International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 6 Issue 6, September-October 2022 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Method Development, Validation and Forced Degradation Studies of Dapagliflozin and Pioglitazone Hydrochlorides in Synthetic Mixtures by RP-HPLC

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ABSTRACT

A simple, sensitive, robust, precise, and efficient RP-HPLC approach for the simultaneous determination of Dapagliflozin and Pioglitazone Hydrochloride in Synthetic Mixture. As per ICH Q2 (R1) guidelines, the final chromatographic conditions were Optimized with a mobile phase ratio of (25:75% v/v) in ACN: Potassium Dihydrogen Phosphate Buffer (pH 4) was adjusted by adding OPA at a flow rate of 1 mL/min, column temperature of 30 °C, injection volume of 20 µL, Kromstar Vertex C18 analytical column, and UV detection at 228 nm wavelength. Dapagliflozin and Pioglitazone Hydrochloride reported retention times of 3 min and 6.5 min, respectively. Validation of a method was found to be linear in the range of 2-10 µg/ml for Dapagliflozin and 3–15 µg/mL for Pioglitazone Hydrochloride. The % Recovery for Dapagliflozin was discovered to be 98.52 - 99.90 %, while for Pioglitazone Hydrochloride, it was found to be 99.67- 99.94 %. The Precision results for both drugs were within the limits while expressed Intraday and Interday. For Dapagliflozin, the LOD and LOQ were reported to be 0.041 µg/mL and 0.13 µg/mL, respectively, and for Pioglitazone Hydrochloride, 0.105 µg/mL and 0.32 µg/mL. As per ICH Q1A (R2) guidelines, the synthetic mixture was subjected to acid, base, oxidation, thermal, and photolysis stress conditions.

KEYWORDS: *RP-HPLC, Dapagliflozin, Pioglitazone Hydrochloride, Forced Degradation, ICH guidelines*

How to cite this paper: Mr. Tarang Patel | Ronak Parikh "Method Development, Validation and Forced Degradation Studies of Dapagliflozin and Pioglitazone Hydrochlorides in Synthetic Mixtures by RP-HPLC" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-6 | Issue-6, October 2022, pp.1858-1869, URL: www.ijtsrd.com/papers/ijtsrd52165.pdf

IJTSRD52165

1. INTRODUCTION

Diabetes is a long-term condition in which the body's ability to produce or respond to the hormone insulin is disrupted, resulting in incorrect carbohydrate metabolism and high blood glucose levels. To maintain a tight check on your blood sugar levels, you should use a combination of medicines, exercise, and food and keep them within a range set by your doctor. By paying close attention to what and when you eat, you can minimize or avoid the "seesaw effect" of rapidly shifting blood sugar levels, which can need quick adjustments in prescription dosages, especially insulin, by paying close attention to what and when you eat.

Dapagliflozin is a sodium-glucose co-transporter 2 (SGLT-2) inhibitor that is used to treat type 2 diabetes. A proliferator-activator receptor agonist is pioglitazone hydrochloride. The combination of Dapagliflozin and Pioglitazone hydrochloride was investigated in a clinical trial context, and it was observed that the synergistic effect improved glycaemic control while also lowering body weight [1-5]. This combination was effective, well-tolerated, and reduced Pioglitazone-induced weight gain without raising hypoglycemia risk. Pioglitazone (15 mg) and dapagliflozin (10 mg) were the medication dosages, both of which are in phase 3 clinical trials for the treatment of type 1 diabetes (insulin-dependent), a chronic illness in which the pancreas produces little or no insulin [6]. The Chemical

Structure of Dapagliflozin and Pioglitazone hydrochloride were shown in Figure 1.

For the simultaneous determination of DAPA and PIO, several analytical techniques have been published for single drugs but not for this combination. UV spectrophotometric methods [7-11] and HPLC [12-20] were among the methods used.

Analytical method validation ensures that different HPLC analytical procedures provide consistent and repeatable results; it is a crucial stage in the development of a method from a synthetic mixture since it provides information on precision, linearity, detection, and quantitation limits. "The goal of validation of an analytical method is to demonstrate that it is suitable for its intended purpose," according to ICH standards. Validation data must now be provided to the appropriate authorities during the pharmaceutical development process. The ICH has validation requirements for analytical procedures [21].

This study developed a new sensitive and quick RP-HPLC technique for identifying Dapagliflozin and Pioglitazone hydrochloride in a synthetic mixture, which was then validated as per ICH Q2 (R1) regulations. The ICH Q1A (R2) recommendations were used to conduct forced degradation tests in which a synthetic mixture was subjected to acid, base, oxidation, heat, and photolysis stress conditions [22].

2. Materials and Methods [23]

2.1. Instrumentation

A Systronics LC-138 device with a pump, Hamilton sampler injector, column compartment, and UV detector was used to conduct the HPLC analysis. To collect and process all chromatographic data, Clarify Software was used as a data acquisition tool.

2.2. Chromatographic Condition

The separation was performed using a Kromstar Vertex C_{18} column (250 ×4.6 mm, 5µm). A mobile phase of Methanol: 10mM potassium dihydrogen phosphate buffer with 10% OPA pH 4.0 (25:75,v/v) was used to equilibrate the column. Orthophosphoric acid was used to adjust the buffer's final pH to 4.0. The UV detector was set to 228nm, the injection volume was 10 liters, the temperature was 30 ° C, and the flow rate was 1 mL/min.

2.3. Chemicals and Reagents

Dapagliflozin standard and API were gifted from Alembic Pharmaceuticals Pvt. Ltd, India. Pioglitazone Hydrochloride standard and API were gifted by Cadila Pharmaceuticals Pvt. Ltd, India. Orthophosphoric acid (AR grade) was purchased from Astron Chemical India, while Potassium Dihydrogen Phosphate Buffer (AR Grade) was purchased from Chemdyes Corporation in Rajkot, India. Hydrogen Peroxide was delivered by LOBA Chemie Pvt. Ltd of Mumbai, India. Acetonitrile and Hydrochloric acid, both HPLC grade solvents, were delivered by Merck Life Science Pvt. Ltd of Mumbai, India. The remaining reagents were of analytical grade.

2.4. Preparation of Solutions

2.4.1. Preparation of Buffer (10mM KH_2PO_4)

1.36 g of potassium dihydrogen hydrogen phosphate (KH_2PO_4) was accurately weighed and transferred to 1000 mL water, dissolved in HPLC grade water, sonicated for 10 minutes, and diluted with HPLC grade Milli Q water. It was filtered through a 0.45µm membrane filter.

2.4.2. Preparation of 10% Ortho-Phosphoric acid

10% Orthophosphoric acid was prepared by diluting 1.0 mL of concentrated OPA in 10 ml HPLC grade water.

2.4.3. Preparation of Sodium hydroxide solution (0.1 N)

Accurately weighed 4.0 gm of Sodium hydroxide was transferred in a 100mL volumetric flask and diluted with methanol up to 100 mL. From this solution take a 10 mL aliquot and transferred it to a 100 mL volumetric flask and diluted up to 100 mL with methanol.

2.4.4. Preparation of Hydrochloric acid solution (0.1 N)

0.86 ml Concentrated HCl transferred in 100 mL volumetric flask and dilute with methanol up to 100 mL.

2.4.5. Preparation of Hydrogen peroxide solution (3%)

From 30 % Hydrogen peroxide (H_2O_2) 10 mL was transferred to a 100 mL volumetric flask and diluted with methanol up to 100 mL.

2.4.6. Preparation of Mobile phase

A mixture of 10 mM Phosphate Buffer (pH 4.0 adjusts with 10% OPA): Methanol (75:25 % v/v) Mobile phase was used after filtering it through a 0.45 µm membrane filter and sonication.

2.4.7. Preparation of standard solutions

2.4.7.1. Preparation of standard stock solution of Dapagliflozin (100 µg/mL)

Accurately weigh 10 mg of Dapagliflozin was transferred into a 100 mL volumetric flask and diluted with Methanol

2.4.7.2. Preparation of standard stock solution of Pioglitazone hydrochloride (100µg/mL)

Accurately weighing 10 mg of Pioglitazone hydrochloride was transferred into a 100 mL volumetric flask and diluted with Methanol.

2.5. Preparation of Calibration Curve

Analyses of standard solutions in the ranges of 2-10 µg/mL and 3-15 µg/mL were used to determine the linearity of Dapagliflozin and Pioglitazone hydrochloride. Pipette out 0.2, 0.4, 0.6, 0.8, and 1.0 mL solutions from the Stock solution of Dapagliflozin (100 µg/mL) and 0.3, 0.6, 0.9, 1.2, and 1.5 mL solutions from the Stock solution of Pioglitazone HCl (100 µg/mL) into a 10 mL volumetric flask and make up with mobile phase to obtain 2, 4, 6, 8, and 10 µg/mL for Dapagliflozin and 3, 6, 9, 12 and 15 µg/mL for Pioglitazone hydrochloride, respectively.

2.6. Preparation of Sample Solution

Equivalent concentrations of Dapagliflozin (10 mg) and Pioglitazone hydrochloride (15 mg) were accurately weighed and transferred to a 100 mL volumetric flask, where they were sonicated and made up to the mark with methanol. Whatman filter paper was used to filter this solution. Methanol was used to dilute the filtrate to the desired concentration. It has 100 µg/mL Dapagliflozin and 150 µg/mL Pioglitazone hydrochloride. Pipette 0.4 ml solution from Dapagliflozin (100 µg/mL) and Pioglitazone hydrochloride (150 µg/mL) into a 10 mL volumetric flask, and adjust the volume with the mobile phase to the desired level. Dapagliflozin had a final concentration of 4 µg/mL, while Pioglitazone hydrochloride had a concentration of 6 µg/mL.

2.7. Preparation of Synthetic Mixture of Dapagliflozin and Pioglitazone Hydrochloride

The Dapagliflozin and Pioglitazone Hydrochloride Synthetic Mixture were prepared in a 10:15 ratio. PVP, Microcrystalline Cellulose, Lactose, Magnesium Stearate, Talc, and the drugs Dapagliflozin 10 mg and Pioglitazone Hydrochloride 15 mg are all frequent excipients. Equivalent weights of Dapagliflozin (10 mg) and Pioglitazone Hydrochloride (15 mg) were accurately weighed and transferred to a 100 mL volumetric flask, where Methanol was used to make up the half mark. This solution was sonicated until the medication disintegrated and then Methanol was added to get it up to the correct concentration. The solution was then filtered using Whatman filter paper. Dapagliflozin was obtained at a concentration of 100 µg/mL, while Pioglitazone Hydrochloride was obtained at a concentration of 150 µg/mL. From the stock solution of the synthetic mixture (Dapagliflozin is 100 µg/mL and Pioglitazone Hydrochloride 150 µg/mL) pipette out 0.4 mL and transferred into a volumetric flask of 10 mL and make up the volume with optimized mobile phase,to get the concentration of 4 µg/mL and 6 µg/mL for Dapagliflozin and Pioglitazone Hydrochloride respectively.20 µL of the above solution was injected to perform the assay and % RSD should be calculated.

2.8. Method Validation

The method was validated according to ICH regulations ICH Q2 (R1), with validation parameters such as specificity, linearity, range, accuracy, precision, LOQ, LOD, and robustness [21].

2.9. Forced degradation studies

A synthetic mixture of Dapagliflozin 4 µg/mL and Pioglitazone hydrochloride 6 µg/mL was stressed under various conditions to conduct forced degradation tests to establish whether the analytical method and assay were stability-indicating. Methanol: 10 mM Potassium Dihydrogen Phosphate Buffer (pH 4.0) (25: 75 % v/v) was utilized as a solvent in all experiments, and the solution of Dapagliflozin and Pioglitazone hydrochloride was prepared in methanol. All forced degradation solutions were prepared with an initial concentration of 4µg/mL Dapagliflozin and 6 µg/mL Pioglitazone hydrochloride in a mixture solution.

2.9.1. Acid degradation

Pipette 1 mL of sample solution from the mixed solution into a 10 mL volumetric flask with precision. Fill each flask with 1 mL of 0.1 N hydrochloric acid. Keep the flask at 40 °C. for 2 hours. Remove the flask from the water bath and chill the contents at 1 and 2-hour intervals. Fill each flask with 1 mL of 0.1 N sodium hydroxide. The mobile phase should be diluted up to volume and mixed properly. Prepare a blank preparation without a sample at the same time.

2.9.2. Base degradation

Pipette 1 mL of sample solution from the mixed solution into a 10 mL volumetric flask with precision. Fill each flask with 1 mL of 0.1 N sodium hydroxide. Keep the flask at 40 ° C. for 2 hours. Remove the flask from the water bath and cool the contents at 1 and 2-hour intervals. Fill each flask with 1 mL of 0.1 N hydrochloric acid. With the mobile Phase, dilute to volume and mix properly. Prepare a blank preparation without a sample at the same time.

2.9.3. Oxidation degradation

Pipette 1 mL of sample solution from the mixed solution into a 10 mL volumetric flask with precision. Fill each flask with 1 mL of 3 % hydrogen peroxide. Keep the flask at 40 ° C. for 2 hours. Remove the

flask from the water bath and cool the contents at 1 and 2-hour intervals. With the mobile phase, dilute to volume and mix evenly. Prepare a blank preparation without a sample at the same time.

2.9.4. Photolytic degradation

Drugs were placed in a photostability chamber and exposed to direct UV light for two hours to assess their photostability. Following removal from the photostability chamber at 1 and 2-hour intervals, the sample was prepared for analysis as previously reported. Prepare a blank preparation without a sample at the same time.

2.9.5. Thermal degradation

Pipette 1 mL of sample solution from the mixed solution into a 10 mL volumetric flask with precision. For 2 hours, it was exposed to heat at 80 °C. Remove the flask from the water bath and cool the contents at 1 and 2-hour intervals. Add about 5 mL of methanol and sonicate to completely dissolve it and bring the volume up to the desired level with the mobile phase.

3. Result and Discussion [24]

3.1. Optimization of Chromatographic Conditions

Different chromatographic conditions were used to develop a suitable RP-HPLC method for simultaneous estimation of Dapagliflozin and Pioglitazone hydrochloride, and optimized chromatographic conditions were developed shown in Figures 2(a) and 2(b) respectively.

Chromatographic conditions for optimized mobile phase trials

Column: Kromstar Vertex C18 (250 ×4.6 mm, 5µm)

Mobile Phase: 10 mM Potassium Dihydrogen Phosphate Buffer (pH 4.0): Methanol (75:25 %v/v)

Detector: UV detector

Flow rate: 1 mL/min

Injection volume: 20 µL

Detection wavelength: 228 nm

Syringe: Hamilton

3.2. Specificity

For Dapagliflozin and Pioglitazone hydrochloride, the specificity of the established analytical approach was tested. To ensure that no contaminants influenced the results, a placebo solution was placed onto a C_{18} column.

3.3. System Suitability Parameter

For System Suitability, the theoretical plates, tailing factor, resolution, peak asymmetry, and % RSD for peak area were considered. The results for DAPA and PIO were evaluated using a specified chromatogram and acceptance criteria. After injecting a stock solution comprising a mixture of (4 μg/mL) DAPA and (6 μg/mL) PIO three times, the chromatograms were recorded. The number of theoretical plates, resolution, and peak asymmetry were determined to see if the result reached the suggested limit described in Table 1.

3.4. Linearity of Dapagliflozin and Pioglitazone HCl

The Linearity of Dapagliflozin and Pioglitazone HCl were taken to be in the range of 2 –10 µg/mL and 3 – 15 µg/mL respectively. The Overlay Chromatogram of Dapagliflozin and Pioglitazone HCl showed in Figure 2(c). The % RSD of Dapagliflozin and Pioglitazone HCl at 228 nm was found to be 0.16–1.23 and 0.15 -1.17 respectively. The correlation coefficient values for Dapagliflozin and Pioglitazone HCl were found to be 0.996 and 0.998 respectively. The regression line equation for Dapagliflozin and Pioglitazone HCl were found to be y = 32.785x - 38.192 and y = 61.134x - 23.013. The results of Linearity for Dapagliflozin and Pioglitazone HCl showed in Table 1. The calibration Curve of Dapagliflozin and Pioglitazone HCl showed in Figures 2(d) and 2(e) respectively.

3.5. LOD and LOQ of Dapagliflozin and Pioglitazone HCl

The LOD and LOQ of Dapagliflozin at 228 were found to be 0.041 µg/mL and 0.13 µg/mL respectively. The LOD and LOQ of Pioglitazone HCl at 228 were found to be 0.105 µg/mL and 0.34 µg/mL respectively. All the results of LOD and LOQ were shown in Table 1.

3.6. Precision of Dapagliflozin and Pioglitazone HCl

The % RSD of Dapagliflozin and Pioglitazone HCl for Intraday precision at 228nm was found to be 0.45 – 1.16 and 0.39 – 1.11, respectively. The % RSD of Dapagliflozin and Pioglitazone HCl for Intraday precision at 228nm was found to be 0.55 – 1.31 and 0.49 – 1.29, respectively. The % RSD of Dapagliflozin and Pioglitazone HCl for Intraday precision at 228nm was found to be 0.81 and 0.72, respectively. The precision of Dapagliflozin and Pioglitazone HCl showed in Table 1.

3.7. Accuracy of Dapagliflozin and Pioglitazone HCl

The % recovery of Dapagliflozin and Pioglitazone HCl was found to be 98.52% – 99.90 % and 99.67% – 99.94% respectively. The recovery of Dapagliflozin and Pioglitazone HCl showed in Table 1.

3.8. Robustness of Dapagliflozin and Pioglitazone HCl

With the change of (± 0.2 ml/min) in flow rate, (± 2 nm) in wavelength, and (± 2 v/v) in mobile phase ratio, we can observe the robustness. The % Assay was found to be 98.62% – 97.37% for Dapagliflozin and 97.25% – 99.75% for Pioglitazone HCl after changing the inflow rate of the mobile phase respectively. The % RSD was found to be 98.75% – 100.12% for Dapagliflozin and 98.75% – 100.25% for Pioglitazone HCl after changing in detection wavelength respectively. The % RSD was found to be 97.75% – 99.75% for Dapagliflozin and 97.75%– 99.75% for Pioglitazone HCl after changing in mobile phase ratio respectively. The robustness of Dapagliflozin and Pioglitazone HCl showed in Table 1.

3.9. Analysis of Synthetic mixture by the developed method

The % assay of Dapagliflozin and Pioglitazone HCl was found to be 99.75% and 99.84%, respectively. Analysis of the synthetic mixture showed in Table 1 and Figure 2(f).

3.10. Forced degradation study

3.10.1. Acid degradation

At 1-2 hours after heating the drug solution with 0.1 N hydrochloric acid at 40°C for 2 hours, Dapagliflozin and Pioglitazone HCl were degraded. Figure 3(a) describes the blank chromatogram of the mobile phase. After 2 hours, three more peaks of degradation were observed described in Figures 3(b) and 3(c), respectively. The results were summarized in Table 2.

3.10.2. Base degradation

At 1-2 hours after heating the drug solution with 0.1 N Sodium Hydroxide at 40°C for 2 hours, Dapagliflozin and Pioglitazone hydrochloride were degraded. After 2 hours, three more peaks of degradation were observed described in Figures 3(d) and 3(e), respectively. The results were shown in Table 2.

3.10.3. Oxidation degradation

After heating the drug solution with % hydrogen peroxide at room temperature for 2 hours, Dapagliflozin and Pioglitazone hydrochloride were degraded. After 2 hours, three more peaks of degradation were observed shown in Figures 3(f) and 3(g). The results were summarized in Table 2.

3.10.4. Photolytic degradation

When the drug solution was exposed to direct UV light for 2 hours, degradation was observed in Dapagliflozin and Pioglitazone hydrochloride. After 2 hours, two more peaks of degradation were described in Figures 3(h) and 3(i), respectively. The results were shown in Table 2.

3.10.5. Thermal degradation

At 1-2 hours after heating the drug solution at 80 °C for 2 hours, Dapagliflozin degradation and Pioglitazone hydrochloride degradation were observed in the chromatogram. After 2 hours, two more peaks of degradation were observed shown in Figures 3(j) and 3(k), respectively. The results were summarized in Table 2.

4. Conclusion

For routine analysis of Dapagliflozin and Pioglitazone HCl, a simple, precise, accurate, and fast simultaneous estimate approach has been devised and validated. The developed approach is suggested for regular and manufacturing standards analysis of the combination of Dapagliflozin and Pioglitazone HCl. A stability-indicating RP-HPLC technique was devised and validated for the determination of Dapagliflozin and Pioglitazone HCl in synthetic combinations. All method validation parameters meet the ICH Q2 (R1) guideline's acceptance criteria. As a result, we can conclude that the procedure is selective, linear, accurate, and accurate. As a result, it can be used to routinely analyze Dapagliflozin and Pioglitazone HCl in synthetic mixtures. There was no indication of any degradation in the major peak, and the results were found to be within acceptable limits. As a result, the proposed stability-indicating RP-HPLC assay method may be used to estimate Dapagliflozin and Pioglitazone HCl in the synthetic mixture.

Ethical approval

This article does not contain any studies with human participants or animals performed by authors.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Conflict of Interest

All authors declare that they have no conflict of interest.

Acknowledgments

Dr. Vishva Patel and Dr. Jitendra O. Bhangale professors, of Smt. N. M. Padalia Pharmacy College, Ahmedabad, Gujarat-382210, India, provided invaluable assistance to the authors. Dr. Mehul Patel, professor of Ramanbhai Patel College of Pharmacy, Charusat, Changa-388-421, India, provided excellent guidance and support to the authors.

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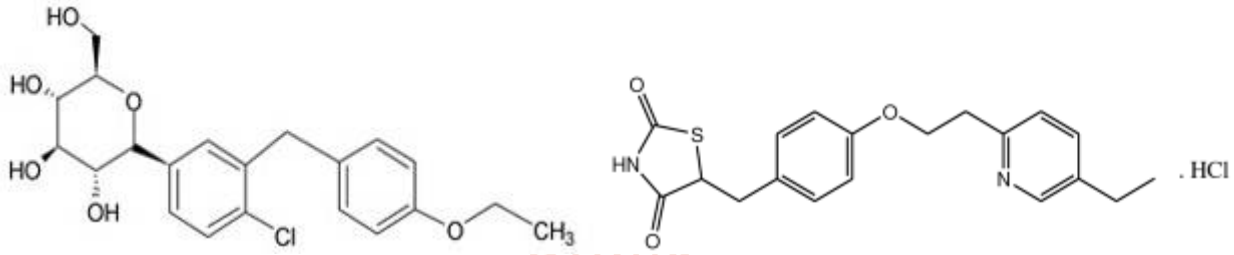
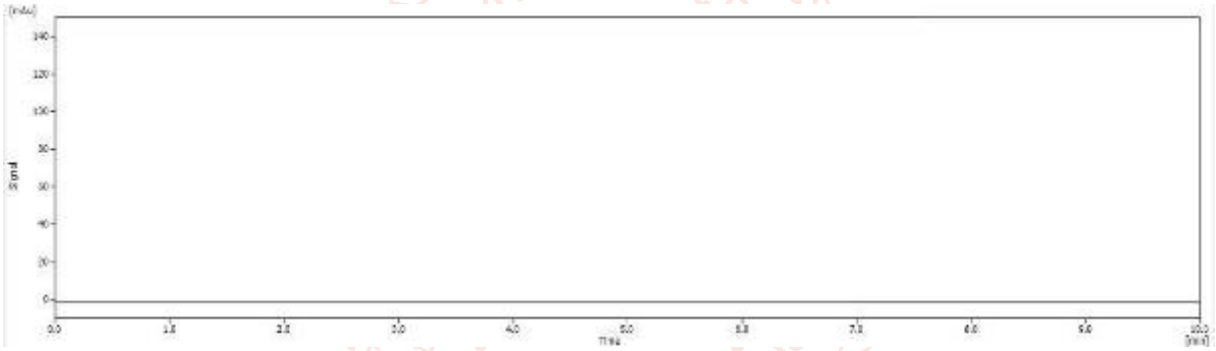
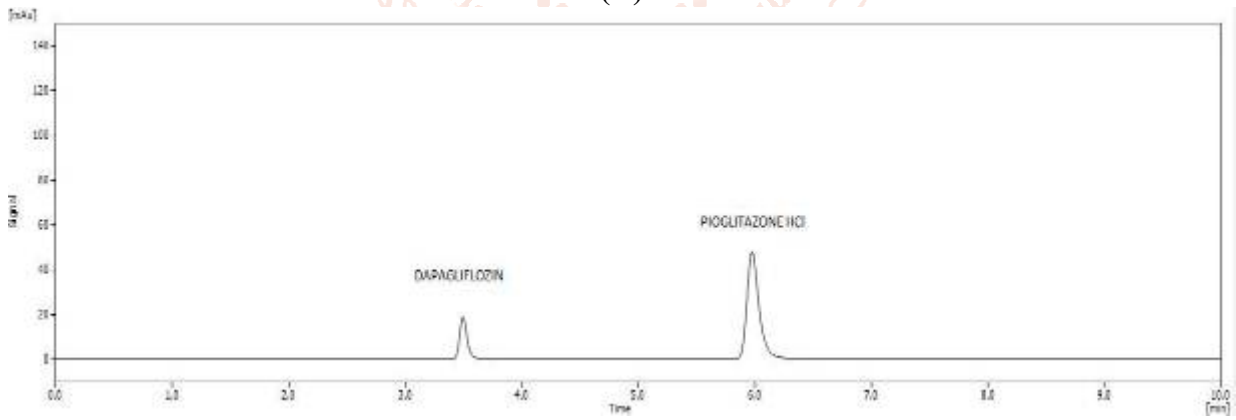


(a) **(b)**

Figure 1: Chemical Structure of Dapagliflozin (a) and Pioglitazone Hydrochloride (b)



(A)



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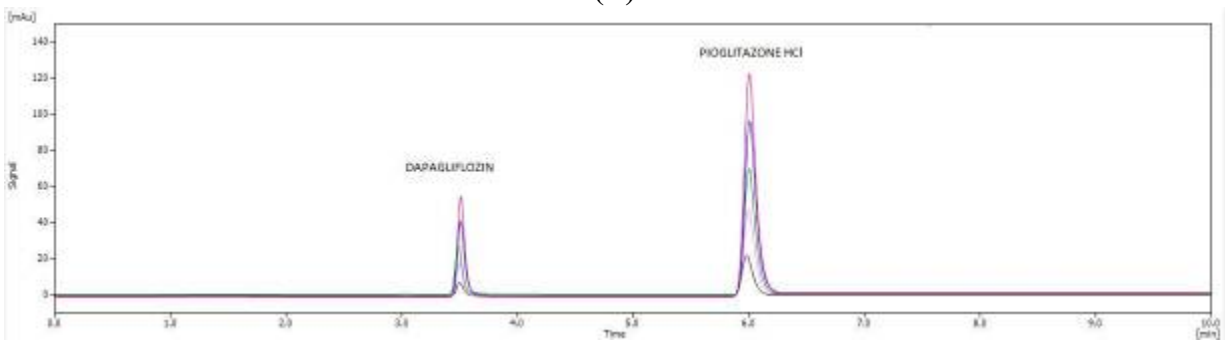
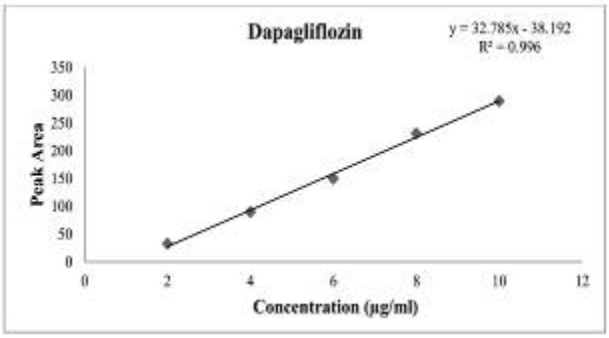
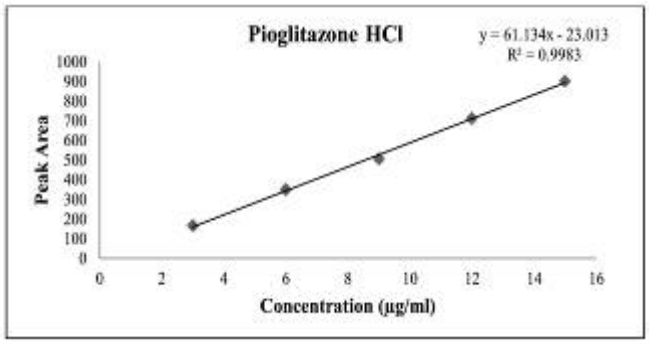


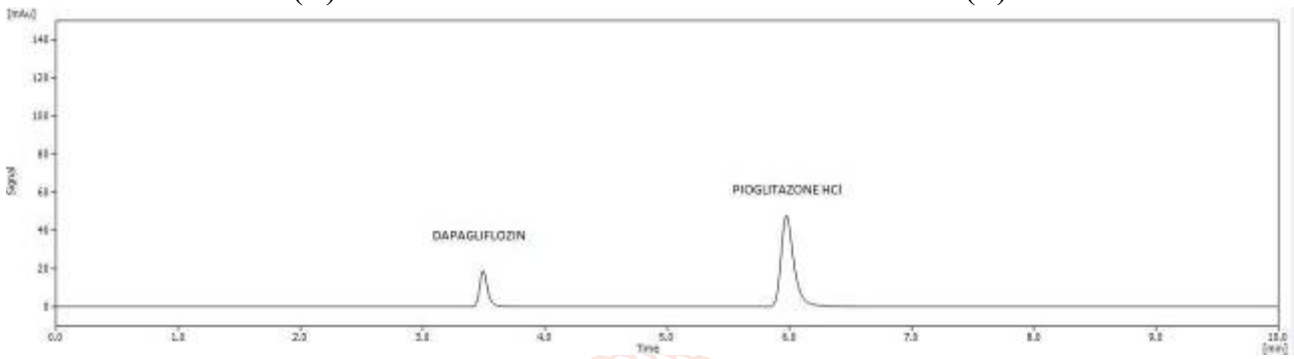
(C)



(D)



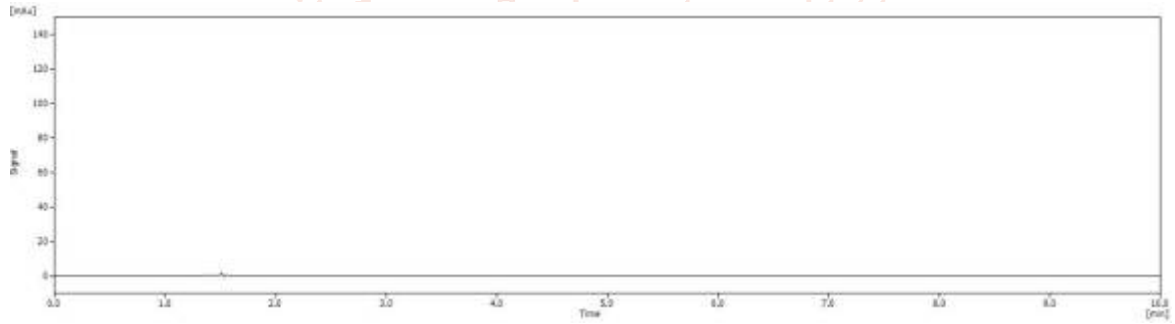
(E)



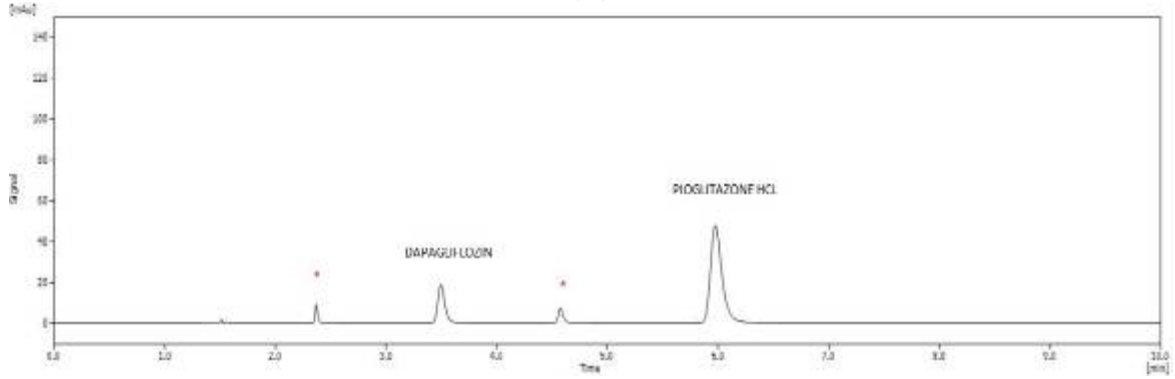
(F)

Figure 2: Chromatograms of Dapagliflozin and Pioglitazone Hydrochloride

(a) RP-HPLC Chromatogram of Blank in Phosphate Buffer (pH 4. 0): Methanol (75: 25 %v/v) at 228 nm
(b) RP-HPLC Chromatogram of Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) in Phosphate Buffer (pH 4. 0): Methanol (75: 25 %v/v) at 228 nm.
(c) Overlay Chromatogram of Dapagliflozin 2-10 (µg/ml) and Pioglitazone HCl (3-15 µg/ml) at 228 nm.
(d) Calibration Curve of Dapagliflozin (2 - 10 µg/ml) at 228 nm.
(e) Calibration Curve of Pioglitazone hydrochloride (3 - 15 µg/ml) at 228 nm.
(f) Chromatogram of Dapagliflozin and Pioglitazone HCl in Synthetic mixture.



(A)



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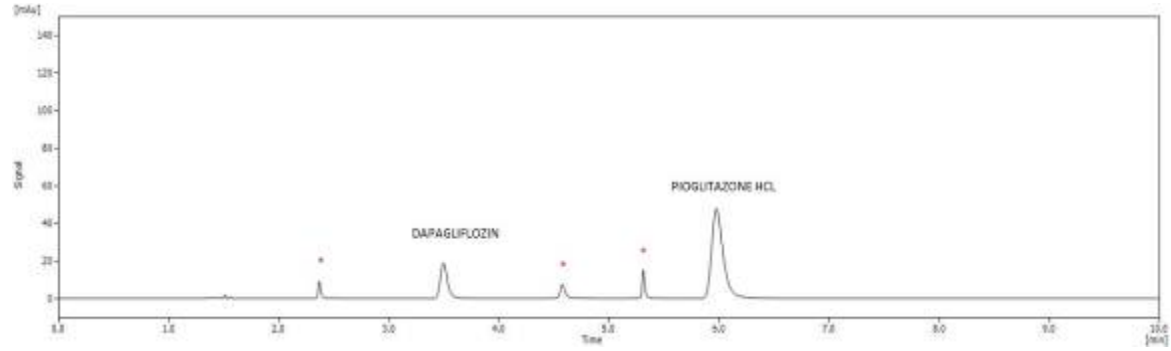
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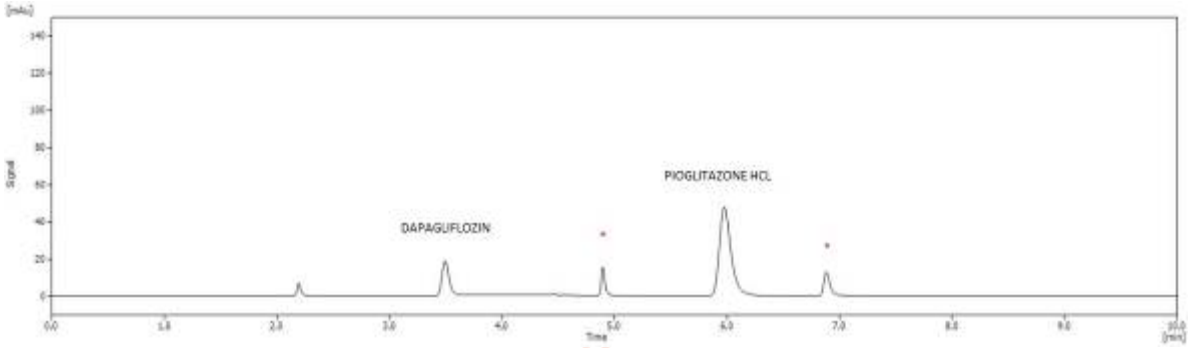
DAPAGLIFLOZIN

(C)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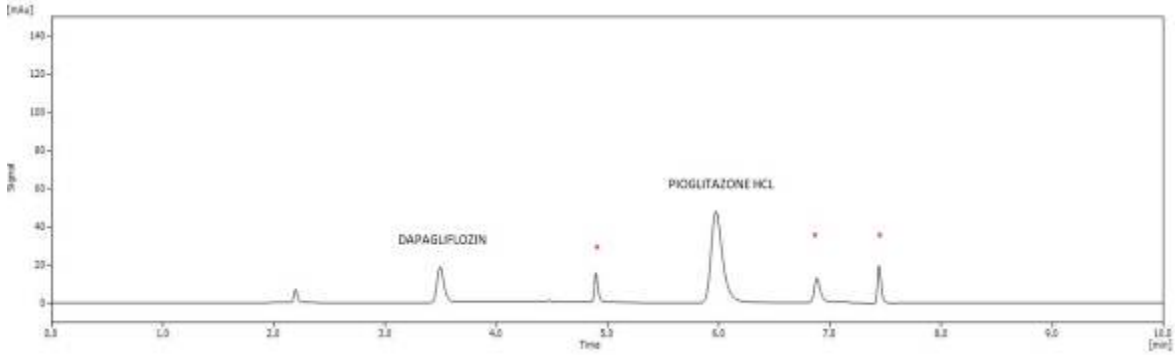
DAPAGLIFLOZIN

(D)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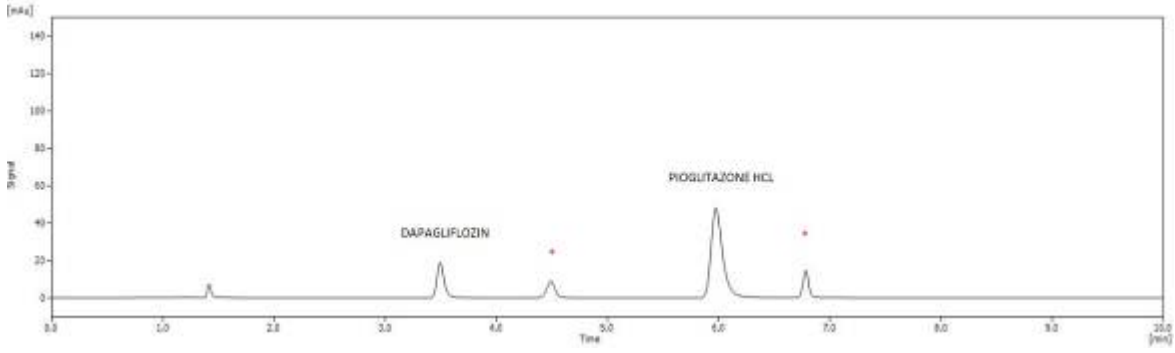
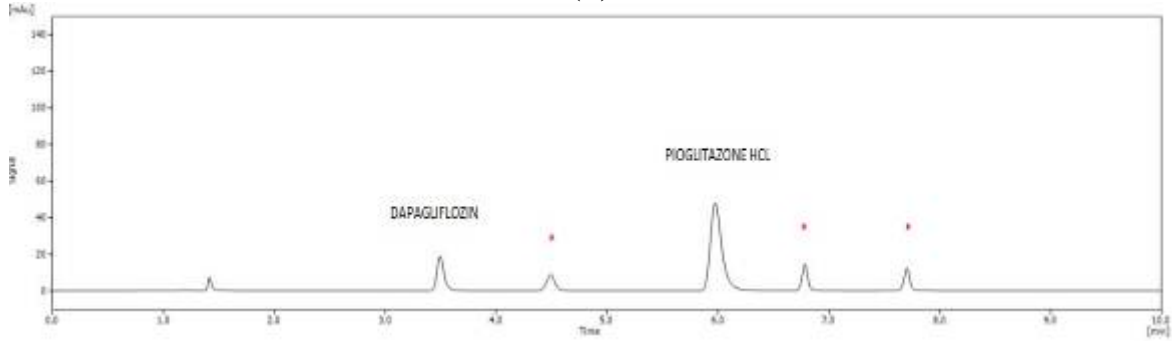
DAPAGLIFLOZIN

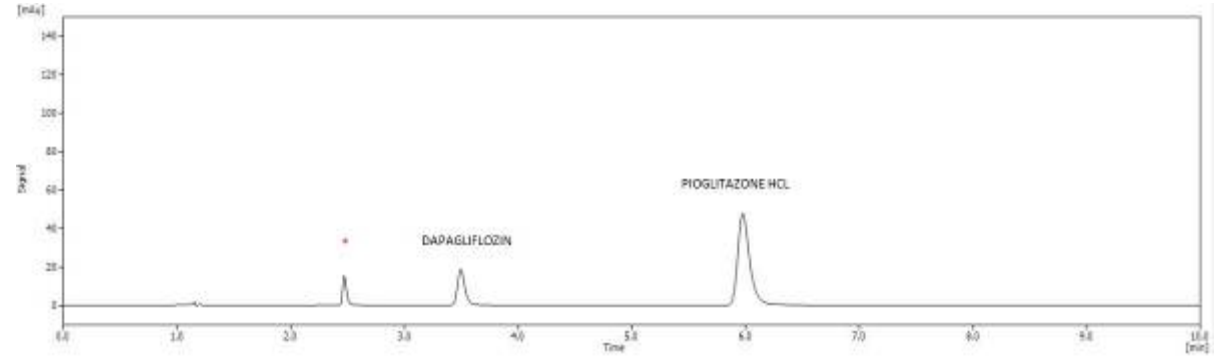
(E)


PIOGLITAZONE HCL
DAPAGLIFLOZIN

(F)


PIOGLITAZONE HCL
DAPAGLIFLOZIN

(G)



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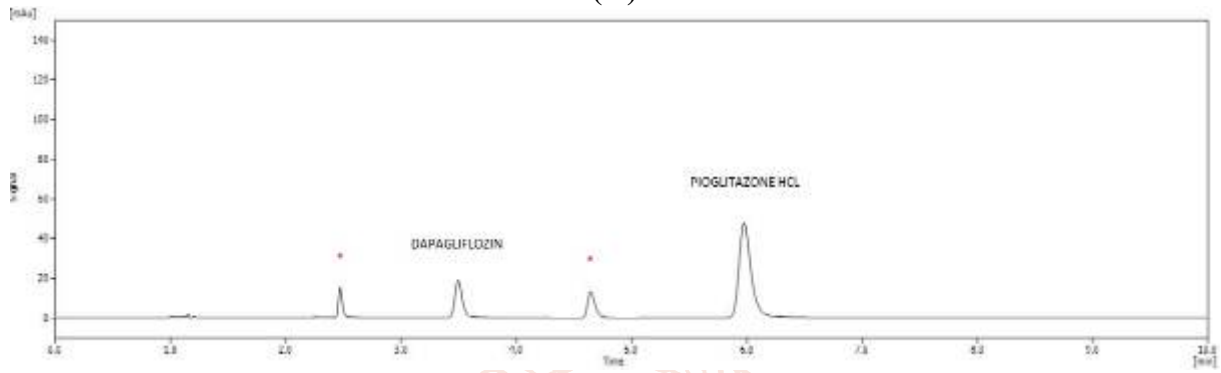
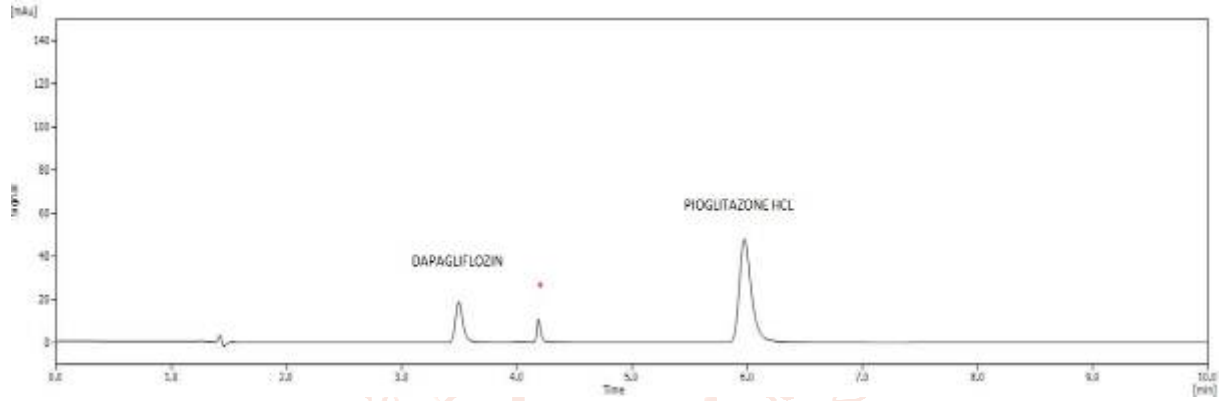
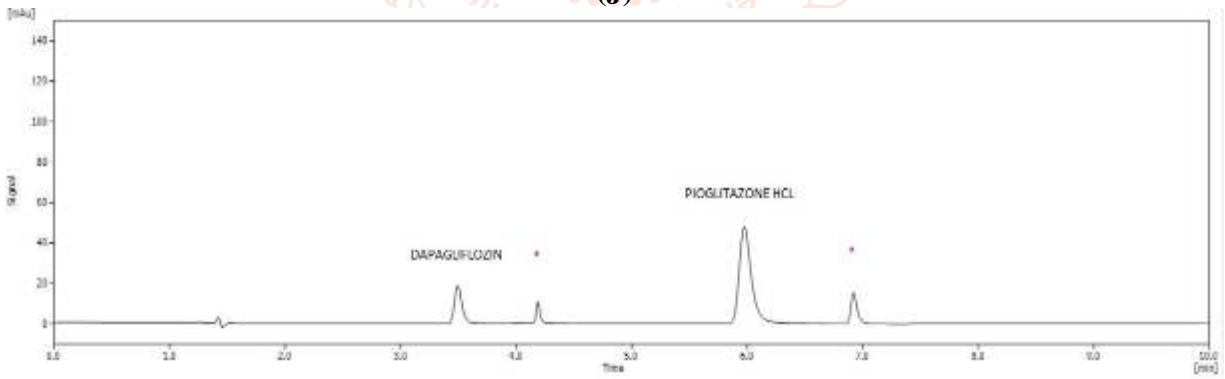


(I)



(J)



(K)

Figure 3: Forced degradation studies of Dapagliflozin and Pioglitazone HCl from Synthetic mixture

(*) Degradant peak formed in Chromatogram

(a) RP-HPLC Chromatogram of Blank in Phosphate Buffer (pH 4. 0): Methanol (75: 25 %v/v) at 228 nm.

(b) RP-HPLC Chromatogram of Acid Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 1 hour 228 nm.

(c) RP-HPLC Chromatogram of Acid Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 2 hour 228 nm.

(d) RP-HPLC Chromatogram of Base Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 1 hour 228 nm.

(e) RP-HPLC Chromatogram of Base Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 2 hour 228 nm.

(f) RP-HPLC Chromatogram of Oxidation Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 1 hour 228 nm.
(g) RP-HPLC Chromatogram of Oxidation Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 2 hour 228 nm.
(h) RP-HPLC Chromatogram of Photo Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 1 hour 228 nm.
(i) RP-HPLC Chromatogram of Photo Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 2 hour 228 nm.
(j) RP-HPLC Chromatogram of Thermal Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 1 hour 228 nm.
(k) RP-HPLC Chromatogram of Thermal Degradation for Dapagliflozin (4 µg/ml) and Pioglitazone HCl (6 µg/ml) Sample after 2 hour 228 nm.

Table 1: Summary results for analytical method validation

Parameters	Results obtained for DAPA	Results obtained for PIO
System suitability parameter		
Retention time	3. 5 min	6. 0 min
Theoretical Plates	6099	12857
Tailing Factors	1. 206	1. 102
Flow Rate	1mL/min	1mL/min
Linearity and Range	2-10 µg/mL	3-15 µg/mL
Linearity equation	Y = 32. 785x - 38. 192	Y = 61. 134x - 23. 013
Regression value	0. 996	0. 998
Slope	32. 785	61. 134
Intercept	38. 192	23. 013
LOD (µg/mL)	0. 041	0. 105
LOQ (µg/mL)	0. 13	0. 34
Precision (%RSD) (n=3)		
Repeatability	0. 81	0. 71
Interday	0. 89	0. 81
Intraday	0. 82	0. 72
Accuracy (n=3)		
80 % (% Recovery)	98. 52	99. 67
80 % (% RSD)	1. 45	0. 91
100% (% Recovery)	99. 63	99. 84
100% (% RSD)	1. 31	1. 02
120% (% Recovery)	99. 90	99. 94
120% (% RSD)	1. 13	0. 95
Robustness (% Assay ± SD) (n=3)		
Change in Flow rate (1 mL ± 0. 2 mL/ min)		
0. 8 mL/min	97. 37±4. 5166	97. 75±2. 3730
1. 0 mL/min	99. 87±4. 2691	99. 75±3. 5545
1. 2 mL/min	98. 62±6. 4770	97. 25±5. 0286
Change in Detection wavelength (228 nm ± 2 nm)		
226	97. 5±6. 1268	97. 25±0. 9454
228	100. 12±6. 4267	100. 25±1. 5055
230	98. 75±7. 0256	98. 75±1. 3762
Change in Mobile Phase ratio (ACN: Phosphate buffer 75: 25 ± 2 % v/v)		
73: 27	98. 12±2. 0784	97. 75±2. 1116
75: 25	99. 75±1. 9421	99. 75±2. 0552
77: 23	97. 75±2. 0143	98. 25±2. 1845
Analysis of Synthetic mixture (% Assay±SD)(n=3)	99. 75±1. 0146	99. 84±1. 1042

Table 2: Summary of forced degradation studies of Dapagliflozin and Pioglitazone HCl from Synthetic mixture

Sr. No	Degradation Type	Degradation Condition	% Assay observed for DAPA (n=3)	% Assay observed for PIO (n=3)
1	Acid Hydrolysis	0. 1 N HCl after 1 hour , 40 ° C	14. 68	6. 36
		0. 1 N HCl after 2 hour, 40 ° C	18. 66	13. 70
2	Alkaline Hydrolysis	0. 1 N NaOH after 1 hour, 40 ° C	16. 30	10. 31
		0. 1 N NaOH after 2 hour, 40 ° C	19. 53	14. 50
3	Oxidative Stress	3% H_2O_2 after 1 hour	13. 31	9. 58
		3% H_2O_2 after 2 hour	17. 89	13. 72
4	Photolytic Stress	Kept in UV light after 1 hour	9. 59	8. 33
		Kept in UV light after 2 hour	15. 65	12. 16
5	Thermal Stress	80° C after 1 hour	8. 35	6. 81
		80 °C after 2 hour	13. 65	10. 44

Declaration of Conflict of Interest

All authors declare that they have no conflict of interest.