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Stability indicating assay method for the simultaneous estimation of Empagliflozin and Metformin HCl by RP-HPLC method

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ABSTRACT

A Specific, Linear and Precise reversed phase- HPLC was developed for the simultaneous estimation of Metformin HCl and Empagliflozin and the column used is Zorbax SB Phenyl with length, Internal diameter and Particle size of 250mm, 4.6 mm and 5μm respectively. The Mobile phase is Phosphate buffer: ACN: Methanol in ratio 45:25:30. 1.0 ml/min was the used flow rate and the wavelength was adjusted to 220nm for detection. The retention time for Empagliflozin was found to be 5.5min and for Metformin was 9.3min. Both the APIs exhibited good linearity revealing correlation coefficient(R) of 0.9999. The percentage recoveries for Metformin and Empagliflozin was found to be 100.0 – 100.9% and 100.3 – 102.4% respectively which was found to be within the limit. Forced degradation studies were performed and the developed method has suitable specificity as no interference is observed with impurity spiked sample and placebo of Drug Product. The proposed drug products were subjected to various types of stress conditions according to ICH Q1 guidelines like acidic, alkaline, neutral, peroxide, and Thermal conditions. The degradation products were well resolved from the main peaks , thus indicating the stability- indicating nature of the method. The method was validated with respect to system suitability, linearity, accuracy, precision and robustness according to ICH guidelines and the proposed RP-HPLC Method was accurate, precise and linear for the simultaneous determination of Metformin and Empagliflozin in bulk and pharmaceutical formulations.

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INTRODUCTION

Metformin hydrochloride (Met HCl) is 1,1-diMethylbiguanide hydrochloride. The molecular formula of Met HCL is $C_4H_{11}N$. HCl with the molecular weight of 165.63 g/mol. It is mainly used for the therapy for type-2 diabetes especially in people with overweight. It is generally used as first choice of drug in the management of type 2 diabetes. Common side effects of Met HCl include diarrhoea, nausea, and abdominal pain. The Structure for Metformin is given in the Figure 1. (BPC, 2000; Indian Pharmacopoeia, 2007).

MOA of Metformin is unique from other class of oral hypoglycaemic drugs. It decreases the blood glucose level by reducing the gluconeogenesis (production of hepatic glucose), thus decreasing the glucose absorption in intestine and results in increased uptake and utilization of peripheral glucose by increasing insulin sensitivity. This process will lead

to the reduction of blood glucose, thus managing type 2 diabetes and exhibiting positive effects on glycaemic control. (Song, 2016). Molecular formula of Empagliflozin (Empa) is $C_{23}H_{27}ClO_7$ and having a molecular weight of 450.91 g/mol. It is used as a medication along with exercise and diet for the therapy of type 2 diabetes. Structure of Empa is given in Figure 2. (Autorů, 2014; Shyamala *et al.*, 2020)

Empagliflozin is sodium glucose co-transporter (SGLT-2) inhibitor. SGLT-2 is responsible for the uptake of glucose from the glomerular filtrate in kidney. It also contributes to the decreased hyperglycaemia and helps in weight loss and reduction of blood pressure. (Zinman *et al.*, 2015). According to the literature survey, many analytical methods have been recorded for the estimation of individual drugs as well as combination by HPLC, UPLC, UV, LC/MS technique in dosage form. The intention of the study is to develop and validate simultaneous Method for two antidiabetic drugs with less time consumption.

MATERIALS AND METHODS

Experimental

Metformin and Empagliflozin, active pharmaceutical ingredients (APIs) were furnished by Dr. Reddy's laboratories, Hyderabad. All the reagents used are of HPLC grade.

Apparatus

The LC system consists of auto sampler HPLC designed by Waters and UV-Vis detector. The studies were carried out on phenyl column having length of 250mm and 4.6 mm of internal diameter and particle size of 5μm and it was monitored at 220 nm. Chromatogram was obtained by empower software. (Bhagyashri *et al.*, 2012; Dange *et al.*, 2018).

Zorbax SB Phenyl with length, internal diameter and particle size of 250mm, 4.6 mm and 5μm respectively were used as a column. PDA detector is used at 220nm.

Reagents & Material

All the chemicals and reagents used are of AR grade. Metformin HCl and Empagliflozin were obtained from Dr. Reddy's Laboratories Pvt. Ltd., Hyderabad as a gift sample.

Preparation of standard stock solution

Metformin HCl and Empagliflozin were weighed in precise measures of 50 mg and 100 mg in two separate 100ml volumetric flasks. 70ml of diluent [water: acetonitrile in 50:50 (percent v/v)] was added to it, and was retained until it dissolved for sonication. Diluent was used to make up the volume, and for a correct blend. (Priya *et al.*, 2016; Padmaja *et al.*, 2016)

Preparation of working solutions

5 ml is pipetted out form the stock solution into 100 ml of volumetric flask and the mark is made up by using water to get a concentration of 25 μg/ml and 50 μg/ml (Met HCl and Empa) respectively. (Kar and Choudhury, 2009)

Chromatographic Conditions

The Table 12 indicating the chromatographic parameters. (Shyamala *et al.*, 2020). The Chromatograms of Blank and Standard are given in Figure 3 and Figure 4.

RESULTS AND DISCUSSION

The main aim of this was to develop a stable HPLC Method that indicated the simultaneous estimation of Metformin HCl and Empagliflozin. HPLC provides a quick and highly accurate Method and efficient when compared to other chromatographic techniques. While developing a Method, different trials were performed by changing the columns like Cyano, Hi chrom Altima, Zorbax phenyl etc., when cyano and Hi chrom Altima columns were used; results were not proper, a shift was observed in RT of Empagliflozin and Metformin peak shape was not good because tailing factor was more than 2. Different buffers such as pH 5.5 and, pH 7 phosphate were used which showed that the two main peaks were eluting at the same retention time, and by altering the organic phase ratio in the mobile phase by which there was a proper separation in both the peaks. The good peak shape was obtained with short rum time in the chromatographic parameters using Zorbax SB Phenyl column with pH 7.5 phosphate buffer: Acetonitrile: Methanol (45:25:30) as a mobile phase, Injection volume of 10μL and 1 mL/min flow rate. It was detected at 220 nm.

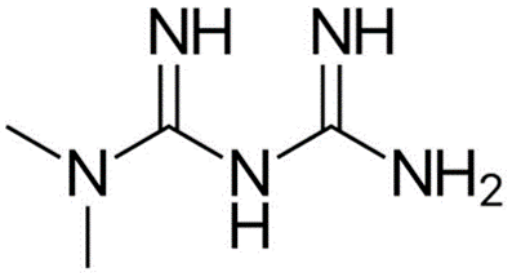

Figure 1: Metformin

System Suitability

Suitability tests are conducted to check the instrument's reproducibility and resolution. The theoreti-

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Table 1: Results for system suitability

System suitability parameters	Observed value		Acceptance Criteria
	Empagliflozin	Metformin HCl	
Tailing factor for Empagliflozin and Metformin peaks from standard solution	1.1	1.5	Not more than 2.0
Theoretical plate count for Empagliflozin and Metformin peaks from standard solution	13007	13853	Not less than 2000
%RSD for peak areas of Empagliflozin and Metformin peaks from five replicate injections of standard solution	0.02	0.2	Not more than 2%

Table 2: Results of Linearity

Linearity Level	Empagliflozin		Metformin	
	Concentration (μg/mL)	Peak Area	Concentration (μg/mL)	Peak Area
Linearity Solution-1	10.0058	260970	2.5065	72268
Linearity Solution-2	20.0116	523639	10.0258	288784
Linearity Solution-3	50.0291	1297452	25.0646	720507
Linearity Solution-4	60.0349	1566713	50.1292	1445975
Linearity Solution-5	90.0523	2335378	60.1550	1742904
Linearity Solution-6	120.0698	3130478	80.2067	2316405

Linearity Parameters	**Empagliflozin**	**Metformin**
Slope	26024.691985	28917.562787
Intercept	114.867417	-1482.834656
Correlation Coefficient	0.9999875	0.9999948
% Bias at 100%	0.01	-0.2
Residual sum of squares	147436852.921254	40073987.162193

Correlation coefficient should be not less than 0.999.

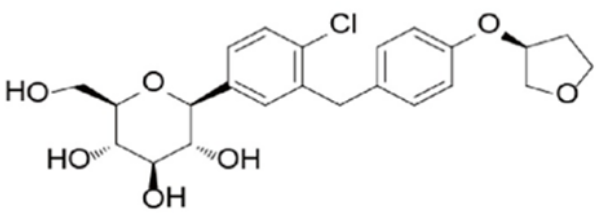

Figure 2: Empagliflozin

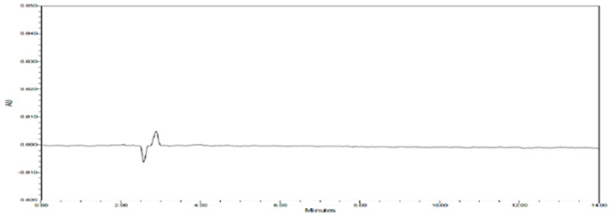

Figure 3: Chromatogram of Blank

Table 3: Results of precision

Sample No.	% Assay	
	Empagliflozin	Metformin
1	100.5	101.3
2	100.6	100.9
3	100.0	100.0
4	99.9	100.5
5	100.2	100.2
6	100.5	100.9
Average	100.3	100.6
*% RSD	0.3	0.5

*% RSD is percentage relative standard deviation and it should be not more than 2.0.

Table 4: Results of Intermediate precision

Analyst-1, Column-1, System-1, Day-1		
S. No.	% Assay	
	Empagliflozin	Metformin
1	100.5	101.3
2	100.6	100.9
3	100.0	100.0
4	99.9	100.5
5	100.2	100.2
6	100.5	100.9
Average	100.3	100.6
Analyst-2, Column-2, System-2, Day-2		
1	100.6	99.8
2	100.2	99.9
3	100.2	99.5
4	100.1	99.6
5	100.3	99.9
6	100.6	100.2
Average	100.3	99.8
% RSD	0.2	0.2
**C. % RSD	0.2	0.6

*% RSD is percentage relative standard deviation and it should be not more than 2.0.
**Cumulative % RSD of % assay is calculated from both Method precision and Intermediate precision.

Table 5: Results of Accuracy (Empagliflozin)

S.No.	Spike level	Amount added 'mg'	Amount found 'mg'	% Recovery
1	50% level	5.92388	6.04548	102.0
2	100% level	124.09796	125.21444	100.9
3	150% level	190.98820	191.87038	100.4

Table 6: Results of Accuracy (Metformin)

S.No.	Spike level	Amount added 'mg'	Amount found 'mg'	% Recovery
1	50% level	250.32834	250.65639	100.1
2	100% level	10000.56878	10068.40012	100.6
3	150% level	15002.68450	15022.93303	100.1

Individual % recovery should be between 97% and 103%.

Table 7: Results of effect of variation in flow rate

System suitability parameters		Observed value			Acceptance Criteria
		0.8 mL/min	1.0 mL/min	1.2 mL/min	
Tailing factor for Empagliflozin and Metformin peaks from standard solution	Empagliflozin	1.2	1.1	1.1	NMT 2.0
	Metformin	1.5	1.5	1.4	
Theoretical plate count for Empagliflozin and Metformin peaks from standard solution	Empagliflozin	13732	13007	11096	NLT 2000
	Metformin	13428	13853	12442	
%RSD for peak areas of Empagliflozin and Metformin peaks from five replicate injections of standard solution	Empagliflozin	0.1	0.02	0.1	NMT 2.0%
	Metformin	0.1	0.2	0.2	

Table 8: Results of effect of variation in column temperature

System suitability parameters		Observed value			Acceptance Criteria
		35°C	40°C	45°C	
Tailing factor for Empagliflozin and Metformin peaks from standard solution	Empagliflozin	1.1	1.1	1.2	NMT 2.0
	Metformin	1.4	1.5	1.4	
Theoretical plate count for Empagliflozin and Metformin peaks from standard solution	Empagliflozin	11343	13007	12467	NLT 2000
	Metformin	11664	13853	13097	
%RSD for peak areas of Empagliflozin and Metformin peaks from five replicate injections of standard solution	Empagliflozin	0.1	0.02	0.1	NMT 2.0%
	Metformin	0.1	0.2	0.1	

Table 9: Results of effect of variation of pH of bufferin Mobile phase composition

System suitability parameters		Observed value			Acceptance Criteria
		pH 7.3	pH 7.5	pH 7.7	
Tailing factor for Empagliflozin and Metformin peaks from standard solution	Empagliflozin	1.3	1.1	1.3	NMT 2.0
	Metformin	1.5	1.5	1.7	
Theoretical plate count for Empagliflozin and Metformin peaks from standard solution	Empagliflozin	7199	13007	7577	NLT 2000
	Metformin	6673	13853	7200	
%RSD for peak areas of Empagliflozin and Metformin peaks from five replicate injections of standard solution	Empagliflozin	0.04	0.02	0.1	NMT 2.0%
	Metformin	0.1	0.2	0.5	

Table 10: Results of Forced degradation studies (Empagliflozin)

Name of Degradation	Conditions	% degradation	Purity Angle	Purity Threshold	Purity Flag	Interference
Sample As such	NA	NA	0.131	0.301	No	Not Applicable
Acid degradation	15mL of 5N HCl, heating at 80°C for 16 Hour	3.4	0.340	0.345	No	No
Base Degradation	5mL of 5N NaOH at bench top for 1 Hour	0.7	0.163	0.323	No	No
Peroxide Degradation	10mL of 30% H2O2 at bench top for 48 Hours	1.3	0.029	0.280	No	No
Photolytic degradation	Visible light for 1.2 million Lux hrs and UV light for 200 watt hour/Meter square	0.2	0.127	0.298	No	No
Humidity Degradation	90% RH at 25°C for 7 days	0.3	0.161	0.394	No	No
Hydrolytic Degradation	5mL of water, heating at 80°C for 2 Hours	0.4	0.145	0.308	No	No
Thermal Degradation	105°C for 22 Hours	0.2	0.168	0.313	No	No

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Table 11: Results of Forced degradation studies (Metformin)

Name of Degradation	Conditions	% degradation	Purity Angle	Purity Threshold	Purity Flag	Interference
Sample As such	NA	NA	0.033	0.198	No	Not Applicable
Acid degradation	5mL of 5N HCl, heating at 80°C for 1 Hour	1.2	0.035	0.200	No	No
Base Degradation	5mL of 5N NaOH heating at 60°C for 45 minutes	5.7	0.034	0.198	No	No
Peroxide Degradation	5mL of 30% H2O2 at bench top for 1 Hour	1.2	0.036	0.198	No	No
Photolytic degradation	Visible light for 1.2 million Lux hrs and UV light for 200 watt hour/Meter square	0.7	0.048	0.198	No	No
Humidity Degradation	90% RH at 25°C for 7 days	0.3	0.035	0.199	No	No
Hydrolytic Degradation	5mL of water, heating at 80°C for 2 Hours	0.9	0.032	0.197	No	No
Thermal Degradation	105°C for 22 Hours	0.8	0.038	0.199	No	No

Net degradation should be between 5% and 20% in atleast one stress condition.
Peak purity – Purity Angle should be less than Purity Threshold and peak should not have any flag in purity table.

Table 12: Final Chromatographic conditions

Chromatographic conditions	Specification
Column	Zorbax SB Phenyl 250*4.6,5μ
Mobile Phase	Ph 7.5 phosphate buffer
Injection volume	10μL
Flow rate	1 ml/min
Column temperature	40°C
Wavelength	220nm

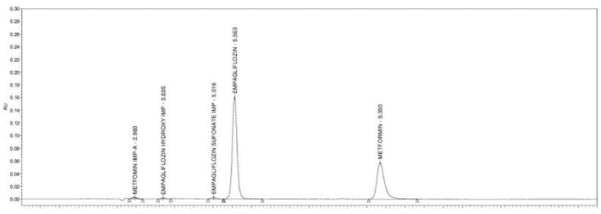
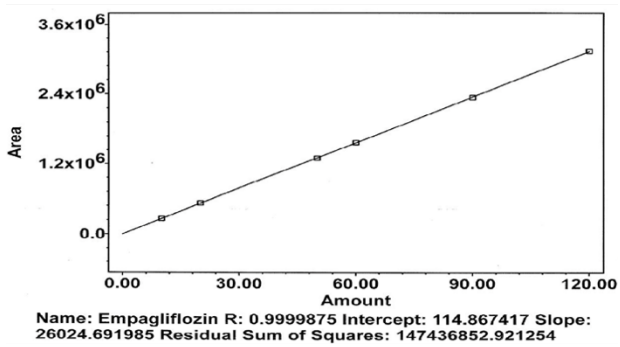


Figure 4: Chromatograms of Metformin HCl and Empagliflozin



Name: Empagliflozin R: 0.9999875 Intercept: 114.867417 Slope: 26024.691985 Residual Sum of Squares: 147436852.921254

Figure 5: Calibration curve of Empagliflozin

cal plate count, the percentage of RSD and the tailing factors shown in Table 1 were within the limit. (Ravisankar *et al.*, 2015)

Linearity

Linearity was performing by taking concentrations of 10-120 μg/mL for Empagliflozin and 2-80 μg/mL for Metformin HCl. The graph was found to be linear when plotted between concentration versus peak area as shown in the Figure 5 and Figure 6,

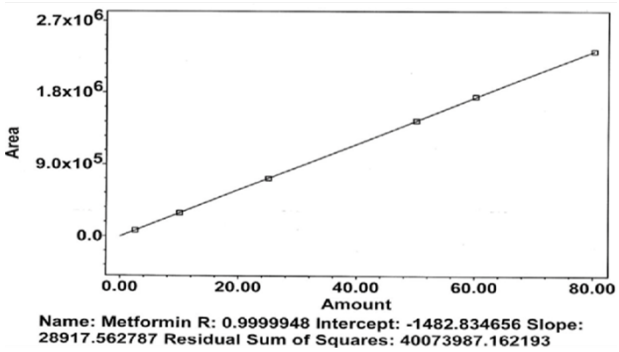


Figure 6: Calibration curve of Metformin

Table 2. (ICH Steering Committee, 1996)

Precision

Precision of a system is given as degree of closeness between the same quantity replicate measurements.

Method Precision

The precision was accomplished by taking six preparations as per the test protocol and evaluating the % assay and % RSD and was found to be within the limit as given in Table 3.

Intermediate Precision

To demonstrate the ruggedness of the method, test was conducted by variability of systems, analyst to analyst and day to day together by performing assay on six samples as per test Method.

The % Assay and % RSD was calculated for both the drugs and was found to be within the limit as given in Table 4.

Accuracy

Accuracy was executed by taking different levels of API concentrations i.e., 50%, 100% and 150% and spiked on three placebo preparations. The percentage recovery was within the limits as given in Table 5 and Table 6.

Robustness

The analytical method's robustness was achieved by altering various chromatographic parameters such as flow rate alterations of 0.8 ml/min and 1.2 ml/min, column temperature alterations of 35°C and 45°C, organic phase and aqueous phase ratios in mobile phase and buffer pH, i.e. pH 7.3 and 7.7, respectively. The findings are presented in Table 7, Table 8, Table 9.

Specificity

Specificity studies were carried for drug product and drug substance by comparing the plots with diluents, placebo and impurities.

Peak purity tests were also carried out. (Quadri *et al.*, 2014)

Forced degradation studies

FDS was carried out on Empagliflozin and Metformin, where the API are subjected to different stress conditions like Acid, Base, Oxidation, Thermal, Photolytic and Humidity. Acid stress was performed by adding 15 ml of 5 N HCl, heating for 16 Hour at 80°C for Empagliflozin, while, Metformin was stressed with 5N of HCl, heated for 1 Hour at 80°C. Base stress was done by adding 5ml of 5N NaOH, kept on bench top for 1 Hour for Empagliflozin, and 5ml 0f 5N NaOH heated at 60°C for 45 minutes for Metformin. Oxidative stress was done by adding 10ml and 5ml of 30% H_2O_2 for Empagliflozin and Metformin respectively and leave for 1 Hour on bench top. Hydrolytic stress was performed by addition of 5ml water and then warmed up at 80°C for 2 Hour for both the drugs. The drugs were stressed with heat at 105°C for 22 Hours and with humidity at 90% RH for 7 days at 25°C. Photolytic degradation was performed by exposing the drugs to visible light in the range 1.2 million Lux hours and UV light of 200 Watt hour/Meter square. The results are given in Table 10 and Table 11. (Rao *et al.*, 2015; Quadri *et al.*, 2014)

Assay of marketed formulation

Accurately weighed 10 tablets were powdered and the weight was taken equivalent to 5 mg and 500 mg of Empa and Met HCl respectively in 250 ml flask. Sonication was done for 60 mins after addition of 200 ml of diluent with occasional shaking. It is then made up to the mark with diluent and centrifuged for 15mins at 4000 rpm. The above solution is then diluted to get a concentration of 5μg/ml of Empa and 25μg/ml of Met.

CONCLUSION

The simultaneous method developed was tested in compliance with the guidelines of ICH Q2 (R1), and was within the limits. It can be assumed that the system is efficient, reliable and consistent and can be used to regular formulation testing.

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Conflict of Interest

None.

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