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Development of Validated Stability Indicating Assay Method for Simultaneous Estimation of Metformin and Dapagliflozin by RP- HPLC

[1,2]T. Deepan, [2]M.V. Basaveswara Rao and [1]M.D. Dhanaraju

[1]GIET School of Pharmacy, Andhra Pradesh, India
[2]Krishna University, Andhra Pradesh, India

Abstract: A simple, precise and stability indicating HPLC method was developed and validated for the simultaneous determination of Metformin hydrochloride (MET) and Dapagliflozin (DAP) in pharmaceutical dosage forms. The separation was achieved on an Inspire (4.6 x 150mm, 5µm)5micro column with isocratic flow. The mobilephase at a flow rate of 1.0mLmin consisted ofAcetonitrile and 0.1M orthophosphoric acid buffer (70:30, v/v). The UV detection was carried out at 260nm. The retention times for MET and DAP were 2.097min and 3.691min, respectively. Parameters such as linearity, precision, accuracy, specificity and ruggedness are studied as reported in the International Conference on Harmonization guidelines.A linear response was observed over the concentration range of 5-25?µg/ mL for DAP and 500-2500?µg/ mL for MET respectively. Limit of detection and limit of quantification for DAP were 2.98 and 9.95?µg/mL and for MET were 3.05?µg/mL and 10.07?µg/mL respectively. Individual drugs (MET and DAP) were exposed to thermal, photolytic, hydrolytic and oxidative stress conditions. The resultant stressed samples were analyzed by the proposed method. The method gave high resolution among the degradation products and the analytes. The analysis concluded that the method was selective for simultaneous estimation of Metformin and Dapagliflozin which will help to improve quality control and contribute to stability studies of pharmaceutical tablets containing these drugs.

Key words: Metformin · Dapagliflozin · Validation · Stability Studies · HPLC

INTRODUCTION

Metformin is chemically 1, 1-dimethyl biguanide hydrochloride (Fig. 1A). Dapagliflozin is chemically known as (1S)-1, 5-anhydro-1-C- [4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol (Fig. 1B). Metformin and Dapagliflozin is a sodium glucose cotransporter 2 (SGLT 2) inhibitor and biguanide antidiabetic combination. The SGLT2 inhibitor works by decreasing the amount of sugarthe body absorbs and increasing the amount of sugar that leaves the body in the urine. A combination of 1000mg of Metformin and 10 mg of Dapagliflozinis available commercially as tablets indicated for the treatment of diabetes mellitus [1, 2].

Literature survey shows that numerous analytical methods are reported for the individual estimation of MET and DAP as well as metformin with other pharmaceutical preparations by various methods such as UV spectrophotometry [3, 4] HPLC [5-9] HPTLC [10] LC MS [11-14] and Capillary electrophoresis [15]. However there is no method reported for metformin and dapagliflozin by HPLC. Hence there is a need for sensitive HPLC method which is stability indicating for MET and DAP. Stability studies was carried out by forcing the drug under variety of stress conditions like thermal, oxidative, light and hydrolysis (Acid and base), The developed HPLC method was validated as per ICH guidelines [16]. The aim of the present study was to develop a HPLC method for Metformin and Dapagliflozin in API and marketed formulation and to perform the stability studies under various stress conditions.

Corresponding Author: M.D. Dhanaraju, GIET School of Pharmacy, Andhra Pradesh, India.

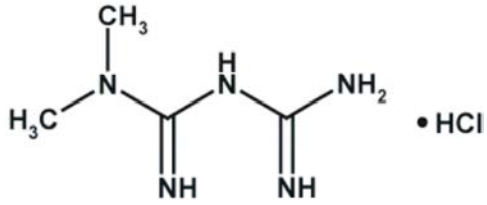

Fig. 1a: Structure of Metformin

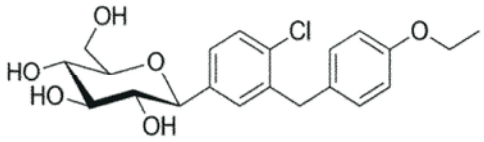

Fig. 1b: Structure of Dapagliflozin

MATERIALS AND METHODS

Metformin hydrochloride and Dapagliflozin reference standard were kindly supplied by Glemnark pharma and Piramalhealthcare respectively and tablets were purchased from local market (Hyderabad) Analytical-grade OPA (Orthophosphoric acid) and HPLC-grade Acetonitrile and water were purchased from Merck (Darmstadt, Germany). A membrane filter of 0.45 µm porosity was used to filter and degas the mobile phase. The tablets of metformin in combination with dapagliflozin were purchased from the Indian market. Double-distilled water was used throughout the experiment. Other chemicals were of analytical or HPLC grade.

Chromatographic Conditions: Waters Corporation (Milford USA) was used for method development, forced degradation and method validation. This system is comprised of a ternary gradient pump and auto sampler (2487 Separation module), column oven and a photo diode array detector. Inspire (4.6 x 150mm, 5µm) column was used. The instrumental settings were a flow rate of 1 mL/min, a column temperature at 40°C and a detector wavelength of 260 nm. The injection volume was 10 µL. Data acquisition was made with the software PC 1000 (ThermoSeparations Products, Riviera Beach, FL).

Mobile Phase (Diluent): The mobile phase consisted of buffer and Acetonitrile in the ratio of 30:70 (v/v). The pH of the mobile phase was adjusted to 3.0with sodium hydroxide. The buffer used in the mobilephase consisted of 0.1%orthophosphoric acid in double-distilled water. The mobile phase waspremixed and filtered through a 0.45-µm filter anddegassed.

Standard Stock Solution: Standard stock solution wasprepared by transferring approximately 1000 mg of MET and 10 mg of DAP reference standards to a 100 mL volumetric flask.About 70 mL of diluent was added. It was then sonicated for10 min and water was added to make up the volume in the flask.Each stock solution was further diluted with the diluent, to produce reference standard and test solutions containingMET (1500ppm) and DAP (15 ppm).

Preparation of Sample Solutions: Ten tablets were weighed and finely powdered. A quantity equivalent to one tablet containing 1000 mg of metformin HCl and10 mg of dapagliflozin was transferred in a 100-mL volumetric flask.To this flask, 70 mL of diluent was added, sonication was performed for 15 min with shaking the flask, the solution was cooled to ambient temperature, diluted to volume with diluentand mixed well. Then it was filtered through 0.45 micron Injection filter. (Stock solution). Further pipetted 1.5 ml of Metformin and Dapagliflozin from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. (1500 ppm MET and 15 ppm of DAP)

Calculation: (For Metformin)

$$\%Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Assay Results: (For Metformin)

$$\frac{94480}{93332} * \frac{1000}{100} * \frac{1.5}{10} * \frac{100}{1250.08} * \frac{10}{1.5} / \frac{1250.08}{1000} * \frac{99.8}{100} * 100 = 101.03\%$$

Calculation: (For Dapagliflozin)

$$\%Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Assay Results: (For Dapagliflozin)

$$\frac{8431}{8414} * \frac{10}{100} * \frac{1.5}{10} * \frac{100}{1250.08} * \frac{10}{1.5} * \frac{1250.08}{10} * \frac{99.8}{100} * 100 = 99.95\%$$

Forced Degradation Study: The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability

characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Metformin and Dapagliflozin using the proposed method.

Preparation of Stock Solution for Degradation Studies: Accurately weighed 10 tablets crush in mortar and pestle and transferred equivalent to 1000 mg of Metformin and 10mg Dapagliflozin in (Marketed formulation = 1250.08 mg of tablet Powder) sample into a 100mL clean dry volumetric flask add about 70 mL of Diluent and sonicated it up to 30 min to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter (Stocksolution).

Hydrolytic Degradation under Acidic Condition: Pipetted 1.5 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then the volumetric flask was kept at 60°C for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filtered the solution with 0.22 microns syringe filters and placed in vials.

Hydrolytic Degradation under Alkaline Condition: Pipetted 1.5ml of above solution into a 10ml volumetric and 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then the volumetric flask was kept at 60°C for 6 hours and then the solution neutralized with 0.1N HCl and made up to 10ml with diluent. The solution was filtered with 0.22 microns syringe filters and placed in vials.

Thermal Induced Degradation: Metformin and Dapagliflozin sample was taken in Petridish and kept in Hot air oven at 110°C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

Oxidative Degradation: Pipetted 1.5ml of above stock solution into a 10ml volumetric flask and 1ml of 3% w/v of hydrogen peroxide was added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. The solution was filtered with 0.45 microns syringe filters and placed in vials.

Photo Degradation: Pipetted 1.5 ml of above stock solution into a 10ml volumetric flask and exposed it to sunlight for 24hrs and the volume was made up to the mark with diluent. The solution was filtered with 0.45 microns syringe filters and placed in vials.

Method Validation: The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, limit ofdetection (LOD), limit of quantification (LOQ), robustness andsystem suitability parameters in accordance with the ICH guidelineQ2 (R1) [16].

Accuracy or Recovery: Accuracy is represented and determined by recovery experiments. In this process, it was tested at three different levels that were 50, 100 and 150% and analyzing Chromatogram.Accuracy was evaluated by determining the analyte in solutionsprepared according to the standard addition method andexpressed in terms of percentage recoveries of MET and DAP from the real samples.

Precision: The precision evaluated at the repeatability of the method was studied by calculating the relative standard deviation (RSD) for 6 determinations performed on the same day andunder the same experimental conditions. These results were summarized in (Table 3).

Linearity: Standard stock solutions of drugs were diluted in the concentration range of 500–2500 µg/mL MET and 5-25 µg/mL DAP respectively for the determination of linearity. 3 sets of such solutions were prepared. Each set was analyzed to plot a calibration curve. Standard deviation (SD), slope, intercept and coefficient of determination (r 2) of the calibration curves were calculated to ascertain linearity of the method.

Determination of the Limits of Detection and Quantitation: For determining the limits of detection (LOD) and quantitation (LOQ), the method based on the residual standard deviation (SD)of a regression line and slope was adopted. To determine the LODand LOQ, a specific calibration curve was studied using samplescontaining the analytes in the range of the detection and quantitationlimits. The LODs for MET and DAP were 3.05 and 2.98 and the LOQs were 10.07 and 9.98, respectively.

System Suitability Study: System suitability tests were carried out on a freshly prepared standard solution of the MET and DAP to scrutinize the various optimized parameters. Such as plate count, resolution and tailing factor. Results are shown in Table 5.

RESULTS AND DISCUSSION

Optimization of Chromatographic Techniques: The chromatographic method was optimized by changing the various variable parameters of the mobile phase. Initially the mobile phase tried was methanol:ammonium acetate buffer and methanol:phosphate buffer with various combination of pH as well as varying proportion but the peaks are eluted upto 20 mts.Finally the mobile phase was optimized with pottassium dihydrogen phosphate with buffer (pH 3.0), Acetonitrile in proportion 30:70 respectively. The peak was eluted at less than 5 mts. The method was performed with various columns like C18 column, Hypersil column, Lichrosorb and Intersil ODS column. Inspire ODS (4.6×150mm, 5mm) were found to be ideal as it gave good peak shape and resolution at 1.0 ml per min flow.

A chromatographic separation was metformin and dapagliflozin was achieved with inspire ODS (4.6×150mm, 5mm) analytical column using Orthophosphoric acid buffer: Acetonitrile (30:70) in isocratic mode at a flow rate of 1ml/min. column at ambient temperature and detection of all the drugs were monitored at 260 nm using PDA detector. The system suitability test was appropriate using 0.1% orthophophoric acid pH 3.0 adjusted with sodium hydroxide in combination with acetonitrile (30:70, v/v)at room temperature, which resulted in a retention time of2.097 min for MET and 3.691 for DAP, as shown in (Fig. 2 and 3) MET and DAP were determined at 260 nm and no interference was observed, therefore, this wavelength was utilized.

Specificity and Forced Degradation Studies: Forced degradations were performed to show the stability indicating properties of the analytical method, particularly when there is no information available about the potential degradation products. MET and DAP were found to be stable under acid, base, photolytic stress and thermal stress conditions. Under acid condition a significant decrease in the area of the MET and DAP was exhibited with two additional peaks at 2.73 and 3.17 respectively shown in (Fig. 4a). For MET and DAP, the basic conditions resulted in a significant decrease in the area with the additional peaks. Under the basic conditions, a significant decrease of the peak area of MET and DAP was observed within 5 min, with two additional peak detected at 2.75 min and 3.48 min respectively shown in (Fig. 4b). Under oxidative condition, a significant decrease of the area of MET and DAP was detected and a small degradation peaks were seen approximately at 2.73 min shown in (Fig. 4d). Under thermal condition, a slight decrease of the area of MET was detected and one smalldegradation peaks were seen approximately at 3.29 min shown in (Fig. 4c). The stressed samples were respectively analyzed for metformin and dapagliflozin and the results are shown in (Table 5).

Linearity: The analytical curves constructed for MET and DAP were found to be linear in the 500–2500 µg/mL and 5-25 µg/mL range respectively. The results are shown in (Table 3a and 3b). The value of correlation coefficient calculated for MET (R 2 = 0.99, y =58.74 x+6509, where x is concentration and y is the peak absolute area) were shown in (Fig. 8a) and DAP correlation coefficient (R 2 = 0.99, y = 554.5x-247) were shown in (Fig. 8b).

Accuracy: Accuracy was evaluated by determining the analyte in solutions prepared according to the standard addition method andexpressed in terms of percentage recoveries of MET and DAP from the real samples. The mean recovery data of MET and VLG were 100.58 % (Table 1a) and 100.54 % (Table 1b), demonstrating that the method is accurate within the desired range.

Precision: The precision of the method was studied by determining theconcentrations of each ingredient in the tablets six times. The RSD value for MET and DAP was 0.6 and 1.0 respectively. The inter-day precision was assessed by analyzing 6 samples on 3 different days. The RSD values obtained for MET and DAP was 1.2 and 1.2 respectively. These results were summarized in (Table3).

Theresults of the precision study (Table 2) indicate that the method is Reliable [relative standard deviation (RSD) percentage < 2].

Limit of Detection and Quantitation: The LOD and LOQ were obtained by using the signal to noiseratio calculations.Theexperimental LOD and LOQ were 3.05 µg/mL and 10.07 µg/mL for MET, 2.98 µg/mL and 9.98 µg/mL for DAP respectively.

System Suitability: For system suitability studies, five replicate injections of mixed standard solutions were injected and the parameters like, column efficiency, resolution and tailing factor of the peaks were calculated. Tailing factor for MET and DAP was found to be 1.82 and 1.84. Theoretical plates for MET and DAP was 2940 and 3415. Resolution was found to be 6.06. The Results are shown in (Table 4a and 4b).

Table 1a: Accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	47085	500	503.48	100.70	100.58
100%	94332	1000	1008.69	100.87	
150%	140531	1500	1502.70	100.18	

Table 1b: Accuracy results for Dapagliflozin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	4224	5	5.01	100.16	100.54
100%	8503	10	10.08	100.81	
150%	12734	15	15.10	100.65	

Table 2: Precision results for Metformin and Dapagliflozin

Injection	Area for Metformin	Area for Dapagliflozin
Injection-1	97532	8829
Injection-2	99523	8695
Injection-3	96832	8755
Injection-4	97332	8523
Injection-5	98332	8762
Injection-6	96332	8645
Average	97649	8702
Standard Deviation	1142.7	107.6
%RSD	1.2	1.2

Table 3a: Linearity results for Metformin

S.No	Linearity Level	Concentration	Area
1	I	500	37517
2	II	1000	63997
3	III	1500	93332
4	IV	2000	123766
5	V	2500	154482
Correlation Coefficient		0.999	

Table 3b: Linearity results for Dapagliflozin

S.No	Linearity Level	Concentration	Area
1	I	5	2647
2	II	10	5201
3	III	15	8029
4	IV	20	10737
5	V	25	13743
Correlation Coefficient		0.999	

Table 4a: System suitability results for Metformin

		System Suitability Results	
S.No	Flow Rate (ml/min)	USP Plate Count	USP Tailing
1	0.8	2075.75	1.86
2	1.0	2978	1.82
3	1.2	2694.04	1.63

Table 4b: System suitability results for Dapagliflozin

		System Suitability Results	
S.No	Flow Rate (ml/min)	USP Plate Count	USP Tailing
1	0.8	4078.38	1.89
2	1.0	3415.94	1.84
3	1.2	3196.52	1.47

Table 5: Degradation studies

	Metformin		Dapagliflozin		Peak purity
Sample Name	Area	% Degraded	Area	% Degraded	-
Standard	93332	-	8418	-	
Acid	84646	9.31	7716	5.44	Passes
Base	88401	5.28	7607	3.39	Passes
Peroxide	87925	5.79	7753	6.42	Passes
Thermal	87133	6.64	7707	3.39	Passes
Photo	88359	5.33	7634	6.42	Passes

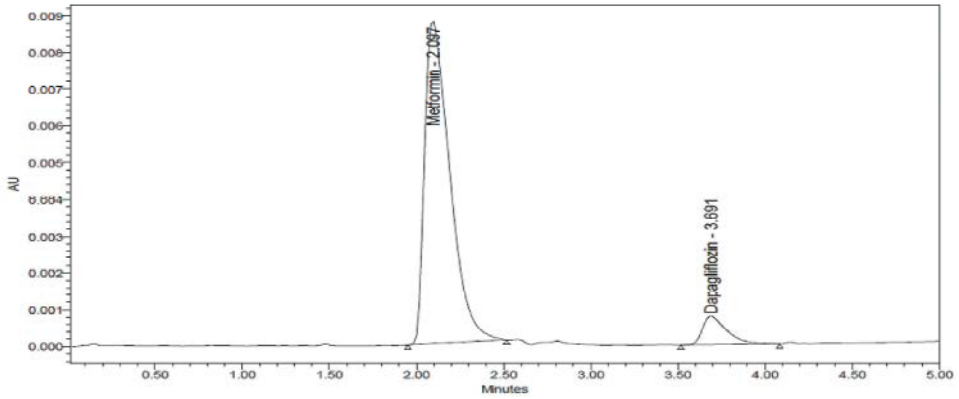

Fig. 2: Standard chromatogram of MET and DAP

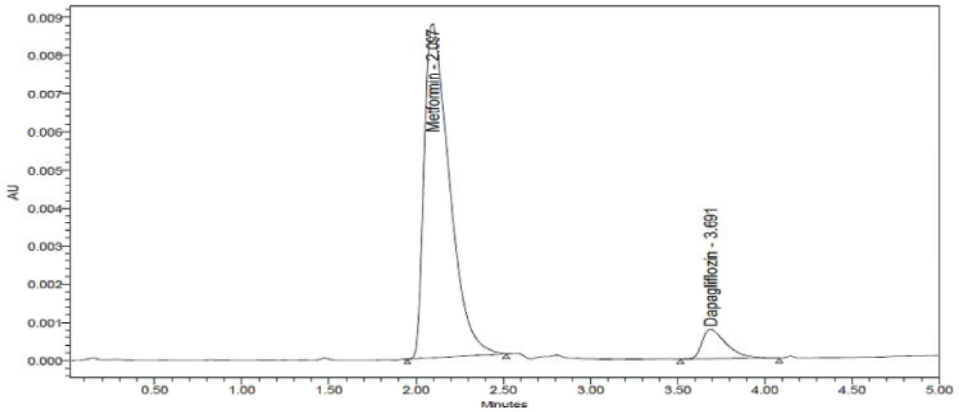

Fig. 3: Sample chromatogram of MET and DAP

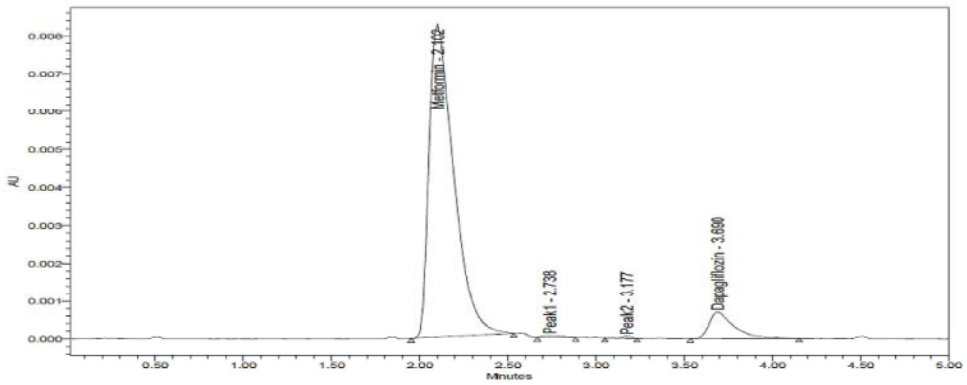

Fig. 4a: Chromatogram of forced degradation studies (acid hydrolysis)

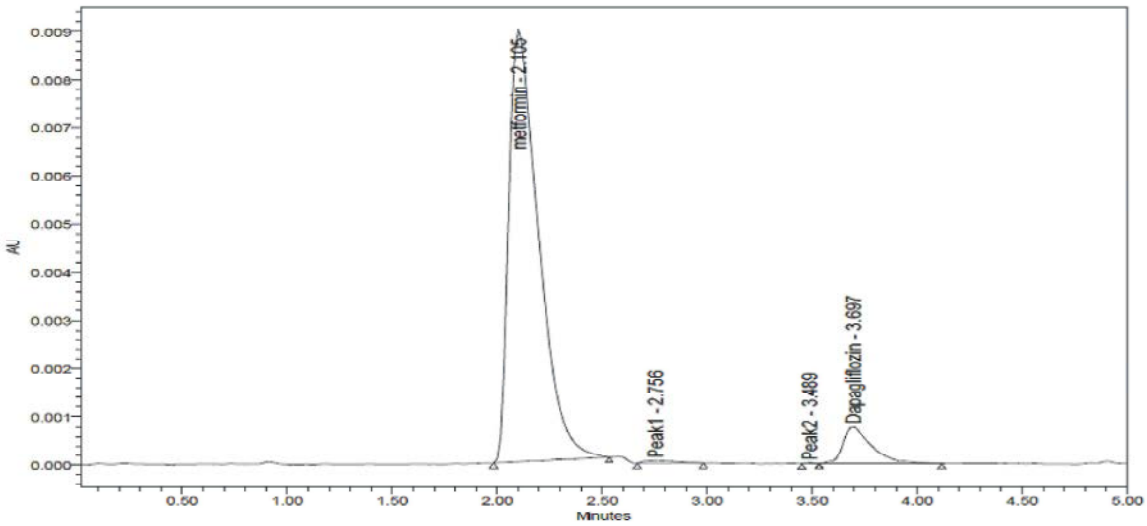

Fig. 4b: Chromatogram of forced degradation studies (alkaline hydrolysis)

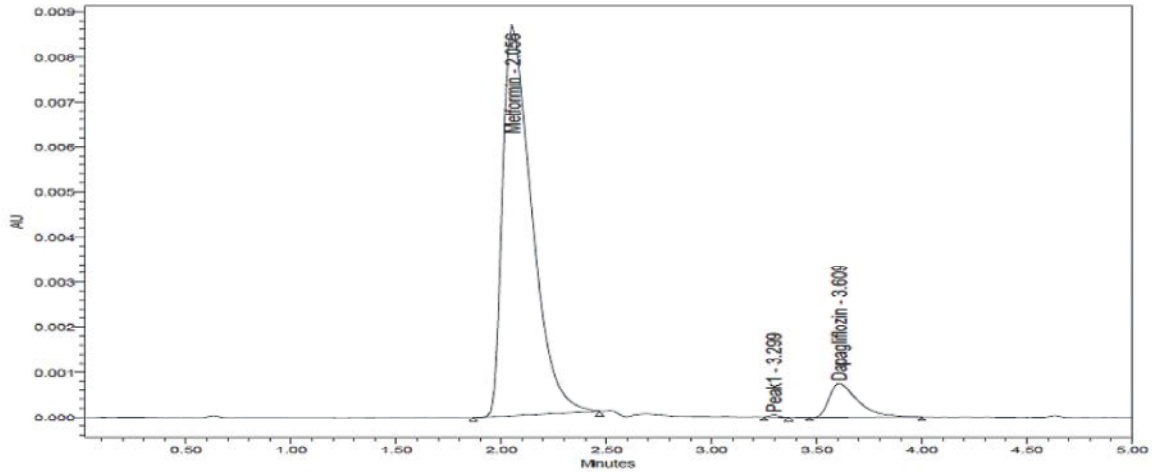

Fig. 4c: Chromatogram of forced degradation studies (Thermal hydrolysis)

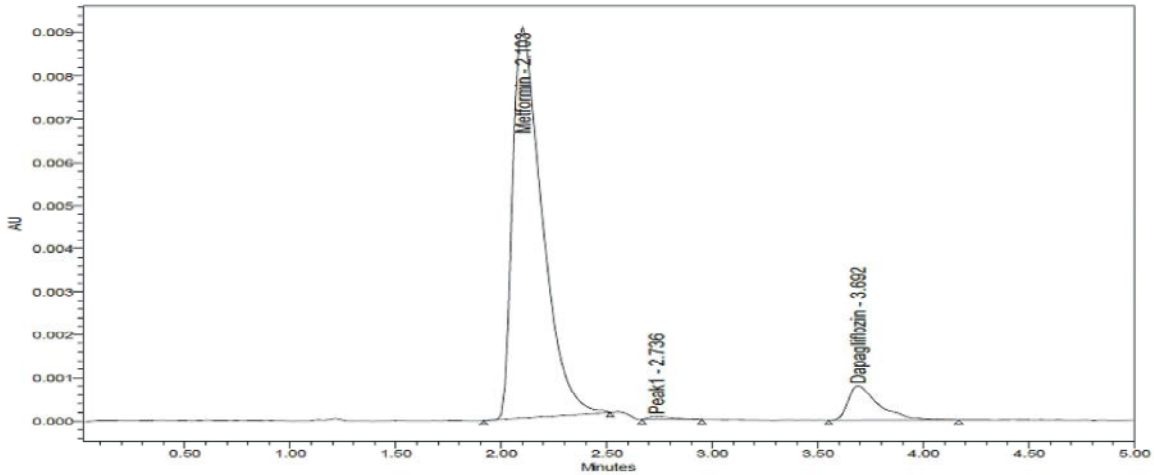

Fig. 4d: Chromatogram of forced degradation studies (Oxidative hydrolysis)

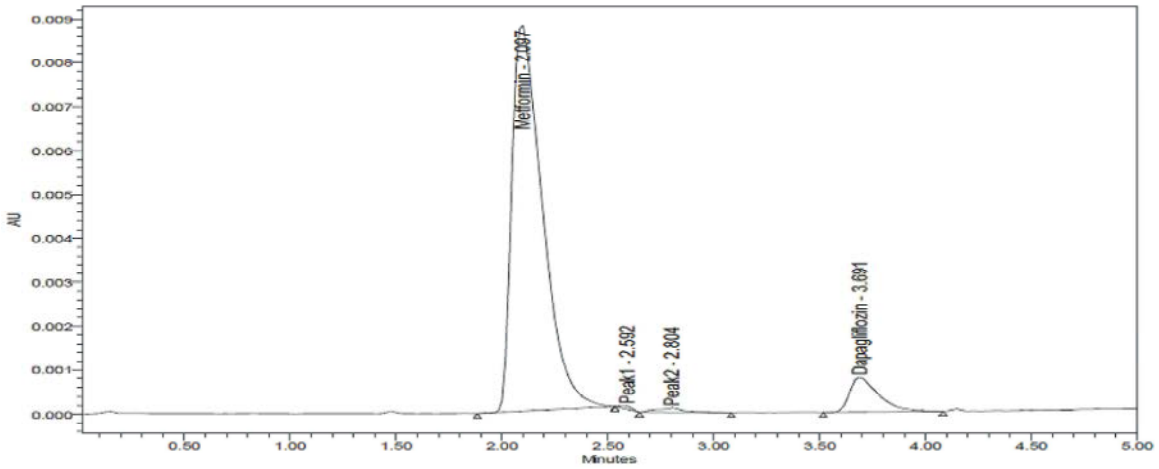

Fig. 4e: Chromatogram of forced degradation studies (Photolytic degradation)

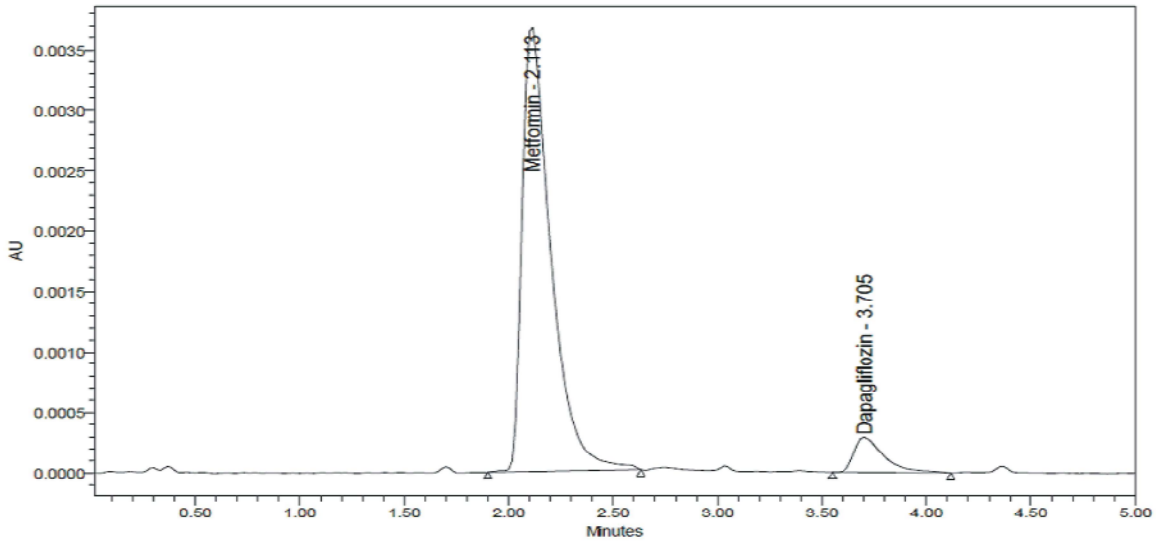

Fig. 5: Chromatogram of Accuracy

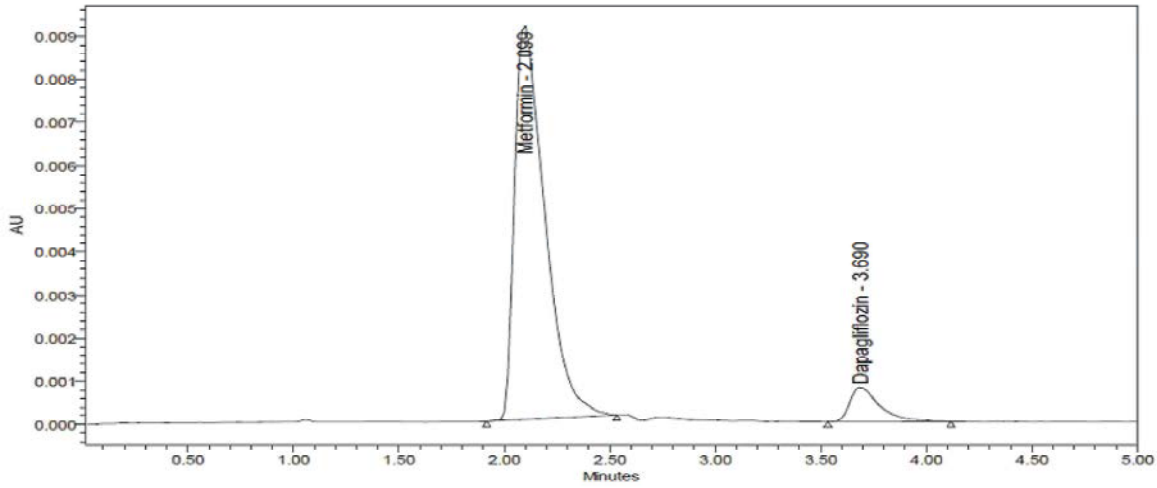

Fig. 6: Chromatogram of Precision

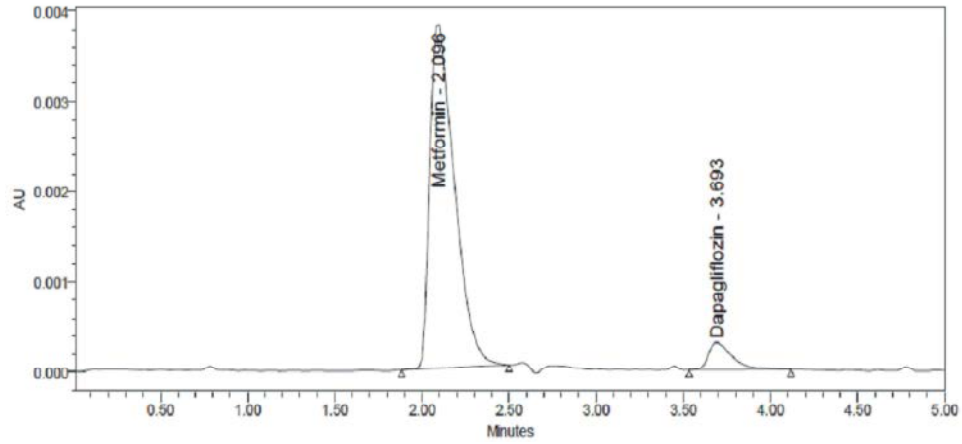

Fig. 7: Chromatogram of linearity

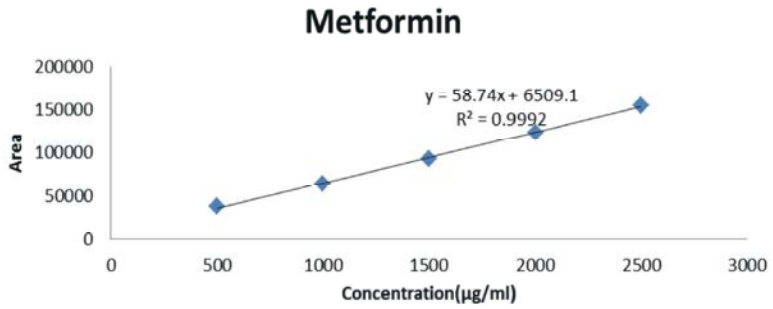

Fig. 8a: Linearity curve of Metformin

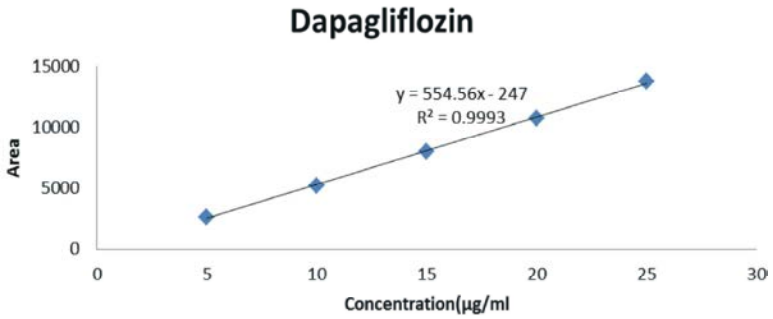

Fig. 8b: Linearity curve of Dapagliflozin

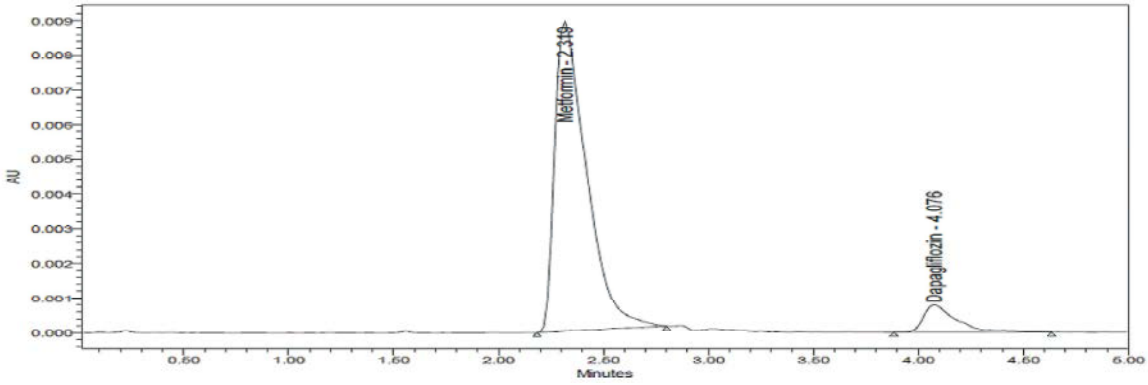

Fig. 9: Chromatogram for system suitability

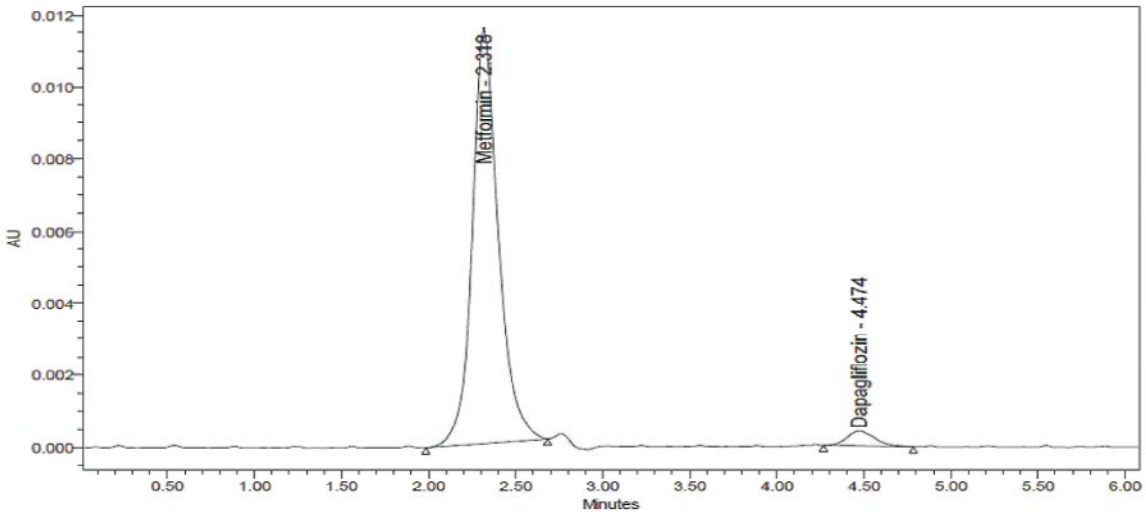

Fig. 10: Chromatogram for Robustness

CONCLUSIONS

This method can be used for the simultaneous determination of Metformin and Dapagliflozin in the pharmaceutical dosage form. The method is validated and shown to be accurate and precise. Results of the validation studies showed that the stability-indicating RP-HPLC method is simple, mass compatible, accurate, robust and specific without any interference from the excipients and degradation products. The proposed method was successfully applied for the quantitative analysis of MET and DAP in tablets. The method may thus be used for routine analysis, quality control and stability studies of the pharmaceutical tablets containing these drugs.

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Conflicts of Interest: There are no conflicts of interest.

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