CHAPTER - IV

DEVELOPMENT OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND METFORMIN

4.1. PHYSICAL, CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF THE DRUGS

4.1.1. Dapagliflozin

IUPAC name	:	(2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol
Molecular formula	:	$C_{21}H_{25}ClO_6$
Molecular weight	:	408.875 g/mol
Appearance	:	white crystalline powder
Solubility	:	Freely soluble in dimethylsulfoxide and ethanol; moderately soluble in water.

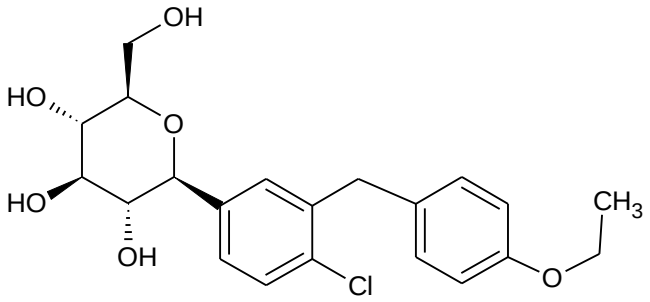

Figure 4.1: Chemical structure of dapagliflozin

Dapagliflozin is an antihyperglycemic agent belonging to the gliflozin class of drugs [1]. In 2014, the US Food and Drug Administration approved dapagliflozin for glycemic control in adult patients with type - II diabetes [2]. Dapagliflozin acts as selective inhibitor for sodium-glucose co-transport subtype 2 proteins [3-5]. These proteins are responsible for the reabsorption of glucose in the kidney. By inhibiting these proteins, dapagliflozin decreases blood sugar levels by causing the kidneys to eliminate more glucose in the urine.

4.1.2. Metformin

IUPAC name	:	3-(diaminomethylidene)-1,1-dimethylguanidine
Molecular formula	:	$C_4H_{11}N_5$
Molecular weight	:	129.167 g/mol
Appearance	:	white to off-white crystalline
Solubility	:	It is freely soluble in water, slightly soluble in alcohol, practically insoluble in acetone and methylene chloride

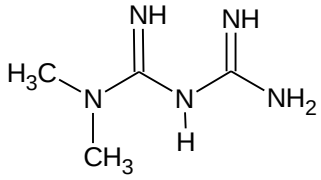

Figure 4.2: Chemical structure of metformin

Metformin is an oral hypoglycemic agent belonging to the biguanides class of compounds. Metformin is prescribed for the management of non insulin dependent diabetes mellitus [6,7]. Metformin exerts hypoglycemic activity by decreasing hepatic production and intestinal absorption of glucose and improving insulin sensitivity. All the effects are mediated by the activation of enzyme adenosine monophosphate (AMP)-activated protein kinase by metformin [8-10].

4.2. REVIEW OF LITERATURE

4.2.1. Dapagliflozin

The therapeutic importance of dapagliflozin initiated several researchers on its determination in both pharmaceuticals formulations and in biological fluids. Gajanan *et al.,* [11] Sanagapati *et al.,* [12,13] and Karuna *et al.,* [14] reported UV

spectrophotometric methods like Area under the curve, First order derivative and Second order derivative method using different solvent systems for the estimation of dapagliflozin in bulk and pharmaceutical formulations.

Jeyabaskaran *et al.,* [15] Sanagapati *et a.,* [16] and Debata *et al.,* [17] estimated dapagliflozin in pure and tablet dosage forms using reverse phase high performance liquid chromatography approach.

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) bioanalytical assay of dapagliflozin in rat plasma and human plasma were reported by Aubry *et al.,* [18] and Qin *et al.,* [19] respectively. Aubry *et al.,* [18] LC–MS/MS method were applied to study the pharmacokinetics of dapagliflozin in rat plasma whereas Qin *et al.,* [19] LC–MS/MS method was used to future clinical studies for the newly approved drug Farxiga or any combination therapy containing dapagliflozin.

4.2.2. Metformin

Metformin is official in United States Pharmacopoeia [20], Indian Pharmacopoeia [21], European Pharmacopoeia [22] and British Pharmacopoeia [23]. Indian Pharmacopoeia and British Pharmacopoeia official method includes UV spectrophotometric method for estimation of metformin. United States Pharmacopoeia and European Pharmacopoeia suggest potentiometric titration with 0.1N perchloric acid for the assay of metformin.

Different analytical methods have been reported in the literature for the assay of metformin in bulk and in its pharmaceutical preparations and include many

techniques as UV spectrophotometry [24-27], visible spectrophotometry [28-30], high-performance liquid chromatography [31], high-performance thin layer chromatography [32,33], conductometric titration [34], Nuclear magnetic resonance spectrometry [35], potentiometry [36], spectrofluorimetry [36], Ion-pair liquid chromatography [37,38].

High performance liquid chromatography [39-43], liquid chromatography–tandem mass spectrometry [44, 45], ion-pair liquid chromatography [46] and capillary electrophoresis [47] are reported in the literature for the assay of metformin in human plasma, urine, and breast milk samples. For the quantification metformin in mouse and rat plasma samples techniques such as ultra performance hydrophilic interaction LC-MS/MS [48] and hydrophilic interaction LC-MS/MS [49] have been reported.

4.2.3. Dapagliflozin and metformin combination

In 2014, the US Food and Drug Administration has approved the combination of dapagliflozin and metformin, along with diet and exercise, to improve blood glucose control in adults with type - II diabetes [50]. The combination of these two drugs is not official in any pharmacopoeias. An extensive literature survey was done and found that there were two UV spectrophotometric methods [51,52] and two RP-HPLC methods [53,54] for the determination of dapagliflozin and metformin simultaneously in bulk [51-54], synthetic mixture [51,52] and tablet dosage forms [53,54].

Jani *et al.,* [51,52] proposed two UV spectrohotometric methods for the simultaneous quantification of dapagliflozin and metformin in bulk and synthetic mixture. The first method involves solving of simultaneous equations based on

measurement of absorbance of dapagliflozin and metformin at two wavelengths 225 nm and 237 nm [51]. The second method involves solving of first order derivative based on measurement of absorbance of dapagliflozin and metformin at two wavelengths 235 nm and 272 nm [52].

In the reverse-phase high-performance liquid chromatography method developed and validated by Mohammad and Gowri [53], chromatographic separation and quantification of the two drugs in the presence of their degradation products was carried out on a Hypersil BDS C18 (250 mm × 4.6 mm id) 5 μm column with a mobile phase of 0.1 % orthophosphoric acid (pH was adjusted to 6.8 with triethylamine) and acetonitrile (50:50 *v/v*) at a flow rate of 1.0 mL/min. The detection was carried out at 240 nm using photodiode array detector.

The second method reported by Shyamala *et al.,* is an RP-HPLC method with UV detection [54]. The separation and quantification of the two drugs were done on a Hypersil BDS C18 column (250 x 4.6 mm, 5 μm). The mobile phase consisted of Phosphate Buffer (pH 6.5), methanol and acetonitrile (50:30:20 *v/v/v*) and the detection was done at 240 nm.

The reported HPLC methods [53,54] suffers from one or more drawbacks like narrow range of linearity, more run time, less precise, less accurate, greater tailing factor, less resolution factor and less sensitive. In the present study, an attempt was made to develop a new stability indicating RP-HPLC method for simultaneous estimation of dapagliflozin and metformin in bulk and tablet dosage form with good sensitivity, selectivity, linearity, precision, accuracy and reproducibility.

4.3. MATERIALS AND METHODS

4.3.1. Instrumentation

1. Waters 2695 alliance HPLC system comprised an auto sampler injector, binary pump and a Waters 2998 Photo Diode Array Detector coupled with Waters Empower2 software.
2. The syringe used for injecting was 10 µL Hamilton syringe
3. Degassing of mobile phase was done with Spectra lab DGA 20 A3 ultrasonic bath sonicator.
4. The chemicals and drugs were weighed using Electronic balance ELB 300.
5. Digisun pH meter was used for all pH measurements.

4.3.2. Materials

Metformin and dapagliflozin were obtained as gift sample from Lara Drugs Private Limited (Telangana, India). Xigduo XR tablets (Astra Zeneca Pharmaceuticals LP, Wilmington) were obtained from the local pharmacy market. Acetonitrile (HPLC grade) and methanol (HPLC grade) were supplied by Merck India Ltd., Mumbai, India. Analytical reagent grade dipotassium hydrogen orthophosphate, hydrogen peroxide, hydrochloric acid and sodium hydroxide were obtained from Sd. Fine Chemicals Ltd., Mumbai, India. Milli-Q water (Millipore, USA) was used in all experiments.

4.3.3. Chromatographic conditions

The chromatographic separation of metformin, dapagliflozin, and their stress degradants were carried out on a Supelco C18 (250 mm × 4.5 mm i.d., particle size 5 µm). The mobile phase was a mixture of 0.1 M dipotassium hydrogen phosphate, acetonitrile and methanol (60:30:10, *v/v/v;* pH 7.5) delivered at a flow rate of 1.2 mL/min. The mobile phase was filtered through 0.45 µm pore size membrane filter and sonicated for 20 min. The analysis was performed at 30 ^{o}C temperature. The elution of metformin and dapagliflozin was monitored by photodiode array detector. The chromatograms were recorded at 285 nm and the injection volume was 10 µL.

4.3.4. Stock and working solutions

A stock standard solution of dapagliflozin (0.1 mg/mL) and metformin (10 mg/mL) was prepared by dissolving 10 mg of dapagliflozin and 1000 mg of metformin in 100 mL of mobile phase in a 100 mL volumetric flask. This stock standard solution was used to prepare the working solutions at different concentrations (dapagliflozin - 2, 3, 4, 5 and 6 µg/mL: metformin – 200, 300, 400, 500 and 600 µg/mL). The stock and working standard solutions were stored in the refrigerator until further use.

4.3.5. Calibration curve

Aliquots of the working standard solutions (10 µL) were injected into the HPLC system. The detector response was determined using the chromatographic conditions (described in section 4.3.3) and plotted against the concentration of drug to

construct the calibration curve. The y-intercept, slope and regression coefficient were calculated to statistically evaluate the linear relationship.

4.3.6. Determination of dapagliflozin and metformin in tablet dosage form

Ten tablets of Xigduo XR (each tablet labelled to contain 10 mg dapagliflozin and 1000 mg metformin) were powdered. An amount equivalent to 10 mg dapagliflozin and 1000 mg metformin was accurately weighed into a 100 mL volumetric flask and mixed with 30 mL of mobile phase. The solution was sonicated for 20 min and filled with mobile phase to obtain a final concentration of 0.1 mg/mL (dapagliflozin) and 10 mg/mL (metformin). The solution was filtered through a 0.45 μm pore size membrane filter.

Aliquot of the above tablet sample stock solution were further diluted with the mobile phase to obtain final concentration of 4 μg/mL of dapagliflozin and 400 μg/mL of metformin. The resulting solution was then subjected to analysis by the proposed RP-HPLC method. The dapagliflozin and metformin content of the tablet dosage form can be calculated either using the calibration curve or using the regression equation.

4.3.7. Stress testing

Stress testing was carried out to induce forced degradation, to identify the stability of the drugs and also to validate the specificity of the proposed RP-HPLC method. Forced degradation was performed by exposing tablet sample solution (metformin - 400 μg/mL and dapagliflozin - 4 μg /mL) to stress conditions of hydrolysis (acid and alkali), oxidation, photo and thermal [55].

4.3.7.1. Acid hydrolysis

Tablet powder equivalent to 10 mg dapagliflozin and 1000 mg metformin was transferred to a 100 mL volumetric flask. The powder was mixed with 10 mL of 0.1 N hydrochloric acid and subjected to sonication for 30 min at room temperature (25±2 °C). The samples were neutralized with an amount of 0.1 N NaOH equivalent to that of the previously added. The flask was made up to the volume with mobile phase. The degraded sample solution was appropriately diluted with mobile phase to obtain a concentration of 400 µg/mL (metformin) and 4 µg/mL (dapagliflozin).

4.3.7.2. Alkali hydrolysis

Tablet powder equivalent to 10 mg dapagliflozin and 1000 mg metformin was transferred to a 100 mL volumetric flask. The powder was mixed with 10 mL of 0.1 N sodium hydroxide and subjected to sonication for 30 min at room temperature (25±2 °C). The samples were neutralized with an amount of 0.1 N HCl equivalent to that of the previously added. The flask was made up to the volume with mobile phase. The degraded sample solution was appropriately diluted with mobile phase to obtain a concentration of 400 µg/mL (metformin) and 4 µg/mL (dapagliflozin).

4.3.7.3. Oxidative degradation

Tablet powder equivalent to 10 mg dapagliflozin and 1000 mg metformin was transferred to a 100 mL volumetric flask. The contents were mixed with 10 mL of 30 % hydrogen peroxide solution. The reaction mixture was allowed to sonication for 30 min at room temperature (25±2 °C) and then the volume of the flask was made up to 100 mL with mobile phase. The degraded sample solution was appropriately

diluted (metformin - 400 µg/mL and dapagliflozin - 4 µg /mL) with mobile phase for analysis by the proposed method.

4.3.7.4. Thermal degradation

Tablet sample powder (dapagliflozin - 10 mg and metformin – 1000 mg) was exposed to 105 ^{o}C for 30 min in oven. After the specified time, the tablet powder was cooled and dissolved in 30 mL of mobile phase in a 100 mL volumetric flask. The solution thus prepared was diluted to volume with the mobile phase. Aliquot of the above degraded solution were further diluted with the mobile phase to obtain final concentration of 4 µg/mL of dapagliflozin and 400 µg/mL of metformin. The resulting solution was then subjected to analysis by the proposed RP-HPLC method.

4.3.7.5. Photolytic degradation

Tablet sample powder (dapagliflozin - 10 mg and metformin – 1000 mg) was exposed to direct sun light for up to 24 hr. After the specified time, the tablet powder was cooled, dissolved in 30 mL of mobile phase in a 100 mL volumetric flask and diluted to volume with the mobile phase. Aliquot of the above degraded solution were further diluted with the mobile phase to obtain final concentration of 4 µg/mL of dapagliflozin and 400 µg/mL of metformin. The resulting solution was then subjected to analysis by the proposed RP-HPLC method.

All the stressed samples were filtered through 0.45 µm pore size membrane filter and analyzed by the proposed RP-HPLC. The metformin and dapagliflozin peaks were checked for the retention times, peaks interference and spectra purity.

4.4. RESULTS AND DISCUSSION

4.4.1. Method development

Supelco C8 and C18 column with different temperatures were tried. The Supelco C18 column (250 mm × 4.5 mm, 5 μm) at 30 ^{o}C temperature was found to be apt for the separation of metformin and dapagliflozin efficiently. Various ratios of 0.1 M dipotassium hydrogen phosphate, acetonitrile and methanol with different flow rates and pH values were tested using a Supelco C18 (250 mm × 4.5 mm, 5 μm) column. Results were evaluated in terms of peak response, resolution, peak symmetry, selectivity and analysis time for drugs. The mobile phase with a composition of 60 % 0.1 M dipotassium hydrogen phosphate, 30 % acetonitrile and 10 % methanol (*v/v/v*) with the flow rate of 1.2 mL/min and pH 7.5 exhibited the appropriate separation of metformin and dapagliflozin with good peak shape and resolution. The detection wavelength for dapagliflozin and metformin was obtained using the PDA detector. A wavelength of 285 nm was selected for the simultaneous determination of dapagliflozin and metformin with good sensitivity.

The typical chromatogram of dapagliflozin and metformin using the optimized chromatographic conditions is shown in Figure 4.3. The proposed method permitted adequate resolution of the dapagliflozin and metformin within reasonable run time (6 min). Dapagliflozin and metformin were eluted at 2.847 min and 3.804 min, respectively. The optimized chromatographic conditions were summarized in the Table 4.1.

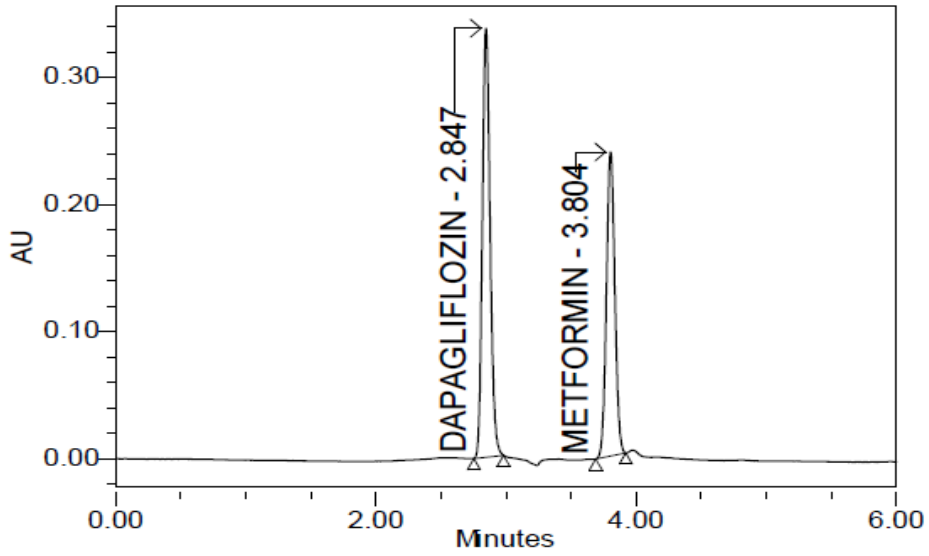

Figure 4.3: Chromatogram of dapagliflozin and metformin with optimized chromatographic conditions

Table 4.1: Optimized chromatographic conditions

S.No	Parameter	Value
1	Column	Supelco C18 (250 × 4.6 mm; 5 µm particle size)
2	Mobile phase	0.1 M dipotassium hydrogen phosphate, acetonitrile and methanol (60:30:10, *v/v/v;* pH 7.5)
3	Flow rate	1.2 mL/min
4	Diluent	Mobile phase
5	Column temperature	30±1 ^{o}C
6	Runtime	6 min
7	Retention time	Dapagliflozin - 2.847 min and metformin - 3.804 min
8	Volume of injection	10 µL
9	Detection wavelength	285 nm

4.4.2. Method validation

The developed RP-HPLC method was validated regarding system suitability, selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, robustness and specificity according to the International Conference on Harmonization [56].

4.4.2.1. System suitability studies

To evaluate the system suitability of the developed RP-HPLC method, five replicate analyses were done at a concentration of 4 μg/mL dapagliflozin and 400 μg/mL metformin. The system suitability parameters (% RSD of retention time, % RSD of peak area, USP plate count and USP tailing factor) were calculated and compared with the accepted criteria (Table 4.2). The results reveal the method suitability.

Table 4.2: System suitability parameters

Parameters	Metformin		Dapagliflozin		Recommended limit
	Value*	RSD (%)	Value*	RSD (%)	
Retention time	3.807	0.093	2.852	0.156	RSD ≤2
Peak area	1074166	0.259	1391792	0.395	RSD ≤2
USP resolution	8.218	0.786	-	-	> 1.5
USP plate count	16276	0.190	11053	0.255	> 2000
USP tailing factor	1.044	0.525	1.162	0.385	≤ 2

**Average of five values*

4.4.2.2. Selectivity

Selectivity was assessed by evaluating the chromatograms of mobile phase blank, placebo blank, working standard solution and tablet sample solution. The solutions of working standard and tablet sample were prepared at a concentration of 4 µg/mL dapagliflozin and 400 µg/mL metformin. The solutions of placebo blank, mobile phase blank, working standard and tablet sample were injected into the HPLC system. The chromatograms of placebo blank and mobile phase did not show any peaks (Figures 4.4 and 4.5). The chromatogram of tablet sample did not show any peaks other than that of metformin and dapagliflozin (Figure 4.7). The retention time of metformin and dapagliflozin in chromatograms of working standard solution and tablet sample solution are same (Figures 4.6 and 4.7). The results confirmed the specificity of the developed RP-HPLC method.

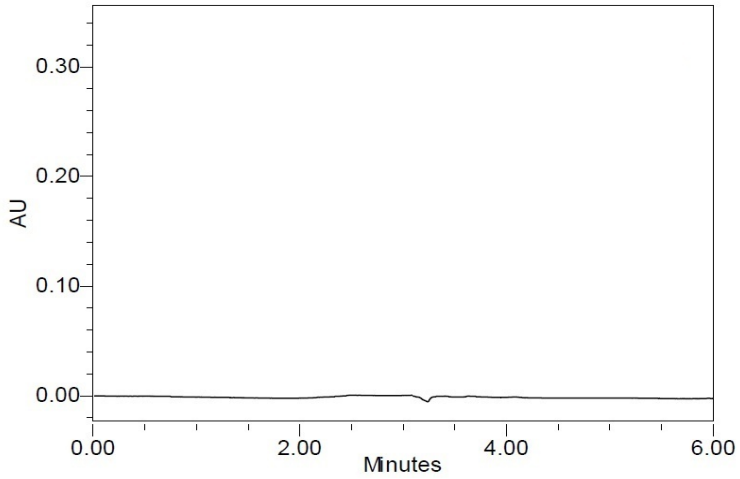

Figure 4.4: Chromatogram of mobile phase blank

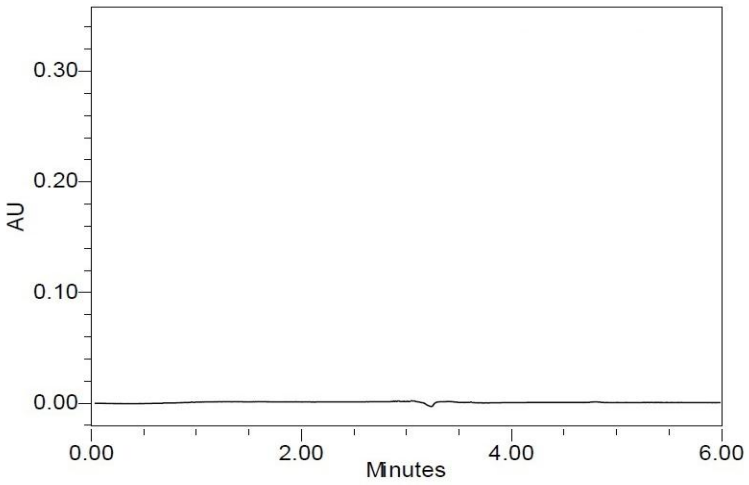

Figure 4.5: Chromatogram of placebo blank

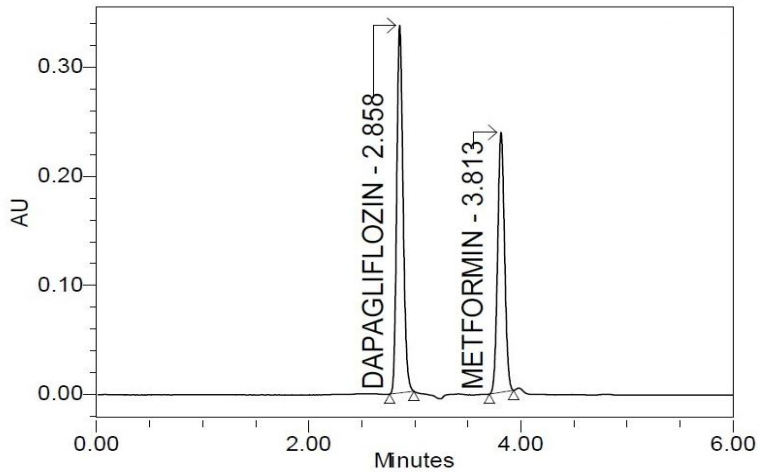

Figure 4.6: Chromatogram of working standard solution

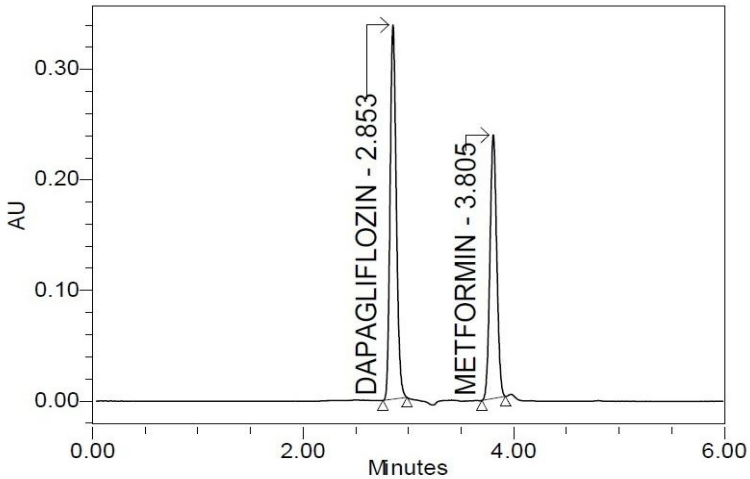

Figure 4.7: Chromatogram of tablet sample solution

4.4.2.3. Linearity

The linearity of the developed RP-HPLC method was obtained in the concentration range of 2-6 µg/mL for dapagliflozin and 200-600 µg/mL for metformin. The regression equations for the calibration curve were:

Metformin: $y = 2694 x – 1680$ ($R^2 = 0.9998$)

Dapagliflozin: $y = 34790 x + 336.0$ ($R^2 = 0.9999$)

Where y = peak area; x = concentration of drug in µg/mL; R^2 = Regression coefficient.

The results are summarized in Figures 4.8, 4.9 and Table 4.3. The results show a good correlation between the peak area of drugs and their concentrations with R^2 value $\geq$ 0.9998. The results demonstrated that the linearity of the method as satisfactory.

Table 4.3: Linearity data for dapagliflozin and metformin

Linearity of dapagliflozin		**Linearity of Metformin**	
Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**
2	695297	200	537561
3	1046886	300	805091
4	1391083	400	1072152
5	1738528	500	1347270
6	2088331	600	1617162
Regression equation: $y = 34790 x + 336.0$ (R^2=0.9999)		Regression equation: $y = 2694 x – 1680$ (R^2=0.9998)	

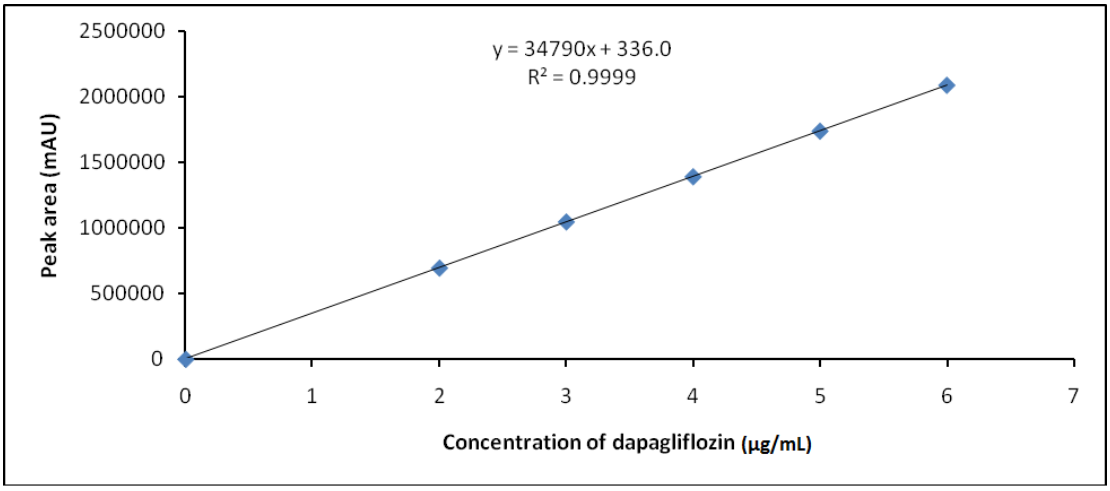

Figure 4.8: Linearity curve of dapagliflozin

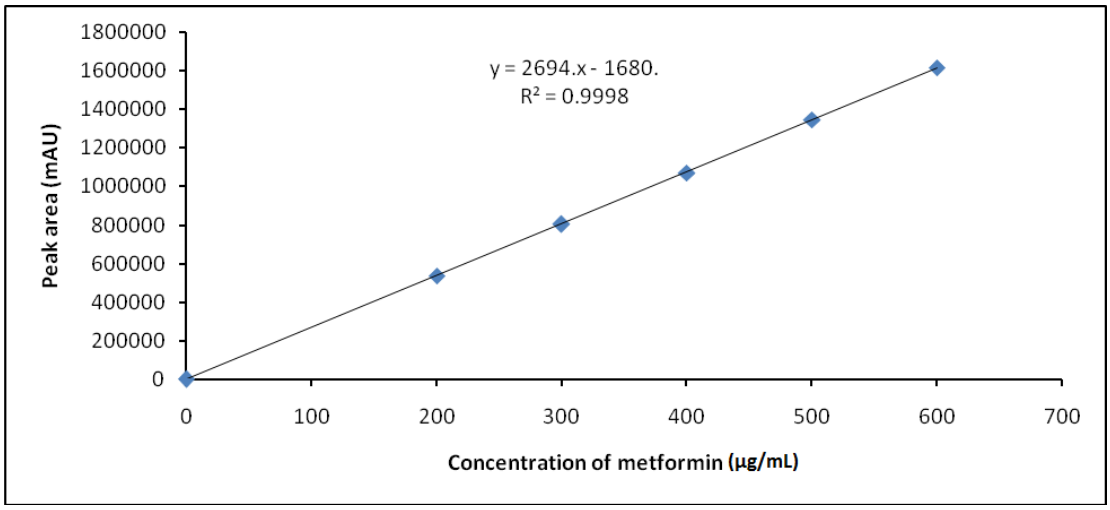

Figure 4.9: Linearity curve of metformin

4.4.2.4. Sensitivity

The sensitivity of the developed method was assessed by determining Limit of quantification (LOQ) and detection (LOD). The LOD and LOQ are defined as a signal-to–noise of more than three and ten-fold, respectively. The LOQ for dapagliflozin and metformin in this method were found to be 0.014 µg/mL and

0.907 µg/mL, respectively (Figure 4.10). The LOD of dapagliflozin and metformin were found to be 0.004 µg/mL and 0.272 µg/mL, respectively (Figure 4.11).

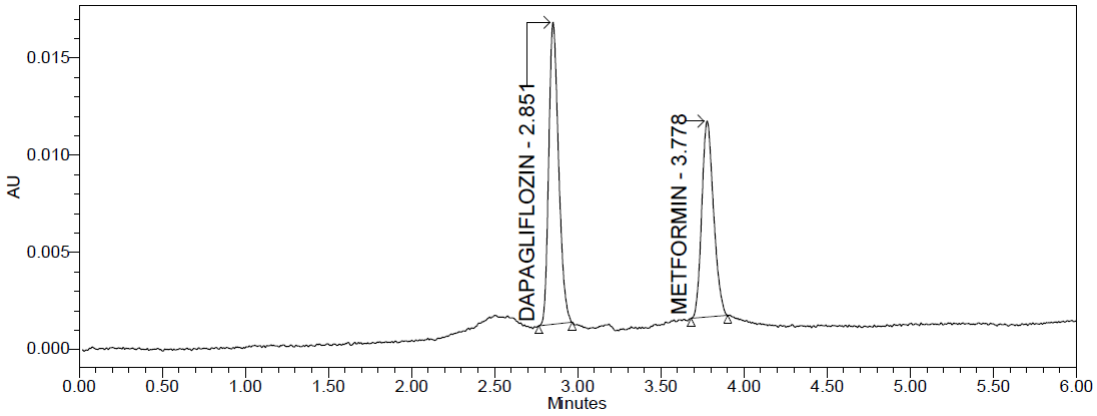

Figure 4.10: Chromatogram of dapagliflozin and metformin at LOD level

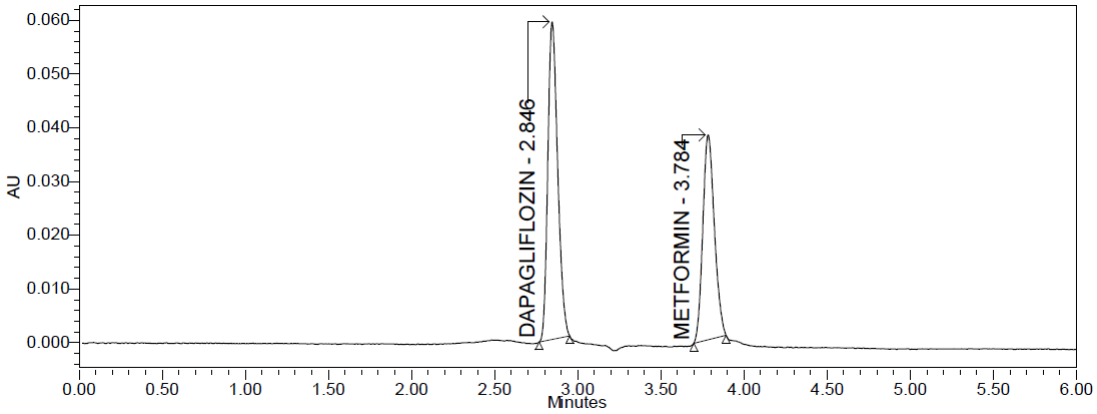

Figure 4.11: Chromatogram of dapagliflozin and metformin at LOQ level

4.4.2.5. Precision

Method precision was evaluated by injecting five independent working standard solutions with concentration 4 µg/mL of dapagliflozin and 400 µg/mL of metformin. The percentage relative standard deviation (% RSD) of peak area response was determined. The % RSD values for dapagliflozin and metformin were

found to be 0.098 % and 0.290 %, respectively. The results (Table 4.4) of precision testing proved that the developed method is precise.

Table 4.4: Precision and accuracy

Sample No.	Dapagliflozin		Metformin	
	Peak area	**Recovery (%)**	**Peak area**	**Recovery (%)**
1	1395556	99.37	1073942	99.68
2	1394761	99.31	1078341	100.1
3	1397868	99.53	1071294	99.43
4	1394339	99.28	1071016	99.41
5	1395322	99.35	1071352	99.44
Mean*	**1395569**	**99.36**	**1073189**	**99.61**
RSD	**0.098**	**0.098**	**0.290**	**0.291**

**Average of five values*

4.4.2.6. Accuracy

For evaluating the accuracy of the method, dapagliflozin and metformin concentration of 4 µg/mL and 400 µg/mL level were determined in five replicates. Table 4.4 illustrates the accuracy data of the method. The percent recovery was calculated and found to be 99.36 % and 99.61 % for dapagliflozin and metformin, respectively. The results confirmed the accuracy of the proposed method.

The accuracy of the method was further determined by spiking preanalyzed tablet sample with known amounts of dapagliflozin and metformin at three concentration levels. The samples were once again analyzed by the proposed method under optimized conditions. The mean recovery rates were found in the range 99.00 % to 99.46 % for dapagliflozin (Table 4.5) and 99.70 % to 99.82 % for metformin (Table 4.6). The results showed that the presence of excipients in the tablet

does not interfere with the determination of dapagliflozin and metformin. The chromatograms of dapagliflozin and metformin at three different concentration levels are shown in Figures 4.12, 4.13 and 4.14.

Table 4.5: Recovery data for dapagliflozin

Level	Concentration of dapagliflozin (µg/mL)		% Recovery	Mean*
	Added	Found		
50 %	2	1.980	99.00	99.00
	2	1.979	98.95	
	2	1.981	99.05	
100 %	4	3.986	99.65	99.46
	4	3.974	99.35	
	4	3.975	99.38	
150 %	6	5.977	99.62	99.29
	6	5.949	99.15	
	6	5.947	99.12	

**Average of three values*

Table 4.6: Recovery data for metformin

Level	Concentration of metformin (µg/mL)		% Recovery	Mean*
	Added	Found		
50 %	200	199.587	99.79	99.73
	200	199.409	99.70	
	200	199.410	99.71	
100 %	400	398.710	99.68	99.70
	400	397.280	99.32	
	400	400.430	100.11	
150 %	600	600.760	100.13	99.82
	600	598.320	99.72	
	600	597.610	99.60	

**Average of three values*

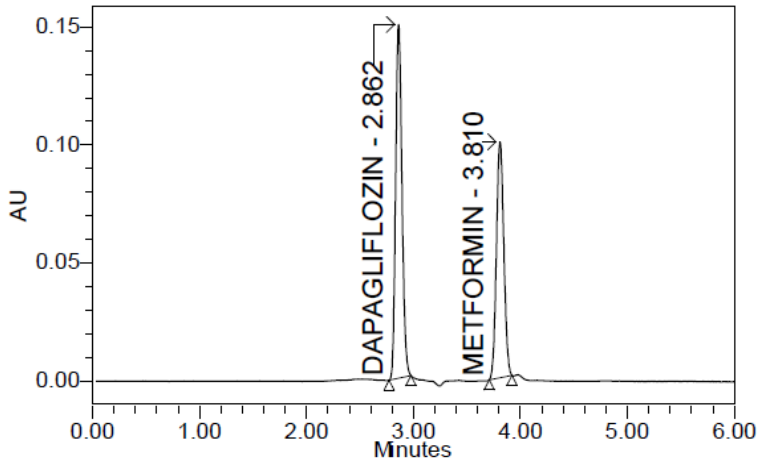

Figure 4.12: Chromatogram of selected drugs at 50 % accuracy level

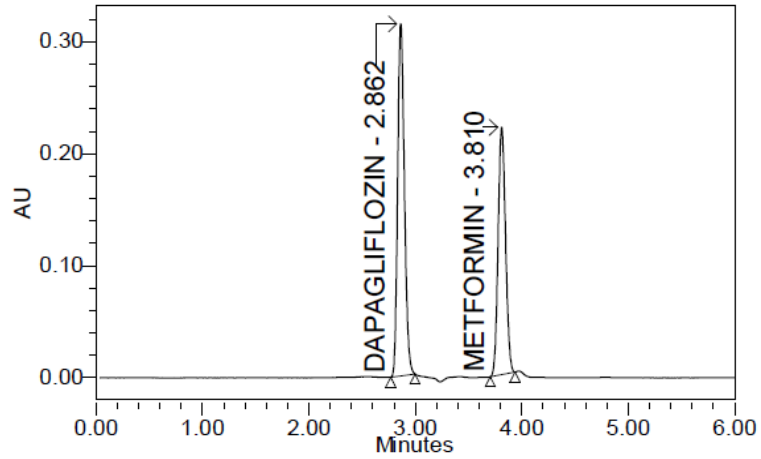

Figure 4.13: Chromatogram of selected drugs at 100 % accuracy level

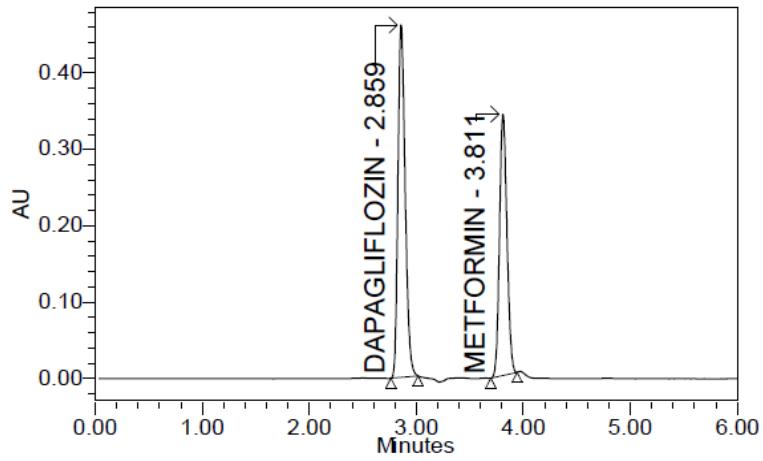

Figure 4.14: Chromatogram of selected drugs at 150 % accuracy level

4.4.2.7. Robustness

To evaluate the robustness of the proposed RP-HPLC method, the effect of minor variations in the flow rate (± 0.1 mL) and column temperature (± 5 ^{o}C) on system suitability parameters were observed. Robustness was studied using working standard solution containing dapagliflozin and metformin at a concentration of 4 µg/mL and 400 µg/mL, respectively. The results are shown in Table 4.7. There was no significant change in the system suitability parameters, thus established the robustness of the proposed RP-HPLC method.

Table 4.7: Robustness data for dapagliflozin and metformin

Parameter	Dapagliflozin			Metformin		
	USP Tailing	USP plate count	USP resolution	USP Tailing	USP plate count	USP resolution
Flow rate 1.0 + 0.1 mL/min	1.17	10889	-	1.04	16527	8.27
Flow rate 1.0 – 0.1 mL/min	1.16	11146	-	1.08	16314	8.21
Temperature 30 + 5 ^{o}C	1.16	11093	-	1.05	16229	8.24
Temperature 30 – 5 ^{o}C	1.15	11210	-	1.07	16323	8.11

4.4.2.8. Specificity

Forced degradation was performed to evaluate the specificity and stability indicating properties of the developed RP-HPLC method, by exposing tablet sample to different stress conditions like hydrolysis (acid and alkali), oxidation, photo and thermal stresses. The degradation results are summarized in Table 4.8. Upon treatment of dapagliflozin and metformin under different stress conditions, it was found that both the drugs were degraded under all the stress conditions applied. From

the percentage of degradation values, it was indicated that the dapagliflozin is less stable than metformin. In all the degradation conditions two peaks, in addition to dapagliflozin and metformin peaks, were detected. From the results, it was observed that there is no interference between the peaks of dapagliflozin, metformin and degradation products under the various stress conditions (Figures 4.15, 4.16, 4.17, 4.18 and 4.19). The peak purity tool was applied to confirm 100 % purity for dapagliflozin and metformin peaks in all cases. The peaks of dapagliflozin and metformin were pure because purity threshold was greater than purity angle under all the forced tests. The results showed that dapagliflozin and metformin peaks are free of coeluting degradation products.

Table 4.8: Summary of degradation studies of dapagliflozin and metformin

Stress condition	Peak Area	Recovery (%)	Degradation (%)	Purity threshold	Purity angle
Dapagliflozin					
Acid induced	1191919	84.87	15.13	0.370	0.241
Alkali induced	1177716	83.86	16.14	0.371	0.244
Oxidative	1192189	84.89	15.11	0.384	0.244
Thermal	1192280	84.89	15.11	0.390	0.248
Photo	1191517	84.84	15.16	0.377	0.245
Metformin					
Acid induced	998763	92.70	7.30	0.375	0.236
Alkali induced	990644	91.95	8.05	0.393	0.238
Oxidative	998149	92.64	7.36	0.407	0.235
Thermal	997657	92.60	7.40	0.405	0.239
Photo	991688	92.04	7.96	0.402	0.234

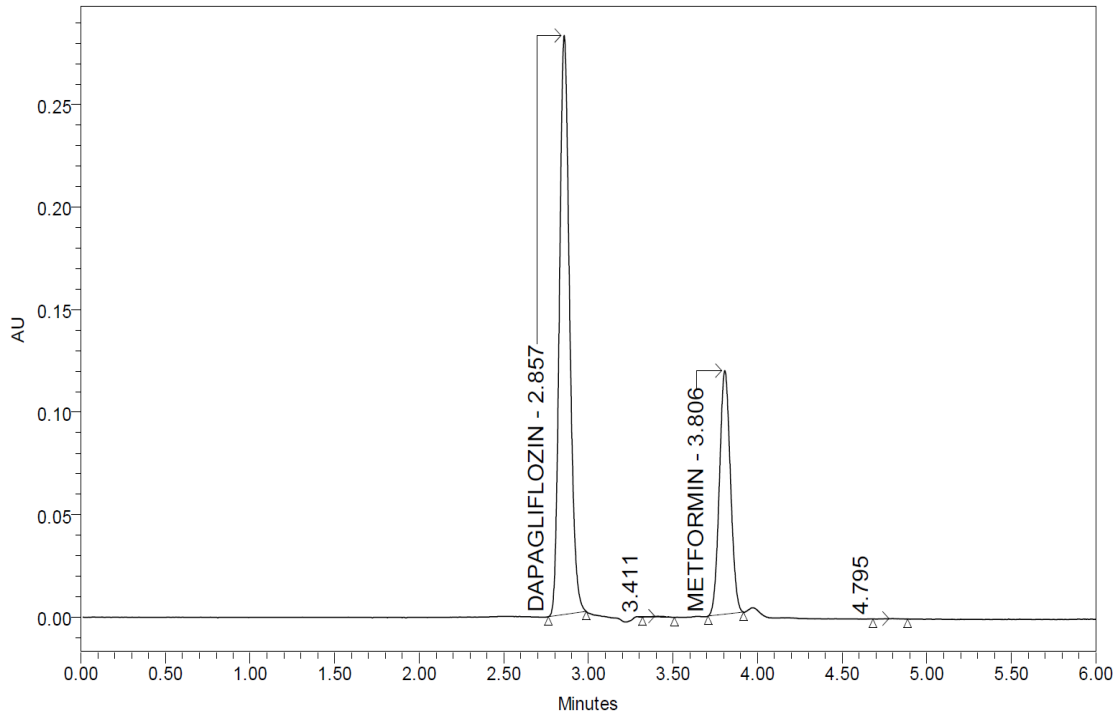

Figure 4.15: Dapagliflozin and metformin in 0.1 N HCl after 30 min at room temperature

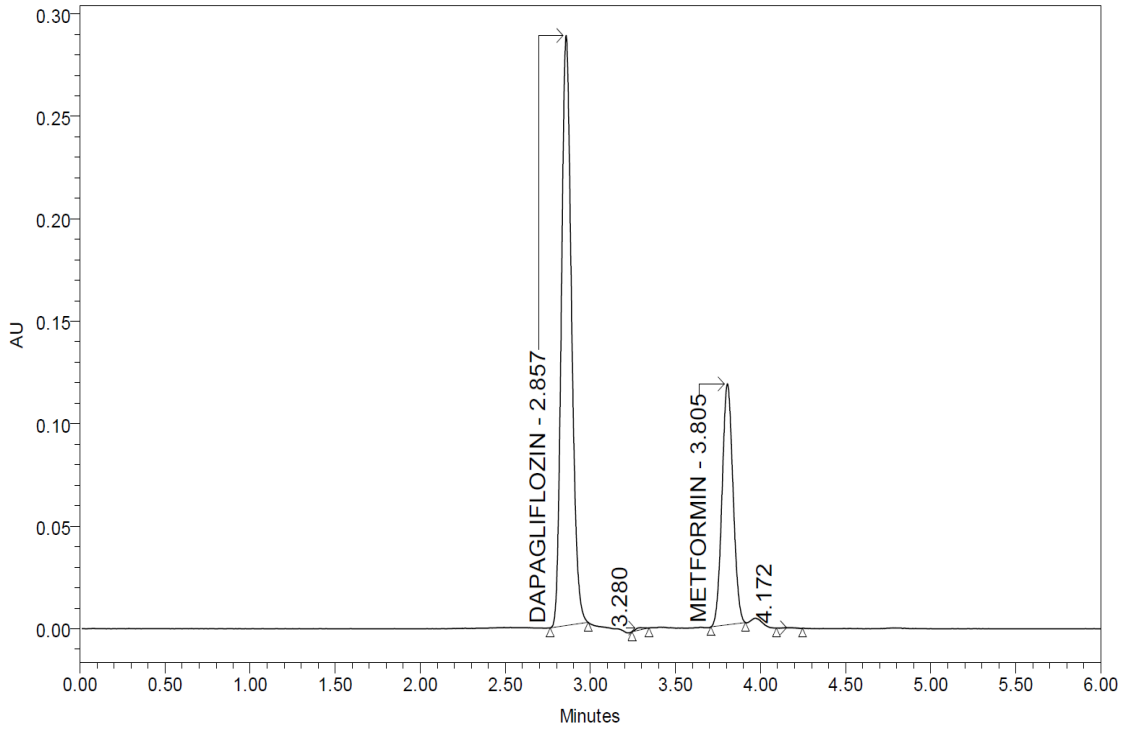

Figure 4.16: Dapagliflozin and metformin in 0.1 N NaOH after 30 min at room temperature

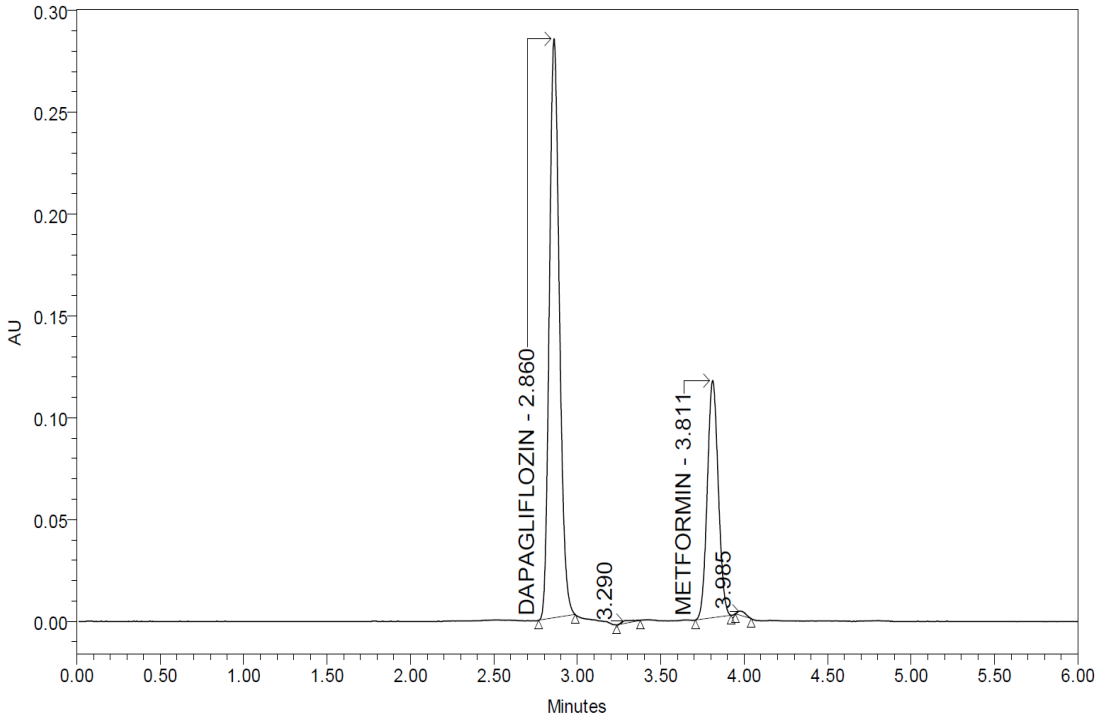

Figure 4.17: Dapagliflozin and metformin in 30 % H_2O_2 after 30 min at room temperature

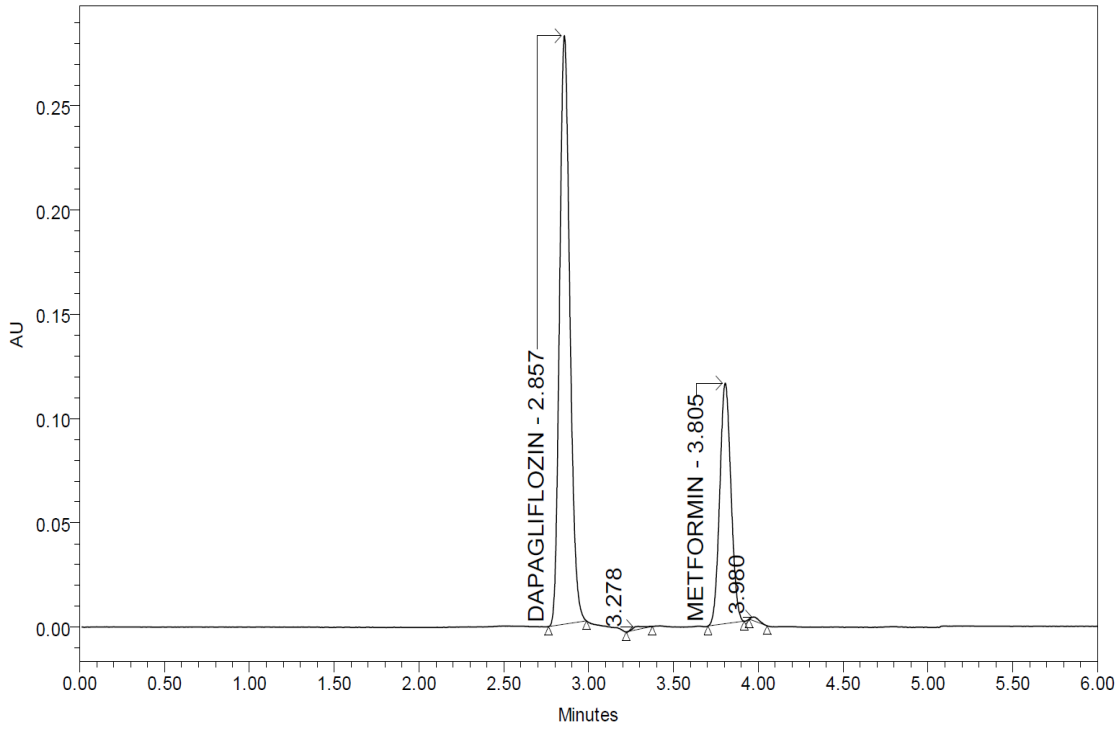

Figure 4.18: Dapagliflozin and metformin after exposure 105 ^{o}C for 30 min

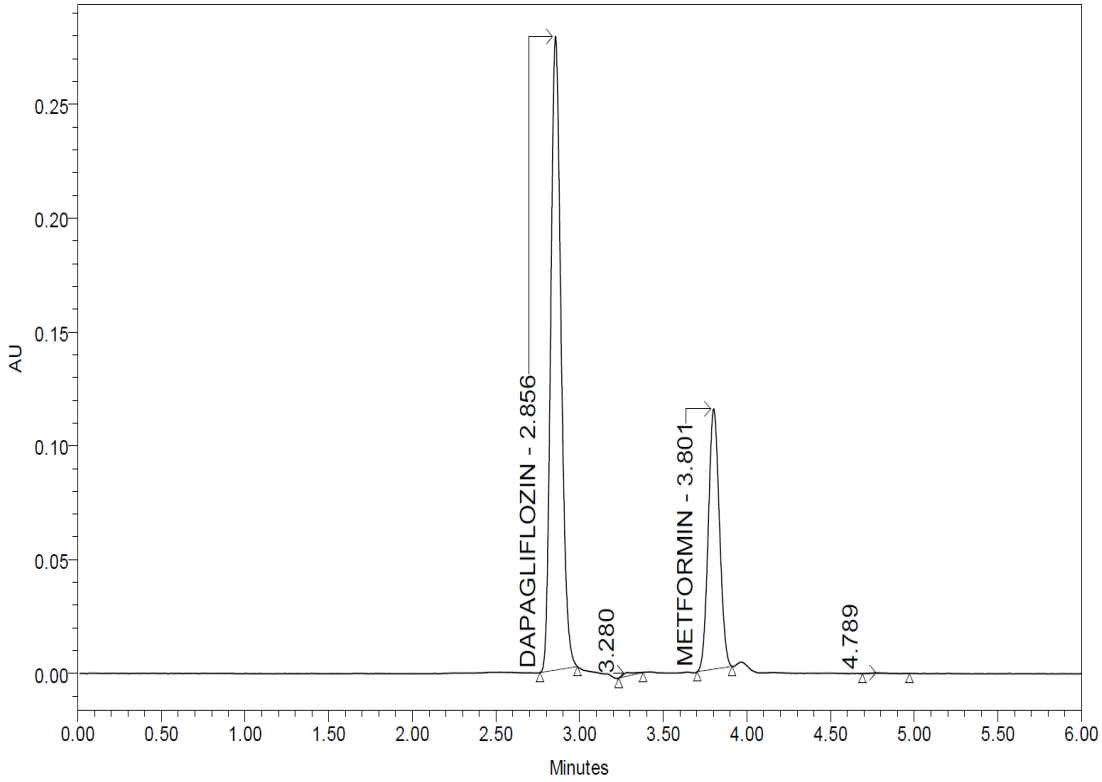

Figure 4.19: Dapagliflozin and metformin after 24 hours of exposure to sunlight

4.5. COMPARISON AMONG THE PROPOSED RP-HPLC METHOD AND OTHER REPORTED METHODS

The present method for the simultaneous determination of dapagliflozin and metformin was compared with other reported UV spectrophotometric and HPLC methods as shown in Table 4.9. Though the UV spectrophotometric methods are simple [51,52] and sensitive [52], they are less selective and are not applied to tablet dosage form. In Jani *et al.*, [51] UV spectrophotometric method, LOD and LOQ were not reported. The proposed method has broad range of linearity [51-54], more

sensitive [53,54], more precise and accurate [51-54] than the reported methods. The volume of sample used for single analysis by the proposed method (10 μL) is less than the reported methods (≥ 20 μL) [51-54]. The less run time makes the proposed RP-HPLC method more rapid than reported HPLC methods [53,54].

Table 4.9: Comparison of the proposed method and other methods in the literature applied to assay of dapagliflozin and metformin

Method	Drug	Detection wavelength (nm)	Run Time (min)	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	RSD (%)	Recovery (%)	Reference
UV spectro photometry	Met	225 & 237	-	25-125	NR	NR	0.484-0.641	100.48-102.96	Jani *et al.*, [51]
	Dapa	225 & 237		0.5-2.5	NR	NR	0.551-0.582	99.10-102.40	
UV spectro photometry	Met	272	-	25-125	0.013	0.041	0.235-0.399	99.34-99.95	Jani *et al.*, [52]
	Dapa	235		0.5-2.5	0.009	0.039	0.760-0.929	98.15-99.66	
RP-HPLC	Met	240	7	85-510	1.32	3.95	0.52-0.83	99.66-100.23	Mohammad and Gowri [53]
	Dapa	240		0.5-3.0	0.43	1.43	0.26-0.37	99.61-100.38	
RP-HPLC	Met	240	7	85-510	2.469	2.468	1.22	99.83-100.65	Shyamala *et al.*, [54]
	Dapa	240		0.5-3.0	3.650	3.649	0.98	99.48-100.54	
RP-HPLC	Met	285	6	200-600	0.272	0.907	0.290	99.70-99.82	Proposed
	Dapa	285		2-6	0.004	0.014	0.098	99.00-99.46	

Met - metformin; Dapa - dapagliflozin; NR - not reported

4.6. SUMMARY AND CONCLUSION

The overall results obtained for the proposed method validation were tabulated in Table 4.10.

Table 4.10: Summary of the proposed method

Parameter	Dapagliflozin	Metformin
Linearity (µg/mL)	2-6	200-600
Regression equation	y = 34790 x + 336.0	y = 2694 x – 1680
Regression coefficient (R^2)	0.9999	0.9998
LOD (µg/mL)	0.004	0.272
LOQ (µg/mL)	0.014	0.907
Precision (%RSD)	0.098	0.290
Accuracy (%Recovery)	99.00-99.82	99.70-99.82

A sensitive, precise and accurate stability indicating RP-HPLC method with a photodiode array detector has been proposed for the simultaneous analysis of dapagliflozin and metformin. The developed RP-HPLC method was validated as per ICH guidelines. The validation results showed that the proposed method was rapid, sensitive, precise, accurate and selective than the reported methods. The proposed method provides a stability-indicating assay for the quantification of dapagliflozin and metformin in bulk powder and tablets, without interference from the common excipients used in the preparation of tablets and in the presence of acidic, alkaline, oxidative, thermal and photolytic degradation products. The degradation products were well separated from the dapagliflozin and metformin signifying the stability indicating nature of the method. Hence the developed RP-HPLC method is a stability indicating assay that can be used for the routine analysis of dapagliflozin and metformin in bulk and tablets without any interference.

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