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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF EMPAGLIFLOZIN AND METFORMIN HCl

Avani M. Patel* and Ankit B. Chaudhary

Saraswati Institute of Pharmaceutical Sciences, Dhanap, Gandhinagar, Gujarat, India.

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***Corresponding Author**
Avani M. Patel
Saraswati Institute of Pharmaceutical Sciences, Dhanap, Gandhinagar, Gujarat, India.

ABSTRACT

One simple, specific, accurate, precise and reproducible and robust method have been developed and validated for the Simultaneous Estimation of Empagliflozin and Metformin HCl. In Reverse Phase High Performance Liquid Chromatography Method the chromatographic system was equipped with Inertsil ODS-2 (250mm x 4.6 mm, 5µ) as stationary phase and UV detector set at 227 nm, in conjunction with a mobile phase of 0.05M Potassium Dihydrogen Phosphate buffer (pH- 3.5, adjusted with 1% Orthophosphoric acid) and Acetonitrile in the ratio of 50:50% v/v at a flow rate of 1.0 ml/min. The described method was linear over a concentration range of 2.5- 18.75 µg/ml and 25-75µg/ml for Empagliflozin and Metformin HCl respectively. The retention time of Metformin HCl and Empagliflozin were 2.635 min and 4.388 min respectively. The % recoveries of Empagliflozin and Metformin HCl were found to be 99.60% - 100.48% and 100.15% - 100.23% respectively. Method was statistically validated for accuracy, precision, specificity, LOD, LOQ and robustness according to ICH guidelines. Developed method was found to be new, accurate, precise and rapid for simultaneous estimation of for Empagliflozin and Metformin HCl.

KEYWORDS: Empagliflozin and Metformin HCl, Stability indicating, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Empagliflozin, is chemically designated 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 has the

molecular wt. 450.91 (figure-1). Empagliflozin is a 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.

SGLT2 co-transporters are responsible for reabsorption of glucose from the glomerular filtrate in the kidney. The glucuretic effect resulting from SGLT2 inhibition reduces renal absorption and lowers the renal threshold for glucose, therefore resulting in increased glucose excretion. Additionally, it contributes to reduced hyper glycaemia and also assists weight loss and blood pressure reduction.[1] Various analytical methods have been reported for the estimation of Empagliflozin as alone as well as in combination with other drugs. They include spectrophotometric methods[2,3] HPLC.[4-6]

Metformin Hydrochloride, is chemically designated as 1-carbamimidamido-N,N-dimethylmethanimidamide and has the molecular wt. 165.625 (figure-2). 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[7] Various analytical methods have been reported for the estimation of Metformin Hydrochloride as alone as well as in combination with other drugs. They include Titrimetric method[8], spectrophotometric methods[9-11], HPLC[12-14] However an extensive literature search didn't reveal any estimation method for Empagliflozin and Metfromin hydrochloride drugs in their combined dosage form. Therefore, attempt was made to stability indicating method development and Validation of simple, precise, and accurate, RP-HPLC method for simultaneous determination of Empagliflozin and Metfromin hydrochloride drugs in their combined dosage form.

MATERIALS AND METHOD

Reagents and Chemicals

Empagliflozin and Metformin hydrochloride were obtained as gift samples from Jigs Chemicals, Ahmedabad and Tuton pharma Ahmedabad respectively. HPLC grade Acetonitrile, Methanol, Water and Ortho-phosphoric acid, and Potassium Dihydrogen Phosphate of analytical grade were obtained from Finar Chemicals Ltd.

Instruments and Chromatographic Conditions

Young lin HPLC system was used for method development and validation. Data acquisition

was performed on YL 9100 HPLC software. The separation were achieved on Inertsil ODS-2 (250 × 4.6 mm, 5µm) column. The column was maintained at room temperature and the eluent was monitored at 227 nm using PDA detector. The mixture of Phosphate buffer pH 3.5 and Acetonitrile in proportion of 50:50 %v/v at a flow rate of 1.0 ml/min was used as a mobile phase. The injection volume was 20µl.

Preparation of Diluent: Mixed water & Methanol in the ratio of (50:50 %, v/v).

Prepration of Standard Stock Solution

Preparation of Metformin hydrochloride standard stock solution (500 µg/mL): A 50 mg of Metformin hydrochloride was weighed and transferred into 100 mL volumetric flask. A 50 mL of HPLC grade water was added and shaken to dissolve. Diluted up to the mark with HPLC grade water and mixed thoroughly.

Preparation of Empagliflozin standard stock solution (625 µg/mL): A 62.5 mg of Empagliflozin was weighed and transferred into 100 mL volumetric flask. A 50 mL of methanol was added and shaken to dissolve. Diluted up to the mark with methanol and mixed thoroughly.

Preparation of Working standard solution of Empagliflozin and Metformin hydrochloride (12.5: 50 µg/mL): A 1.0 mL of Empagliflozin standard stock solution and 5.0 mL of Metformin hydrochloride standard stock solution was pipetted out in to 50 mL volumetric flask, diluted up to the mark with diluent and mixed thoroughly.

Preparation of sample Stock Solution

Preparation of sample stock solution: Physical mixture equivalent to 12.5 mg Empagliflozin and 500 mg of Metformin hydrochloride was transferred to 100 ml volumetric flask. A 50 ml Diluent was added and sonicated to 30 minutes with intermitted shaking. Cooled to room temperature, filtered this solution to another 100 ml volumetric flask and diluted up to the mark with diluent and mixed.

Preparation of sample solution for Empagliflozin: A 10 ml of sample stock solution was pipetted out to 100 ml volumetric flask and diluted up to the with diluent and mixed.

Preparation of sample solution for Metformin Hydrochloride: A 5 ml of sample solution for Empagliflozin was pipetted out to 50 ml volumetric flask and diluted up to the mark with

diluent and mixed.

Forced Degradation

Forced Degradation Studies of the drugs, in combination, were performed under different stress conditions as mentioned in ICH guideline Q1A (R2). The standard solution containing 12.5µg/ml Empagliflozin and 50µg/ml Metformin HCl was subjected to acidic, alkaline and oxidative stress condition. Acidic and alkaline degradation were performed using up to 0.1 N to 2 N strength of acid/base at different temperature. Oxidative stress studies were carried out for using 3-10% H2O2.

A) **Acid degradation:** The solution containing 12.5µg/ml Empagliflozin and 50µg/ml Metformin HCl was subjected to different strengths of Hydrochloric acid (HCl) like 0.1N to 2N. The solution was neutralised with 0.1 N and 2 N NaOH and volume was made up using the diluent. The maximum degradation was obtained with 2N HCl at 80ºC. The representative results are shown in Table 4.

B) **Alkali degradation:** The solution containing 12.5µg/ml Empagliflozin and 50µg/ml Metformin HCl was subjected to different strengths of NaOH like 0.1N to 1N. The solution was neutralised with 0.1 N to 1 N hydrochloric acid and volume was made up using the diluent. The maximum degradation was obtained with 1N HCl at 80ºC. The representative results are shown in Table 4.

C) **Peroxide degradation:** The solution containing 12.5µg/ml Empagliflozin and 50µg/ml Metformin HCl was subjected to different strengths of Hydrogen peroxide (H2O2) like 3%, 6% and 10%. For each strength of H2O2. Final volume was made up using the diluent. The maximum degradation was obtained with 10% H2O2 at 80°C. The representative results are shown in Table 4.

Method Validation

1) Specificity

Specificity of method can be termed as absence of any interference at retention times of samples. Specificity was performed by injecting blank, placebo and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes. Specificity graph of Empagliflozin and Metformin HCl given in figure 3-6.

2) Linearity and Range

A series of standard solutions 2.5-18.75µg/ml of Empagliflozin and 25-75µg/ml of

Metformin hCl were prepared. Plot of average peak area versus the concentration is plotted and from this the regression coefficient and regression equation were generated. The calibration data of empagliflozin and Metformin HCl is given in Table 5 and 6, while Figure 7-9 represents linearity graphs of both drugs respectively.

3) Precision

The method was validated in terms of intra-day inter-day precision. The solution containing 12.5µg/ml of Empagliflozin and 50µg/ml of Metformin HCl was injected six times for repeatability study. Inter-day and Intra-day study was performed by injecting 10, 12.5 and 15µg/ml of Empagliflozin and 40, 50 and 60µg/ml of Metformin HCl solutions three times for each aliquots. The % RSD for precision study was found less than 2% as shown in Table 7.

4) Accuracy

Accuracy was determined by calculating recovery of Empagliflozin and Metformin HCl by the standard addition method. Known amounts of standard solutions of Empagliflozin (6.25, 12.5 and 18.75µg/ml) and Metformin HCl (25, 50 and 75µg/mL) were added to a pre quantified test solutions of cilnidipine (3.125µg/ml) and valsartan (10µg/ml). Each solution was injected in triplicate and the recovery was calculated by measuring peak areas. Results obtained are shown in Table 8-9.

5) LOD and LOQ

LOD and LOQ for Empagliflozin and Metformin HCl were calculated as suggested by ICH guidelines using equations LOD = 3.3 σ/s and LOQ = 10 σ/s, respectively. Where, σ is the SD of the response and S is the slope of the calibration curve.

6) Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by making two small changes. Robustness of the method was studied by changing pH by ± 0.1, flow rate ± 0.02 mL/minutes and mobile phase composition ± 2 ml. After each changes sample solution was injected and system suitability parameters were observed. The results were shown in Table 10.

RESULT AND DISCUSSION

System suitability study

The detection was carried out in the UV region at 227 nm. The different composition of mobile phase was testing and the composition giving retention time of 2.635 min for

Metformin HCl and 4.388 min for Empagliflozin with good resolution and theoretical plates was selected, that optimized mobile phase was 0.05 M KH_2PO_4 Buffer (pH 3.5): Acetonitrile (50:50% v/v). A chromatogram of the mixture in optimized conditions is shown Figure 5-6 and the system suitability parameters are shown in Table 2.

Forced degradation study

The mentioned percent degradation of both Empagliflozin and Metformin HCl is with respect to their decrease in the areas. Peak purity of the drug is not affected.. Thus, the conditions subjected to the drugs make them undergo forced degradation thereby being able to detect any difference in the response in terms of their areas and impurities. The final results for the stress conditions are shown in Table 4.

Method Validation

A) Specificity

The method was found to be specific as there was no interference observed in any of the parameters under observation.

B) Linearity and Range

The linearity of Empagliflozin and Metformin HCl were found between 2.5-18.75 µg/ml and 25- 75 µg/ml, respectively. The results are shown in Table 5and 6.

C) Precision

The % RSD for repeatability study for Empagliflozin and Metformin HCl was found to be 0.21 and 0.32 respectively. The Inter-day and Intra-day study also show % RSD value for Empagliflozin and Metformin HCl within the acceptable limit. Results for precision study are shown in Table 7.

D) Accuracy

Accuracy of the method was confirmed by recovery study at three levels (50%, 100% and 150%) of standard addition. Percentage recovery for Empagiflozin was found to be 99.60-100.48%**,** while for Metformin HCl it was found to be 100.15-100.23% as shown in Table 8.

E) LOD and LOQ

The LOD was found to be 0.09µg/ml for Empagiflozin and 0.52µg/ml for Metformin HCl, while the LOQ was found to be 0.27µg/ml for Empagiflozin and 1.59µg/ml for Metformin HCl.

F) Robustness The typical variations studied under this parameter were mobile phase composition and detection wavelength. Overall % RSD was found to be less than 2% for all

the variations which indicates that the proposed method is robust. Robustness data are shown in Table 9.

G) Analysis of Physical mixture by proposed method

Applicability of the proposed method was tested by analyzing the Physical mixture. The percentage of Empagliflozin and Metformin HCl was found to be 100.16% for Empagliflozin and 99.90% for Metformin HCl. Results as % Assay are shown in Table 10.

Table 1: Preparation of Physical mixture.

Ingredients	Quantity (mg)
Empagliflozin	12.5 mg
Metformin Hydrochloride	500 mg
Lactose Anhydrous	131.5 mg
Talc	7 mg
Magnesium Stearate	14 mg
PVPK dry	35 mg
Total	700 mg

Table 2: System Suitability parameters.

	Metformin hydrochloride	Empagliflozin
Retention time (min) (n=3)	2.635 ±0.003	4.388±0.005
Theoretical plate (n=3)	4516 ± 78.824	9634 ± 24.131
Average area ±SD (n=3)	8708708 ± 33095.15	1476313 ±3038.36
Asymmetry (n=3)	1.71 ± 0.025	0.99 ±0.018
Resolution (n=3)	10.21 ± 0.3305	

Table 3: Preparation of calibration curve.

Linearity solution number	Linearity solution		Volume of standard stock solution of Empa (250 µg/mL) in mL	Volume of standard stock solution of Met (500 µg/mL) in mL	Dilute up to the mark with diluent
	Empa (µg/mL)	Met (µg/mL)			
1	2.5	25	1	5	100
2	5	40	2	8	100
3	7.5	50	3	10	100
4	12.5	60	5	12	100
5	18.75	75	7.5	15	100

Table 4: Degradation result.

Degradation method	Optimized Condition	% Degradation	
		Empagliflozin	Metformin hydrochloride
Acid	5 mL 2N HCl (30 min 80ºC)	7.37	4.83
Base	5 mL 1N NaOH (30 min 80ºC)	No Degradation	36.25
Oxidation	1 mL 10 % H2O2 (30 min 80ºC)	13.72	17.65

Table 5: Data for linearity and range of Empagliflozin.

Sr. No.	Empagliflozin			
	Conc. (in µg/mL)	Average Area (n=3)	SD	%RSD
1	2.5	291894.00	1036.198	0.355
2	5	583091.67	846.200	0.145
3	7.5	882364.67	1881.198	0.213
4	12.5	1474173.33	1882.189	0.128
5	18.75	2196419.33	1196.351	0.054

Table 6: Data for linearity and range of Metformin Hydrochloride.

Sr. No.	Metformin Hydrochloride			
	Conc. (in µg/mL)	Average Area (n=3)	SD	%RSD
1	25	4682598.67	2580.089	0.055
2	40	7362563.67	22541.368	0.306
3	50	9121489.00	5281.214	0.058
4	60	10830692.33	66990.876	0.619
5	75	13917596.67	12481.154	0.090

Table 7: Precision study result.

Parameters	Concentration (µg/mL)		% RSD	
	Empagliflozin	Metformin HCl	Empagliflozin	Metformin HCl
Repeatability	12.5	50	0.21	0.32
Intraday	10	40	0.52	0.60
	12.5	50	0.24	0.15
	15	60	0.29	1.21
Interday	10	40	0.81	1.35
	12.5	50	0.29	0.31
	15	60	0.28	0.48

Table 8: Accuracy Study for Empagliflozin.

% accuracy Level	Amount of Empa Taken (125µg/mL)	Amount of Std Empa added (250µg/mL)	Total Amount of Empa (µg/mL)	Amount of Empa Found (µg/mL) ±SD (n=3)	Mean % Recovered ±SD (n=3)
50	3.125	3.125	6.25	6.28 ± 0.08	100.48 ± 1.31
100	3.125	9.375	12.5	12.45 ± 0.12	99.60 ± 0.94
150	3.125	15.625	18.75	18.79 ± 0.10	100.21 ± 0.53

Table 9: Accuracy Study for Metformin Hydrochloride.

% accuracy Level	Amount of Met Taken (500µg/mL)	Amount of Std Met added (500µg/mL)	Total Amount of Met (µg/mL)	Amount of Met Found (µg/mL) ±SD (n=3)	Mean % Recovered ±SD (n=3)
50	10	15	25	25.06 ± 0.11	100.23 ± 0.43
100	10	40	50	50.09 ± 0.16	100.17 ± 0.32
150	10	65	75	75.11 ± 0.12	100.15 ± 0.15

Table 10: Robustness study for Empagliflozin and Metformin Hydrochloride.

Parameters		Area (n=3)	
		Empagliflozin	Metformin Hydrochloride
pH (±0.1)	3.4	1427091	9099451
	3.5	1438549	9121151
	3.6	1430039	9169480
	Mean ± SD	1431893 ± 5949.742	9130027.33 ±35848.39
	% RSD	0.42	0.39
Flow Rate (±0.02 ml/min)	0.98 ml/min	1459709	9284815
	1.0 ml/min	1438549	9121151
	1.02 ml/min	1409121	8993531
	Mean ± SD	1435793.00± 25406.36	9133165.67 ± 146013.21
	% RSD	1.77	1.60
Mobile Phase Composition Buffer: ACN (± 2 mL)	48:52	1439132	9183513
	50:50	1438549	9121151
	52:48	1400838	9194229
	Mean ± SD	1426173.00 ±21942.69	9166297.67 ±39463.58
	% RSD	1.54	0.43

Table 11: Analysis of Physical mixture of Empagliflozin and Metformin hydrochloride by Proposed Method.

Empagliflozin			Metformin Hydrochloride		
Labelled Amount mg	Amount Found (mg)	% Assay	Labelled Amount mg	Amount Found (mg)	% Assay
12.5 mg	12.55	100.40	500 mg	50.02	100.04
	12.32	98.56		49.94	99.88
	12.69	101.52		49.89	99.78
Mean ± SD	12.52 ± 0.1868	100.16± 1.4945	Mean ± SD	49.95 ± 0.0656	99.90 ±0.1311
% RSD	1.49	1.49	% RSD	0.13	0.13

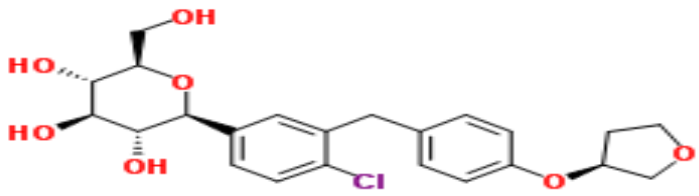

Figure 1: Structure of Empagliflozin.

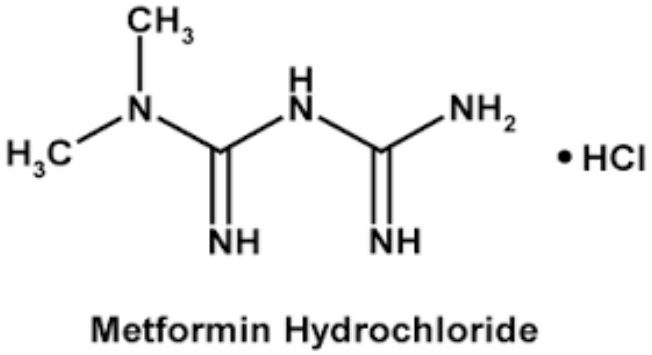

Figure 2: Structure of Metformin Hydrochloride.

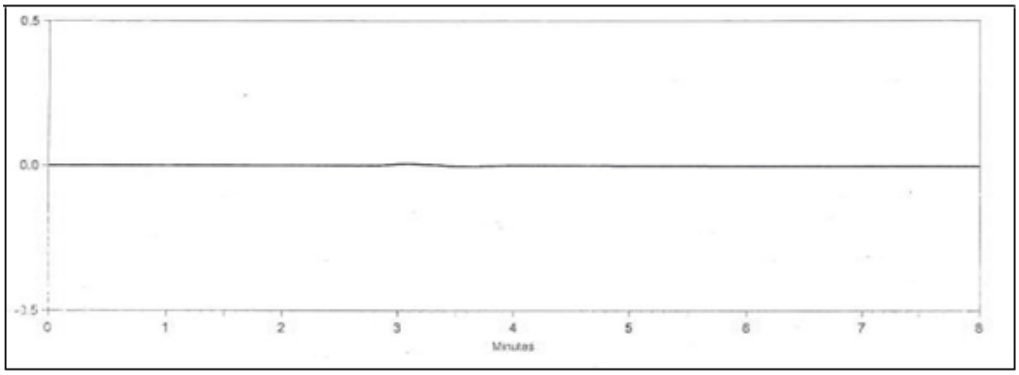

Figure 3: Chromatogram of Blank.

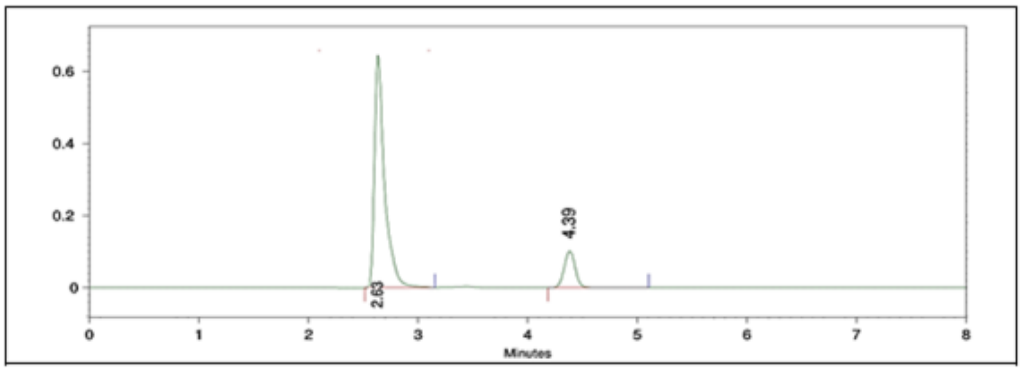

Figure 4: Chromatogram of Standard Solution.

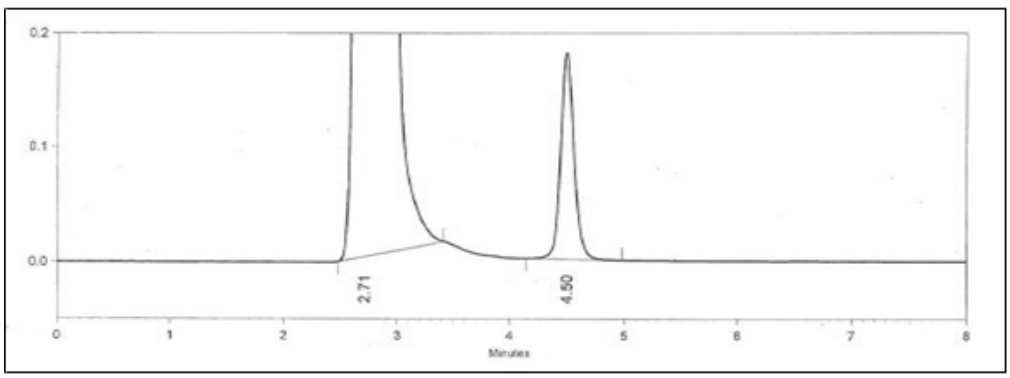

Figure 5: Chromatogram of Empagliflozin sample solution.

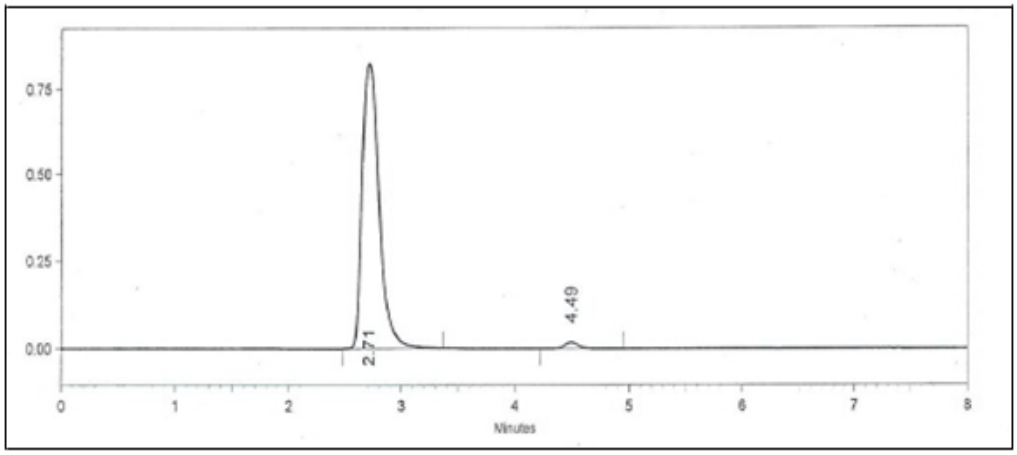

Figure 6: Chromatogram of Metformin HCl sample solution.

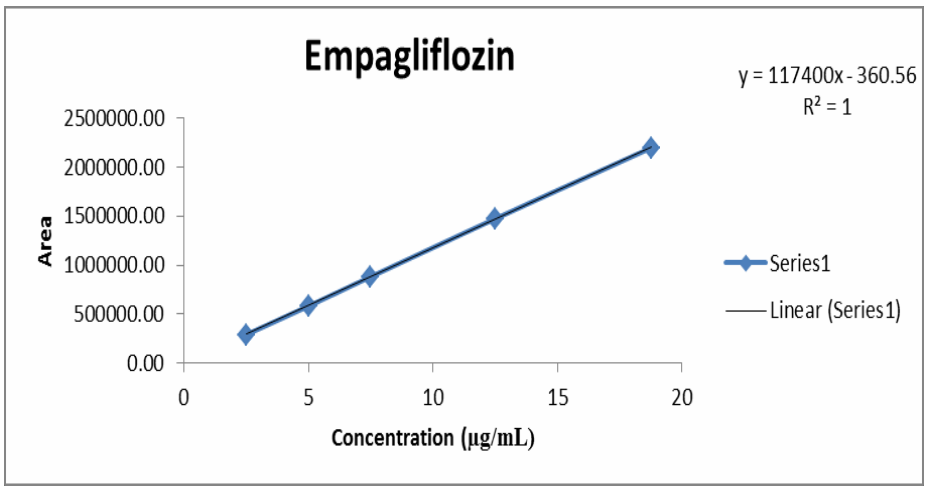

Figure 7: Linearity Graph of Empagliflozin.

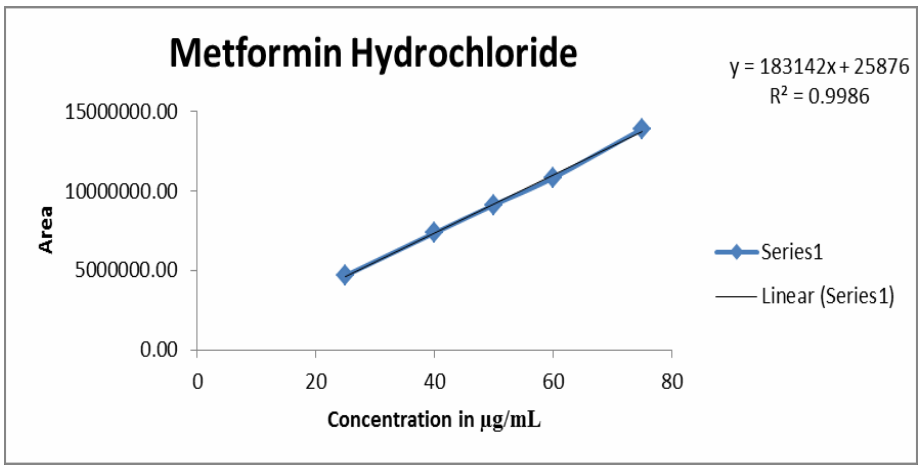

Figure 8: Linearity Graph of Metformin Hydrochloride.

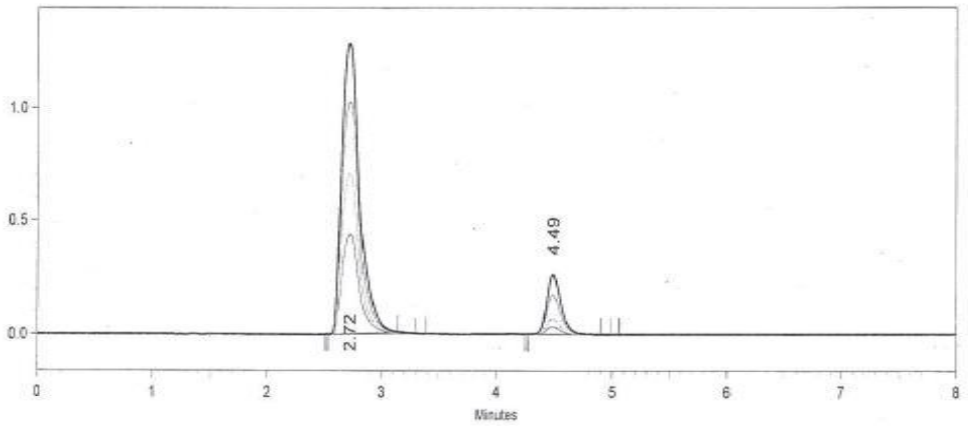

Figure 9: Overlay Chromatogram of Linearity Study.

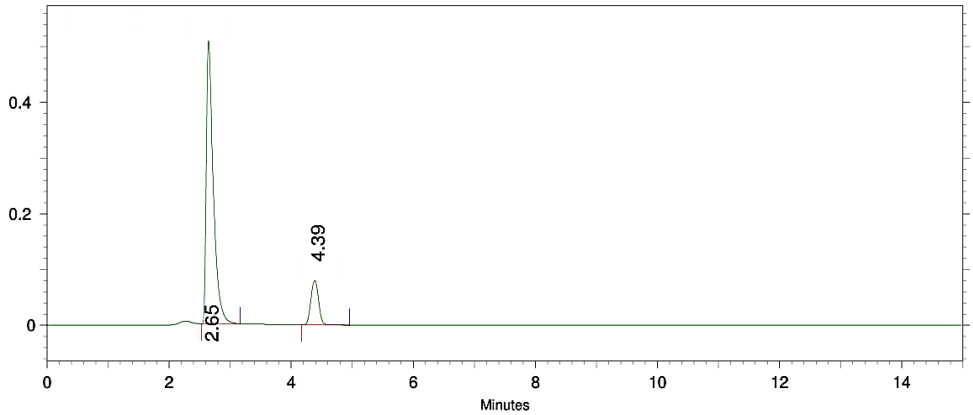

Figure 10: Chromatogram of acid degradation.

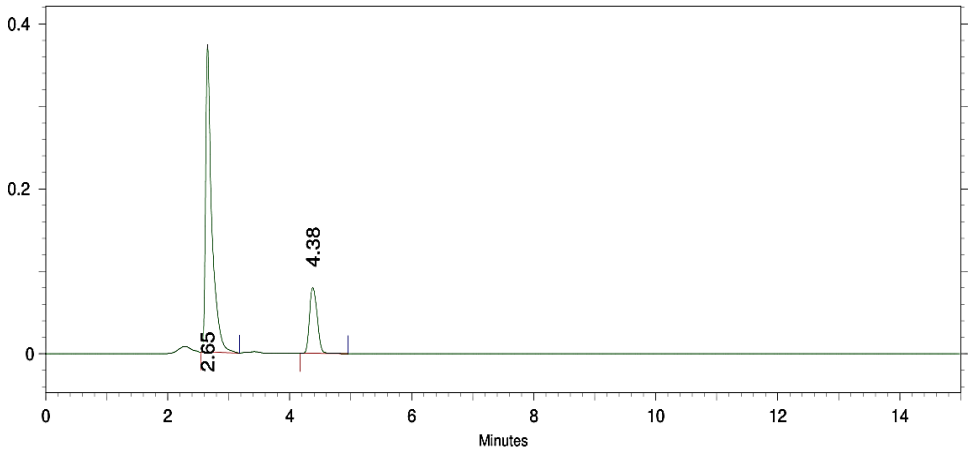

Figure 11: Chromatogram of Alkali degradation.

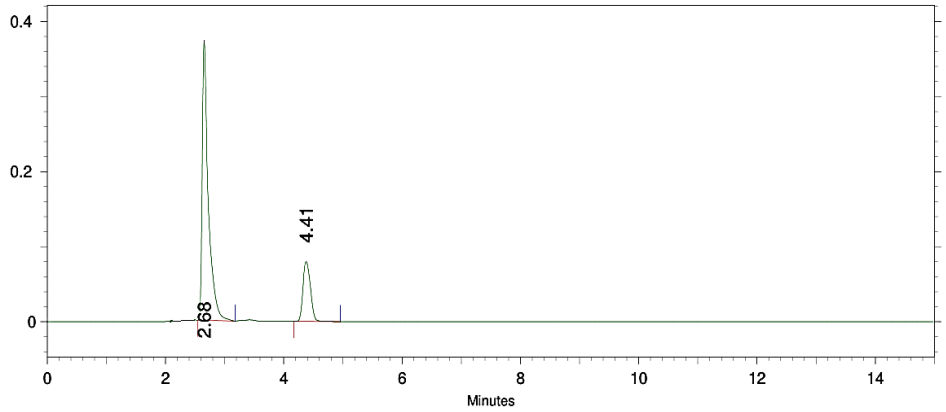

Figure 12: Chromatogram of peroxide degradation.

CONCLUSION

From the above Research work it can be concluded that the proposed method is precise, accurate and stability indicating. Results are in good agreement with label claim which indicates there is no interference of exicipients. Therefore the proposed method can be used for routine analysis of Empagliflozina nd Metformin HCL in Physical mixture.

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