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A NEW VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF METFORMIN HCL AND EMPAGLIFLOZIN IN ITS BULK AND PHARMACEUTICAL DOSAGE FORMS

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Keywords:

Metformin, Empagliflozin, HPLC, Methanol

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ABSTRACT: A New method was established for simultaneous estimation of Metformin and Empagliflozin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Metformin and Empagliflozin by using Symmetry C18 column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer (KH_2PO_4 and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength used was Waters HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.403 mins and 3.907 mins. The % purity of Metformin and Empagliflozin was found to be 99.87% and 100.27% respectively. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1). The linearity study of Metformin and Empagliflozin was found in concentration range of 50µg-250µg and 5µg-25µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.3and 0.3, % RSD for intermediate precision was 1.3 and 0.4respectively. LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

INTRODUCTION: Empagliflozin is a drug of the gliflozin class, approved for the treatment of type 2 diabetes in adults in 2014. The chemical name of empagliflozin is (empagliflozin; 1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran – 3 – yl -oxy) benzyl]-benzene (**Fig. 1)**. Empagliflozin is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), which is found almost exclusively in the proximal tubules of nephronic components in the kidneys[1].SGLT-2 accounts for about 90 percent of glucose reabsorption into the blood. Blocking SGLT-2 reduces blood glucose by blocking glucose reabsorption in the kidney and thereby excreting glucose (i.e., blood sugar) via the urine.

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The side effects of this drug is a higher frequency of urinary tract infections. There are concerns it may increase the risk of diabetic ketoacidosis. Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM) [3]. The chemical name of Metformine Dimethylimidodicarbonimidic diamide (**Fig. 2**).

It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake [4]. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients.

When used alone, metformin does not cause hypoglycemia; however, it may potentiate the hypoglycemic effects of sulfonylureas and insulin. Its main side effects are dyspepsia, nausea and diarrhea [5].

FIG. 1: STRUCTURE OF EMPAGLIFLOZIN

FIG. 2: STRUCTURE OF METFORMINE

Various analytical methods were reported in literature for the determination of empagliflozin and metformin in pure drug, pharmaceutical dosage forms and in biological samples using High performance liquid chromatography [7-12], High performance thin layer chromatography [13-14], UV-Spectrophotometry [15-17], Ultra performance liquid chromatography [18], either in single or in combined forms. Lack of any published method for RP-HPLC simultaneous estimation of Metformin and Empagliflozin in API, therefore, provoked us to investigate the application of method development and validation of RP-HPLC method for the simultaneous estimation of Metformin and Empagliflozin in API. The new method developed and validated for Metformin and Empagliflozin in its bulk and tablets will help as a research interest to formulate different advanced drug delivery dosage forms and further its analyses.

MATERIALS AND METHODS:

Instrumentation: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 240 nm with an Intersil C18, 150mmx40mm, 5 µm) dimensions at ambient temperature

Chemicals and reagents: Empagliflozin and Metformine were supplied as gift sample from Mylon laboratories, Hyderabad. KH_2PO_4 was analytical grade supplied by FINER chemical LTD, Mumbai, Orthophosphoric acid (Merck), Acetonitrile (Molychem, HPLC grade) and Water for HPLC (Lichrosolv (Merck).

Preparation of solutions:

Preparation of buffer: Accurately weighed 6.8 grams of KH2PO4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase: Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

The diluents: The Mobile phase was used as the diluent.

Preparation of standard stock solution: Accurately weigh and transfer 1000 mg of Metformin and10 mg of Empagliflozin working standard into a 100 ml clean dry volumetric flask add about 70 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.(1500 ppm MET and 15 ppm of EMPA)

Preparation of Sample stock solution: Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 1000 mg of Metformin and 10mg Empagliflozin (marketed formulation) sample into a 100mL clean dry volumetric flask add about 70 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1.5 ml of Metformine and Empagliflozin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. (1500 ppm MET and 15 ppm of EMPA).

Procedure: Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Metformin and Empagliflozin peaks and calculate the %Assay by using the formulae.

Method development selection of wavelength: Stock solution of 100 mg/ml was prepared for Empagliflozin and Metformine further diluted to get the concentration of 10µg/ml of Empagliflozin and Metformine was prepared with methanol. The wavelength was selected by scanning the above standard drug solution between 200 to 400nm. The scanned results showed that reasonable maximum absorbance was recorded at 240nm. Therefore 240nm was selected as the detection wavelength for the RP-HPLC investigation **Fig. 3.**

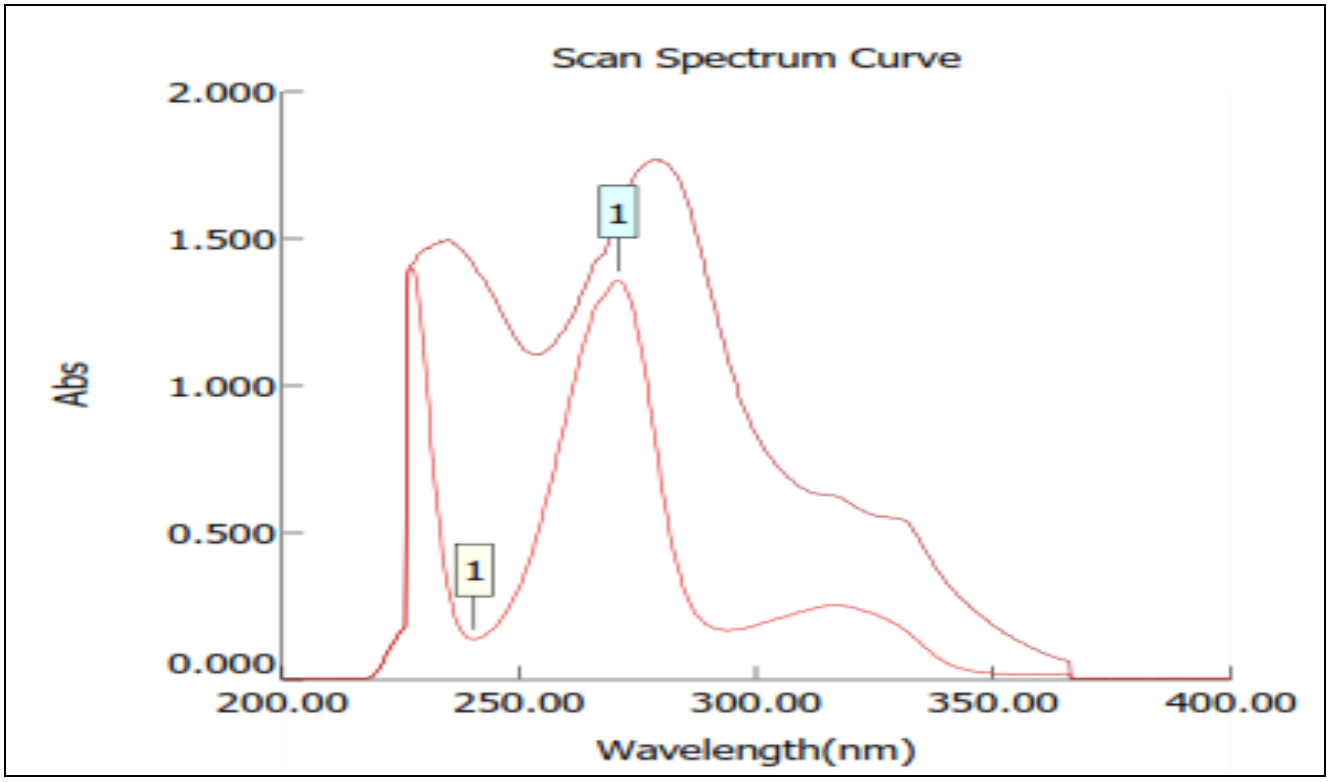

FIG. 3: UV SPECTRA OF EMPAGLIFLOZIN AND METFORMINE

Construction of calibration curve: Aliquots of different concentrations of standard solution were prepared and their chromatograms were recorded at the optimized chromatographic conditions. The mean peak areas at different concentration levels were calculated from the chromatograms. Then the linearity plot was constructed using the mean peak areas at their respective concentrations.

Method validation: The developed method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness and system suitability parameters as described in ICH guidelines.

Linearity: From the stock solution, 25, 50, 75, 100, 125, 150µg/ml solutions were made and their chromatograms were recorded. From the recorded chromatograms, their respective mean peak areas were calculated and the linearity plot was constructed using the mean peak areas at their respective concentrations. The correlation coefficient was found to be 0.999. The linearity data of Empagliflozin and Metformine was shown in **Table 1** and **Table 2**, the calibration plot.

RESULTS AND DISCUSSION: The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Metformin and Empogliflozin by RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Metformin and Empogliflozin by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Metformin and Empogliflozin in pharmaceutical dosage form.

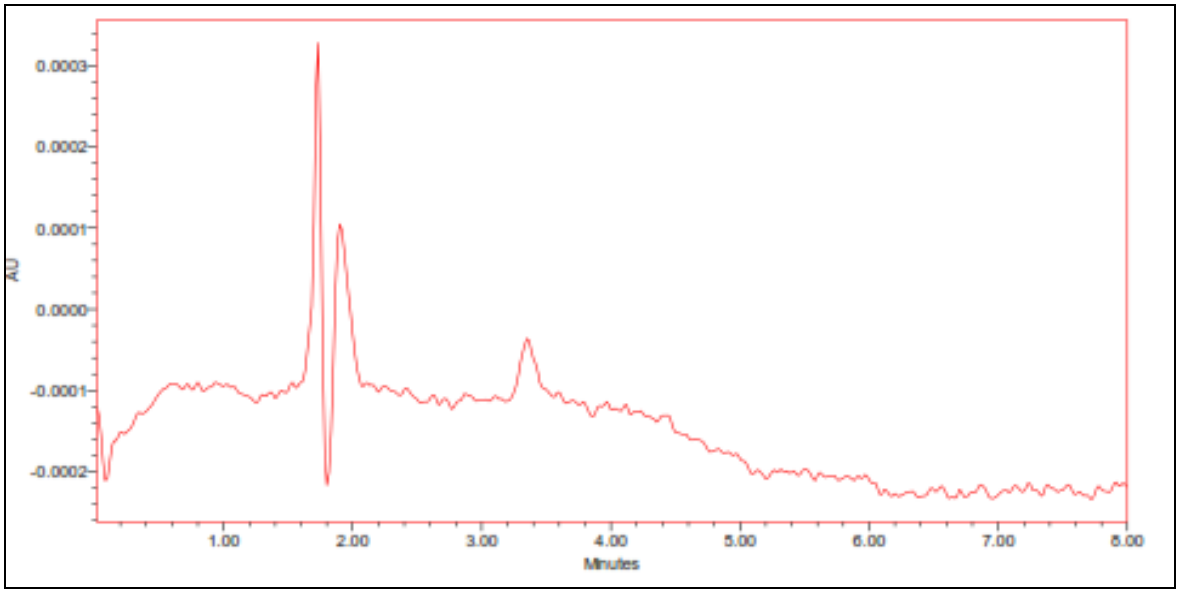

FIG. 4: CHROMATOGRAM SHOWING BLANK PREPARATION (MOBILE PHASE)

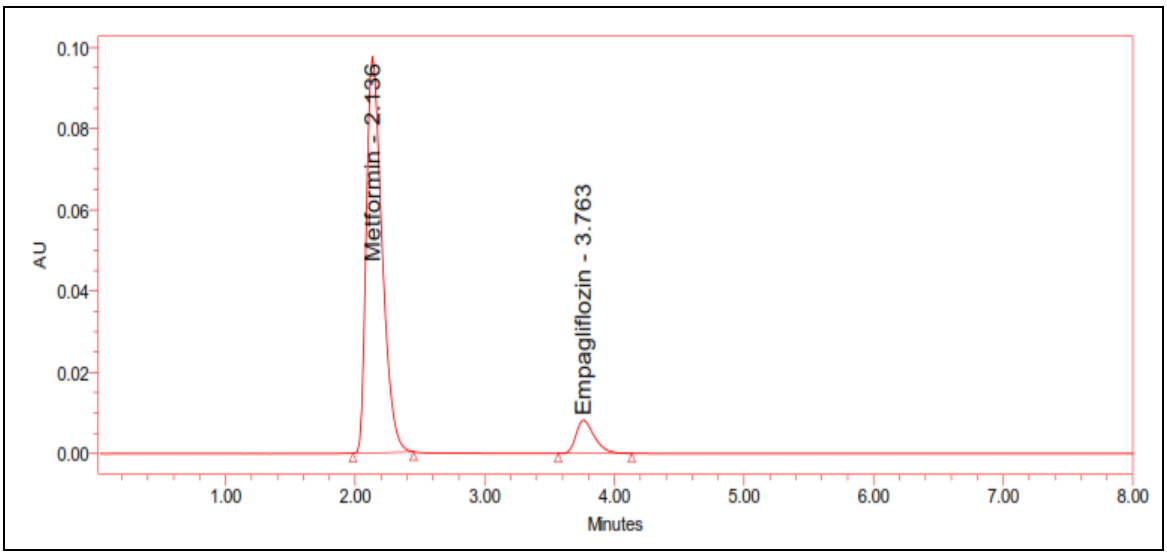

FIG. 5: CHROMATOGRAM SHOWING ASSAY OF SAMPLE INJECTION

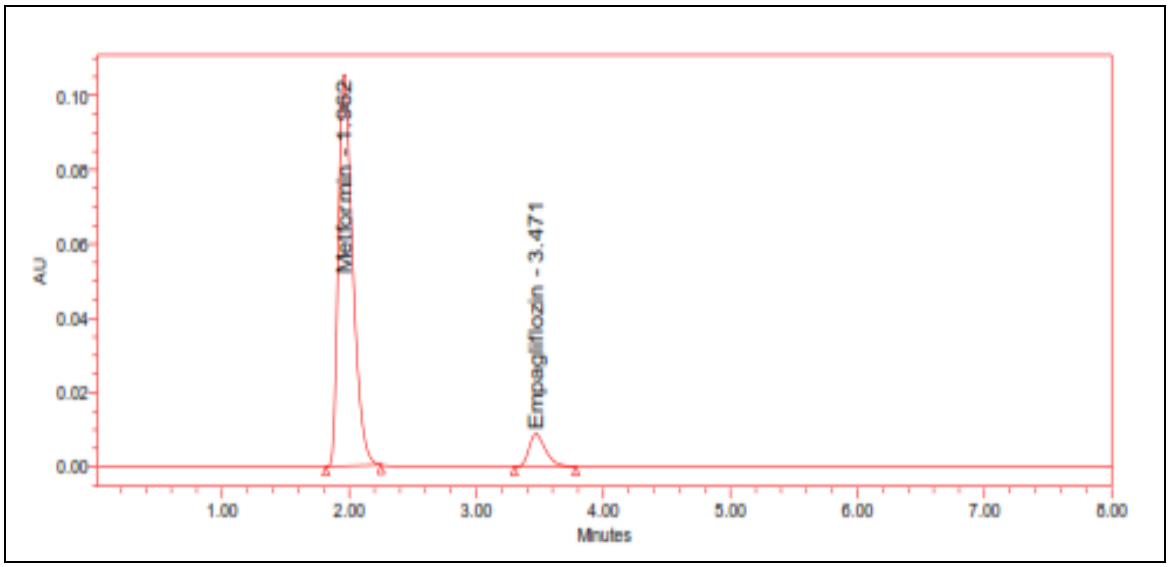

FIG. 6: CHROMATOGRAM SHOWING STANDARD OF SAMPLE INJECTION

TABLE 1: SHOWING ASSAY RESULTS

S. no	Name of compound	Amount taken(mg)	%purity
1	Metformin	1000mg	98.21
2	Empogliflozin	10mg	100.47

Linearity:

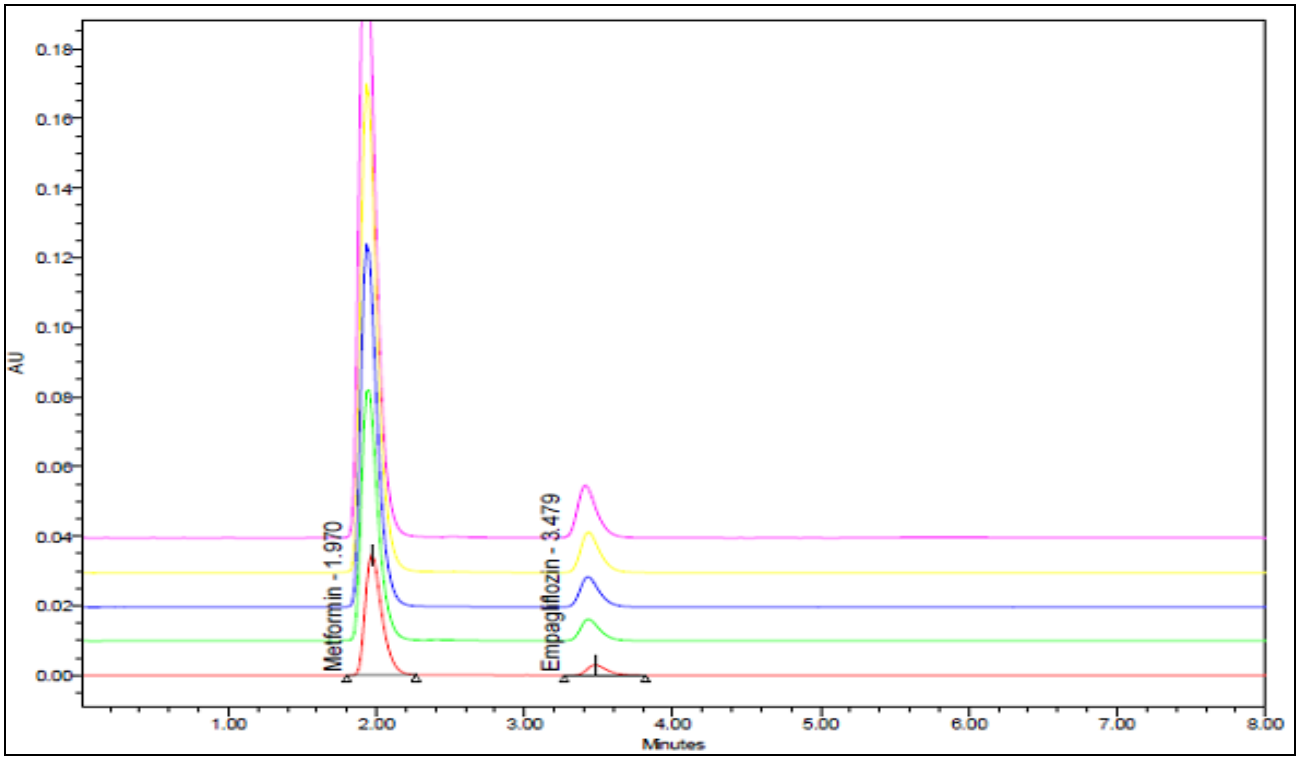

FIG. 7: CHROMATOGRAMS SHOWING LINEARITY OVERLAY

TABLE 2: LINEARITY RESULTS FOR METFORMINE

S. No	Linearity Level	Concentration(ppm)	Area
1	I	500	270141
2	II	1000	558098
3	III	1500	798449
4	IV	2000	1080708
5	V	2500	1369144
	Correlation Coefficient		0.999

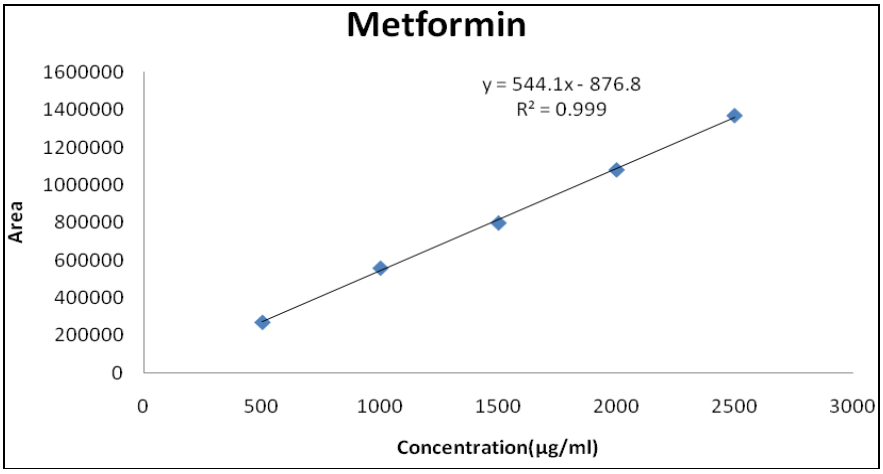

FIG. 8: SHOWING CALIBRATION GRAPH FOR METFORMINE

TABLE 3: LINEARITY RESULTS FOR EMPAGLIFLOZIN

S. No	Linearity Level	Concentration(ppm)	Area
1	I	5	27213
2	II	10	55506
3	III	15	78707
4	IV	20	105801
5	V	25	133244
	Correlation Coefficient		0.999

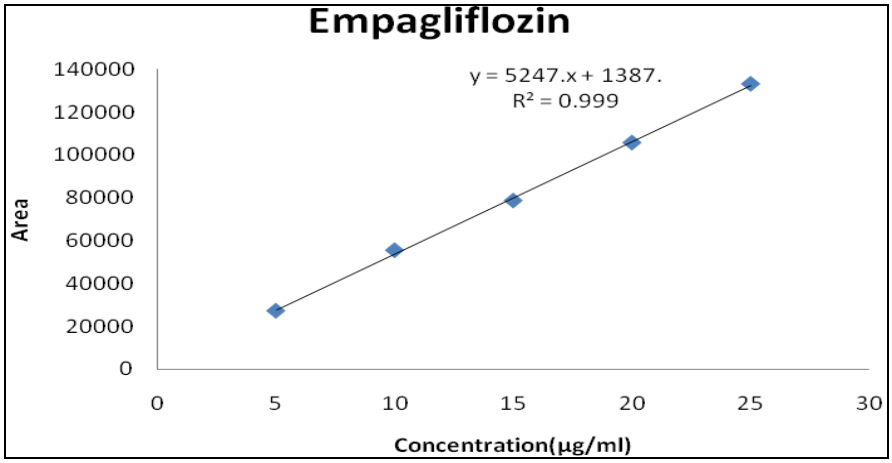

FIG. 9: SHOWING CALIBRATION GRAPH FOR EMPOGLIFLOZIN

Accuracy: The accuracy study was performed for 50%, 100% and 150 % for Metformin and Empogliflozin. Each level was injected in triplicate into chromatographic system. The area of each leve l was used for calculation of % recovery.

TABLE 4: SHOWING ACCURACY RESULTS FOR METFORMIN

%Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	428731	75	74.96	99.91%	
100%	851297	150	149.98	99.18%	99.56%
150%	1275904	225	224.02	99.60%	

TABLE 5: SHOWING ACCURACY RESULTS FOR EMPAGLIFLOZIN

%Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	40731	25	24.99	99.53%	
100%	82456	50	49.05	99.38%	99.47%
150%	122414	75	74.495	99.52%	

The accuracy study was performed for % recovery of Metformin and Empogliflozin. The % recovery was found to be 99.18% and 99.91% respectively (NLT 98% and NMT 102%)

Precision:

TABLE 6: SHOWING% RSD RESULTS FOR METFORMIN AND EMPAGLIFLOZIN

S.no.	Metformine		Empagliflozin	
	RT	Area	RT	Area
1	1.956	829858	3.457	81017
2	1.962	824838	3.471	79440
3	1.980	834631	3.501	81571
4	1.992	845317	3.523	81704
5	1.996	849490	3.557	82457
6	2.002	841368	3.562	82471
Mean		837583.7		81443.3

Std. Dev	9452.4	1128.1
% RSD	1.1	1.4

Intermediate precision/Ruggedness:

TABLE 7: SHOWING RESULTS FOR INTERMEDIATE PRECISION OF METFORMIN AND EMPAGFLIFLOZIN

S.no.	Metformine		Empagliflozin	
	RT	Area	RT	Area
1	1.910	854596	3.368	83674
2	1.921	864089	3.387	84042
3	1.926	852527	3.400	83222
4	1.931	857218	3.407	83523
5	1.940	862365	3.426	83218
6	1.946	845558	3.435	82581
Mean		856058.7		83376.4
Std. Dev		6789.4		497.0
% RSD		0.8		0.6

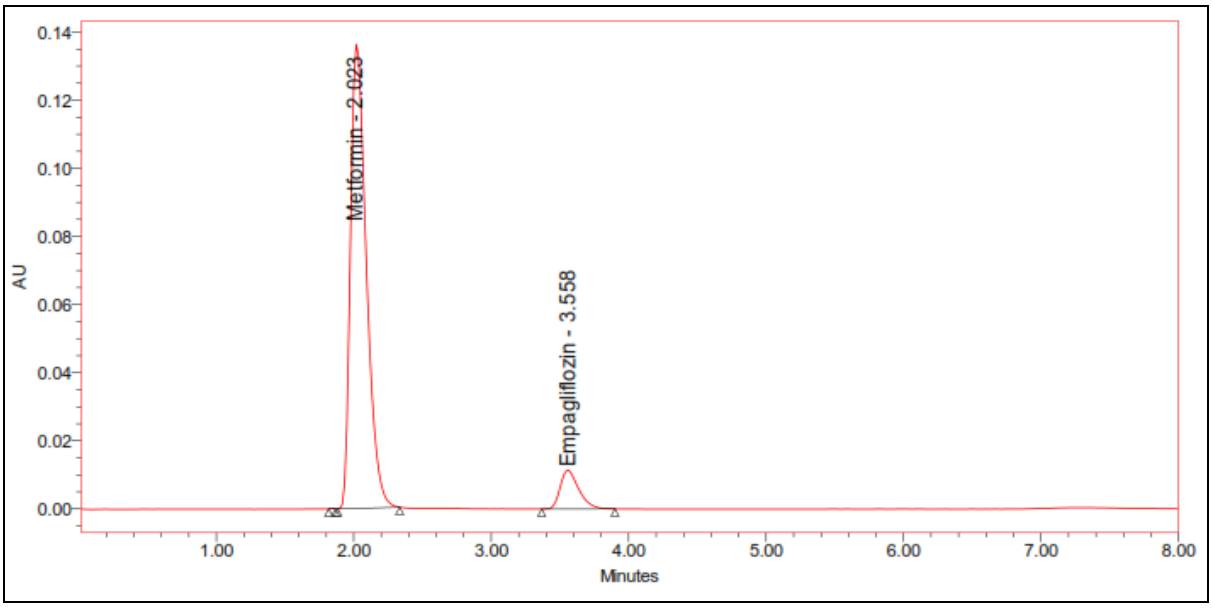

FIG. 10: CHROMATOGRAM SHOWING LESS FLOW

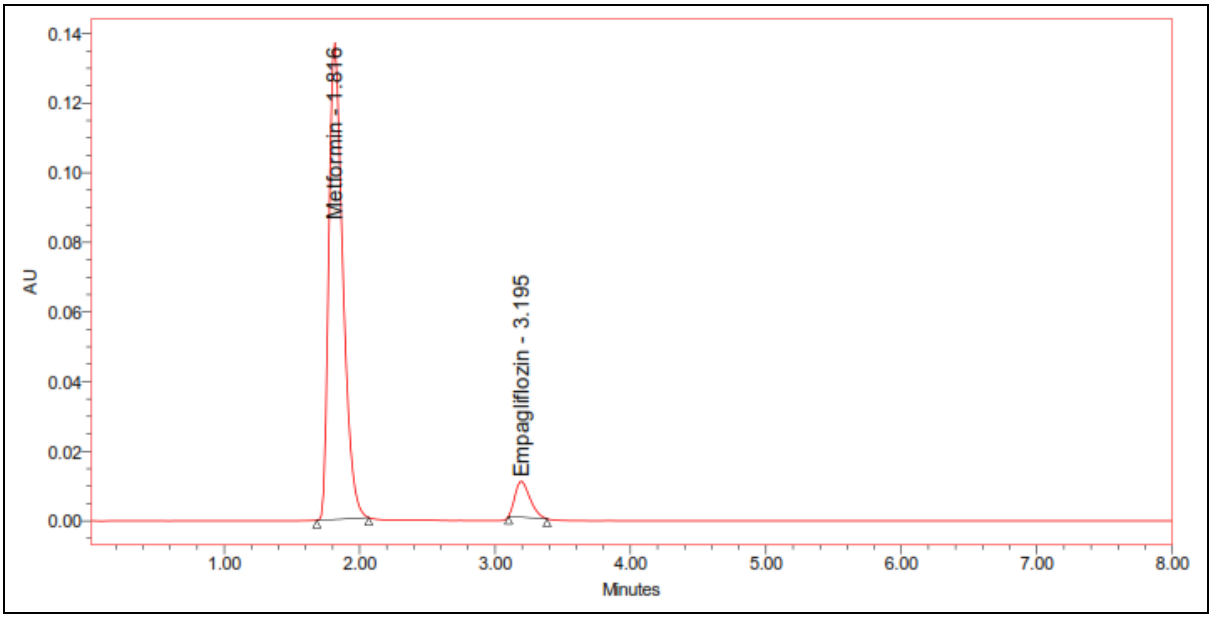

FIG. 11: CHROMATOGRAM SHOWING MORE FLOW

TABLE 8: SYSTEM SUITABILITY RESULTS FOR METFORMIN

S. no.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2418.2	1.5
2	1.0	2415.75	1.43
3	1.2	2424.1	1.4

TABLE 9: SYSTEM SUITABILITY RESULTS FOR EMPAGLIFLOZIN

S. no.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3318.5	1.4
2	1.0	3310.51	1.36
3	1.2	3698.9	1.4

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

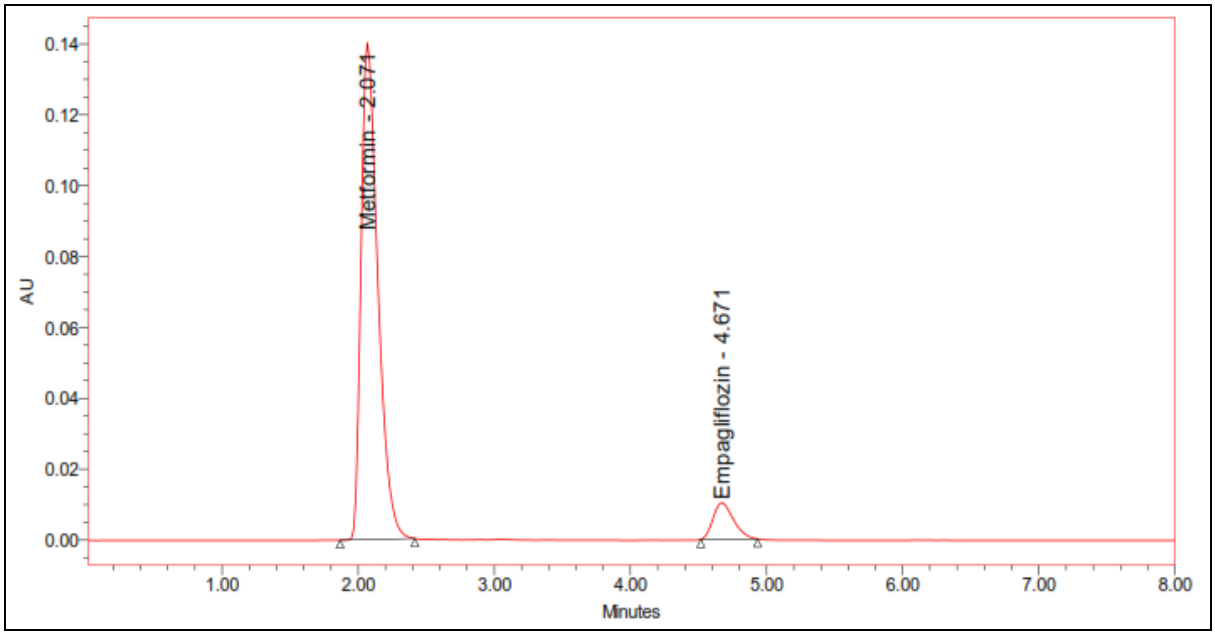

FIG. 12: CHROMATOGRAM SHOWING LESS ORG

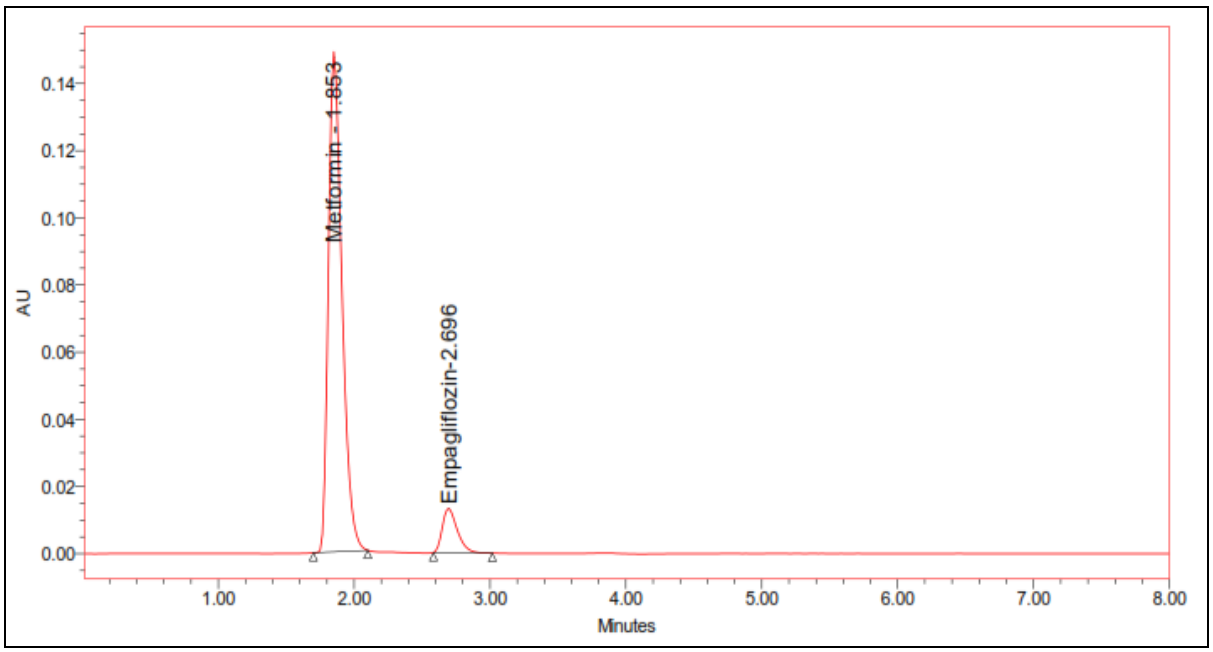

FIG. 13: CHROMATOGRAM SHOWING MORE ORG

TABLE 10: SHOWING SYSTEM SUITABILITY RESULTS FOR METFORMIN

S. no.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2318.6	1.5
2	*Actual	2415.75	1.43
3	10% more	2563.0	1.4

TABLE 11: SHOWING SYSTEM SUITABILITY RESULTS FOR EMPOGLIFLOZ

S. no.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4406.0	1.3
2	*Actual	3310.51	1.36
3	10% more	2775.5	1.4

Detection limit:

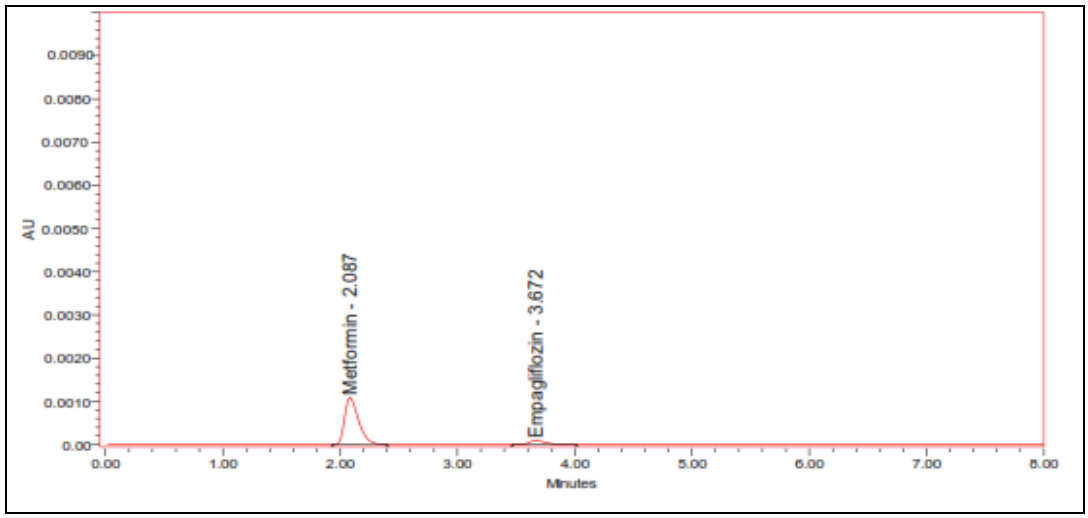

FIG. 14: CHROMATOGRAM SHOWING LOD

TABLE 12: SHOWING RESULTS FOR LIMIT OF DETECTION

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Metformin	371827.90	563365963	2.17
Empogliflozin	5401.60	479884400	0.0372

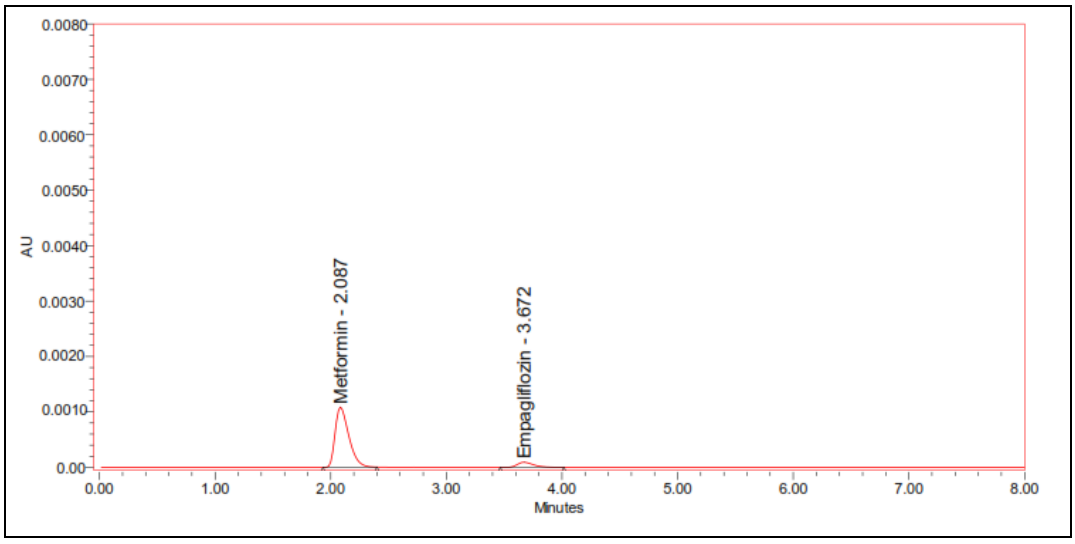

FIG. 15: CHROMATOGRAM SHOWING LOQ

TABLE 13: SHOWING RESULTS FOR LIMIT OF QUANTITATION

Peak Name	RT	Area
Metformine	2.087	892474
Empagliflozin	3.672	87176

CONCLUSION: The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Metformin and Empagliflozin in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Metformin and Empagliflozin in pure and its pharmaceutical dosage forms. The new method developed and validated for Metformin and Empagliflozin in its bulk and tablets will help as a research interest to formulate different advanced drug delivery dosage forms and further its analyses.

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REFERENCES:

1. Grempler R, Thomas L, Eckhardt M, Himmelsbach F, Sauer A, Sharp DE, Bakker RA, Mark M, Klein T, Eickelmann P. "Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors". Diabetes Obes Metab, 2012, 14 (1), 83–90.
2. FDA (2015-05-15), SGLT2 inhibitors: Drug Safety Communication - FDA Warns Medicines May Result in a Serious Condition of Too Much Acid in the Blood". 2015.
3. "Metformin Hydrochloride". The American Society of Health-System Pharmacists. Retrieved Jan 2016.
4. Hirst JA1, Farmer AJ, Ali R, Roberts NW, Stevens RJ, Quantifying the effect of metformin treatment and dose on glycemic control, Diabetes Care, 2012 Feb, 35(2), 446-54.
5. Lilian Beatriz Aguayo Rojas, Marilia Brito Gomes., Metformin: an old but still the best treatment for type 2 diabetes.2013
6. Arayne, M. SAEED, Najma Sultana, and M. HASHIM Zuberi, Development and validation of RP-HPLC method for the analysis of metformin, Pak J Pharm Sci, 2006, 19.3 231-5.
7. Shyamala, K. Nirmala, J. Mounika and B. Nandini, Validated stability-indicating RP-HPLC method for determination of Empagliflozin. Der Pharmacia Lettre, 2016, 8 (2), 457-464
8. Dnyaneshwar Thakare, Patil Vikas, Kalkotwar Ramesh, Jadhav Vijay, Chandra K Sekhar2, a new RP–HPLC method for simultaneous estimation of metformin HCL and linagliptin in tablet dosage form, 2013, 2(3), 1332-1341
9. Kavitha. K. Y, Geetha. G1, Hariprasad, Kaviarasu, Venkatnarayanan, Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form. Journal of Chemical and Pharmaceutical Research, 2013, 5(1), 230-235
10. Pandya, Rutvik H., Rajeshwari Rathod, and Dilip G. Maheswari, Bioanalytical method development and validation for simultaneous determination of linagliptin and metformin drugs in human plasma by RP-HPLC method, Pharmacophore , 2014, 5(2), 202-218.
11. Sri, S. G., et al, A new RP-HPLC method development for simultaneous estimation of Metformin and Alogliptin in bulk as well as in pharmaceutical formulation using PDA detector, World J Pharm Pharm Sci, 2013, 6(2), 6720-43.
12. Ranetti, Maria-Cristina, et al, Validation of a HPLC method for the simultaneous analysis of metformin and gliclazide in human plasma, Farmacia, 2009, 57(6), 729-735.
13. Modi, Darshana K, Punit B. Parejiya, Bhavesh H. Patel, A simple and sensitive HPTLC method for simultaneous determination of Metformin hydrochloride and Sitagliptin phosphate in tablet dosage form, Journal of Chemistry, 2013 (2012).
14. Srivani, Jillala, Balekari Umamahesh, Ciddi Veeresham, Development and Validation of stability indicating HPTLC method for simultaneous determination of linagliptin and metformin, International Journal of Pharmacy and Pharmaceutical Sciences, 2015, 8(1) 112-115.
15. Jani, B. R., K. V. Shah, P. P. Kapupara, Development and Validation of UV spectroscopic method for simultaneous estimation of dapagliflozin and metformin hydrochloride in synthetic mixture.
16. Chirag , Amrita Parle, Development and validation of UV spectrophotometric method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms, Der Pharma Chemica, 2014, 6(1), 303-311
17. N. Padmaja, G. Veerabhadram , Development and validation of analytical method for Simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy, Der Pharmacia Lettre, 2015, 7 (12), 306-312
18. Ayoub, Bassam M, UPLC simultaneous determination of empagliflozin, linagliptin and metformin, RSC advances, 2015, 5.116.

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