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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR METFORMIN AND EMPAGLIFLOZINE IN TABLET DOSAGE FORM

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ABSTRACT

The purpose of the investigation was to develop a new HPLC method for simultaneous estimation of Metformin and Empagliflozine in pharmaceutical dosage forms. Chromatography was carried out on an ODS 250mm x 4.6 mm, 5µ particle size with a isocratic mobile phase composed of Buffer, Acetonitrile and methanol at a flow rate of 1mL/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 233 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. The retention times for Metformin and Empagliflozine and were 2.211 min and 4.592 *min respectively. The percentage recoveries of Metformin and Empagliflozine were* 100.39 %and 100.58% *respectively. The relative standard deviation for assay of tablets found to be less than 2%. The method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and pharmaceutical industries.*

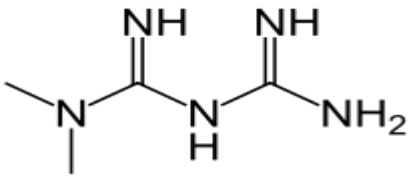
KEYWORDS: Metformin, Empagliflozine, Simultaneous estimation,Stability, ICH guidelines.

1. INTRODUCTION

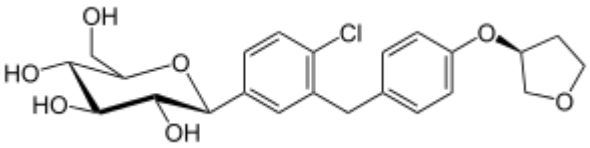
Metformin (MET) is chemically named as N,N-dimethylimidodicarbonimidic diamide (Fig.1). Metformin is a first line oral pharmacotherapy for type 2 diabetes. Activation of the energy-regulating enzyme AMPactivated protein kinase (AMPK), principally in muscle and the liver, is considered a major mode of metformin action.[1-3]

Empagliflozine (EMPA) is chemically named as (3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol (Fig.2).

Empagliflozine is used for type 2 diabetes. Empagliflozine is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), and causes sugar in the blood to be excreted by the kidneys and eliminated in urine. Various UV & HPLC assay methods are also reported in the literature for the estimation of Metformin and Empagliflozine individually and in-combination with other drugs.[4-6] According to literature survey there is no official method for the simultaneous estimation of Metformin and Empagliflozine by RP-HPLC in combined tablet dosage forms. Hence, an attempt has been made to develop new method for simultaneous estimation and validation of Metformin and Empagliflozine in tablet formulation in accordance with the ICH guidelines.



Metformin



Empagliflozine

Figure 1 & 2. Chemical structures of drugs investigated in this study.

2. MATERIALS AND METHODS

2.1 Materials:

Metformin, Empagliflozinee, Synjardy® (500mg Metformin + 5mg of Empagliflozinee) tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic

acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid all are from **Rankem chemicals**.

2.2 Instrument

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Metformin, Empagliflozinee solutions.

2.3 Standard Preparation: (500µg/ml Metformin & 5µg/ml Empagliflozinee)

Accurately Weighed and transferred 125mg&5mg of Metformin and Empagliflozinee working Standards into a 25ml and 100ml clean dry volumetric flask respectively, add 20ml and 75ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

2.4 Sample Preparation

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 70ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents.

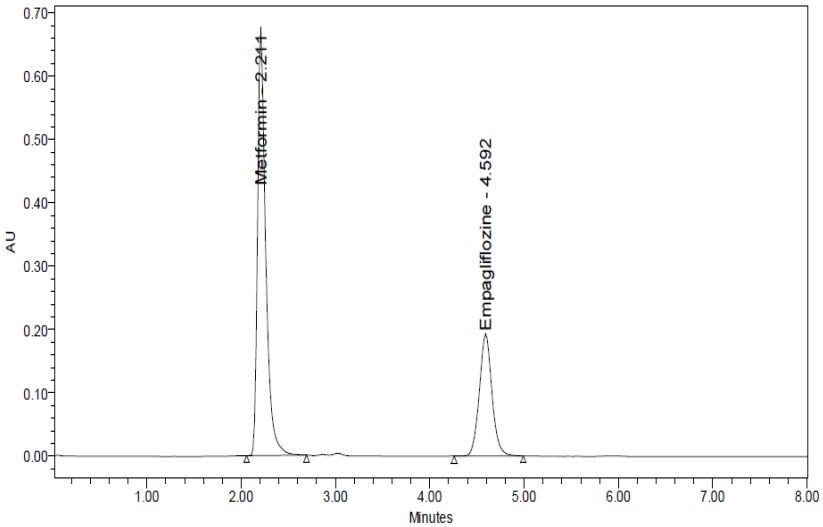
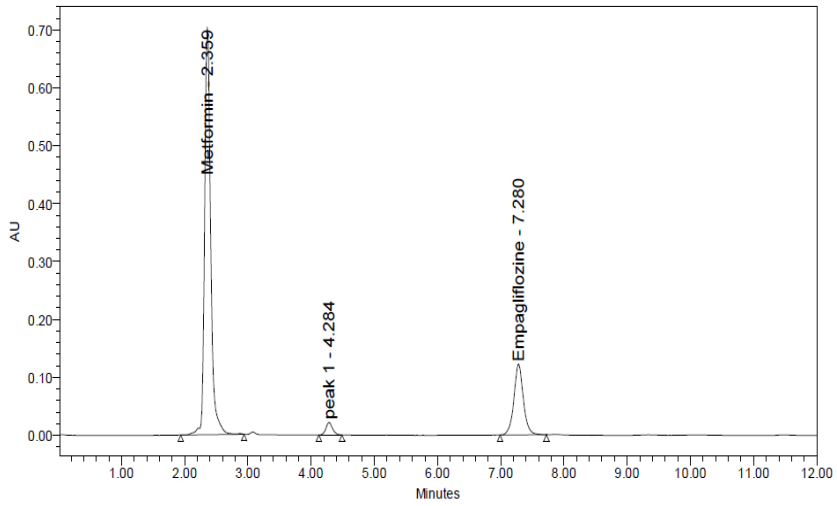


Figure 3: A typical Chromatogram of Metformin and Empagliflozine.

2.5 Degradation studies

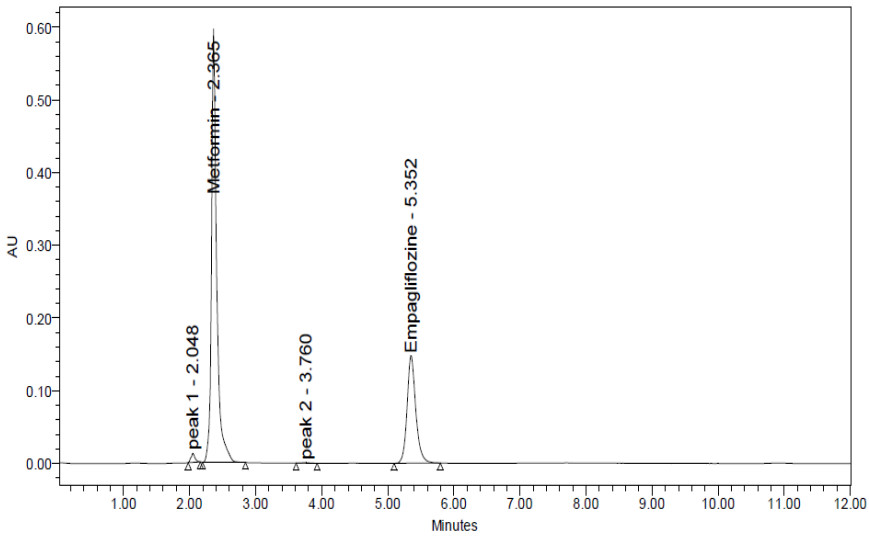
Oxidation

To 1 ml of stock solutionof Metformin and Empagliflozine, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLCstudy, the resultant solution was diluted to obtain 500µg/ml&5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.



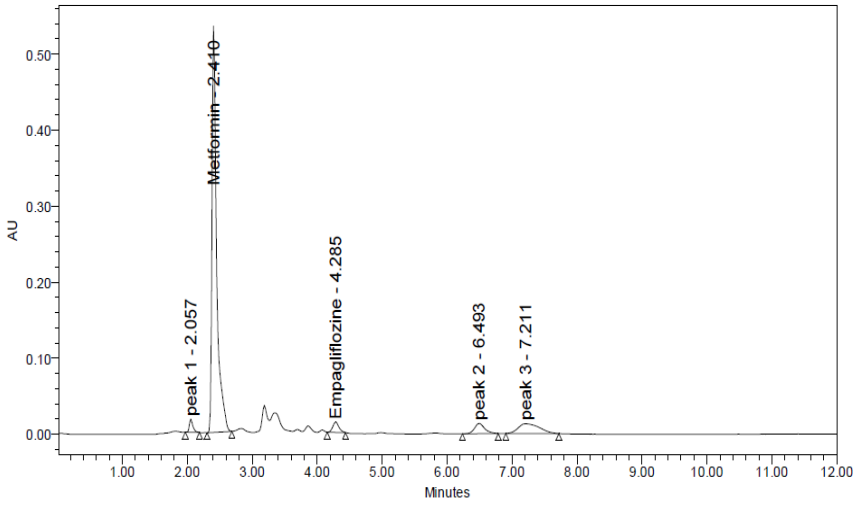
Acid Degradation Studies

To 1 ml of stock ssolution Metformin and Empagliflozine, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 500µg/ml&5µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.



Alkali Degradation Studies

To 1 ml of stock solution Metformin and Empagliflozine, 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 500µg/ml&5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.



Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 500µg/ml&5µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

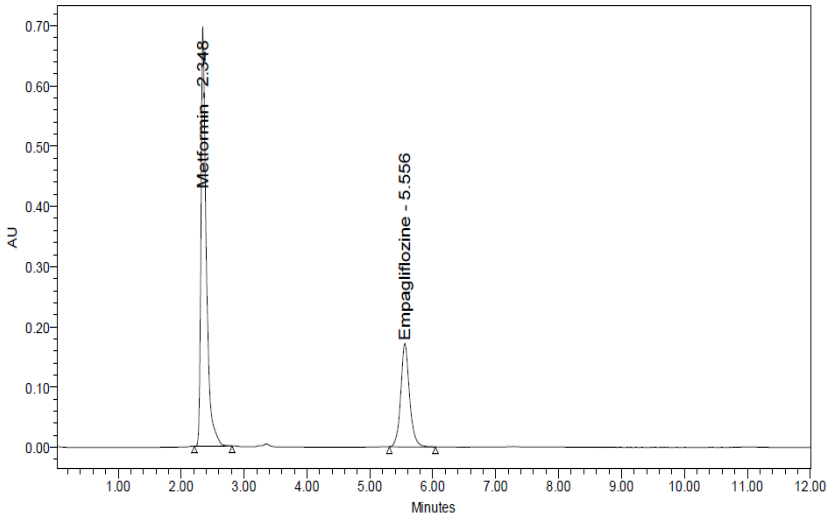
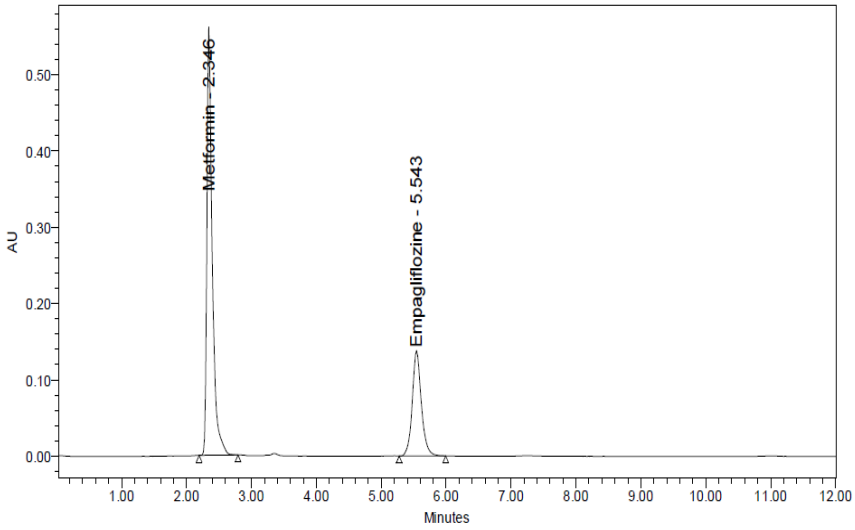


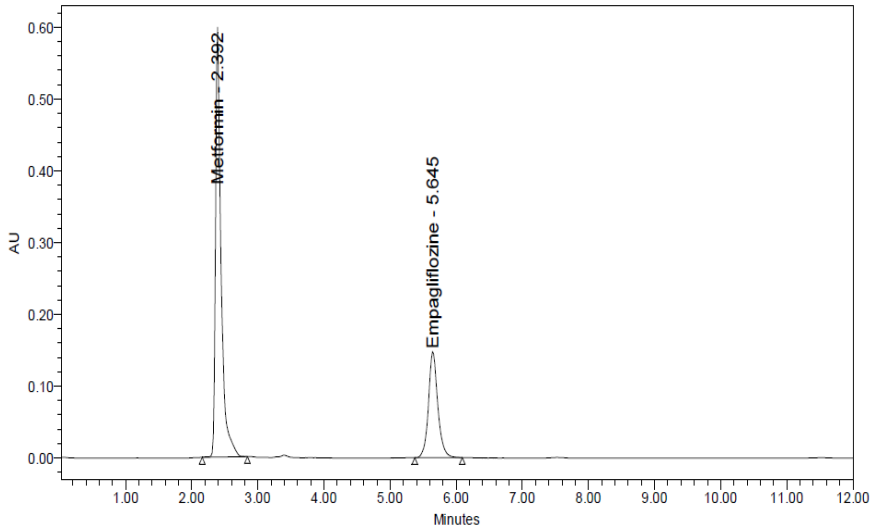
Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 5000µg/ml&50µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 500µg/ml&5µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.



Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 500µg/ml&5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**



3. RESULTS AND DISCUSSION

3.1 Method development

Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH: 4.8 both drugs eluted with better separation. Thereafter, buffer: Acetonitrilel were taken in isocratic ratio: %buffer / %Acetonitrile : 70/30 and with flow rate of 1mL/min was employed. ODS 250mm x 4.6 mm, 5µ. particle size was selected as the stationary phase to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 205nm to 280nm. Both MET and EMPA showed maximum absorption at 233nm of wavelength and 233 nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.211 min and 4.592 min for MET and EMPA, respectively. The chromatogram obtained was shown in the Fig. 2.

Table 1: System suitability of MET and EMPA

SYSTEM SUITABILITY PARAMETERS	MET	EMPA
No of theoretical plates	3246	5814
Tailing Factor	1.46	1.08
Resolution	11.3	
RT	2.211 min	4.592 min
Mean Area	3479262	155807
RSD	0.86	0.2

Table 2: Results of accuracy of MET and EMPA

Conc.	Metformin			Empagliflozine		
	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Amount added (µg/ml)	Amount recovered (µ g/ml)	% Recovery
50%	250	252.8973	101.16	2.5	2.533139	101.33
	250	251.3102	100.52	2.5	2.501912	100.08
	250	252.0607	100.82	2.5	2.490416	99.62
100%	500	497.9804	99.60	5	5.029409	100.59
	500	505.6918	101.14	5	5.00816	100.16
	500	499.9807	100.00	5	5.111012	102.22
150%	750	745.3798	99.38	7.5	7.578994	101.05
	750	760.3527	101.38	7.5	7.541373	100.55
	750	746.5356	99.54	7.5	7.472364	99.63

3.2 Method Validation

3.2.1 System suitability and Specificity

System suitability parameters such as number of theoretical plates, peak tailing, and retention time and resolution factor were determined. The total run time required for the method is only 8 minutes for eluting both MET and EMPA. The results obtained were shown in Table 1.

3.2.2 Linearity

MET showed a linearity of response between 125-750 µg/mL and EMPA showed a linearity of response between 1.25-7.5µg/mL. These were represented by a linear regression equation as follows: y (MET) = 6525.4x + 536.95.3 (r2=0.9992), y (EMPA) = 30967x + 196.32(r2=0.9992) and regression line was established by least squares method and correlation coefficient (r^2) for MET and EMPA is found to be greater than 0.98. Hence the curves established were linear.

3.2.3 Accuracy

To pre analyzed sample solution, a definite concentration of standard drug (50%, 100% & 150 % level) was added and recovery was studied. The % Mean recovery for MET and EMPA are 100.39 and 100.58 respectively and these results are within acceptable limit of 98-102. The % RSD for MET and EMPA are 0.78 and 0.84 respectively and %RSD for MET and EMPA are within limit of ≤2. Hence the proposed method is accurate and the results were summarized in Table 2.

Table 3: Results of Precision for MET and EMPA

	Repeatability		Intermediate precision	
S. No.	**Metformin**	**Empagliflozine**	**Metformin**	**Empagliflozine**
1	3491527	158326	3473455	157111
2	3482621	159292	3481807	158128
3	3512489	159824	3479262	158221
4	3524749	157206	3473434	155807
5	3559453	159178	3494364	158907
6	3509399	158432	3551210	158746
Mean	3513373	158710	3492255	157820
Std. Dev.	27175	925.8	29886	1170
%RSD	0.77	0.6	0.86	0.7

Table 4: Results of Robustness for MET

Analytical conditions Evaluation parameters	Flow rate (1ml/min)		Column temperature (oc)		Mobile phase composition	
	1.1	**0.9**	**35**	**25**	**+5%**	**-5%**
Mean RT	2.20	2.58	2.20	2.34	2.40	2.34
Mean area	3451382	3553238	3557054	3565571	3511812	3768335
SD	29250	24334	25812	25945	12373.9	22383
RSD	0.85	0.68	0.7	0.7	0.4	0.59
Tailing factor	1.59	1.62	1.59	1.67	1.62	1.67
No. of theoretical plates	3339	4386	3344	4106	4595	4105

Table 5: Results of Robustness for EMPA

Analytical conditions Evaluation parameters	Flow rate (1ml/min)		Column temperature (oc)		Mobile phase composition	
	1.1	**0.9**	**35**	**25**	**+5%**	**-5%**
Mean RT	5.13	5.83	5.13	5.33	5.91	5.33
Mean area	158243	157481	156843	157907	157402	157577
SD	622	2325	1987	1779	1894	2479
RSD	0.4	1.5	1.3	1.1	1.2	1.6
Tailing factor	1.07	1.14	1.07	1.17	1.10	1.17
No. of theoretical plates	7042	8388	7046	8071	7933	8066

3.2.4 Precision

Repeatability

Six replicates injections in same concentration were analyzed in the same day for repeatability and the % RSD for MET and EMPA found to be 0.77 and 0.6 respectively and % RSD for MET and EMPA found to be within acceptable limit of ≤2 and hence method is reproducible and the results are shown in Table 3**.**

Intermediate Precision

Six replicates injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for MET and EMPA is found to be 0.86 and 0.7 and it is within acceptable limit of ≤2. Hence the method is reproducible on different days withdifferent analyst and column. This indicates that the method is precise and the results are as shown in Table 3.

3.2.5 Robustness

The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean Rt and RSD is within limit of ≤2 .The tailing factor, resolution factor and no. of theoretical plates are found to be acceptable limits for both MET and EMPA. Hence the method is reliable with variations in the analytical conditions and the results of MET are shown in Table 4 and results of EMPA shown in Table 5.

Table 6: Results of HPLC Analysis of Tablet for MET and EMPA

No. of sample assayed	Label amount (mg)		Amount found(mg)		%Assay (Mean±SD)		RSD	
6	MET	EMPA	MET	EMPA	MET	EMPA	MET	EMPA
	500	5	501.89	5	100.38±0.776	100.09±0.583	0.77	0.58

3.2.6 Stability of sample solution

The sample and standard solutions injected at 0 hr (comparison sample) and after 24 hr (stability sample) by keeping at ambient room temperature 30^{O}c. The RSD for 0 hr. and 24 hr. for sample and standard solutions of MET are 0.77 and 0.86 respectively. The RSD for 0 hr. and 24 hr. for sample and standard solutions of EMPA are 0.58 and 0.7 respectively. RSD results for both MET and EMPAare within limit of ≤2 and hence the sample and standard stock are stable for 24 hr in ambient room temperature and the results are shown in Table 6.

3.2.7 LOD and LOQ

LOD and LOQ for MET were 0.05 and 0.16 µg/mL respectively and for EMPA were 0.01 and 0.03 µg/mL, respectively. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive.

3.3 Tablet Analysis

The Content of MET and EMPA in the tablets was found by the proposed method. RSD results for both MET and EMPA is within limit of ≤2 and the results were shown in Table 6.

4. CONCLUSION

A new precise accurate and simple HPLC method was developed and validated for simultaneous estimation of Metformin and Empagliflozine tablet dosage form. This method is

fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in QC laboratories and industries.

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