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Research article

Development and validation of RP-HPLC method for Empagliflozin and Metformin HCL

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Key words: Empagliflozin, Metformin HCL, RP-HPLC, Effect of pH, Analytical method validation.

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Abstract

A simple, specific and accurate RP-HPLC method was developed and validated for simultaneous determination of Empagliflozin and Metformin hydrochloride in bulk and Pharmaceutical dosage form. Chromatographic separation was carried out on Grace C18 column (250×4.6mm, 5µm), Methanol: Water pH 3.0 in the ratio of 80:20 % v/v, Flow rate is 0.8 ml/min and UV detection at 227nm.The Retention times of Empagliflozin and Metformin hydrochloride were found to be 2.630 min and 5.133min, respectively. The validation parameter studied were linearity, accuracy, precision, specificity and robustness i.e. pH of the mobile phase on the retention behavior. The proposed method was validated as per ICH guideline.

Introduction

Chemically MET is N, N- dimethyl biguanide [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].

EMPA belongs to the class of Sodium glucose co-transporter-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 an orally administered selective sodium glucose cotransporter-2 (SGLT-2) inhibitor, which lowers blood glucose in people with type 2 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 concentration and GFR. Inhibition of SGLT2 in patients with type 2 diabetes and hyperglycaemia leads to excess glucose excretion in the urine [3].

Literature survey revealed several quantitative analytical method for simultaneous estimation of MET and EMPA in pharmaceutical formulation [5-13].

The aim of the present paper was to develop a new and validated RP-HPLC method for simultaneous estimation of these two antidiabetic drugs, MET and EMPA in tablet formulation.

Experimental

Material and Methods

Chemical and Reagents

Pharmaceutical grade Empagliflozin batch no.CRD/1141/3/15/193 and Metformin hydrochloride PRL-3/1246/002/17/2 were kindly supplied as gift sample from Macleod Pharmaceutical Pvt. Ltd. Daman (Gujrat). Methanol, Water and Phosphoric acid used in analysis were of HPLC grade and all chemicals and reagents were purchased from SD fine chemicals Mumbai, Maharshtra. Mobile phase used after filtering through 0.45µ membrane filter paper purchased from Millipore (India) Pvt. Ltd., Bengaluru, Karnataka. Empagliflozin and Metformin hydrochloride containing pharmaceutical dosage forms were prepared in house.

Instrumentation and chromatographic condition

Chromatographic analysis was carried out using an HPLC system consisting of pump P-3000-M Reciprocating (40MPa)equipped with 100 µL Rheodyne loop injector (7725*i*) and detection was carried out on UV-3000-M detector (JASCO Corporation, Tokyo, Japan) using HPLC Workstation Chromatography software. The mobile phase composed of methanol: Water (pH 3) 80:20 v/v. Isocratic elution was carried out on Grace C18 (250×4.6×5µ) at flow rate of 0.8 ml/min. the wavelength was fixed at 227 nm.

Preparation of standard solution
Quantity equivalent 10 mg of Empagliflozin and Metformin hydrochloride were weighed and transferred to 10 ml volumetric flask and volume made up to the mark with methanol. The resulting solution were of 1000 µ/ml. Further dilution take 0.01 ml of EMPA and 0.68 ml of MET volume make up to 10 ml.

Calibration curve standard
The prepared standard stock solution of Empagliflozin and Metformin hydrochloride (1000 µg/mL) was appropriately diluted with Methanol : Water 80:20 to get five different working standard solutions for Metformin hydrochloride of concentrations; 68, 136, 204, 272 340 µg/mL, and for Empagliflozin 1, 2, 3, 4, 5 µg/mL, respectively. The calibration curves were analyzed in triplicates and the mean peak area were plotted on *y axis* against concentration on *x axis*. The intercept, slope and co-efficient of regression were determined.

Method validation
Linearity, Range
Different concentration each of Empagliflozin and Metformin hydrochloride in the range of 1-5 µg/mL and 68-340 µg/mL, respectively.
The solutions were injected to the HPLC-UV system for analysis. Average peak area at each concentration level was subjected to linear regression analysis with the least square method. Linearity was described by slope, intercept and correlation coefficient obtained from regression equation [1, 6, 14].

Precision
The intra-day precision was assessed by performing three analyses using standard stock solution containing 4 µg/mL of Empagliflozin and 272 µg/mL Metformin hydrochloride. Similarly inter-day precision was assessed by performing replicate analysis using same concentration of all the analytes for three consecutive days under the same experimental conditions. The mean of percentage recovery and % RSD were calculated [1, 6, 14].

Accuracy
The accuracy of the method was determined by standard addition technique. Three different levels (50,100 and 150%) of standards were added to formulation containing Empagliflozin and Metformin hydrochloride. the percentage recoveries of all the compounds at each level and each replicate were determined. The mean of percentage recoveries and % RSD was calculated [1, 6, 14].

Robustness
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness was determined by analyzing the sample solution containing 5 µg/mL each of Empagliflozin and 340 µg/mL Metformin hydrochloride under variety of conditions of the method parameters, such as flow rate, pH of mobile phase. The mean of percentage recoveries and the % RSD was calculated from three replicates [1, 6, 14].

Specificity
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.
It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination). In this case a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination
The samples were chromatographed to determine the extent to which the mobile phase components and excipients could contribute to the interference with analytes [1, 6, 14].

Analysis of marketed formulation
For analysis of tablet formulation, tablet containing 68 mg of MET and 1 mg of EMPA were prepared inhouse. Twenty tablets were weighed and finely powdered. A tablet power equivalent amount was transfer to 100 ml of volumetric flask and shaken with methanol: water 1:1 for 10 min. After filtration the excipients were separated and the volume was made up to the 100 ml with same solvent. From this stock solution, suitable aliquot was diluted with mobile phase to get concentration of 68 µg/ml of MET and 1 µg/ml of EMPA and subjected to chromatographic analysis. From the calibration curve standards the amount of MET and EMPA was estimated [4, 14].

Result and Discussion

Optimization of HPLC-UV conditions:
Using isocratic mobile phase composition of methanol: water (80:20 *v/v*) at a flow rate of 0.8 ml/min gave good peak shapes with short separation times. The retention time was found to be 2.6 min for Metformin hydrochloride and 5.4 min for Empagliflozin with total chromatographic run time of 8 min. chromatogram obtained from mix standards (68 µg/ml and 5 µg/ml) for MET and EMPA respectively.

Table 1. Summary of validation parameters of proposed RP-HPLC Method Parameters

Parameter	EMPA	MET
Theoretical plates	**8209**	**5680**
Asymmetry factor	**0.99**	**1.06**
Resolution	**4.62**	

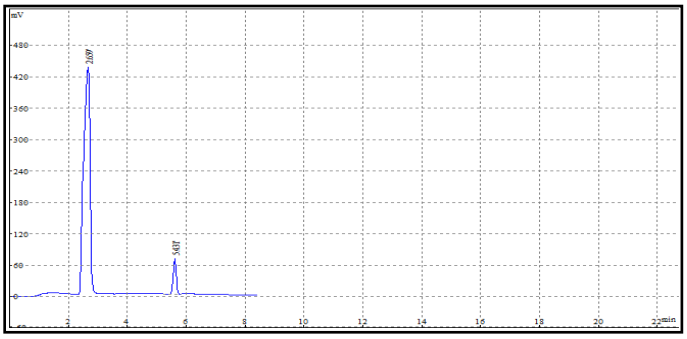

Figure 1. Representative Chromatogram 0f MET (2.6 min) and EMPA (5.4min).

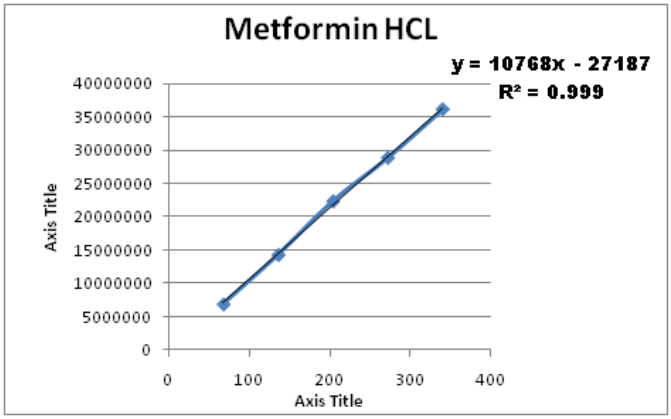

Figure 2. Calibration curve of MET.

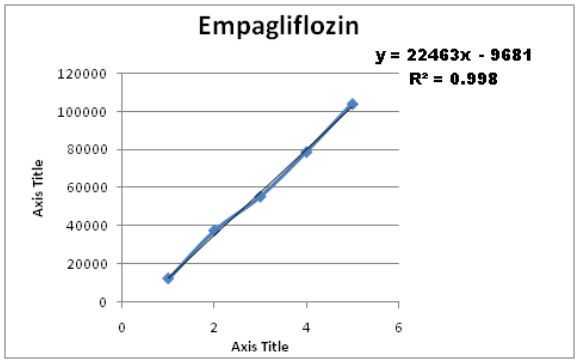

Figure 3. Calibration curve of EMPA.

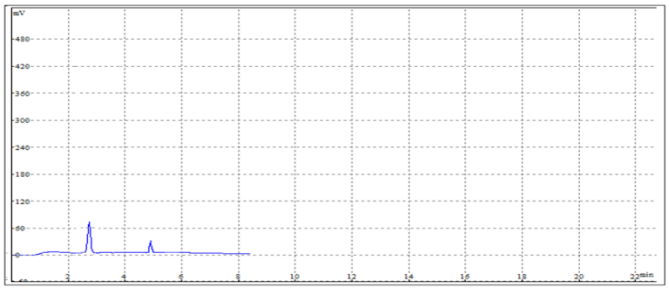

Figure 4. Robustness High Flow (2.5 MET, 3.5 EMPA).

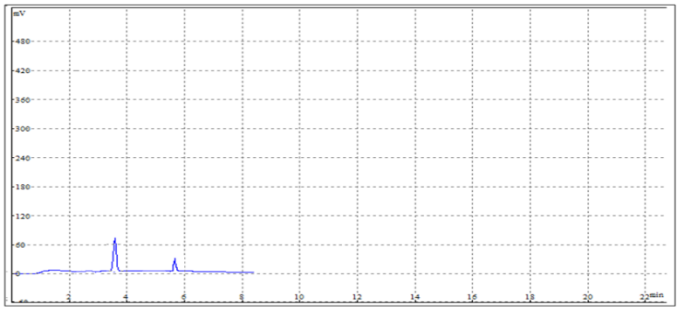

Figure 5. Robustness Low Flow (2.8 MET, 5.9 EMPA).

Table 2. Accuracy study for EMPA and MET

Sr. No.	Conc. EMPA	SD	% SD	% RSD	Conc. MET	SD	% SD	% RSD
	1				68			
1	1	76	0.614	0.614	68	39464.7	0.583	0.583
	1				68			
	3				204			
2	3	366.127	0.645	0.645	204	144320.1	0.625	0.625
	3				204			
	5				340			
3	5	443.744	0.403	0.403	340	303162.1	0.816	0.816
	5				340			

Table 3. % Recovery of EMPA

Sr No.	Level of Spiking	Concentration Added	Total Concentration	Concentration Found	% Recovery	SD	% RSD
1	50%	1	3	2.8772	99.11	53.144	0.0012
2	100%	2	4	3.9273	99.5647	412.8	0.0052
3	150%	3	5	5.1773	102.3955	3154.7	0.0295

Table 4. % Recovery of MET

Sr No.	Level of Spiking	Concentration Added	Total Concentration	Concentration Found	% Recovery	SD	% RSD
1	50%	68	204	203.1409	100.3035	22891.6	0.0069
2	100%	136	272	271.4566	99.6540	90575.7	0.0314
3	150%	204	340	339.7980	99.9078	94282.3	0.0261

Table 5. Robustness of EMPA at different flow rate and pH

Sr. no	Flow rate	Retention Time	Area	Plates	Asymmetry	% RSD
1	**0.8**	5.9	104124	8209	0.99	0.63
2	**1**	3.5	103306	3226	0.76	0.52
Sr. no	**pH**	**Retention Time**	**Area**	**Plates**	**Asymmetry**	**% RSD**
1	**3**	5.431	104124	8209	0.99	0.64
2	**4.5**	5.464	103306	8155	0.98	0.52

Table 6. Robustness of MET at different flow rate and pH

Sr. no	Flow rate	Retention Time	Area	Plates	Asymmetry	% RSD
1	**0.8**	2.853	3616432	5731	0.58	1.49
2	**1**	2.518	3615769	3226	0.77	0.16
Sr. no	**pH**	**Retention Time**	**Area**	**Plates**	**Asymmetry**	**% RSD**
1	**3**	2.991	3616433	4225	0.66	1.03
2	**4.5**	2.990	3615769	3226	0.76	0.18

Table 7. Precision study of EMPA and MET

Sr. No.	Precision	EMPA % RSD	Drug found (mg)
1	**Interday**	1.13%	3.872116
	Intraday	1.54%	3.868116
		MET	
	Precision	**% RSD**	**Drug found (mg)**
2	**Interday**	0.76%	271.4292
	Intraday	1.88%	268.573

Table 8. Summary of validation parameters of proposed RP-HPLC Method

Parameter	EMPA	MET
Linearity Range (µg/mL)	**1-5**	**68-340**
Correlation co-efficient	**0.998**	**0.999**
Slope (m)	**22463**	**10768**
Accuracy (% Recovery)	**100.34**	**99.95**
Precision (% RSD)		
Interday	**1.13**	**0.76**
Intraday	**1.54**	**1.88**
Robustness		
Different flow rate	**0.52**	**0.16**
Different pH	**0.52**	**0.18**

The results obtained for accuracy and precision are summaries in table 2 and table 7 for MET and EMPA respectively. Mean values of concentration found were close to the concentration added and low values of % RSD indicates the acceptable accuracy and precision of the method.

When blanked tablets were analyzed as per the mentioned chromatographic conditions, No peak was obtained at the retention times of MET and EMPA.

During robustness studies it was observed that there was no significant effect on number of therotical plate, asymmetry and retention times of EMPA (last eluted peak) by small but deliberate changes in flow rate and change in pH.

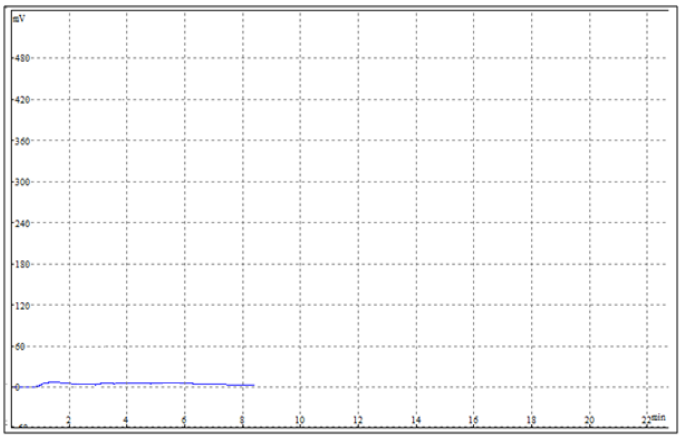

Figure 6. Blank and Placebo.

Analysis of the marketed formulation

The chromatograms of the drug samples extracted did not show any change in the retention time. There was no interference from excipients, which are commonly present in the tablets. The drug content was found to be % with a % RSD for Empagliflozin and Metformin hydrochloride, respectively as shown in Table 9. Therefore it was concluded that, Empagliflozin and Metformin hydrochloride not affected by the excipient in formulation. The % RSD value indicated the suitability of

the method for the routine analysis of Empagliflozin and Metformin hydrochloride in marketed formulation.

Table 9. Amount found in Formulation

Amount per tablet(mg)		Amount Found (mg/mL)		% Found	
MET	**EMPA**	**MET**	**EMPA**	**MET**	**EMPA**
68	1	66.67	0.991	99.68	99.71

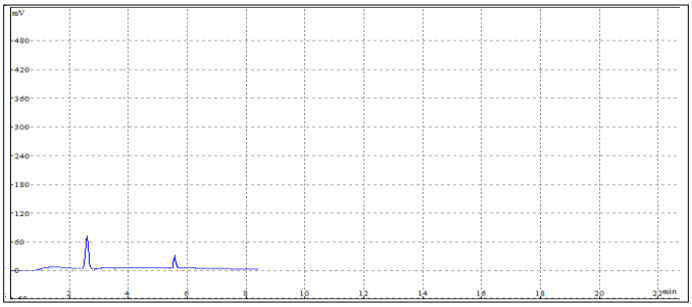

Figure 7. Chromatogram of Metformin Hydrochloride and Empagliflozin tablet formulation.

Conclusion

A new simple and accurate method was developed for simultaneous estimation of MET and EMPA in tablets all parameter of validation are within the acceptable range .The developed method is specific as there was no any interfering peak at the retention time of the drug. Hence the developed method was accurate and can be used for routine analysis of MET and EMPA in tablet.

Drawbacks

We developed this method at RAP analytical lab. We found that UPLC & such other techniques can be used to improve the sensitivity, speed and more accuracy of developed analytical method.

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Conflict of Interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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