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DEVELOPMENT AND VALIDATION OF Q-ABSORBANCE RATIO UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND EMPAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT: A simple, robust, precise, UV spectroscopic method has been developed for the simultaneous estimation of Metformin HCL and Empagliflozin in bulk and tablet dosage forms. In this paper the estimation of those drugs was carried out by absorbance ratio method. This method is based on measurement of absorption at 235nm and 258nm i.e., λ max of Metformin and Isoabsorptive point of both Metformin HCL and Empagliflozin respectively. The linearity observed for Metformin HCL is in the range of 10-50µg/ml and for Empagliflozin is in the range of 3-11µg/ml. The accuracy of methods was assessed by recovery studies and was found to be within the range of 99.5%-100.5% for both Metformin HCL and Empagliflozin. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents. The results were validated as per ICH guidelines.

KEYWORDS: Empagliflozin, Metformin HCL, Absorption Ratio Method, ICH, Validation etc.

INTRODUCTION:

By curing various fatal diseases, drugs play a vital role in the progress of the human civilization. Diabetes is one of the fatal and severe disease threat for the entire world. In India, more than 62 million Indians are affected by diabetes. Hence, it is necessary to control diabetes epidemic. Metformin HCL and Empagliflozin combination is the new drug combination used to cure and control diabetes.

Chemically, Empagliflozin is known as (2S,3R,4R,5S,6R)-2-[4-Chloro-3-[(4-(3S)-Oxolan-3-yl] Oxyphenyl] Methyl] Phenyl-1] (-6-Hydroxymethyl)Oxane-3,4,5-Triol. It is an orally administered sodium glucose co-transporter 2 receptor (SGLT-2) inhibitor. Sodium glucose co-transporter 2 is situated in the proximal tubule of the nephron in kidneys, which lowers the blood glucose level by blocking the glucose reabsorption in kidneys, and excretes excess glucose through urine.

Chemically, Metformin HCL is 1, 1- dimethylbiguanidine Metformin's hydrochloride main effect is to decrease liver glucose production. It also increases insulin sensitivity, which increases peripheral glucose uptake. Metformin decreases increased blood pressure, primarily by suppressing liver glucose production (hepatic gluconeogenesis). inhibition of mitochondrial respiratory chain (complex-1) activation of AMP activated protein kinase (AMPk), inhibition of glucagon-induced elevation of cyclic adenosine monophosphate (cAMP) with reduced activation of protein kinase A (PKA), inhibition of mitochondrial glycerophosphate dehydrogenase and an effect on gut microbiota have been proposed as potential mechanisms. Activation of AMPk was required for metformin's inhibitory effect on liver glucose production. AMPk is an enzyme that plays an important role in insulin signaling, whole body energy balance and the metabolism of glucose and fats.

The reason behind the combination of Metformin HCL and Empagliflozin is that, because Metformin lowers glucose production by liver and its absorption in the intestine. Empagliflozin is a SGLT2 inhibitor. SGLT2 is a protein that facilitates the reabsorption of glucose from the kidney into the blood. By inhibiting SGLT2, empagliflozin lowers blood glucose levels and increases glucose excretion.

Metformin HCL and Empagliflozin is a new drug combination. Therefore, there is only one report of Simultaneous Equation Method for this new combination. Simultaneous Equation Method is also known as Vierodt's method. This method is typically applying for the estimation of drug combinations containing two or more than two drugs. This method is easy, simple and gives reproducible results as compared to other UV methods. Therefore, this is a humble attempt to develop simple, robust, reproducible method for the determination of efficacy and safety of Metformin HCL and Empagliflozin combination. This method was fully validated according to International Conference on Harmonization (ICH) and ready for the application in routine analysis without interference of excipients.

Published Papers on this drug combination and in combination with other drug by UV[1,2,3,4,5,6,7], HPLC[8,9,10,11,12,13,14].

Fig.No.1. Structure of Metformin[11]

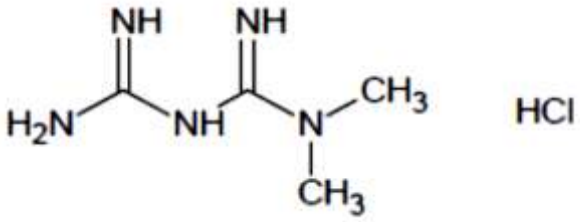
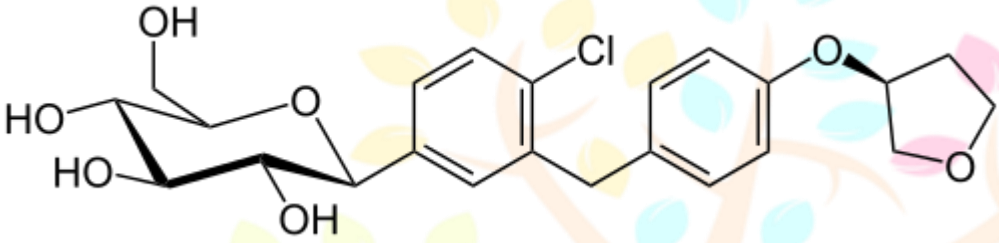


Fig.No.2. Structure of Empagliflozin[11]



MATERIALS AND METHODS

Instruments

Shimadzu UV-1800 double beam spectrophotometer was used to record the spectra of sample and reference solutions using pair of Quartz cells of 10mm path length. All weighing was carried out on Sansui Vibra DJ-150S-S weighing balance. Sonicator of Fast Clean is used for the purpose of sonication, Filter papers of Sartorius Stedim Biotech of grade 292 are used for the filtration purpose.

Chemicals

Empagliflozinand Linagliptin pure drug samples were obtained as gift sample from Boehringer Ingelheim Pharmaceuticals Canada. The combined formulation Synjardy® (12.5mg/500mg) purchased from Vikram Pharmacy Jalgaon. Analytical grade methanol purchased from Merck Chemicals Pvt. Ltd. Mumbai.

Preparation of stock solution and selection of wavelength

Metformin HCL stock solution

An accurately weighed quantity of Metformin (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (8 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get Metformin standard stock solution (1 mg / mL).

Metformin HCLworking solution

Metformin HCL standard stock solution (0.1 mL) was diluted to 10 mL using methanol to get working standard solution (10 µg / mL)

Empagliflozin stock solution

An accurately weighed quantity of Empagliflozin (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (8 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get Empagliflozin standard stock solution (1 mg / mL).

Empagliflozin working solution

Empagliflozin standard stock solution (0.3 mL) was diluted to 10 mL using 77% v/v methanol to get working standard solution (3 µg / mL)

Determination of λ Max of Individual Component

An appropriate aliquot portion of Metformin HCL (0.1mL) and Empagliflozin (0.3 mL) were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using methanol to obtain Metformin (10 µg/mL) and Empagliflozin (3 µg/mL). Drug solutions were scanned separately between 200 nm to 400 nm. Metformin shows the λ max at 235nm while Empagliflozin shows λ max at 258nm.

Overlay spectra of Metformin and Empagliflozin

The overlay spectra of both drugs were recorded and two wavelengths 235nm (λ max of Metformin HCL) and 258nm (λ max of Empagliflozin) were selected for further study.

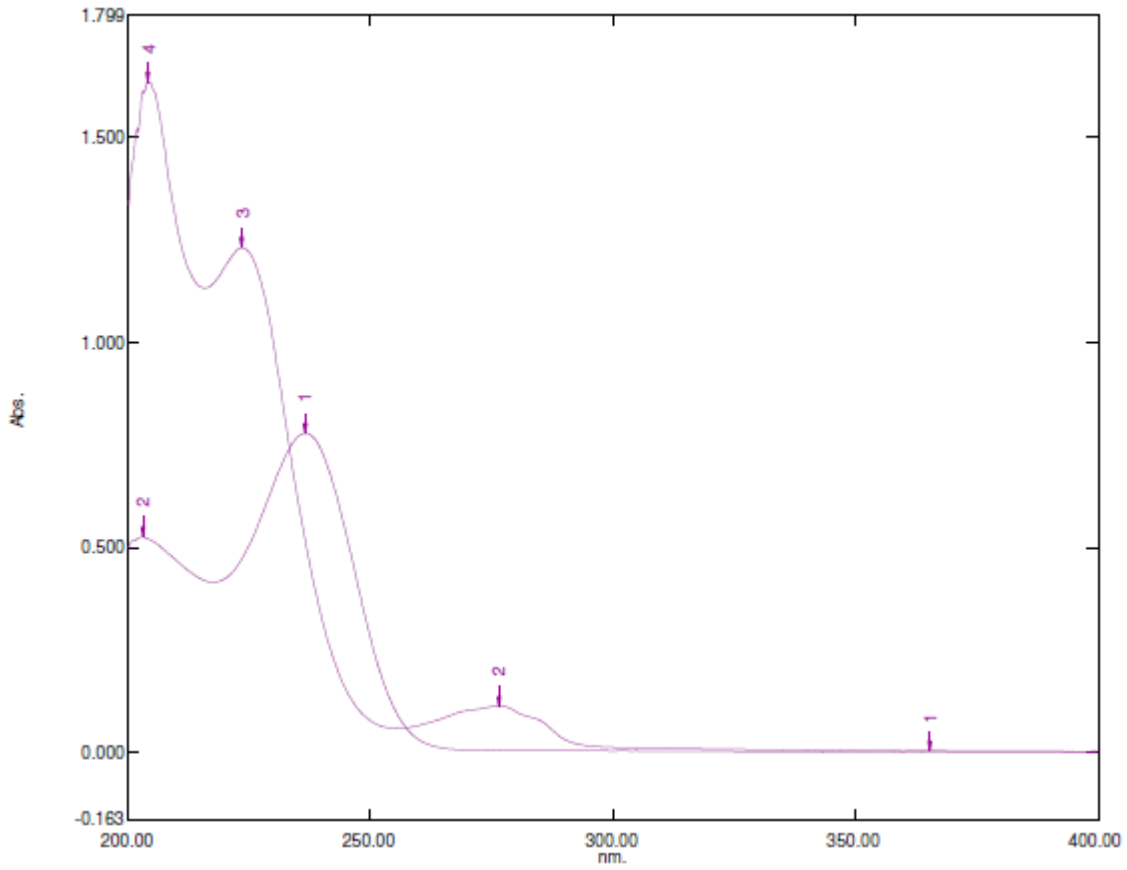


Fig. 3 An Overlay spectra of Metformin HCL and Empagliflozin

Linearity study for Metformin HCL

An accurately measured aliquot portion of working standard solution of Metformin HCL was transferred to five separate 10 mL volumetric flasks. The volume was made up to the mark using methanol to obtain concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml). Absorbance of these solutions was measured at 235 nm and Calibration curve was plotted as Absorbance Verses Concentration. The results are shown in Table No. 1.

Linearity study for Empagliflozin

Accurately measured aliquot portions of working standard solution of Empagliflozin were transferred to five separate 10 mL volumetric flasks. The volume was made up to the mark using 77% v/v methanol to obtain concentrations (3µg/ml, 5µg/ml, 7µg/ml, 9µg/ml, 11µg/ml). Absorbance of these solutions was measured at 258 nm. Calibration curve was plotted, absorbance Verses concentration as measured at 258 nm. The results are shown in Table No.1.

(**Table No. 1) Regression and Optical characteristics of MET HCL and EMPA**

Parameters	**Value for Metformin HCL**	**Value for Empagliflozin**
Beer's law limit (µg/ml)	10-50µg/ml	3-11µg/ml
Regression Coefficient(R^2)	0.9987	0.9984
Regression equation	y= 0.0222x + 0.706	y= 0.0158x – 0.0198
Slope	0.0222	0.0158
Intercept	0.706	0.0198

The study of regression and optical characteristics of MET and EMPA are carried out in which Regression Coefficient (R^2) of MET is 0.997 and of EMPA is 0.9984. The slope of MET 0.0222 and slope of EMPA is 0.0158 with Intercept of MET 0.706 and for EMPA 0.0198. Therefore, Concentration verses Absorbance are fairly linear between both co-ordinates by statistical manner and obey ICH guidelines.

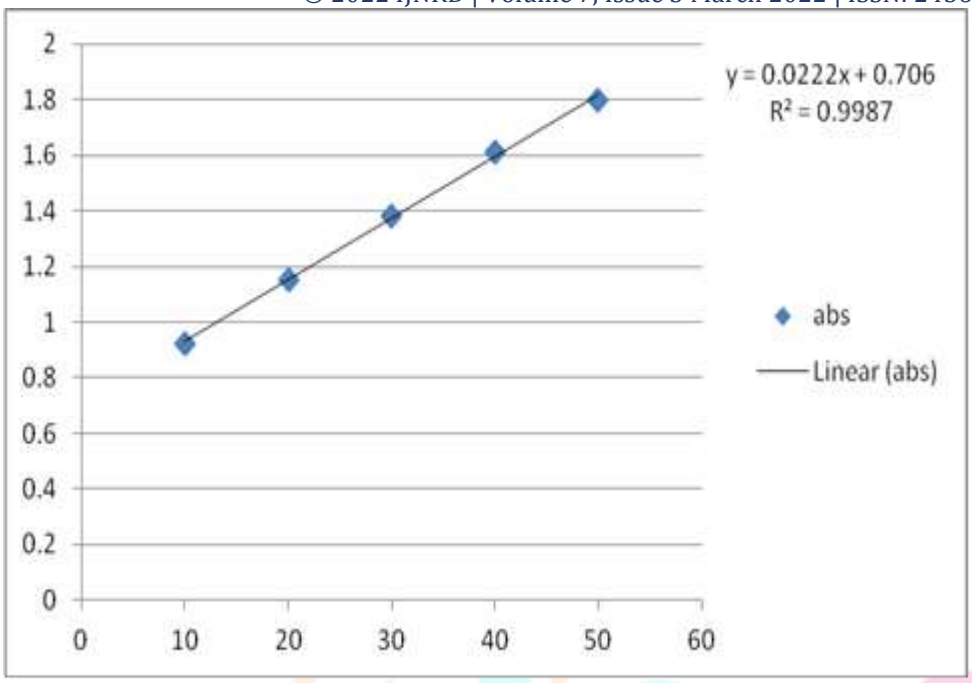


Fig. 4 Calibration curve of Metformin at 235nm

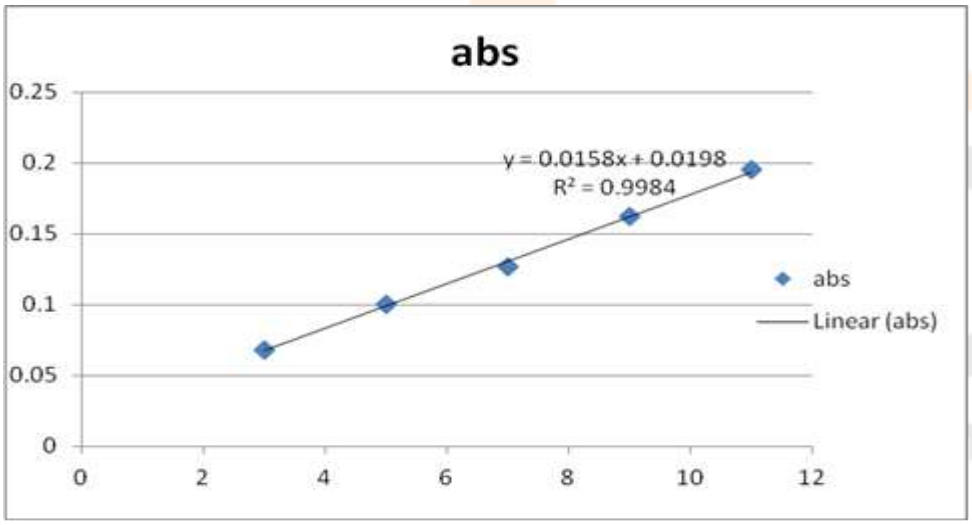


Fig. 5 Calibration curve of Empagliflozin at 258nm

Estimation of Laboratory mixture by proposed method

Method: Absorbance Ratio Method

The absorbance ratio method is a modification of the simultaneous equations procedure. It depends on the property that, for a substance which obeys Beer's Law at all wavelengths, the ratio of absorbance's at any two wavelengths is a constant value independent of concentration or path length.

Absorbance method uses the ratio of absorbance at two selected wavelengths, one at isoabsorptive point and other being the λ max of one of the two drugs. Metformin HCL and Empagliflozin have λ max at 235 and 276 nm respectively and isoabsorptive point 258 nm. The wavelengths selected for analysis were 235 and 258 nm, respectively. E (1%,1cm) values of Metformin and Empagliflozin were determined at 235 and 258 nm.

The concentration of two drugs in mixture was calculated by using following equations

$$C_{MET} = \frac{Qm - Qy}{Qx - Qy} X \frac{A}{ax1} \quad (1)$$

$$C_{EMPA} = \frac{Qm - Qx}{Qy - Qx} X \frac{A}{ay1} \quad (2)$$

Where,

$$Qm = \frac{\text{Absorbance of sample at 258 nm}}{\text{Absorbance of sample at 235 nm}}$$

$$Qx = \frac{\text{E (1 \% 1cm) of MET at 258 nm}}{\text{E (1 \% 1cm) of MET at 235 nm}}$$

$$Qy = \frac{\text{E (1 \% 1cm) of EMPA at 258 nm}}{\text{E (1 \% 1cm) of EMPA at 235 nm}}$$

The results are displayed in the Table No. 2

(**Table No. 2) Results of Estimation of MET HCL and EMPA in standard laboratory mixture**

Analyte	% Concentration estimated (Mean ± S.D)	% R.S.D
Metformin	99.90± 0.1139	0.114
Empagliflozin	99.88± 0.0912	0.091

The estimation of MET HCL and EMPA in standard Laboratory Mixture are carried out in which % concentration of MET HCL and EMPA were found to be 99.90 and 99.88 respectively. These values are fairly accurate by statistical manner and are as per ICH guidelines.

Application of proposed method for Estimation of drugs in tablets

Twenty 'SYNJARDY' Tablets containing Metformin (500mg) and Empagliflozin (12.5 mg) were weighed and ground to fine powder. A quantity of sample equivalent to Metformin (50 mg) Empagliflozin (12.5 mg) was transferred into 100 mL volumetric flask containing methanol (60 mL), sonicated for 10 min and the volume was made up to the mark and filtered through Whatmann filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The absorbance's of the solutions were measured at 235 nm and 258 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations**.** The results are reported in the Table No.3

Table No. 3 Results of Estimation of MET HCL and EMPA in tablets dosage form.

Analyte	Label claim(mg/tab)	% Label claim estimated(Mean±S.D)	% R.S.D
Metformin	500	99.91± 0.101	0.101
Empagliflozin	12.5	99.88± 0.091	0.091

The results of Estimation of MET HCL and EMPA in tablets dosage shows form the % purity 99.91 to 99.88 with SD and RSD bellow 2 which is fairly accurate by statistical manner and are as per ICH guidelines.

Validation of proposed method

The proposed method was validated as per ICH guidelines.

Accuracy (Recovery study)

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions was added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula.

% Recovery = [A - B/ C] X 100

Where,

A = Total amount of drug estimated

B = Amount of drug found on pre-analyzed basis

C = Amount of Pure drug added.

The results are reported in the Table No. 4

Table No. 4 Recovery study

Tablet- SYNJARDY					**Average Weight of Tablet-570 mg.**			
Sr. No.	**Quantity Tablet Powder Taken (mg)**	**Percentage %**	**Amount of Pure Drug Added (mg)**		**Total Amount of Drug Recovered (mg) ± S.D. (n = 3)**		**% of Drug Recovered (n = 3)**	
			MET	**EMPA**	**MET**	**EMPA**	**MET**	**EMPA**
1.	570	80	400	10	339.783 ± 0.005774	9.2473 ± 0.000577	99.75	99.86
2.	570	100	500	12.5	499.683 ± 0.005774	12.4983 ± 0.000577	99.68	99.6
3.	570	120	600	15	599.923 ± 0.005774	15.9973 ± 0.000577	99.92	99.85
						Mean	99.78	99.77
						SD	0.123	0.147
						% RSD	0.123	0.147

The results of Recovery study of MET HCL and EMPA are found to be fairly accurate between 99.60 to 101.5 % for MET HCL and 99.6 to 100 % for EMPA between various concentrations under observation by statistical way and are obey ICH guidelines.

Precision

Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing Metformin HCL (10, 30, and 50 µg/mL) and Empagliflozin (3, 7, and 11 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week. The results are shown in the Table No. 5

Table No. 5. Precision study

Drug	Conc. [µg/mL]	Intra-day Amount Found		Inter-day Amount Found	
		Mean ±S.D [*n* = 5]	% R.S.D.	Mean ± S.D. [*n* =5]	% R.S.D.
MET	10	9.992 ± 0.004472	0.044	9.994 ± 0.005477	0.054
	30	29.96 ± 0.004472	0.014	29.97 ± 0.005477	0.018
	50	49.88 ± 0.005477	0.010	49.91 ± 0.047223	0.094
EMPA	3	2.966 ± 0.005477	0.184	2.978 ± 0.00836	0.280
	7	6.978 ± 0.004472	0.064	6.99 ± 0.00707	0.101
	11	10.99 ± 0.005477	0.049	10.92 ± 0.00836	0.076

The Precision Study of MET HCL and EMPA were carried out and Results are found to be fairly accurate by statistical manner as per ICH guidelines.

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst using same operational and environmental conditions. The results are reported in the Table No. 6

Table No. 6. Ruggedness Study

	MET HCL 500 mg		EMPA 12.5 mg	
	Amount Found in mg Mean ± S.D (n = 3)	% RSD	Amount Found in mg Mean ± S.D (n = 3)	% RSD
Analyst-I	499.93 ± 0.05773	0.115	12.86 ± 0.000577	0.046
Analyst-II	499.93 ± 0.05773	0.115	12.89 ± 0.001	0.080
Day-I	498.83 ± 0.05773	0.115	12.96 ± 0.000577	0.046
Day-II	499.96 ± 0.05507	0.110	12.91 ± 0.001	0.080
Instrument-1	499.92 ± 0.10969	0.219	12.93± 0.000577	0.046
Instrument-2	499.96 ± 0.05507	0.110	12.97 ± 0.001	0.080

The Ruggedness study of MET HCL and EMPA are carried out and results are found to be fairly accurate by statistical manner and obey ICH guidelines.

LOD: Limit of detection of Metformin and Empagliflozin was found to be 2.545µg and 0.476µg respectively.

LOQ: Limit of Quantitation of Metformin and Empagliflozin was found to be 7.714µg and 1.443µg respectively.

Results and Discussion

An Absorbance Ratio Method in UV Spectroscopy was developed for Metformin HCL and Empagliflozin. The method employs 235 nm as λ1 and 258 nm as λ2 for formation of equations. Metformin HCL and Empagliflozin obeys Beer' slaw in the concentration range 10-50 µg/ml(R^2=0.9982) and 3-11 µg/ml (R^2=0.9984) respectively. The mean recovery for Metformin HCL and Empagliflozin was found to be 99.78 % and 99.77 % respectively. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis was found to be in good accordance with the prescribed values.

Conclusion

The proposed Absorbance Ratio Method in UV Spectroscopy presented in this paper hasadvantages of simplicity, accuracy, precision andconvenience for quantitation of Metformin HCL and Empagliflozin. Theproposed method can be used for the quality control of Metformin HCL Empagliflozin in typical laboratories.

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