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Simultaneous For the Estimation of Metformin and Empagliflozin in Pharmaceutical Dosage Form by HPLC Method

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Abstract: *To develop accurate, fast, simple and precise reversed-phase high-pressure liquid chromatography method for simultaneous determination of the binary mixture of metformin and empagliflozin in dosage form. Thechrom atographic separation was conducted on Agilent 1200 with DA detector using X-Select-HSS C18 SB (4.6-mm x 25-cm; 5-µm) as stationary phase. Isocratic mobile phase consisted of Phosphate Buffer : Acetonitrile in ratio (60:40 v/v); The buffer solution is composed of 6.8 gm of potassium dihydrogen orthophosphate in 1000 ml with water and filtered through 0.45 µm nylon membrane filter. The flow rate of 1.0 ml min^{-1} was used. An injection volume of 10 µL was used for both metformin and empagliflozin. The detection wavelength (λ_{max}) was 255 nm using a diode array detector. Linearity of the method was established over the concentration ranges of 250 – 750 µg ml^{-1} for metformin, with a retention time of 2.6 minutes and 3.13 – 9.38 µg ml^{-1} for empagliflozin, with a retention time of 6.4 minutes. Correlation coefficients were greater than 0.999. The relative standard deviation (RSD) was found to be < 2. The method was 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: *HPLC, Metformin, Empagliflozin, Method validation.*

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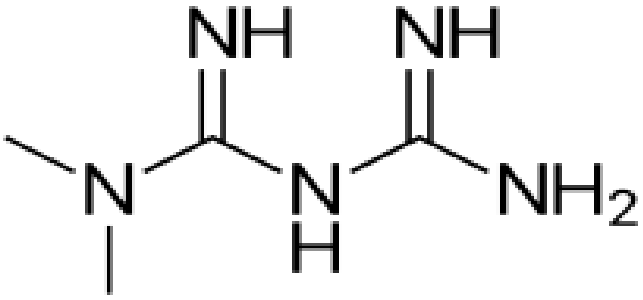
I. Introduction

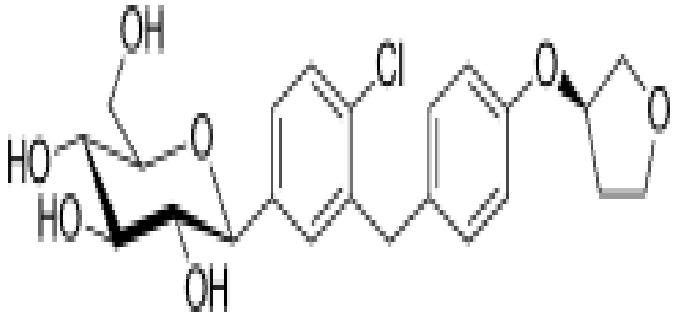
Metformin is chemically named as 4 1-carbamimidamido-N, N-dimethylmethanimidamide and it is as shown in Fig. 1 (A). Metformin hydrochloride decreases the gluconeogenisis while increasing the glucose uptake by muscles and fat cells. Pioglitazone hydrochloride is a thiazolidine dione derivative. It is one of the PPAR-alpha agonist, insulin sensitizer, used to reduce the insulin resistance [1,2]. It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption, and increasing insulin-mediated glucose uptake. MET may induce weight loss and is the drug of choice for obese NIDDM patients. Use of MET is associated with modest weight loss.

Empagliflozin is chemically named as (2S,3R,4R,5S,6R)- 2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy]phenyl}methyl)phenyl]-6- (hydroxymethyl)oxane-3,4,5-triol and it is as shown in Fig. 1 (B). EMPA sodium glucose co-transporter-2 (SGLT-2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with Type 2 diabetes. SGLT-2 cotransporters are responsible for reabsorption of glucose from the glomerular filtrate in the kidney. The glucuretic effect resulting from SGLT-2 inhibition reduces renal absorption and lowers the renal threshold for glucose, therefore resulting in increased glucose excretion [3,4]. In addition, it contributes to reduced hyperglycemia and also assists weight loss and blood pressure reduction.

Literature survey revealed that few analytical methods are reported for analysis of both the drugs alone as well as in combination using ultraviolet (UV) spectrophotometry [5], high-pressure liquid chromatography (HPLC) [6-12], and ultra-pressure liquid chromatography [13]. The proposed method was validated according to ICH guidelines [14].



(A)



(B)

Fig. 1 Chemical structures of Metformin (A) and Empagliflozin (B).

II. Materials and Methods

Chemicals and reagents

Metformin and empagliflozin were obtained from Hetero Drugs, Hyderabad, (India). Acetonitrile 200 far UV was HPLC grade from Romil. Water for chromatography was purchased from Merck. Potassium dihydrogen orthophosphate was from EL Nasr Pharmaceuticals Chemicals. Mobile phase was filtered using 0.45 µm nylon membrane filter, ChromTech.

Equipment and chromatographic conditions

Agilent 1200 with DA detector was used. The HPLC separation and quantitation were achieved on a X-Select-HSS C18 SB (4.6-mm x 25-cm; 5-µm). The mobile phase was prepared by Phosphate Buffer : Acetonitrile in ratio (60:40 v/v); The buffer solution is composed of 6.8 gm of potassium dihydrogen orthophosphate in 1000 ml with water which was run isocratic. The mobile phase was delivered to the system at a flow rate of 1.0 ml/min. All determinations were performed at ambient temperature. The injected volume was 10 µl. The detector was set at 255 nm. The run time was set for 1.0 min. The optimized chromatographic condition is shown in Table 1.

Table 1 Optimized chromatographic conditions

Parameters	**Conditions**
Stationary phase	X-Select-HSS C18 SB, 250 x 4.6 mm, 5 µm
Mobile phase	Phosphate Buffer : Acetonitrile (60:40 v/v)
Flow rate (mL min^{-1})	1.0
Run time (min)	8.0
Column temperature (^{0}C)	Ambient (25 ^{0}C)
Injection volume (µL)	10
Detection wavelength (nm)	255nm

Preparation of standard solution

Accurately weighed 62.5 mg of empagliflozin standard into 200 ml volumetric flask, Add 100 ml 50% methanol, sonicate for 5 min then cool and complete the volume with 50% methanol (S1).

Accurately weigh 125 mg of metformin HCl standard into 50 ml volumetric flask, add 25 ml of 50% methanol, sonicate for 5 min then cool and complete the volume with 50% methanol (S2).

Dilute 1 ml of (S1 solution) and 10 ml of (S2 solution) to 50 ml volumetric flask then complete to volume with mobile phase.

Linearity

Linearity of an analytical procedure is its ability, within a given range, to obtain test results which are directly proportional to the concentration of analyte in the sample. Linearity suitable for single point standardization should extend to at least 20% beyond the specification range and include the target concentration. Linearity is defined by the correlation coefficient, which should be found to be 0.99, using peak area responses.

Linearity was performed by preparing a minimum five different concentrations (50, 80, 100, 120& 150 %) of working standard solution and then making three replicates of each concentration.

Accuracy

Accuracy was evaluated by spiking standard solution. The measurements are made at a concentration of empagliflozin and metformin HCl in Empagliflozin / Metformin F.C.Tablets, which is found to be the target concentration, and at suitable intervals around this point.

Experimental procedure

Placebo except the active ingredient was spiked with known quantities of empagliflozin and metformin HCl working standard. Accuracy was assessed using 4 determinations over three concentrations level covering the specified range (i.e. three concentrations and three replicates). The measurements were made at a concentration, which is to be the (100%) specification, and at suitable concentration intervals around this concentration.

Specificity

It provides an indication of the selectivity and specificity of the procedure. The method is to be selective, if the main peak is well resoluted from any other peak by resolution of minimum 2. This was done by injecting placebo and comparing it with that of standard and placebo; spiked with standard and sample. Then the peak purity was ascertained using of PDA.

System suitability

System suitability was performed by injecting six replicates of standard solution at 100% of the test condition at a 100% level to verify the precision of the chromatographic system. The purposed HPLC method permits the concurrent determination of metformin and empagliflozin in sample drug; through having different retention times. System suitability data are given in Table 2.

Table 2 System suitability parameters for Metformin and Empagliflozin

S. No.	Parameters	Metformin	Empagliflozin
1	Tailing factor	2.66	6.37
2	Retention time	0.88	2.10

Ruggedness

It's defined the degree of reproducibility of test results obtained by the analysis of the same samples under variety of conditions such as different analysts, different columns, different days etc.

- **Day to day**: Five replicates of a single sample of powder material (100%) were used for each determination. On the first day; five replicates was analyzed. Then, on the second day, another five replicates of freshly prepared test from the same sample were analyzed by same analyst.
- **Analyst to analyst**: It determines ruggedness between different analysts. Five replicates of a single sample were analyzed. Then, a second person analyzed five replicates from the same sample, prepared by him.
- **Column to column**: The same analytical method was performed on columns of the same packing material and length but of different batch number.

Robustness

Robustness is determined by observing how a method stands up to slight variations in normal operating parameters. For instance, for HPLC, this could change if slight variation in sonication time or in aliquot stability.

Limit of detection (LOD) and limit of quantitation (LOQ)

Detection and quantitation limits were determined by the signal-to-noise (S/N) approach. In order to examine the limit of quantitation and limit of detection, solutions of different concentrations were prepared by spiking known amounts of metformin and empagliflozin. Each solution was prepared according to the defined protocol and analyzed repeatedly to determine the S/N ratio. The average S/N ratio from all the analyses at each concentration level was used to calculate the limit of quantitation and limit of detection. The concentration level that gives an S/N ratio of 10:1 at which analytes can be readily quantified with accuracy and precision was reported as the limit of quantitation. The concentration level that gives an S/N ratio of 3:1 at which analytes can be readily detected was reported as the limit of detection.

III. Results and Discussion

The proposed HPLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of metformin and empagliflozin was shown in Fig. 2. There was clear resolution between metformin and empagliflozin with retention time of 2.6 and 6.4 minutes; respectively. The developed chromatographic method was validated using ICH guidelines [14]. Validation parameters include linearity, accuracy, precision, robustness, specificity, limit of detection and quantitation.

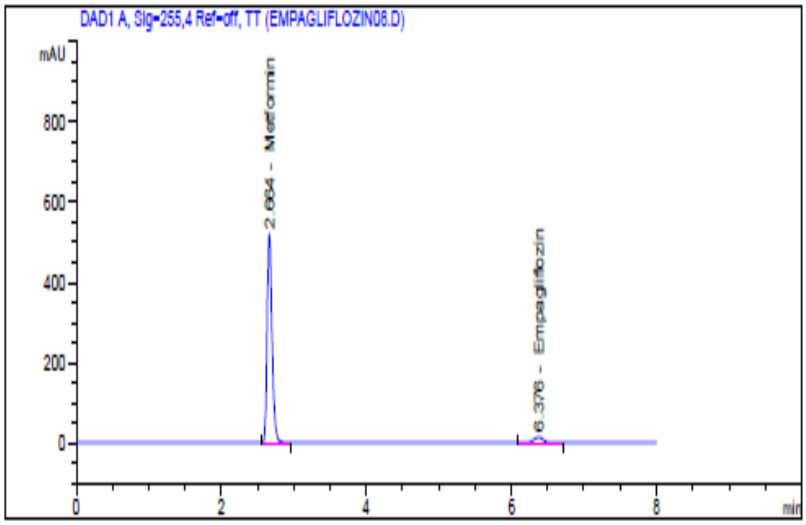


Fig 2. HPLC chromatogram for Metformin and empagliflozin.

Linear calibration plots for the proposed method were obtained in concentration ranges of 250-750 µg mL^{-1} (250, 400, 500, 600 and 750 µg mL^{-1}) for metformin as shown in Fig. 3 and data are shown in Table 3 and 3.13-9.38 µg mL^{-1} empagliflozin (3.13, 5.0, 6.25, 7.5 and 9.38 µg mL^{-1}) as shown in Fig. 4 and data are shown in Table 4.

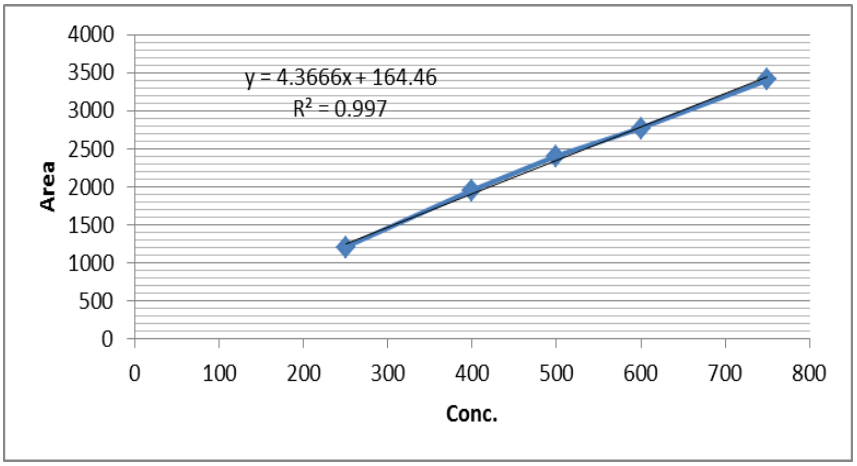


Fig. 3 Calibration curve of Metformin.

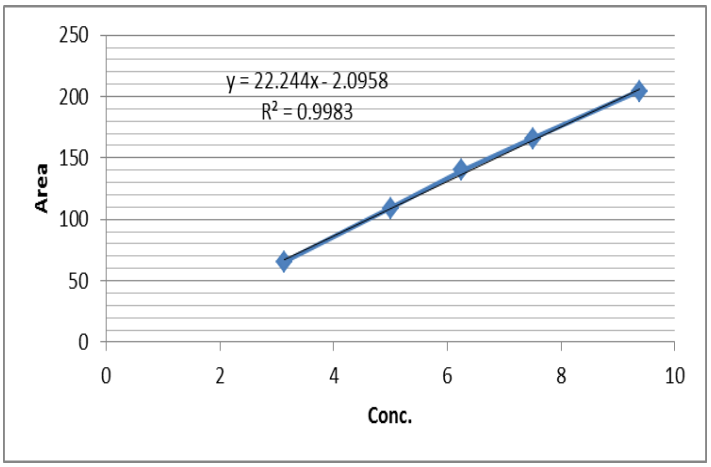


Fig. 4 Calibration curve of empagliflozin.

Table 3 Statistical data of calibration curves of Metformin

Working Conc. (%)	.Working Conc. (µg/ml)	Observed peak areas		
		Mean	SD	RSD
50%	**250.00**	1204.67	16.06	1.33
80%	**400.00**	1949.50	15.74	0.81
100%	**500.00**	2404.39	3.97	0.16
120%	**600.00**	2771.82	2.97	0.11
150%	**750.00**	3408.35	45.67	1.34
Slope	**4.3666**			
Intercept	**164.46**			
R 2	**0.997**			

Table 4 Statistical data of calibration curves of empagliflozin

Working Conc. (%)	.Working Conc. (µg/ml)	Observed peak areas		
		Mean	SD	RSD
50%	**3.13**	65.63	0.81	1.23
80%	**5.00**	109.25	0.70	0.64
100%	**6.25**	139.97	0.25	0.18
120%	**7.50**	165.69	0.25	0.15
150%	**9.38**	204.33	1.70	0.83
Slope	**22.244**			
Intercept	**-2.0958**			
R 2	**0.998**			

Each of the concentrations was injected in triplicate to get reproducible response. Calibration curves were constructed by plotting peak area versus concentration. Each reading was average of three determinations. They were represented by the linear regression equation.

$Y_{Metformin} = 4.3666x + 164.46$, $r^2 = 0.997$
$Y_{Empagliflozin} = 22.244x - 2.0958$, $r^2 = 0.998$

Slopes and intercepts were obtained by using regression equation (Y = mx + c) and least square treatment of the results used to confirm linearity of the method developed.

The limit of detection (LOD) and quantitation (LOQ) were determined by making serial dilutions. LOD was found to be 36.80 $\mu g.ml^{-1}$ and 0.352 $\mu g.ml^{-1}$ for metformin and empagliflozin; respectively (signal to noise ratio of 3:1). LOQ was found to be 110.401 $\mu g.ml^{-1}$ and 1.055 $\mu g.ml^{-1}$ for metformin and empagliflozin; respectively (signal to noise ratio of 10:1).

Accuracy was calculated by addition of standard drugs to preanalyzed sample at three different concentration levels and computing percentage recoveries. Standard limit of % recovery study is 98 - 102 % as per ICH guideline. From the studies it was concluded that % recovery study of metformin and empagliflozin complies with standard limit of ICH guideline. Results of accuracy were proven by the Table 5,6.

Table 5 Accuracy and recovery results for determinations of Metformin

Level (%)	Actual µg/ml	Theoretical µg/ml	(%) Recovery (n=3)
80	402.56	403.41	99.79
100	504.63	504.26	100.07
120	602.42	605.11	99.56
Average Recovery			**99.81**
SD			**0.255**
% RSD			**0.256**

Table 6 Accuracy and recovery results for determinations of empagliflozin

Level (%)	Actual µg/ml	Theoretical µg/ml	(%) Recovery (n=3)
80	5.00	5.04	99.21
100	6.23	6.30	98.89
120	7.45	7.56	98.54
Average Recovery			**98.88**
SD			**0.335**
% RSD			**0.339**

Specificity of a method is its suitability for the analysis of a compound in the presence of potential impurities. Placebo, standards, and sample test solutions were all injected at the same wavelength of 255 nm to demonstrate the specificity of the optimized method. The proposed method is suitable for the quantification of the active ingredients in tablet formulation.

IV. Conclusion

A simple, sensitive and accurate RP-HPLC method is described for simultaneous determination of metformin and empagliflozin in pharmaceutical formulations. Finally, it was concluded that the method is simple, sensitive and has the ability to separate the drug from products and excipients found in the dosage form.

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