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METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF METFORMIN HCL AND EMPAGLIFLOZIN IN ITS BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-ULTRA PERFORMANCE CHOMATOGRAPHY METHOD

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ABSTRACT

A simple, rapid and sensitive analytical procedure was developed for the simultaneous estimation of Metformin and Empagliflozin by RP-UPLC method in bulk drug and pharmaceutical dosage form. The quantification was carried out isocratically using BEH C18 column (2.5×50mm) 3µ with a flow rate 0.3 ml/min. The mobile phase comprises of 70:30 v/v Acetonitrile: phosphate buffer pH: 3. the eluents were detected by UV detector at 220 nm. The retention times for Metformin and Empagliflozin were found to be 0.879 mins and 1.294 mins respectively. The method was validated as per ICH guidelines for linearity, accuracy, precision and robustness. The developed method shows good linearity over the concentration range of 50µg/ml-150µg/ml and 5µg/ml-25µg/ml with the average percentage recoveries were in the range of 99.56% and 99.48% for Metformin and Empagliflozin respectively. The limits of detection (LOD) value were 0.12 and 0.015 ppm and whereas limits of quantification (LOQ) value was 0.42 and 0.05 ppm for Metformin and Empagliflozin respectively. Therefore, the proposed method can be applied for routine analysis of the bulk drugs as well as combined pharmaceutical dosage forms of Metformin and Empagliflozin.

KEYWORDS: **Reverse phase Ultra Performance liquid chromatography, Metformin, Empagliflozin, method development and method validation.**

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INTRODUCTION

Metformin is chemically known as 1, 1-dimethyl biguanide hydrochloride (fig 1) a biguanide derivative which is the most commonly prescribed drug to the patients with type-2 diabetes.[1] it bring down the blood glucose levels by decreasing the hepatic glucose production, declining intestinal absorption of glucose and enhancing insulin sensitivity by elevating peripheral glucose utilization and uptake. [2, 3] Empagliflozin chemically 1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran -3- yl -oxy) benzyl]-benzene (fig.2) which belongs to gliflozin class used in the treatment of type-2 diabetes.[4] Empagliflozin is an inhibitor of the sodium glucose co transporter -2 (SGLT-2) thus SGLT-2 reduces blood glucose by blocking glucose reabsorption in the kidney and thereby excreting glucose (i.e., blood sugar) via the urine.[5] As per literature a wide variety of analytical methods were published for the determination of empagliflozin and Metformin in pure drug, pharmaceutical dosage forms and in biological samples by using High performance liquid chromatography[6] , UV- Spectrophotometry [7,8]. But there are very few methods found performed by using RP-UPLC simultaneous estimation of Metformin and Empagliflozin in API, therefore, the present study investigate the application of method development and validation of RP-UPLC method for the simultaneous estimation of Metformin and empagliflozin in API. Therefore, the proposed method can be applied for routine analysis of the bulk drugs as well as combined pharmaceutical dosage forms with lower retention time and flow rate and high accuracy and precision.

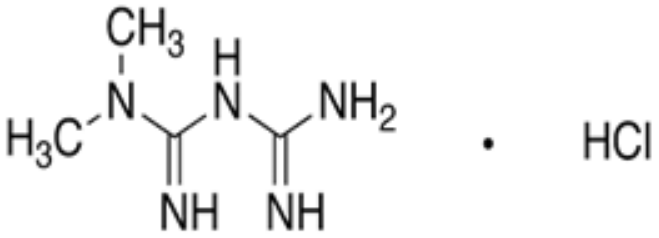

Figure 1
Structure of Metformin Hydrochloride

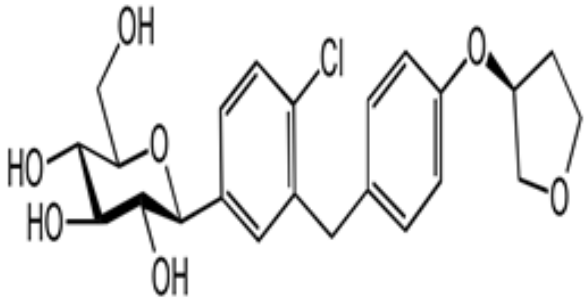

Figure 2
Structure of Empagliflozin

MATERIALS AND METHODS

Instrumentation

The chromatography was performed on Acquity UPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 220 nm with a BEH C18, 50mmx2.5mm, (3µm) dimensions at ambient temperature. The mobile phase was 70:30 (v/v) mixture of Acetonitrile: phosphate buffer pH: 3 delivered at a flow rate of 0.3 mL/min. The column was maintained at 25°C and the injection volume of 10 µL.

Chemicals and reagents

Working reference standards of empagliflozin and Metformin were gifted by Mylon laboratories. HPLC grade Acetonitrile and water were purchased from Merck and analytical grade KH_2PO_4 and Orthophosphoric acid were supplied by Finer chemical LTD and Merck, respectively.

Preparation of standard and sample solution

Preparation of standard solution

A standard solution containing 1500 µg/ml of Metformin and 15 µg/ml of Empagliflozin were prepared by dissolving empagliflozin and Metformin Hcl in 70:30Acetonitrile and phosphate buffer (P^H 3.0).

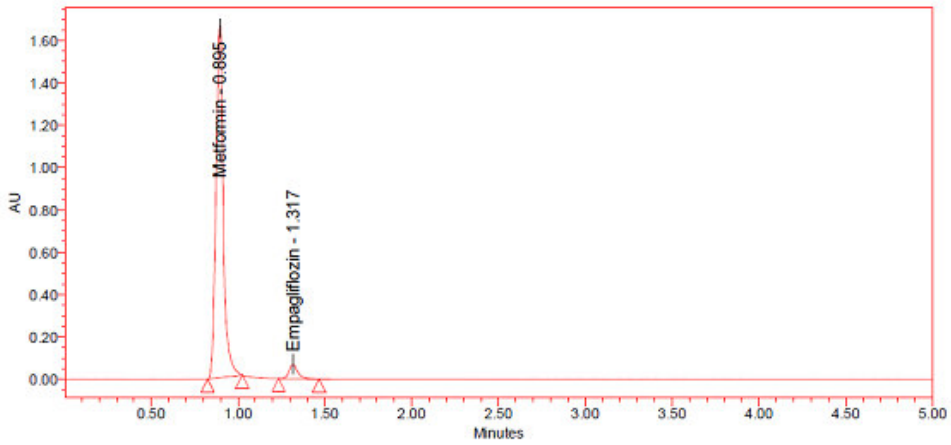

Figure 3
Chromatogram showing standard injection

Preparation of Sample solution
ten tablets, containing each 1000 mg of Metformin Hcl and10 mg of empagliflozin were weighed to determine the average weight individually and powdered in a mortar. Quantity of powder equivalent to 1000mg of Metformin Hcl and 10 mg of empagliflozin were weighed, transferred 100 ml dry volumetric flask and initially 70 ml of diluent was added and sonicated for 30 minutes with shaking and finally made up to the volume with diluent.

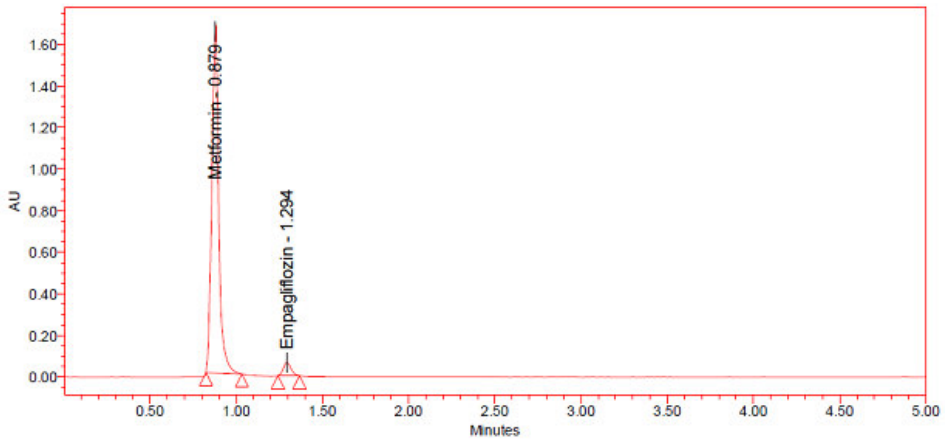

Figure 4
Chromatogram showing assay of sample injection

Procedure for calibration curve
The calibration curve of the proposed method was constructed by considering concentration on X-axis and peak area on Y-axis. For the both methods % RSD was found to be within the acceptable limits ≤ 2.

RESULTS AND DISCUSSION

Validation procedures
The developed method was validated for linearity, accuracy, precision, and limit of detection, limit of Quantitation, and system suitability parameters as described in ICH guidelines.

Linearity
The response for the detector was determined to be linear over the range of 500-3000 µg/ml for Metformin and 5-25 µg/ml for empagliflozin. Each of the concentration was injected in duplicate to response. The linearity range of the method was found more accurate and precise over the reported methods. And the results for calibration curves were shown in fig 6 & 7 Metformin and empagliflozin respectively.

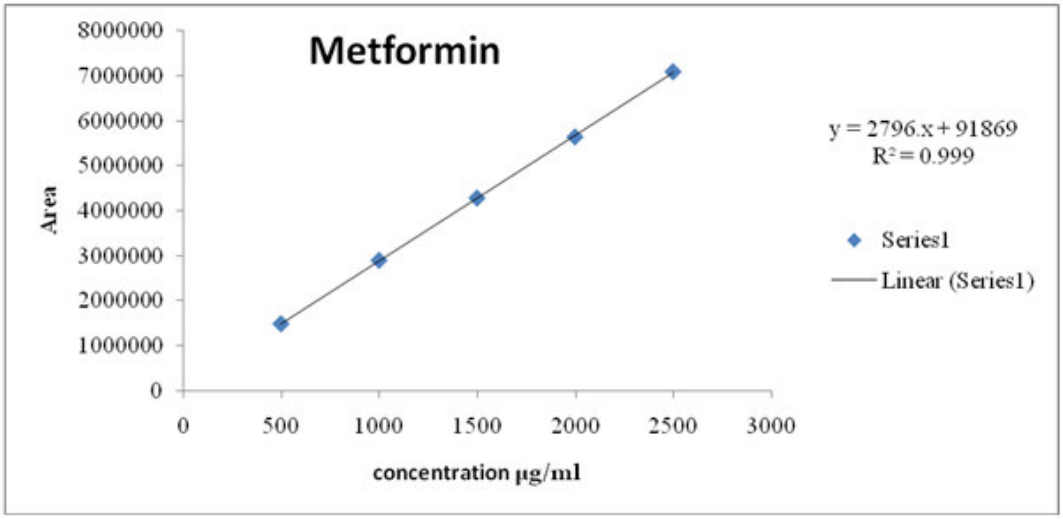

Figure 5
Calibration graph for metformin

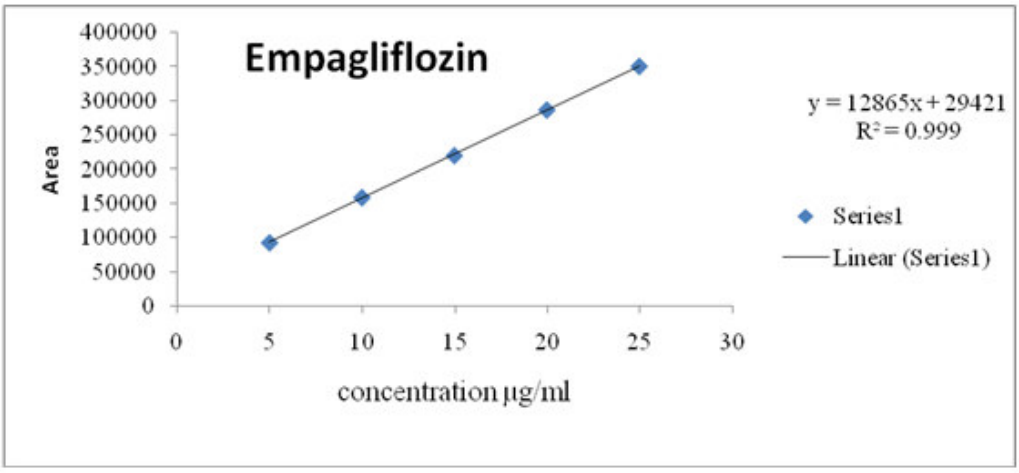

Figure 6
Calibration graph for Metformin

Table 1
Linearity results for Metformin

S.no	Linearity level	Concentration (ppm)	Peak Area
1	I	500	1487625
2	II	1000	2900790
3	III	1500	4287985
4	IV	2000	5651773
5	V	2500	7102614

Table 2
Linearity results for Empagliflozin

S.no	Linearity level	Concentration (ppm)	Peak Area
1	I	5	93475
2	II	10	159362
3	III	15	220699
4	IV	20	287360
5	V	25	351109

Accuracy

Accuracy expressed as standard deviation. Accuracy was evaluated by analyzing six different standard samples at each of 50%, 100%, 150% concentrations on same day. The accuracy study was performed for % recovery of Metformin and empagliflozin. The % recovery was found to be 99.18% and 99.91% (NLT 98% and NMT 102%) Metformin and empagliflozin

respectively.

Precision

The intraday precision were calculated by analyzing six different standard samples at each low, medium and high concentrations on the same day. Intraday precision expressed as relative standard deviation, The results are depicted in table 1&2.

Table 3
Showing% RSD results for metformin and empagliflozin

S.No	Metformine		Empagliflozin	
	Area	RT	Area	RT
1	4669547	0.866	205555	1.277
2	4633682	0.870	213714	1.281
3	4711857	0.873	202403	1.285
4	4586290	0.873	191233	1.286
5	4690109	0.874	197507	1.287
6	4674377	0.876	205189	1.290
Mean	***4660977.0***		***192600.0***	
Std. Dev	***44751.8***		***13583.0***	
% RSD	***1.0***		***1.1***	

LOD & LOQ

Limit of detection was obtained by injecting each standard sample solution at different concentrations to get the signal to noise ratio ≥3 which was found to be 52.86 µg/ml and 3.55 µg/ml for Metformin and empagliflozin respectively. Further, limit of quantification, with a signal to noise ratio ≥10 was found to be 160.01 µg/mL and 10.77µg/mL for Metformin and empagliflozin respectively shown in table.

System suitability

The robustness of the method is determined under normal operating conditions different conditions such as change in mobile phase and flow rate and detection wave length. 10 µl of standard and sample solutions are injected with different mobile phases (less, normal and more organic phases) the flow rate (0.24 ml/min, 0.30 ml/min, 0.36 ml/min) and the chromatograms are recorded and changes were observed in parameters no much variation noticed.

CONCLUSION

The newly developed UPLC method of Metformin Hcl and Empagliflozin was found to be novel precise, simple, accurate and effective method. The assay determination was found to be faster retention time with good resolution in present study. Hence the developed method is suitable for routine analysis I individual and combined dosage forms.

CONFLICT OF INTEREST

Conflict of interest declared none.

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