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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HCL AND EMPAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The present study describes the development and validation of new stability indicating RP-HPLC method for simultaneous estimation of Metformin Hcl and Empagliflozin in bulk and pharmaceutical dosage form. The chromatographic separation was done by using reverse phase Inertsil-ODS column- 3V (250x4.6mm; 5µm) . The mobile phase used was mixture of Ortho Phosphoric Acid (OPA) buffer (pH 3.2) : Methanol (70:30) at flow rate of 1ml/ min and detection was carried out using PDA detector at 254nm. And these were eluted at retention times of 1.970 and 2.979 min respectively.The developed method showed a good linearity in the range of 250-750µg/ml for Metformin Hcl and 2.5-7.5µg/ml for Empagliflozin with regression co-efficient of 0.999 and 0.999 for both the drugs. The proposed method was accurate, precise, robust, stable and specific. The developed method was validated in accordance with ICH guidelines.

KEYWORDS: Metformin HCl, Empagliflozin, RP-HPLC, methanol, Inertsil-ODS column.

INTRODUCTION TO DRUG PROFILES

Metformin Hcl: Chemically MET is N, N- dimethyl biguanide; hydrochloride.[1] It is biguanide class having hypoglycemic activity and used in treatment of diabetes mellitus.

Biguanide lowers blood glucose. They increase glucose uptake and utilization in skeletal muscle there by reducing insulin resistance and reduce hepatic glucose production (gluconeogenesis). Additionally MET reduces low density and very low density lipoprotein (LDL and VLDL, respectively).[2] It is soluble in water, 95% alcohol, practically insoluble in ether and chloroform. Metformin is a biguanide anti-hyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain.

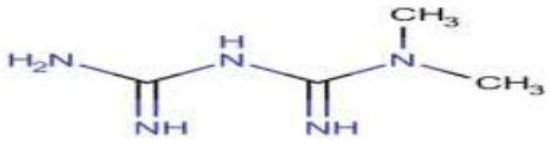

Fig. No. 1: Structure of Metformin HCL.

Empagliflozin

EMPA belongs to the class of Sodium glucose cotransporter-2 (SGLT-2) inhibitor. Empagliflozin chemically (1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4- ([S]-tetrahydrofuran-3-yl-oxy) benzyl]-benzen. It is very slightly soluble in water, soluble in methanol. And it is an orally administered selective sodium glucose cotransporter-2 (SGLT-2) inhibitor, which lowers blood glucose in people with type2 diabetes by blocking the reabsorption of glucose in the kidneys and promoting excretion of excess glucose in the urine. In patients with type 2 diabetes and hyperglycaemia a higher amount of glucose is filtered and reabsorbed. Empagliflozin improves glycaemic control in patients with type 2 diabetes by reducing renal glucose reabsorption. The amount of glucose removed by the kidney through this glucuretic mechanism is dependent on blood glucose.

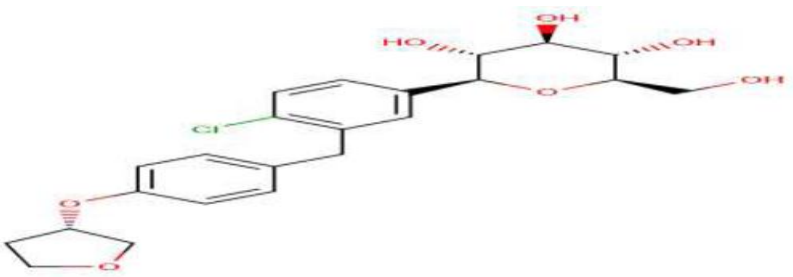

Fig No. 2: Structure of Empagliflozin.

MATERIALS AND METHODS

Hplc Instrumentation and Chromatographic Condition: The HPLC was carried out on the Waters with Empower 2695 separation module, auto Sampler and photo diode array (PDA) detector. Ultraviolet-visible spectrophotometer (Lab india), Electronic analytical balance (SHIMADZU- AY220 Max d=0.1mg), pH meter GLOBAL Digital pH meter (DPH-500) and sonicator (Frontline FS 4, Mumbai, India) were used.

Chromatographic Conditions

Instrument used: Waters HPLC with auto sampler and PDA detector

Mobile Phase: Ortho Phosphoric Acid (OPA) buffer (pH 3.2): Methanol (70:30)

Column: Inertsil-ODS-3V (250x4.6mm; 5µm)

Flow Rate: 1 ml/min

Column temperature: Ambient

Injection Volume: 10 µl

Detection wavelength: 254 nm

Runtime: 10 min

Chemicals and Reagents

HPLC grade potassium Di-hydrogen Orthophosphate, Methanol, HPLC grade analytical water, Di- Potassium hydrogen Phosphate, Ortho-Phosphoric Acid, Membrane filter paper-0.45µm were used.

Drug Samples

The working standard drugs, Metformin HCl and Empagliflozin of purity 99.5% and 100% respectively were obtained from Rainbow Pharma Train; Hyderabad, India. marketed formulation selected for the analysis was Synjardy containing Metformin HCl (500mg) and Empagliflozin (5mg) was purchased from local pharmacy and employed for study.

Preparation of Mobile Phase

Preparation of Ortho Phosphoric Acid (OPA) buffer: Transfer 2 ml of ortho phosphoric acid into 1000 ml of beaker dissolve and diluted volume with Water and adjust pH to 3.2. To prepare mobile phase: Transfer above solution 700ml of Ortho phosphoric Acid buffer was added to 300ml of Methanol, sonicated for 10 minutes, degassing and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of standard stock solutions of Metformin and Empagliflozin

Accurately weighed quantity of 500mg of Metformin and 5mg Empagliflozin was taken in a 100 ml volumetric flask, 10 ml of methanol added. The mixture was subjected to sonication for 10 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was make up to mark with mobile phase. From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with mobile phase.

Estimation of Pharmaceutical Formulation

For the analysis of drugs, 10 tablets were weighed and titurated in a glass mortar and quantity of powder equivalent to 841.92mg (500mg of Metformin and 5mg Empagliflozin) was transferred in to a 100mL volumetric flask and 10mL of methanol was added. Sonicated for about 10min and volume was made up to mark with mobile phase. To obtain a stock solution of 5000µg/mL of Metformin HCl and 500µg/mL of Empagliflozin. This solution was then filtered through 0.45µm membrane filter. Transfer 1mL of above solution in 10 ml volumetric flasks with mobile phase to get required test concentrations of 500µg/mL of Metformin HCl and 5µg/mL for Empagliflozin. This solution was injected 6 times into the column and chromatograms were recorded and respective peak areas were measured.

Development and Validation of Rp- Hplc Method for Metformin Hcl And Empagliflozin

The present study was conducted to obtain a new, rapid and sensitive cost-effective and convenient for Metformin and Empagliflozin in tablet dosage form. The proposed method was validated according to ICH guidelines. The method was validation of parameters like system suitability, accuracy, precision, linearity, specificity, and robustness, limit of detection (LOD) and limit of quantification (LOQ)

System Suitability: For assessing system suitability, six replicates of working standard samples were injected and studied the parameters like plate Number (N), Tailing Factor (K), Resolution (R), Relative Retention Time.

Linearity

The linearity of the method was determined by constructing calibration curves. Standard linearity curves were constructed for the component separately. Different volumes of stock solutions were accurately transferred into 10mL volumetric flasks and diluted to yield the concentration range250-750µg/ml for Metformin HCl and 2.5-7.5µg/ml for Empagliflozin.

Accuracy: The accuracy of an analytical method is the closeness of that results obtained by that method to the true value. The accuracy of the method was calculated at three intervals such as 50%, 100%, 150% of standard drugs were spiked to the test sample.

Precision

Precision of the method was determined with the standard and the test sample. System precision and Method precision was determined by injecting six replicates of the standard and drug sample solutions in to the chromatographic system. The retention times and peak areas of six replicates are recorded. The % RSD values were obtained within the limits.

Specificity

Specificity of the proposed method can be done by injecting blank and placebo using the chromatographic conditions. It was found that there is no interference due to blank and excipients in tablet formulation.

Robustness

The robustness was studied by analysing the same samples of Elbasvir and Grazoprevir to give unaffected results for small deliberate changes in system parameters and method parameters. Like Flow rate , Mobile phase composition, temperature variation was made to evaluated the impact on the method.

Limit of Detection (Lod) and Limit of Quantification (Loq)

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ)The limit of detection (LOD) limit of quantification (LOQ) of the drug calculated by using the following equation as per international conference harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

σ = standard deviation

S = slope.

RESULTS AND DISCUSSIONS

Optimized Chromatographic Condition

The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the simultaneous analysis of Metformin HCl and Empagliflozin in bulk and in

pharmaceutical dosage forms. In order to effect separation of drugs and the reference standard under isocratic conditions, mixtures of phosphate buffer and Acetonitrile in different combinations were tested as mobile phase on Inertsil ODS column-3V (250x4.6mm; 5µm) analytical column. A binary mixture of Ortho Phosphoric Acid (OPA) buffer (pH 3.2) : Methanol, 70:30 v/v proportion was proved to be most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing and fronting. The retention times obtained for Metformin HCl was 1.970 and for Empagliflozin were 2.979. Hence the optimized conditions were taken into consideration for the method

Estimation of pharmaceutical formulation

The formulation solution with the required test concentrations is prepared and injected to determine the assay of the marketed formulation. The results were tabulated as below.

Table No.1: Results of marketed Formulation Analysis:

Drug	Labelled claim (mg)	% Assay
Metformin HCl	500mg	100.61%
Empagliflozin	5mg	99.34%

System Suitability

This test was carried out to find out the resolution and reproducibility of the system for the analysis. The total results of system suitability studies summarized in Table 1. In this studies %RSD value of retention times, peak areas, asymmetry and theoretical plate count were found to be less than 2% for Metformin HCl and Empagliflozin respectively.

Table No.2: System suitability parameters.

S. No	Parameter	Metformin Hcl	Empagliflozin	Acceptance criteria
1	Retention time	1.968	2.978	……
2	Theoretical plates	3366	3688	>2000
3	Tailing factor	1.28	1.19	<2.00
4	Resolution	……	5.97	>1.5

Linearity and Range

In order to test the linearity of the method, dilutions of the working standard solutions of drugs were prepared in the range of 250-750µg/ml and 2.5-7.5µg/ml for Metformin HCl and Empagliflozin. A good linear relationship (R^2 = 0.999) was observed between the concentrations of Metformin HCl and Empagliflozin and the corresponding peak areas.

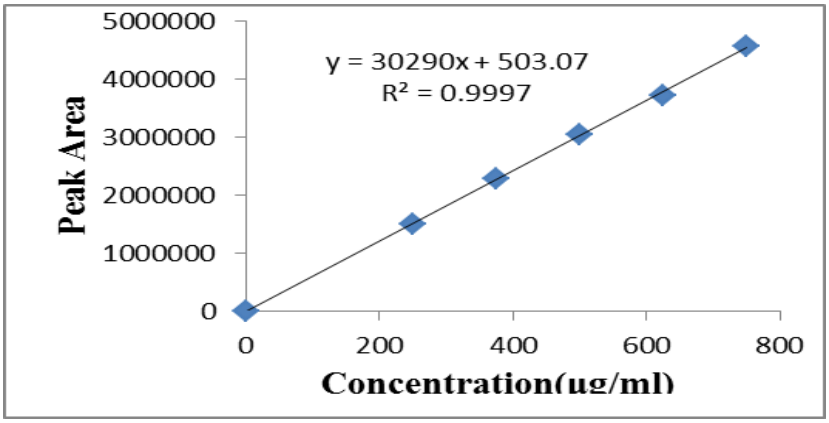

Fig. No.1: Linearity curve for Metformin.

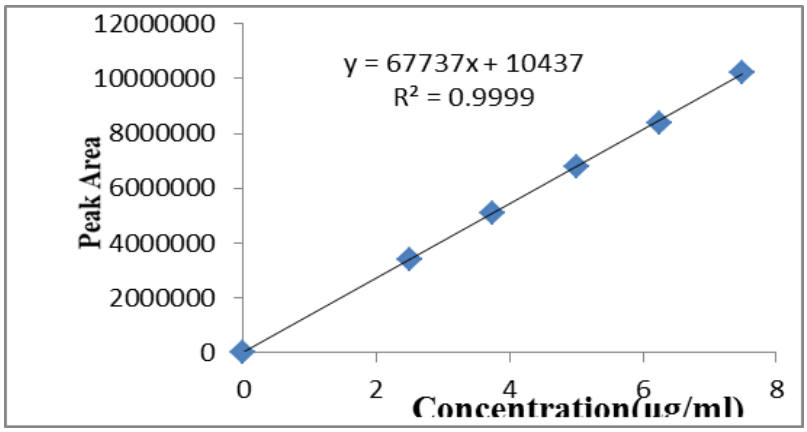

Fig. No.2: Linearity curve for Empagliflozin.

Accuracy: The recovery studies were carried out at three intervals and the percentage recovery and percentage relative standard deviation of the recovery were calculated. The drug content in the formulation was quantified using the proposed method of analysis and the mean amount of Metformin HCl and Empagliflozin obtained in dosage form were in the range of 100.02% – 99.38% and the accuracy were tabulated below.

Table.No.3: Accuracy (%Recovery) data for Metformin HCl.

S.NO	Recovery Level	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean	SD	%RSD
1	50%	247.500	247.42	99.96	100.02	0.10	0.09
		247.500	247.89	100.15			
		247.500	247.42	99.96			
2	100%	495.000	496.70	100.34	100.33	0.04	0.03
		495.000	496.42	100.28			
		495.000	496.87	100.37			
3	150%	742.500	732.46	98.64	99.71	0.96	0.96
		742.500	746.23	100.50			
		742.500	742.500	100			

Table.No.4: Accuracy (%Recovery) data for Empagliflozin.

S. No	Recovery Level	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean	SD	%RSD
1	50%	2.500	2.49	99.6	99.46	0.23	0.23
		2.500	2.49	99.6			
		2.500	2.48	99.2			
2	100%	5.000	4.97	99.4	99.33	0.11	0.11
		5.000	4.96	99.2			
		5.000	4.97	99.4			
3	150%	7.500	7.46	99.46	99.37	0.07	0.07
		7.500	7.45	99.33			
		7.500	7.45	99.33			

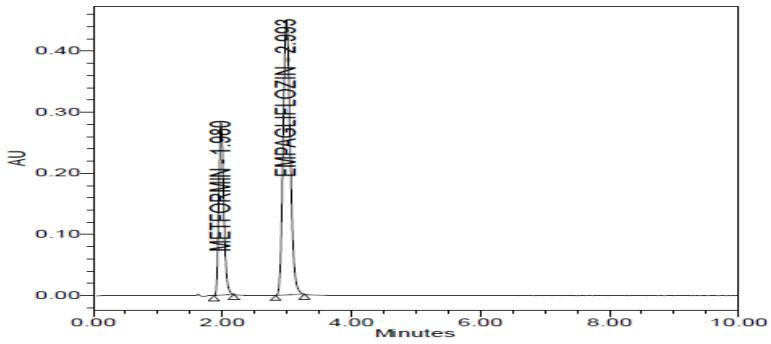

Fig.No.4: 50% Recovery level of MET and EMPA.

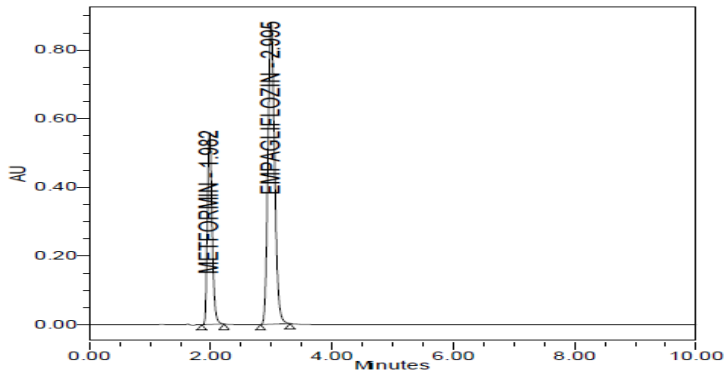

Fig.No.5: 100% Recovery level of MET and EMPA.

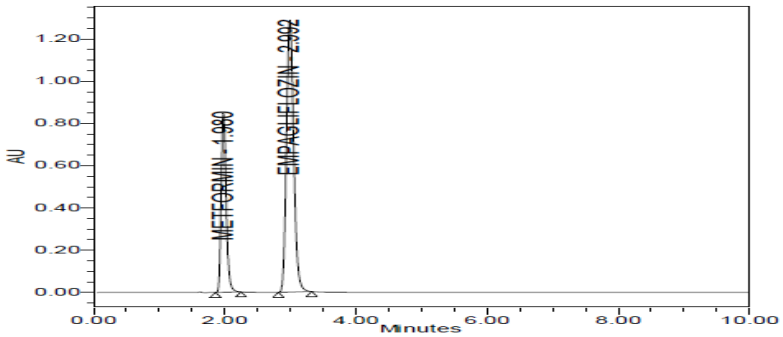

Fig.No.6: 150% Recovery level of MET and EMPA.

METHOD PRECISION

The solution of same preparation was injected for 6 times in 6 different vials and the peak areas along with % assay were observed and the results were tabulated as folows.

Table No.5: Method precision of Metformin HCl and Empagliflozin.

S.No	Conc(µg/ml)		Peak area (n=6)		% Assay	
	MET	EMPA	MET	EMPA	MET	EMPA
1	500	5	3030592	6812728	100.03	100.42
2	500	5	3047349	6802805	100.58	100.27
3	500	5	3051205	6801060	100.71	100.24
4	500	5	3058051	6806079	100.94	100.32
5	500	5	3049714	6816555	100.66	100.47
6	500	5	3053532	6809260	100.79	100.37
Mean			3048407	6808081	100.61	100.34
SD			9458.36	5931.81	0.31	0.08
% RSD			0.31	0.08	0.30	0.07

Robustness

The robustness of the method is deterrmine under normal operating conditions different conditions such as change in flow rate and temperature. 10µl volume of sample solutions are injected the flow rate (0.8ml/min, 1.0ml/min and 1.2ml/min) and temperature (20^{o}C, 30^{o}C, 40^{o}C), and the chromatograms are recorded and changes in parameters are observed. The results for robustness were tabulated below.

Table No. 6: Robustness studies of Metformin HCl and Empagliflozin.

Parameter	Condition	Retentiontime (min)		Plate count		Tailing	
		MET	EMPA	MET	EMPA	MET	EMPA
Flow rate	0.8ml/min	1.980	2.993	3145	3534	1.22	1.16
	1.0ml/min	1.970	2.979	3105	3563	1.27	1.20
	1.2ml/min	1.971	2.979	3245	3594	1.22	1.17
Temperature	20^{o}C	1.981	2.997	3194	3533	1.22	1.16
	30^{o}C	1.970	2.979	3105	3563	1.27	1.20
	40^{o}C	1.967	2.973	2987	3442	1.24	1.18

Limit of detection & limit of quantification

The standard solutions of Metformin and Empagliflozin were prepared in known concentrations to determine the LOD and LOQ, where the LOQ values are three times to LOD values obtained. LOD and LOQ of the method were calculated basing on standard deviation of the response and the slope(s) of the calibration curve. The results obtained were tabulated below.

Table No. 7: LOD and LOQ Parameters of Metformin and Empagliflozin.

Parameter	Metformin				Empagliflozin			
	µg/ml	Area	Rt	Plate count	µg/ml	Area	Rt	Plate count
LOD (µg/ml)	0.445	22313	1.974	3011	0.0029	35462	2.982	4152
LOQ (µg/ml)	1.485	82818	1.972	3109	0.0095	183993	2.980	3522

CONCLUSION

A simple and sensitive stability-indicating RP-HPLC method was explored for the simultaneous determination of Metaformin HCL and Empagliflozin in pure form and in commercially available tablet dosage forms. The method was validated as per ICH guidelines. The proposed stability indicating RP-HPLC method has been evaluated over the accuracy, precision, linearity and LOD and LOQ. The method proved the selectivity, precision, accuracy and mobile phase used to provide simple and economic application to be more convenient and effective for the quality control and identity to Metaformin HCL and Empagliflozin in pharmaceutical dosage forms.

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