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Novel Approach of Stability Indicating Method Development for Determination of Metformin and Empagliflozin by High Performance Liquid Chromatography

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Abstract

A simple, accurate, precise RP-HPLC method was developed for the simultaneous estimation of the Metformin and Empagliflozin in the tablet dosage form. ***Materials and Methods:*** Chromatogram was run through Std Agilent 18 (150 x 4.6mm, 5µm). Mobile phase taken as 0.1% OPA Buffer: Acetonitrile in 60:40% v/v ratio, and pumped through the column at a flow rate of 1mL/min. The buffer used in this method was 0.1% OPA buffer. The temperature was maintained at 25°C. ***Results and Discussion:*** Optimized wave length selected was 245 nm. The retention times of Metformin and Empagliflozin were found to be 2.193 min and 2.668 min respectively. %RSD of the Metformin and Empagliflozin was found to be 1.4 and 0.8 respectively. % Recovery was obtained as 99.66% and 100.24% for Metformin and Empagliflozin respectively. LOD, LOQ values obtained from regression equations of Metformin and Empagliflozin were 0.02, 1.48, and 0.05, 4.93 respectively. Regression equation of Metformin is y = 20952x + 9914.5 and y = 41842x + 571.79 of Empagliflozin. Retention times were decreased and that run time was decreased, The Reverse Phase HPLC isocratic method for Metformin and Empagliflozin is developed and validated as per ICH guidelines. ***Conclusion:*** The test method is found to be sensitive, accurate, precise, linear, convenient, and economical that can be adopted in regular quality control tests in Industries.

Keywords: Metformin; Empagliflozin; RP-HPLC; Validation.

INTRODUCTION

Metformin (Fig. 1) is chemically 1,1-dimethyl biguanide hydrochloride. Metformin is an antihyperglycemic agent that decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization.[1]

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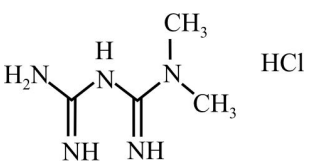

Fig. 1: Molecular structure of Metformin Hydrochloride

Empagliflozin (Fig. 2) 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. It is a sodium glucose co-transporter-2 (SGLT-2) inhibitors are responsible for prevention of reabsorption of glucose from the glomerular filtrate in the kidney.[2]

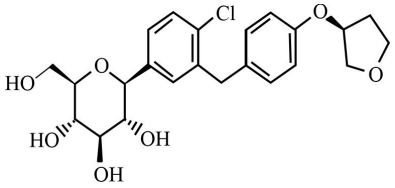

Fig. 2: Molecular structure of Empagliflozin

Literature survey reveals that there is five HPLC method was reported for simultaneous estimation of Metformin and Empagliflozin in pharmaceutical formulations.[3-7] Therefore, an attempt has been made to develop a novel, simple, rapid, accurate, and precise stability indicating RP-HPLC method for simultaneous estimation of Metformin and Empagliflozin and validated in accordance with ICH guidelines.[8]

MATERIALS AND METHODS

Instrumentation: To develop a high-performance liquid chromatographic method for simultaneous estimation of Metformin and Empagliflozin using Waters HPLC 2695 system on Agilent 18 (150 x 4.6mm, 5µm particle size) column was used. The instrument is furnished with an autosampler and UV-Visible detector. A 10µL rheodyne injector port was used for injecting the samples. Data were analyzed by using Empower 2 software. A BVK digital pH meter was used for pH measurements.

Chemicals and solvents: The working standards of Metformin and Empagliflozin were provided as gift samples from Spectrum Labs, Hyderabad, India. The marketed formulation of SynjardyR tablets (Metformin 500mg, Empagliflozin 5mg) was procured from the local market. HPLC grade water and acetonitrile were procured from E.Merck (India) Ltd., Mumbai, India. Methanol and potassium dihydrogen phosphate of analytical reagent grade was acquired from S.D. Fine Chemicals Ltd., Mumbai, India.

Chromatographic conditions: 0.1% OPA Buffer: Acetonitrile taken in the ratio 60:40%v/v was found to be the most suitable mobile phase for ideal chromatographic separation for simultaneous estimation of Metformin and Empagliflozin. Filtration of the solvent mixture was done through a 0.45µm membrane filter and sonicated before use, and the flow rate of the column was adjusted to 1.0mL/min. The injection volume was 10µL and the column was maintained at a temperature of 250C. The column was stabilized by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 245nm. The run time was set as 5 min.

Diluent preparation: Based upon the drugs solubility, acetonitrile and water taken in the ratio of 50:50v/v is selected as diluent.

Standard stock solutions preparation: 50mg of Metformin, 0.5mg of Empagliflozin were accurately weighed and transferred to 25mL and 100mL volumetric flasks separately. The diluent was added in the proposition of 3/4th to both of these flasks and sonicated for 10min. The final volumes of flask were adjusted with diluents and labeled as standard stock solution 1 and 2. (1000µg/mL of Metformin and 10µg/mL of Empagliflozin)

Sample stock solutions preparation: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into 100mL volumetric flask, add up 50mL of diluents, and sonicated for 25min, then the volume was made up with diluent and filtered by HPLC filters. (1000µg/mL of Metformin and 10µg/mL of Empagliflozin).

Sample working solutions (100% solution) preparation: 1mL of filtered sample stock solution was transferred to a 10mL volumetric flask and made up with diluent. (100µg/mL of Metformin and 1µg/mL of Empagliflozin).

PROCEDURE

The column was set at a temperature of 250C. The run time was set at 5 min. The column was stabilized by pumping the mobile phase through the column for at least 30 min before the injection of the drug solutions. Inject 10µL of the standard and sample solutions six times into the chromatographic system at a flow rate of 1.0mL/min and the corresponding chromatograms were obtained. From obtained chromatograms, the average area under the peak of each dilution was evaluated.

Table 1: Optimized chromatographic conditions

Mobile phase	**0.1% OPA Buffer: Acetonitrile taken in the ratio 60:40%v/v**
Column	Agilent 18 (150 x 4.6mm, 5µm)
Flow rate	1.0mL/min
Column temperature	Room temperature (20-25°C)
Sample temperature	Room temperature (20-25°C)
Wavelength	245nm
Injection volume	10µL
Run time	5min
Retention time	2.190 min for Metformin and 2.668 min for Empagliflozin

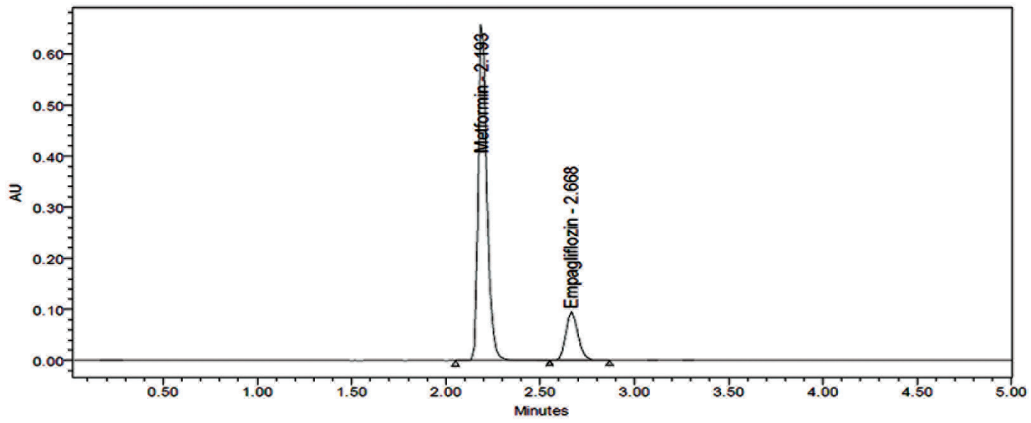

Fig. 3: Typical chromatogram of Metformin and Empagliflozin

METHOD VALIDATION

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Metformin (100ppm) and Empagliflozin (1ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were computed. The %RSD for the area of six standard injections results should not be more than 2%.

Table 2: System suitability parameters of proposed method

S. No.	**Parameters**	**Metformin**	**Empagliflozin**
1	Linearity (µg/mL)	50-150	0.5-1.5
2	Correlation coefficient	0.999	0.999
3	Retention time (min.)	2.190	2.668
4	Resolution	-	4.7
5	Tailing factor	1.256	1.148
6	Theoretical plates (N)	11294	8440
7	LOD (µg/mL)	1.48	0.02
8	LOQ (µg/mL)	4.93	0.05

Specificity: Examine the interference in the optimized method. We should not detect interfering peaks in blank and placebo at retention times of these drugs in this method. So, the developed method was said to be specific.

Precision: Precision for Metformin and Empagliflozin was determined in terms of system precision, repeatability and intermediate precision. Every sample was injected six times. The measurements for peak areas were communicated in terms of %RSD.

Table 3: Precision data of Metformin and Empagliflozin

	S. No.	Area of Metformin	Area of Empagliflozin
System Precision	1	2089058	42641
	2	2120395	41846
	3	2106527	42080
	4	2073485	42284
	5	2039013	42084
	6	2132852	41925
Repeatability	1	2105984	42036
	2	2105343	42057
	3	2123514	41914
	4	2145788	42455
	5	2060546	41728
	6	2084500	42599
Intermediate precision	1	2067994	40596
	2	2043300	40633
	3	2058775	39443
	4	2081426	40604
	5	2089351	39139
	6	2065357	39485

Linearity: Several aliquots of standard solutions of Metformin and Empagliflozin were taken in six different 10mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations were in the range of 25-150µg/mL for Metformin and 0.25-1.5µg/mL for Empagliflozin. The above series of solutions were injected into the HPLC column by keeping the injection volume constant. The drugs were eluted with UV detector at 245nm, peak areas was recorded for all the peaks. The linearity curves were drawn by plotting concentration of the drugs against peak areas. The regression equation of this curve was calculated. This regression equation was used to estimate the amount of drugs in tablet dosage forms.

Accuracy: The accuracy of the method was assessed by recovery studies of Metformin and

Table 4: Linearity results of Metformin and Empagliflozin

S. No.	Metformin		Empagliflozin	
	Concentration (µg/mL)	Mean peak area	Concentration (µg/mL)	Mean peak area
1	25	522703	0.25	11344
2	50	1079904	0.5	21634
3	75	1599117	0.75	32388
4	100	2095288	1	42415
5	125	2620383	1.25	52877
6	150	3151947	1.5	63015

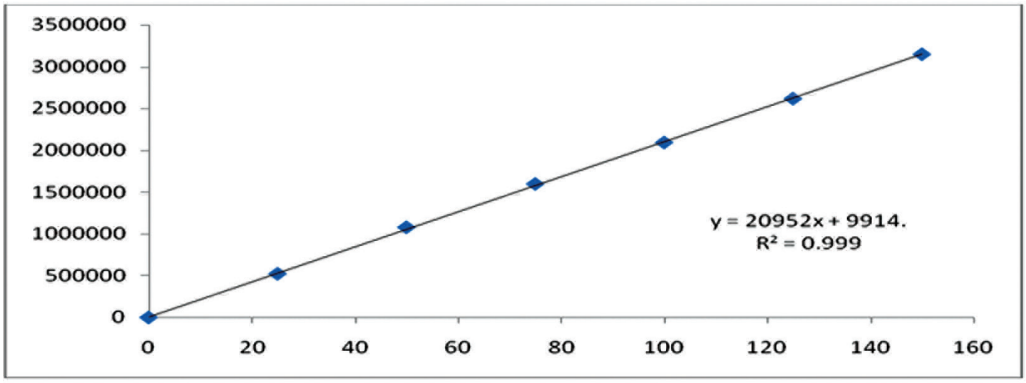

Fig. 4: Calibration curve of Metformin

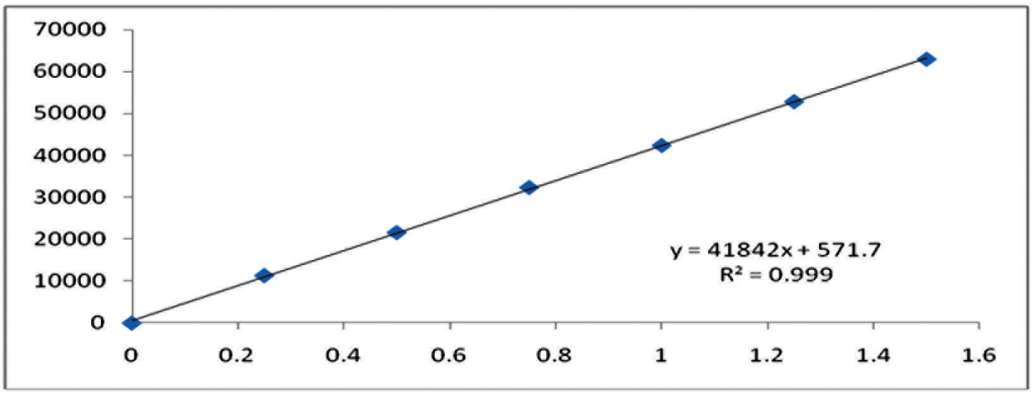

Fig. 5: Calibration curve of Empagliflozin

Empagliflozin at three concentration levels 50%, 100% and 150%. Fixed amount of pre-analyzed sample was spiked with known amount of Metformin and Empagliflozin. Each level was repeated three times. The % Recovery of Metformin and Empagliflozin were calculated.

Robustness: Measure of its capacity to remain unaffected by small, but deliberate variations in method parameters like flow rate, mobile phase ratio, and temperature are made but there were no conceding change in the result data and that are within range as per ICH guidelines.

Table 5: Accuracy studies of Metformin and Empagliflozin

% Concentration level	Metformin			Empagliflozin		
	Conc. added (µg/mL)	Conc. found (µg/mL)	% Recovery	Conc. added (µg/mL)	Conc. found (µg/mL)	% Recovery
50%	50	49.86	99.71	0.5	0.49	99.79
100%	100	100.19	100.19	1.0	0.99967	99.967
150%	150	149.73	99.82	1.5	1.509	100.60

Robustness situations are like flow minus, flow plus, mobile phase minus, mobile phase plus, temperature minus and temperature plus were nurtured and samples were injected in repeated manner. System suitability parameters were not much affected and all the parameters were in acceptable range. %RSD was within the limit.

Table 6: Robustness data for Metformin and Empagliflozin

S. No.	Condition	% RSD of Metformin	% RSD of Empagliflozin
1	Flow rate (–) 0.9mL/min	1.1	1.0
2	Flow rate (+) 1.1mL/min	1.2	1.8
3	Mobile phase (–) 45B:55A	1.2	0.3
4	Mobile phase (+) 55B:45A	1.4	1.2
5	Temperature (–) 25°C	1.5	0.6
6	Temperature (+) 35°C	1.5	0.6

with the same diluents. Inject 10µL of solutions six times into the chromatographic system at a flow rate of 1.0mL/min and the corresponding chromatograms were obtained. From the obtained chromatograms, the average area under the peak of each dilution was calculated.

Sample preparation for LOQ: 0.25mL each from two standard stock solutions was pipetted out and shifted to two separate 10mL volumetric flask and made up with diluent. From the above solutions 0.3mL each of Metformin, Empagliflozin solutions respectively were shifted to 10mL volumetric flasks and made up with the same diluent. Inject 10µL of solutions six times into the chromatographic system at a flow rate of 1.0mL/min and the corresponding chromatograms were obtained. From the obtained chromatograms, the average area under the peak of each dilution was calculated.

Sample preparation for LOD: Pipetted out 0.25mL from 2 standard stock solutions and transferred to separate 10mL volumetric flasks and made up the final volume with diluents. From the above solutions, 0.1mL of Metformin and Empagliflozin solutions were shifted into 10mL volumetric flasks and made up the final volume

Assay: The bulk drug was used for standard preparations and commercial formulation was used for preparation of sample solution. The standard and sample solutions were injected as six homogeneous samples. 10µL of sample solution was injected and from the peak areas of Metformin and Empagliflozin amount of each drug in the sample were computed. The results were compared with the label claim of Metformin and Empagliflozin in tablet dosage forms. From the obtained results the average % Assay was measured.

Degradation Behavior

To test the developed method for degradation behavior, the formulation sample was subjected to acid, base, thermal, photo, peroxide and water

Table 7: Assay results of marketed formulations

S. No.	Metformin		Empagliflozin	
	Standard Area	Sample Area	Standard Area	Sample Area
1	2089058	2105984	42641	42036
2	2120395	2105343	41846	42057
3	2106527	2123514	42080	41914
4	2073485	2145788	42284	42455
5	2039013	2060546	42084	41728
6	2132852	2084500	41925	42599
Avg	2093555	2104279	42143	42132
Label amount	500	5		
Amount found (mg)	502.05	4.9935		
Assay (%purity)	100.41	99.87		

exposure were carried with the aim of detection of degradants in the chromatogram. Acid degradation was carried out by adding 20mL of 0.1N HCl to the stock solution and from that 1mL was removed and added to a 1000mL volumetric flask and the volume adjusted to the mark. In the same manner, 2mL 1N NaOH was added to test for base degradation. To test for thermal degradation, the sample was subjected to heat at 105°C for 3 hrs and the sample prepared as per the assay procedure. For photo degradation study, the sample was exhibited to ultraviolet light with NLT 2000 lux power intensity for 6 hrs and the sample prepared as per the procedure of assay. For peroxide degradation, 2mL H_2O_2 were added to the stock 1000mL volumetric flask, 1mL was removed and added to a 1000mL flask, the volume adjusted to the mark with the diluent, and the sample was injected. Stress study under neutral conditions was carried by refluxing the drug in water for 1hrs at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 90µg/mL & 5µg/mL and from that 10µL was injected into the system and the chromatograms were generated to assess the stability of the sample.

Statistical Analysis

The data were computed through the Q Sight software, and the results were generated as mean and ± SD for the accuracy and the RSD for the precision. The coefficient of regression was also

Table 8: Degradation Data of Metformin and Empagliflozin

S. No.	Degradation Condition	Metformin		Empagliflozin	
		% Drug Degraded	% Drug Un-degraded	% Drug Degraded	% Drug Un-degraded
1	Acid	94.80	5.20	94.83	5.17
2	Alkali	95.69	4.31	95.96	4.04
3	Oxidation	96.61	3.39	96.21	3.79
4	Thermal	97.75	2.25	97.24	2.76
5	UV	98.54	1.46	98.22	1.78
6	Water	99.15	0.85	99.23	0.77

computed in the linearity parameter.

Results And Discussion

The HPLC procedure was optimized with a view to develop an accurate, precise and reproducible method for simultaneous estimation of Metformin and Empagliflozin in tablet dosage form Waters HPLC 2695 system on Agilent 18 (150 x 4.6mm, 5µm particle size) column in isocratic mode with mobile phase composition 0.1% OPA Buffer: Acetonitrile taken in the ratio 60:40%v/v resulted in peak with maximum separation, good shape and resolution. Between 0.8 to 1.2mL/min flow rates were studied. A flow rate of 1.0mL/min gave an optimum signal to noise ratio with reasonable separation time, the retention times for Metformin and Empagliflozin were found to be 2.193min and 2.668min respectively. Total run time was 5 min. The drug components were measured with UV detector at 245nm. The optimized chromatographic condition results were shown in Table 1.

Linearity was obtained in the range of 25-150µg/mL for Metformin and 0.25-1.5µg/mL for Empagliflozin. The correlation coefficient (r2) was found to be 0.999 for both Metformin and Empagliflozin. The regression equation of the linearity plot of concentration of Metformin over its peak area was found to be y = 20952x + 9914, where x is the concentration of Metformin (µg/mL) and y is the corresponding peak area. The regression equation of the linearity plot of concentration of Empagliflozin over its peak area was found to be y = 41842x + 571.7, where x is the concentration of Empagliflozin (µg/mL) and y is the corresponding peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. The linearity results were shown in Table 2 and the calibration curves were shown in Fig. 3 and Fig. 4.

The %RSD for system precision, repeatability and intermediate precision for Metformin and Empagliflozin were found to be 1.6 & 0.7, 1.4 & 0.8 and 0.8 & 1.7 respectively (limit %RSD<2.0%) and hence the method is precise. The precision data of Metformin and Empagliflozin were furnished in Table 3.

The % Recovery of the drugs Metformin and Empagliflozin were found to be 98.55 to 100.49% and 99.07 to 101.42% respectively and the high percentage of recovery of Metformin and Empagliflozin indicates that the proposed method is highly accurate. The results of accuracy studies of Metformin and Empagliflozin were shown in Table 4.

The retention times for the drugs Metformin and Empagliflozin was 2.193min and 2.668min respectively. The number of theoretical plates calculated for Metformin and Empagliflozin was 11294 and 8440 respectively. The tailing factor for Metformin and Empagliflozin was 1.256 and 1.148 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantification (LOQ) for Metformin were found to be 1.48µg/mL and 4.93µg/mL; 0.02µg/mL and 0.05µg/mL for Empagliflozin respectively, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 5.

The robustness studies indicated that no considerable effect on the determination of the drugs. Therefore the test method is robust for the quantification of the drugs. In all deliberately varied conditions, the %RSD for replicate injections of Metformin and Empagliflozin were found to be within the acceptable limits and the results are shown in Table 6.

Validated method was applied for the simultaneous estimation of Metformin and Empagliflozin in commercial tablet dosage forms. The % Assay of Metformin and Empagliflozin were found to be 100.41% and 99.87% respectively. The results for the drugs assay showed good agreement with label claims. No interfering peaks were found in the chromatogram of the tablet formulation within the run time indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the drugs Metformin and Empagliflozin by the proposed HPLC method. The assay results are shown in Table 7.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common formulation excipients and impurities. Also the peak areas were compared with standard and were found to be within limits. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks. The typical chromatogram of Metformin and Empagliflozin standard were shown in Fig. 5.

The method was applied to degraded samples to verify its usefulness within the shelf

life period (stability indicating nature). The method detected degradants successfully in all the degradation conditions and the results are shown in Table 8.

CONCLUSION

It is very clear that the proposed Reverse Phase HPLC isocratic method developed for the simultaneous estimation of Metformin and Empagliflozin and validated as per ICH guidelines is sensitive, accurate, precise, linear, and convenient can be reliably adopted for routine quality control analysis in its tablet dosage forms for intended applications in any pharmaceutical industries.

Source of Support: None

Conflict of Interest: Nil

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