabsorption of glucose in the kidneys and promoting excretion of excess glucose in the urine [1-6]. In patients with type 2 diabetes and hyperglycaemia a higher amount of glucose is filtered and reabsorbed. Empagliflozin improves glycaemic control in patients with type 2 diabetes by reducing renal glucose reabsorption. The amount of glucose removed by the kidney through this glucuretic mechanism is dependent on blood glucose concentration and GFR. Inhibition of SGLT2 in patients with type 2 diabetes and hyperglycaemia leads to excess glucose excretion in the urine[7-9].

Metformin hydrochloride

Metformin is an oral anti-diabetic drug in the biguanide class. It is chemically N, N dimethylimidodicarbonimidic diamide hydrochloride (1, 1 dimethyl biguanide hydrochloride) (Fig .2) It lowers blood glucose concentrations in type 2 diabetes without causing overt hypoglycemia. Metformin is also frequently described as an insulin sensitizer leading to reduction in insulin resistance and significant reduction of plasma fasting insulin level. The improvement in insulin sensitivity by metformin could be ascribed to its positive effects on insulin receptor expression and

tyrosine kinase activity [10]. Metformin reduces hepatic glucose production. Metformin is small highly polar compound (pKa=2.8, 11.5) so it has a great solubility in water and poor solubility in lipids so it is very difficult to extract it from the aqueous plasma matrix. HPLC methods for the determination of metformin in human plasma include ion-exchange, ion-pair or normal –phase extraction [11-12]. Metformin inhabits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via decrease in hepatic energy state. [13].

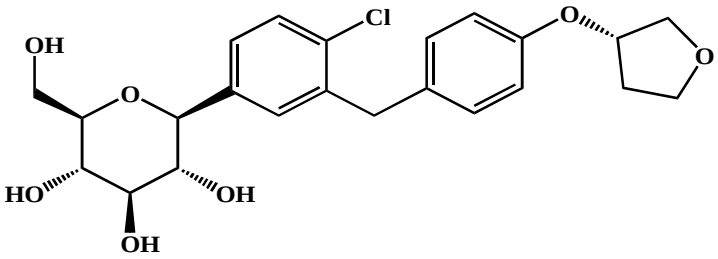

Figure .1:Structure of Empagliflozin

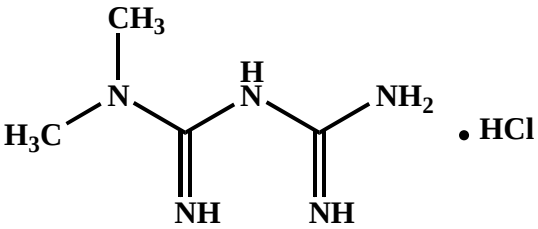

Figure .2:Structure of Metformin

Handful clinical trials were reported in the literature for Empagliflozin and Metformin [1-13]. Only few analytical methods have been reported for method development and validation of RP-HPLC method for estimation of Empagliflozin in API with PDA detection [14], stability indicating RP-HPLC method for determination of Empagliflozin [15] and spectrophotometric determination of Empagliflozin and Linagliptin in bulk and combined dosage form [16]. RP-HPLC method development and validation for simultaneous determination of Linagliptin and Empagliflozin in tablet dosage form [17].

Literature survey shows that there are many methods for the estimation of Empagliflozin and Metformin separately and in combination with other drugs. To our knowledge, spectrophotometric determination of Empagliflozin and Metformin in bulk and combined dosage form has not been developed and reported so far. So, an attempt was made to develop and validate an economic and spectrophotometric determination of Empagliflozin and Metformin in bulk and combined dosage form. The method was validated as per ICH guidelines.

Instrument

UV double beam spectrophotometer of Incarp Instruments Pvt. Ltd. (Hyderabad) Sican 2301with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm was used for analytical work along with matched quartz cell of length 1 cm. The analysis was carried by using UV win software. All the weighing was carried out on the ER 200A weighing balance and manufacture name Ascoset. Sonication samples carried out on the SE60USmodel sonicater.

MATERIALS AND METHODS

Preparation of stock solution and selection of wavelength for analysis:

Standard stock solutions of Empagliflozin and Metformin hydrochloride were prepared separately by adding 10mgof drug to methanol taken in 10ml volumetric flasks and then sonicated for five minutes and the volume was made up with methanol. The resulting solutions contain 1mg/ml of the drug. The stock solutions of Empagliflozin and Metformin were further diluted with water to obtain the concentration of 30µg/ml. The resulting solutions were then scanned in UV spectrophotometer from 400 to 200nm. From the resulting spectra λmax for Empagliflozin and

Metformin were calculated separately .The overlay spectra of Empagliflozin and metformin was also recorded. From the overlay spectra isoabsorptive point of Empagliflozin and metformin was calculated.

Method A: Simultaneous equation method (Vierodt's Method)

If a sample contains two drugs with reasonably dissimilar λmax, each of which exhibits absorbance at the λmax of other, then it is possible to determine the drugs by simultaneous equation method (Vierodt's Method). Two equations are constructed based on the fact that the absorbance at a particular λmax is sum of individual absorbance of two components.

The scanning spectra of 30µg/ml solution of Empagliflozin and Metformin show clear peaks at 272nm and 234nm respectively for Empagliflozin and Metfomin (Fig.3, 4). The λmax of each drug was selected for analysis.

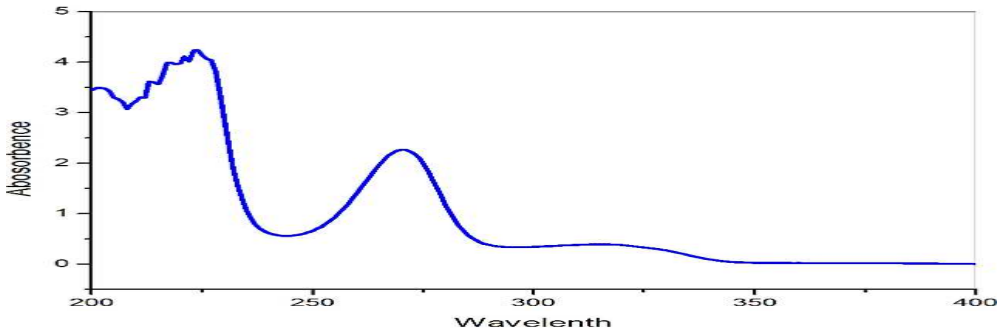

Fig. 3: UV spectra of Empagliflozin

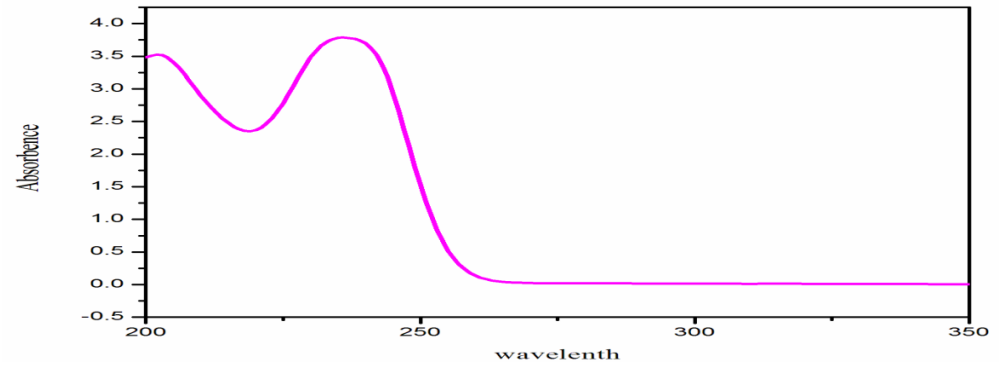

Fig. 4: UV spectra of Metformin

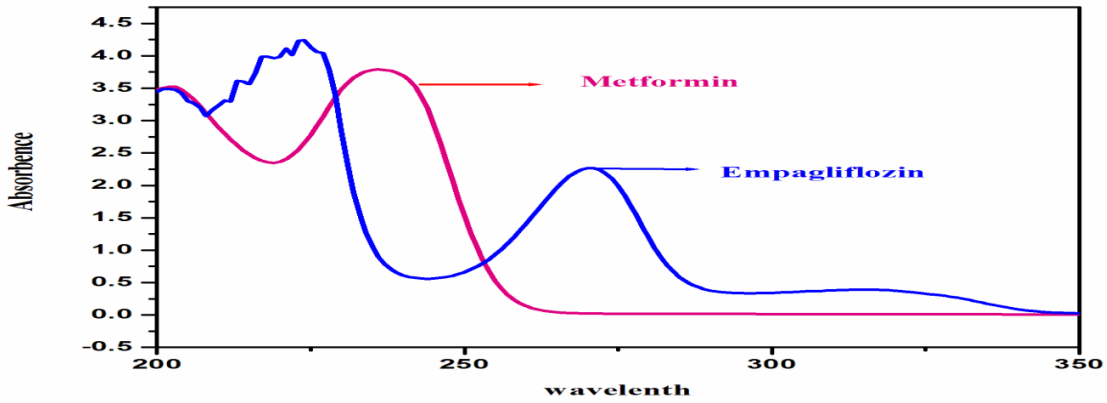

Fig.5: Overlay spectra of Empagliflozin and Metformin

The stock solution of Empagliflozin and Metformin was then diluted absorbance of these solutions was measured at 272(Fig, 5) verify the Beer's law and the absorptivity values for the Two simultaneous equations as given below.

$A_1 = ax_1C_1 + ay_1C_2$
$A_2 = ax_2C_1 + ay_2C_2$
Where
$A_1 = (43.24)\ C_1 + (76.27)\ C_2$ (I)
$A_2 = (51.36)\ C_1 + (98.12)$ (II)

C_1 and C_2 are the concentrations of Empagliflozin and Metformin in mg/100ml respectively in sample solution. A_1 and A_2 are the absorbances of mixture at 272nm and 234nm respectively. Solving equation 1 and 2, C_1 and C_2 are calculated as absorbances of mixture at 234nm and 272nm.

$C_1 = A_2\ ay_1 - A_1\ ay_2 / ax_2\ ay_1 - ax_1\ ay_2$
$C_2 = A_1\ ax_2 - A_2\ ax_1 / ax_2\ ay_1 - ax_1 ay_2$
$C_1 = A_2\ (43.24) - A_1\ (98.12) / -325.48$ (III)
$C_2 = A_1\ (76.27) - A_2\ (98.12) / -325.48$ (IV)

Preparation and assay of tablet formulation
Fixed dose combination of Empagliflozin and Metformin is approved for marketing in USA (Synjardy ® tablets). 20 Synjardy ® tablets were weighed and triturated in a mortar pestle and powder equivalent to5mg of Empagliflozin was taken. To this powder 500mg of Metformin was added, to make concentration of Empagliflozin/ Metformin in ratio of 1:5. A quantity of sample equivalent to 500 mg of Metformin and 5mg of Empagliflozin was transferred into 100 ml volumetric flask containing 40 ml of methanol and sonicated for 10 min Final volume was made up to the mark and filtered through whatman filter paper (No. 41). 0.1 ml of resulting solution was diluted with methanol to 100ml. 1ml of the resulting solution was again transferred to 100 ml volumetric flask diluted with methanol and the volume was adjusted up to the mark. The absorbance was taken at 234nm and 272 nm against blank .The concentrations of Empagliflozin and Metformin was calculated by equation III, IV. The results are reported in the Table 1.

Method validation
The UV Spectrophotometric method was validated as per ICH guidelines for method validation. The performance parameters like linearity, precision and accuracy were evaluated.

Linearity:
Linearity was studied by diluting standard stock solution of Empagliflozin to 5-25µg/ml and Metformin 2-12µg/ml concentrations (n=3). Calibration curves with concentration verses absorbance were plotted at their respective wavelengths and the obtained data was subjected to regression analysis using the least square method. The standard curves for Empagliflozin and Metformin are shown in (Fig. 6, 7) respectively and data is presented in Table 2.

Accuracy:
To check the accuracy of the developed methods and to study interference of formulation additives, analytical recovery experiments were carried out by using standard addition method. Reference standard solution of each drug was added to tablet samples at three different concentrations level (50, 100 and 150%). At each level, samples were prepared in triplicate and the mean percentage recoveries and % RSD value were calculated. Table .6 shows the result for accuracy of the method.

Precision
Repeatability: A mixture containing 10µg/ml each of Empagliflozin and Metformin was prepared and analyzed both by method A and B (n=6). The data is represented in Table 3. Intermediate precision: intermediate precision is studied in terms of intraday and inter-day precision. Three concentrations of Empagliflozin and Metformin was selected in a mixture and analyzed by method A and B (n=3). For intraday, the analysis was carried out at different intervals on the same day and for inter day, the analysis was carried on different days. Table 4 and 5 give the results for intraday and inter-day studies respectively.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slots by different analysts using similar operational and environmental conditions. The results are shown in Table.8

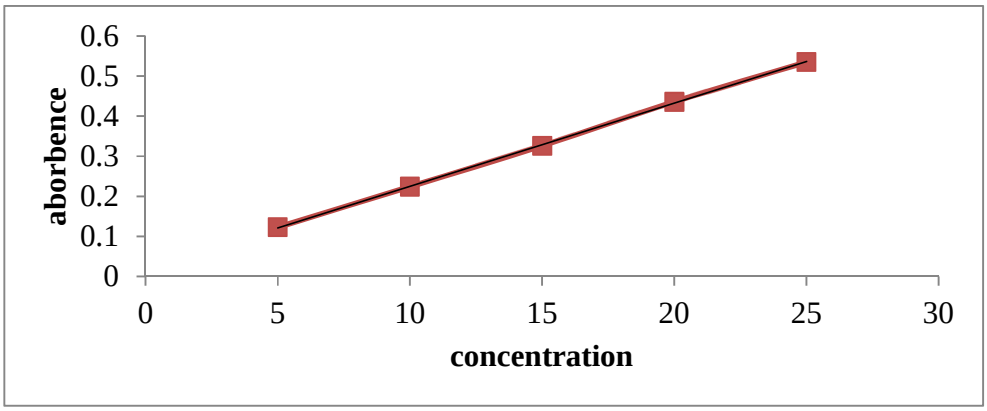

Fig.6: Standard curve of Empagliflozin at 272

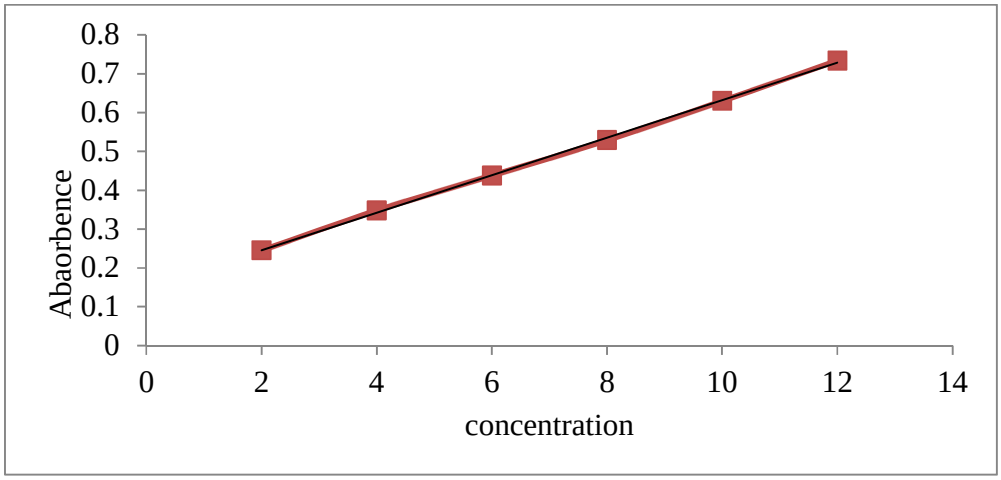

Fig.7: Standard curve of Metformin at 234

Table 1: **Optical characteristics and linearity data**

Parameter	Empagliflozin	Metformin
Absorption maximum (nm)	233	277
Beer's law limit(µg/ml)	5-25	2-12
Correlation coefficient	0.999	0.999
Regression equation Y=mX+C	0.0207x + 0.018	0.0483x + 0.1491
Intercept	0.018	0.1491
Slope(m)	0.0207	0.0483

Table 2: *E (1%, 1 cm) for Empagliflozin and Metformin

*E(1%,1 cm)at 233nm±SD		*E(1%,1 cm)at 277nm±SD	
Empagliflozin	Metformin	Empagliflozin	Metformin
Ax_1=43.24±0.78	Ay_1=76.24±0.64	Ax_2=51.36±0.34	Ay_2=98.12±0.96

Table 3: Assay of formulation (n=6)

Brand (Synjardy)		*%Amount found ±SD	% RSD
Metformin + Empagliflozin	Empagliflozin	101.74± 0.25	0.24
	Metformin	100.11± 0.86	0.85

Table 4: Repeatability study data for mixture of Empagliflozin and Linagliptin (n=6)

Drug	Concentration taken (µg/ml)	% Found	% RSD
Empagliflozin	10	98.58± 1.245	1.25
Metformin	50	100.05± 0.1245	0.12

Table 5: Intraday precision data for mixture of Empagliflozin and Metformin (n=3)

Drug	Concentration taken (µg/ml)	% Found	% RSD
Empagliflozin	5	98.16± 0.452	0.46
	10	99.11± 0.8221	0.82
	15	100.1± 0.8121	0.81
Metformin	2	99.45± 0.7869	0.79
	4	99.56± 0.4452	0.44
	6	100.23± 0.5621	0.560

Table 6: Interday precision data for mixture of Empagliflozin and Metformin (n=3)

Drug	Concentration taken (µg/ml)	% Found	% RSD
Empagliflozin	5	100.23± 0.6152	0.61
	10	99.86± 0.6121	0.61
	15	100.75± 0.4759	0.47
Metformin	2	99.45± 0.5214	0.52
	4	99.98± 0.8852	0.88
	6	101.56± 0.7452	0.73

Table 7: Recovery study data for Empagliflozin and Metformin (n=3)

Drug	Pre-analyzed sample solution	Drug added	% Recovery	% RSD
Empagliflozin	8	0		
		4	99.45±0.1452	0.14
		5	99.78±0.4261	0.14
		6	100.57±0.4582	0.45
Metformin	4	0		
		8	101.12±0.5770	0.57
		10	99.15±0.8524	0.85
		12	98.99±0.452	0.45

Table 8: Ruggedness data for Empagliflozin and Metformin (n=3)

Drug	Parameter	% Found	%RSD
Empagliflozin	Analyst 1	102.14± 0.72	0.70
Metformin	Analyst 2	99.12± 0.821	0.82

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient, precise and accurate way for simultaneous analysis of Empagliflozin and Metformin in its bulk and pharmaceutical dosage form. Absorbance maxima of Empagliflozin at 272nm and Metformin at 234nm were selected for the analysis. Regression analysis shows linearity over the concentration range of 5-15µg/ml for Empagliflozin and 2-6µg/ml for Metformin with respective correlation coefficients of 0.999 and 0.999 respectively. The % RSD for repeatability (n=3), intraday and interday (n=3) precision was found to be less than 2% indicating the precision of method. The amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The Accuracy of the proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. % Recovery for A Empagliflozin and Metformin was found within the range of 98.99 % and 101.12%. Values of standard deviation and coefficient of variation were satisfactorily low indicating the accuracy of both the methods. The assay for Metformin and Empagliflozin was found to be 101.74 % and metformin was 100.11%. The % RSD value for both Empagliflozin and Metformin was found to be less than 2%. The results did not show any statistical difference between operators suggesting that methods developed were rugged. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical formulations containing both these drugs.

CONCLUSION

Based on the results obtained, it is found that the developed UV-Spectrophotometric technique is quite simple, accurate, precise, reproducible, sensitive and economical. They can become effective analytical tools for routine quality control of Empagliflozin and Metformin bulk drug combinations and their combined pharmaceutical dosage form without any prior separation of components.

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