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# STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF METFORMIN AND DAPAGLIFLOZIN BY RP-HPLC

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**ABSTRACT**

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Dapagliflozin in Tablet dosage form. The chromatogram was run through Kromasil 250 x 4.6 mm, 5µ. Mobile phase containing Buffer and Acetonitrile in the ratio of 60:40A was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.1% OPA buffer and the Temperature was maintained at 30°C. Optimized wavelength for Metformin and Dapagliflozin was 266nm. The retention time of Metformin and Dapagliflozin were found to be 2.330min and 3.098 min. %RSD of the Metformin and Dapagliflozin were and found to be 0.7 and 0.8 respectively. %assay was obtained as 99.57% and 99.76% for Metformin and Dapagliflozin respectively. LOD, LOQ values are obtained from regression equations of Metformin and Dapagliflozin were 0.90ppm, 2.73ppm and 0.02ppm, 0.07ppm respectively. Regression equation of Metformin & Dapagliflozin is y = 2433.x + 15236 and y = 13061x + 672.2. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

**Key Words:** Metformin, Dapagliflozin, RP-HPLC, Validation, Retention time

## 1. Introduction:

### 1.1 Metformin

Metformin is a biguanide antihyperglycemic specialist utilized for treating non-insulin-subordinate diabetes mellitus (NIDDM).[1,2]It enhances glycemic control by diminishing hepatic glucose creation, diminishing glucose assimilation and expanding insulin-interceded glucose take-up. Metformin is the main oral antihyperglycemic operator that is not related with weight pick up. Metformin may prompt weight reduction and is the medication of decision for corpulent NIDDM patients. At the point when utilized alone, metformin does not cause hypoglycemia; notwithstanding, it might potentiate the hypoglycemic impacts of sulfonylurea and insulin. Its fundamental reactions are dyspepsia, queasiness and the runs.[3]

### Mechanism action:

Metformin's components of activity vary from different classes of oral antihyperglycemic operators. Metformin diminishes blood glucose levels by diminishing hepatic glucose creation,

diminishing intestinal ingestion of glucose, and enhancing insulin affectability by expanding fringe glucose take-up and use. These impacts are interceded by the underlying initiation by metformin of AMP-enacted protein kinase (AMPK), a liver chemical that assumes an essential part in insulin flagging, entire body vitality adjust, and the digestion of glucose and fats. Actuation of AMPK is required for metformin's inhibitory impact on the creation of glucose by liver cells. Expanded fringe usage of glucose might be because of enhanced insulin official to insulin receptors. Metformin organization likewise builds AMPK action in skeletal muscle. AMPK is known to make GLUT4 arrangement the plasma layer, bringing about insulin-autonomous glucose take-up. The uncommon symptom, lactic acidosis, is believed to be caused by diminished liver take-up of serum lactate, one of the substrates of gluconeogenesis. In those with solid renal capacity, the slight overabundance is basically cleared. In any case, those with serious renal debilitation may aggregate clinically huge serum lactic corrosive levels. Different conditions that may encourage lactic acidosis incorporate extreme hepatic illness and intense/decompensated heart disappointment. [4-5]

**1.2 Dapagliflozin :** A medication called dapagliflozin is used to treat type 2 diabetes. It was created in collaboration with AstraZeneca by Bristol-Myers Squibb. Clinical trials are only now beginning to include individuals with type 1 diabetes, despite the fact that dapagliflozin's mechanism of action would work on both forms of diabetes and other illnesses causing hyperglycemia. [6-8]

**Mechanisam of action:** Sodium-glucose cotransporter 2 (SGLT2), communicated in the proximal renal tubules, is in charge of most of the reabsorption of separated glucose from the tubular lumen. Dapagliflozin is an inhibitor of SGLT2. By repressing SGLT2, dapagliflozin diminishes reabsorption of sifted glucose and brings down the renal limit for glucose, and accordingly increments urinary glucose discharge.[9-12]

## 2. MATERIALS AND METHODS

### 2.1 Materials:

Metformin and Dapagliflozin, Combination Metformin and Dapagliflozin tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

### 2.2 Instrument:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Metformin and Dapagliflozin solutions.

### 2.3 Methods:

#### 2.2.1 Preparation of buffer:

**OPA buffer: 0.1%**

Con. OPA is dissolved in a 1000 ml volumetric flask that has been diluted with distilled water to the prescribed concentration. Triethylamine was used to bring the pH down to 2.8.

**Typical Preparation**

Accurately weighed and transferred 50 mg and 5 mg of the working standards for the drugs metformin and dapagliflozin into a 10 ml clean, dry volumetric flask, respectively. Add 5 ml

of the diluent, sonicate the mixture for 30 minutes, and then add diluents to make up the remaining volume. 1ml was pipette out into a 10ml volumetric flask from the aforementioned stock solutions, and the remaining volume was then made up with diluent.

**Preparation of a Sample**:

In a volumetric flask with a volume of 100 ml and 50 ml of diluent, the weight equivalent to 1 tablet was transferred after 5 tablets were weighed to determine their average weight.

**2.2.2 Linearity**

Linearity solutions are made so that 0.25, 0.5, 0.75, 1.00, 1.25, and 1.5 millilitres from the Stock solutions are used. 2.2.3 Accuracy: Metformin and Dapagliflozin are placed into 6 distinct volumetric flasks and diluted to 10 ml with diluents to obtain concentrations of 125 ppm, 250 ppm, 375 ppm, 500 ppm, 625 ppm, and 750 ppm of Metformin and 1.25 ppm, 2.5 ppm, 3.75 ppm, 5 ppm, 6.25 pp

**Typical Preparation**

Accurately weighed and transferred 50 mg and 3 mg of the working standards for the drugs metformin and dapagliflozin into a 10 ml clean, dry volumetric flask, respectively. Add 5 ml of the diluent, sonicate the mixture for 30 minutes, and then add diluents to make up the remaining volume. 1ml was pipette out into a 10ml volumetric flask from the aforementioned stock solutions, and the remaining volume was then made up with diluent.

Creating 50% spiked alcohol

## 3. RESULTS AND DISCUSSIONS

### 3.1 Method Development

**Method Development:** Many trials were done by changing columns and Mobile phases and were reported below.

**Trial: 1**

**Column Used** : Discovery 250 x 4.6 mm, 5µ.
**Mobile phase** : water: methanol (50:50)
**Flow rate** : 1ml/min
**Wavelength** : 266nm
**Temperature** : 30°C
**Injection Volume** : 10µl

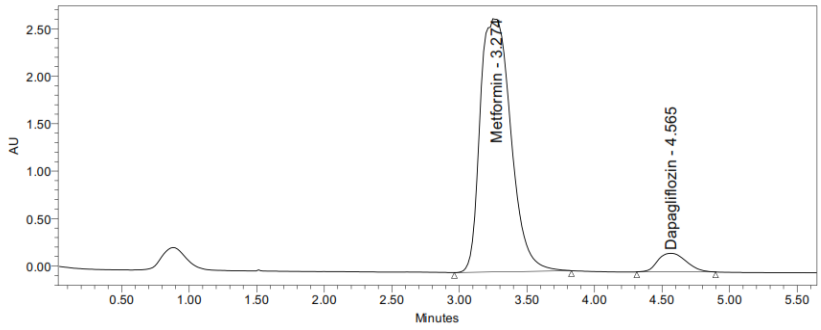



**Fig 3.1 Trial chromatogram 1**

### 3.2 Method validation:

**1. System suitability**: All the system suitability parameters are within range and satisfactory as per ICH guidelines

**Table: 3.2.1 System suitability studies of Metformin and Dapagliflozin method**

| Property | Metformin | Dapagliflozin |
|---|---|---|
| Retention time ($t_R$) | 2.330 min | 3.098 min |
| Theoretical plates (N) | 5609 ± 63.48 | 6566 ± 63.48 |
| Tailing factor (T) | 1.30 ± 0.117 | 1.20± 0.117 |

**2. Linearity:** Six Linear concentrations of Metformin (125-750ppm) and Dapagliflozin (1.25-7.5ppm) are prepared and Injected. Regression equation of the Metformin and Dapagliflozin are found to be, y = 2433.x + 15236, and y =13061.x + 672.2 and regression co-efficient was 0.999.

**Table: 3.2.2 Calibration data of Metformin and Dapagliflozin method.**

| S.N | Concentration Metformin acid(µg/ml) | Response | Concentration Dapagliflozin (µg/ml) | Response |
|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 |
| 2 | 125 | 332374 | 1.25 | 16854 |
| 3 | 250 | 624703 | 2.5 | 35577 |
| 4 | 375 | 938383 | 3.75 | 48957 |
| 5 | 500 | 1239067 | 5 | 65212 |
| 6 | 625 | 1514200 | 6.25 | 81670 |
| 7 | 750 | 1846852 | 7.5 | 99293 |

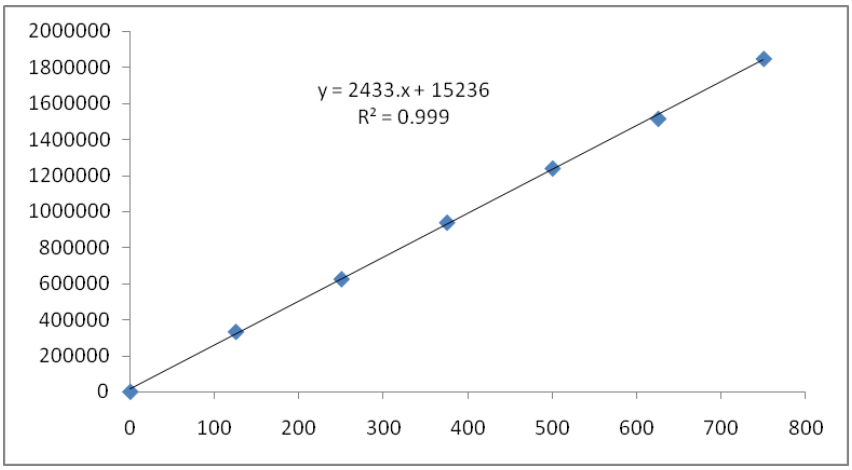



**Fig: 3.2.3 Calibration curve of Metformin**

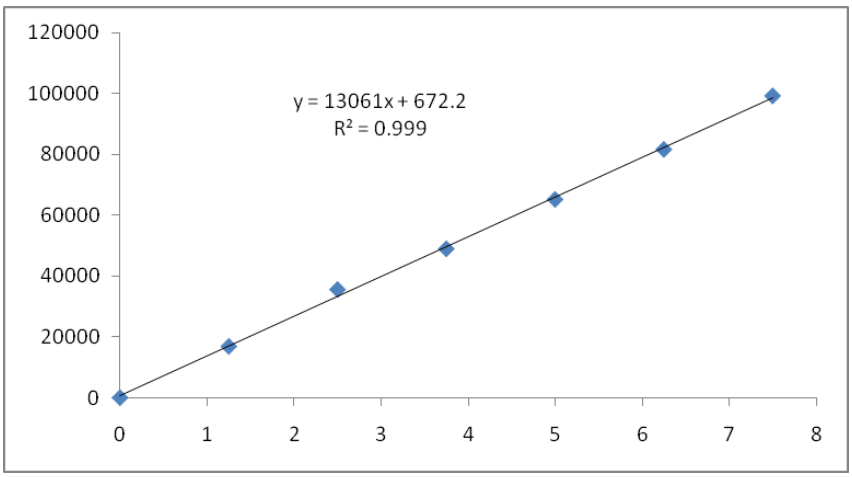



**Fig: 3.2.4 Calibration curve of Dapagliflozin**

**3. Precision:**

**Inter day precision:** Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Metformin and Dapagliflozin were 0.3% and 0.3%.

**Table 3.2.3 Inter day precision results for Metformin and Dapagliflozin.**

| SN.No. | Metformin | Dapagliflozin |
|---|---|---|
| 1 | 1297928 | 66347 |
| 2 | 1302666 | 66511 |
| 3 | 1301611 | 66618 |
| 4 | 1309694 | 66671 |
| 5 | 1297097 | 66864 |
| 6 | 1303058 | 66480 |
| Mean | 1302009 | 66582 |
| Std. Dev. | 4506 | 178.6 |
| %RSD | 0.3 | 0.3 |

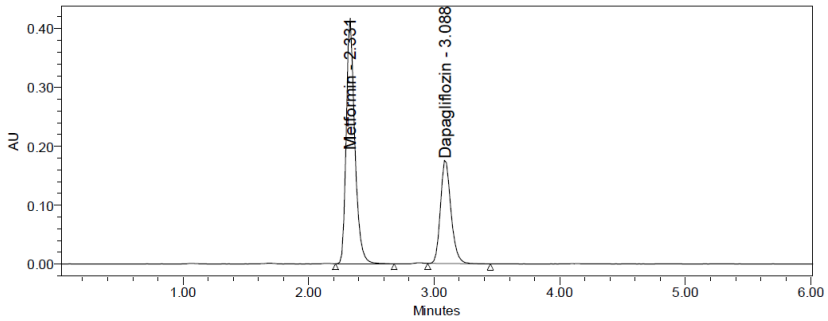
**4. Accuracy:** Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 3.4.& 3.5

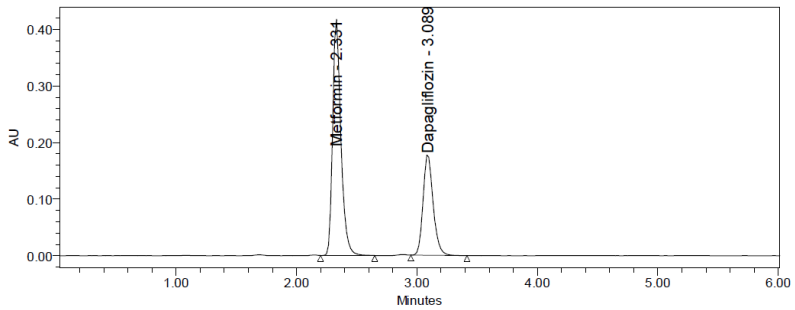
**Table: 3.2.4 Table of Accuracy for Metformin**

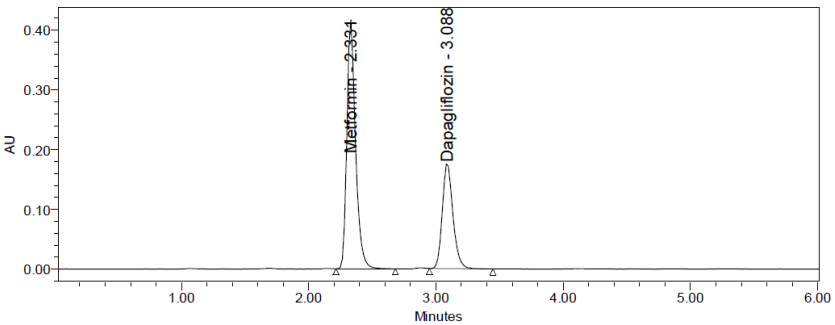
| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 250 | 251.4414 | 100.58 | 99.59% |
| | 250 | 248.7957 | 99.52 | |
| | 250 | 250.0843 | 100.03 | |
| 100% | 500 | 502.0423 | 100.41 | |
| | 500 | 492.7637 | 98.55 | |
| | 500 | 506.2474 | 101.25 | |
| 150% | 750 | 736.6482 | 98.22 | |
| | 750 | 737.8463 | 98.38 | |
| | 750 | 745.4024 | 99.39 | |

**Table: 3.2.5 Table of Accuracy for dapaglifozin**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 2.5 | 2.473762 | 98.95 | 99.55% |
| | 2.5 | 2.45041 | 98.02 | |
| | 2.5 | 2.482184 | 99.29 | |
| 100% | 5 | 4.955654 | 99.11 | |
| | 5 | 5.035051 | 100.70 | |
| | 5 | 5.025251 | 100.51 | |
| 150% | 7.5 | 7.501937 | 100.03 | |
| | 7.5 | 7.36 | 98.16 | |
| | 7.5 | 7.58945 | 101.19 | |



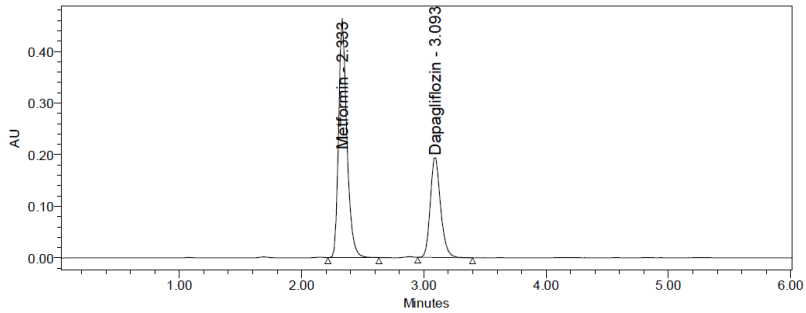



**Fig: 3.2.6 Accuracy 50% Chromatogram of Metformin and Dapagliflozin**

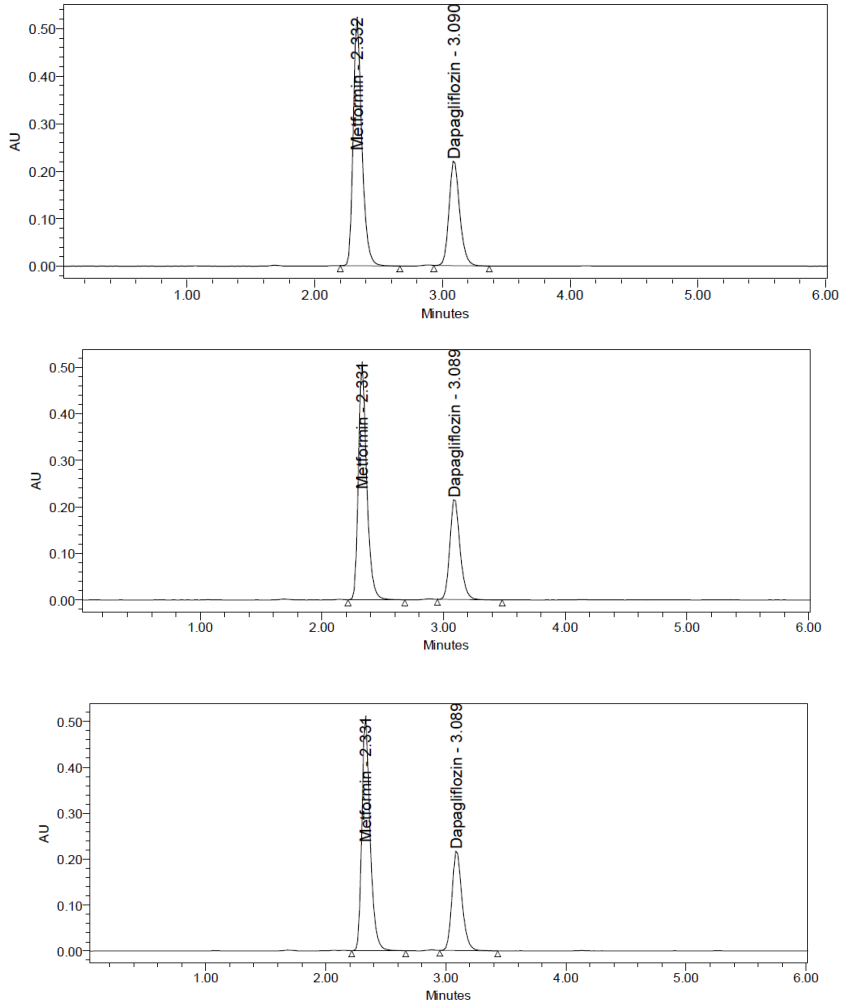
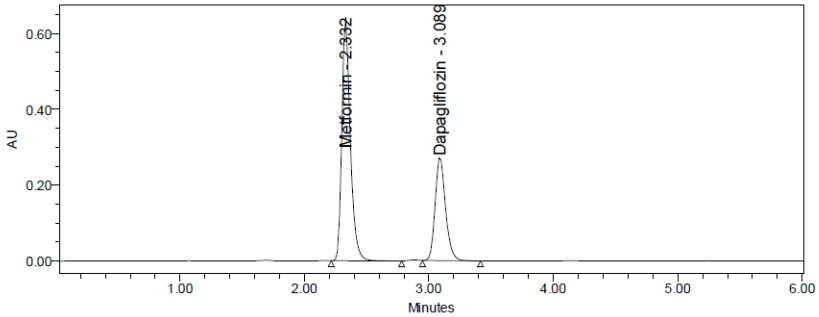



**Fig: 3.2.7 Accuracy 100%  Chromatogram of Metformin and Dapagliflozin**



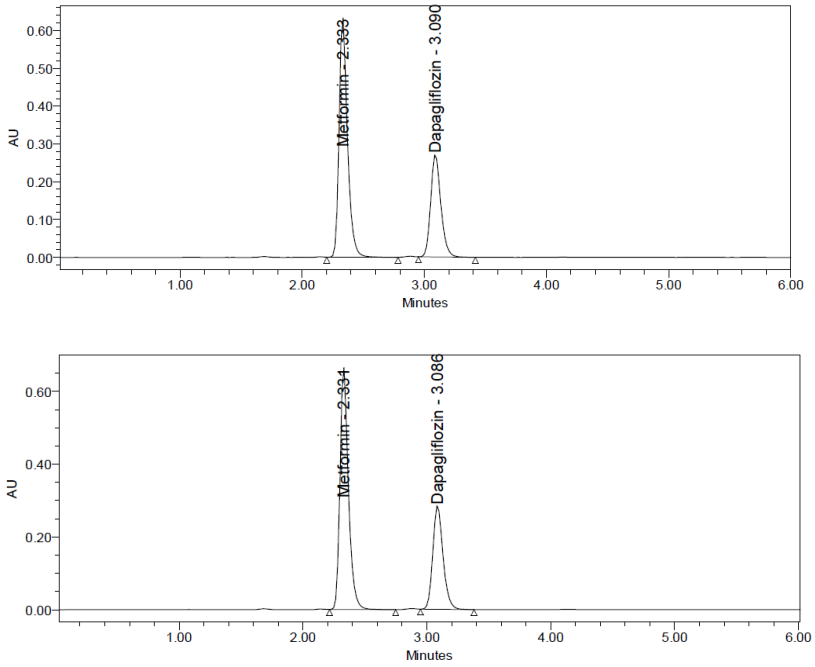



**Fig: 3.2.8 Accuracy 150% Chromatogram of Metformin and Dapagliflozin**

**5. LOD:** Limit of detection was calculated by std deviation method Metformin and Dapagliflozin and LOD for Metformin and Dapagliflozin were found to be 0.90and 0.02respectively.

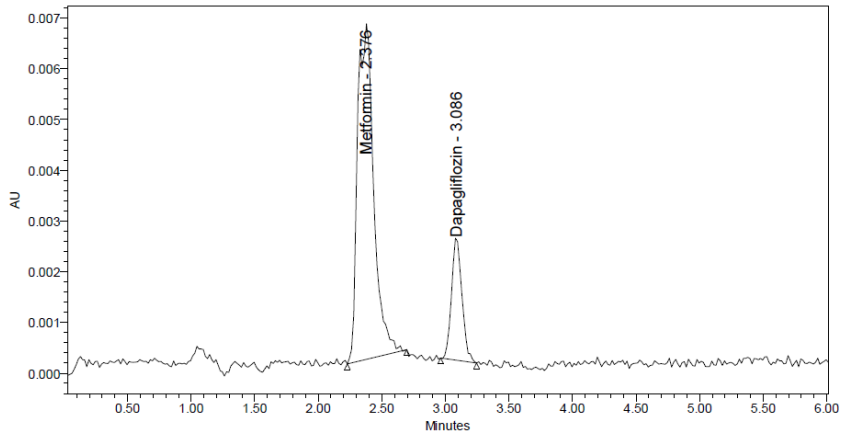



**Fig: 3.2.9 LOD Chromatogram of Metformin and Dapagliflozin**

**6. LOQ:** Limit of Quantification was calculated by std deviation method Metformin and Dapagliflozin and LOQ for Metformin and Dapagliflozin were found to be 2.73 and 0.07 respectively.

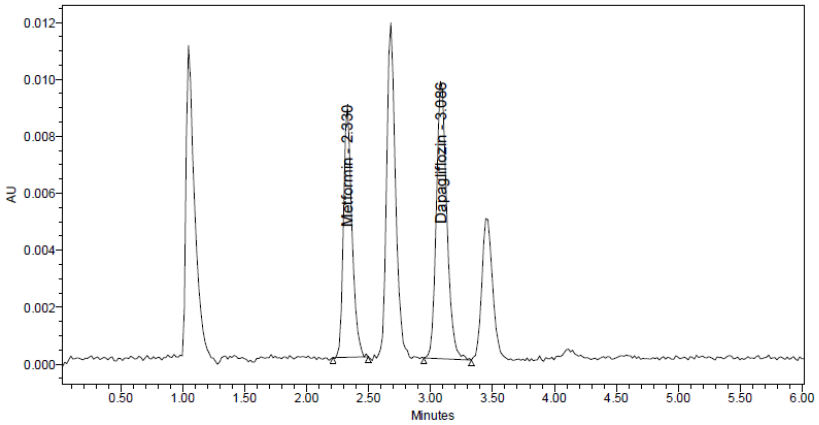



**Fig : 3.2.10 LOQ Chromatogram of of Metformin and Dapagliflozin**

**7. Robustness:** Although minor, purposeful adjustments were made to the flow rate, mobile phase ratio, and temperature of the procedure, the results remained within acceptable limits as prescribed by ICH Guide lines**.**

**Table 3.2.7 Robustness statistics for Dapagliflozin and Metformin**

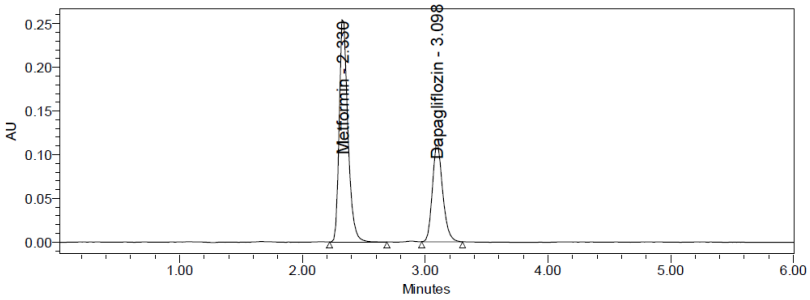
| S.NO | Robustness condition | Metformin %RSD | Dapagliflozin %RSD |
|---|---|---|---|
| 1 | Flow minus | 0.4 | 0.3 |
| 2 | Flow Plus | 1.1 | 0.8 |
| 3 | Mobile phase minus | 0.1 | 0.1 |
| 4 | Mobile phase Plus | 0.3 | 0.5 |
| 5 | Temperature minus | 0.1 | 0.1 |
| 6 | Temperature Plus | 0.3 | 0.3 |

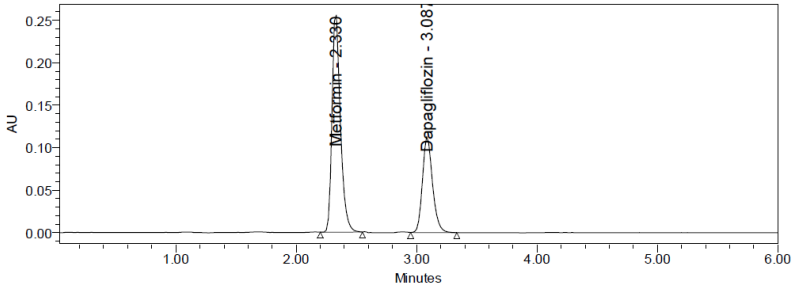
**Assay:** The API is used to make standard preparations, and formulation is used to make sample preparations. Six homogenous samples are injected, including the sample and the standards. By using the standard as a reference, the amount of drug in the formulation was estimated. The Average%Assay was determined to be 99.57% for Metformin and 99.76% for Dapagliflozin, respectively.

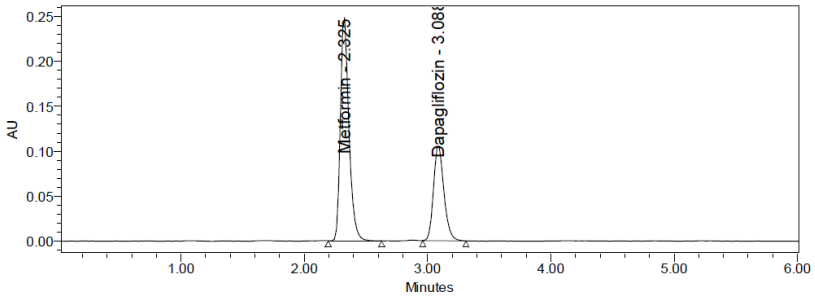
**Table 3.2.8 Assay of Tablet**

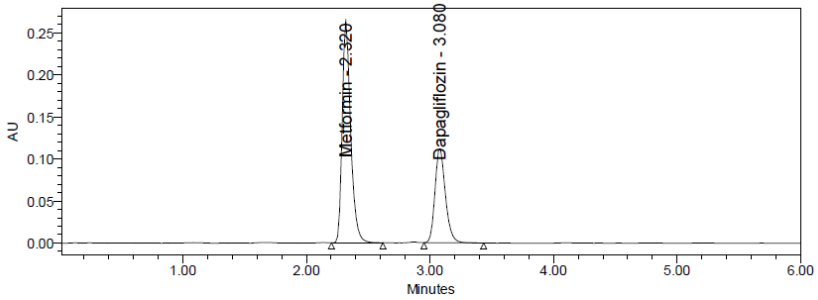
| S. No. | Metformin %Assay | Dapagliflozin %Assay |
|---|---|---|
| 1 | 100.30 | 100.35 |
| 2 | 100.37 | 100.84 |
| 3 | 99.25 | 98.69 |
| 4 | 99.41 | 99.74 |
| 5 | 99.38 | 100.01 |

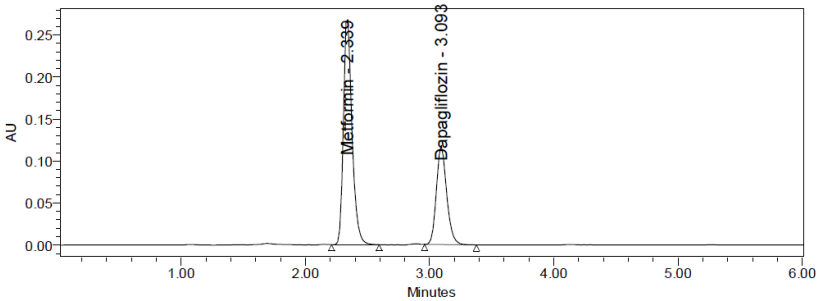
| | | |
|---|---|---|
| 6 | 98.69 | 98.96 |
| AVG | 99.57 | 99.76 |
| STDEV | 0.65 | 0.82 |
| %RSD | 0.82 | 0.82 |



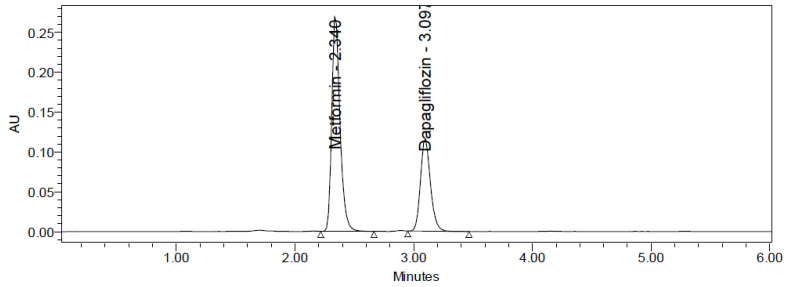



**Fig: 3.2.11 Assay of Tablet**

### 3.2.9. Degradation data Table

| Type of degradation | Metformin | | | Dapagliflozin | | |
|---|---|---|---|---|---|---|
| | AREA | %RECOVERED | % DEGRADED | AREA | %RECOVERED | % DEGRADED |
| **Acid** | 1217888 | 96.77 | 3.23 | 63603 | 96.76 | 3.24 |
| **Base** | 1231073 | 97.81 | 2.19 | 63819 | 97.09 | 2.91 |
| **Peroxide** | 1236181 | 98.22 | 1.78 | 64676 | 98.39 | 1.61 |
| **Thermal** | 1249878 | 99.31 | 0.69 | 65328 | 99.38 | 0.62 |
| **UV** | 1253418 | 99.59 | 0.41 | 65115 | 99.46 | 0.54 |
| **Water** | 1257610 | 99.92 | 0.08 | 65130 | 99.08 | 0.92 |

**Acid degradation chromatogram**

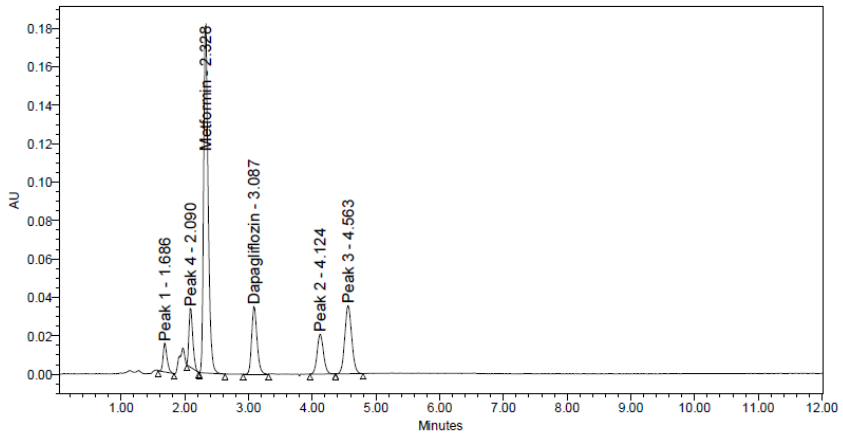



**Fig 3.2.12 Acid degradation chromatogram**

**Base degradation chromatogram**

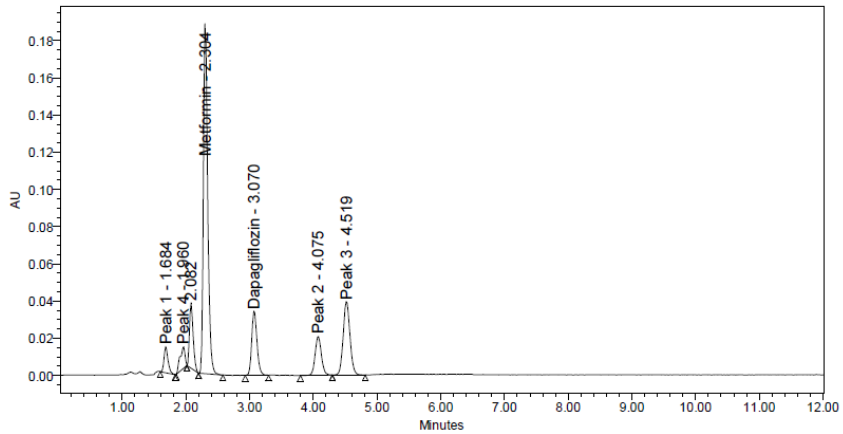



**Fig. 3.2.13 base degradation chromatogram**

**Peroxide degradation chromatogram**

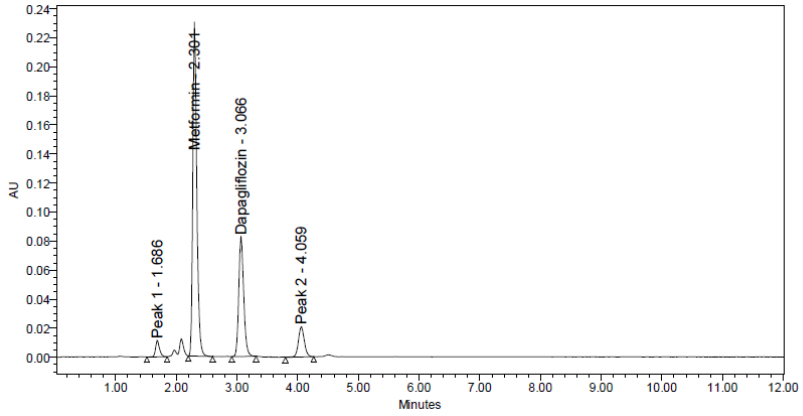



**Fig. 3.2.14 peroxide degradation chromatogram**

**Thermal degradation chromatogram**

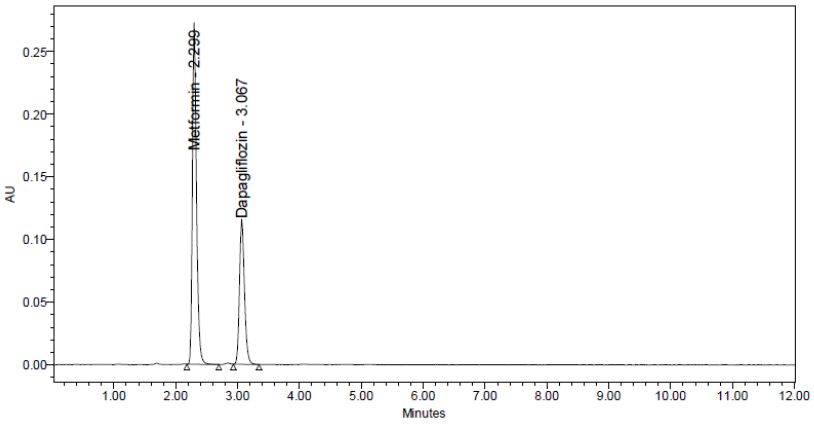



**Fig. 3.2.15 thermal degradation chromatogram**

**Water degradation chromatogram**

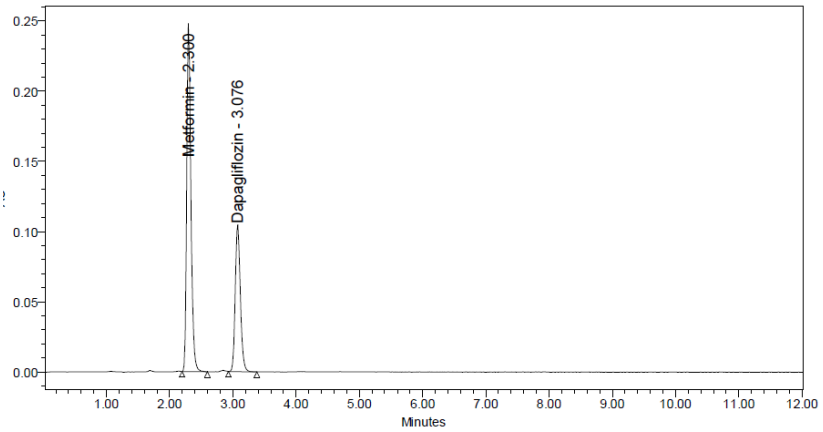



**Fig. 3.2.16 water degradation chromatogram**

## 4. SUMMARY AND CONCLUSION

**4.1 Summary Table**

| **Parameters** | **Metformin** | **Dapagliflozin** | **LIMIT** |
|---|---|---|---|
| **Linearity** Range (µg/ml) | 125-750µg/ml | 1.25-7.5 µg/ml | R< 1 |
| Regressioncoefficient | 0.999 | 0.999 | |
| Slope(m) | 2433 | 13061 | |
| Intercept(c) | 15236 | 672.2 | |
| Regression equation (Y=mx+c) | y = 2433.x + 15236 | y = 13061x + 672.2 | |
| **Assay (% mean assay)** | 99.57% | 99.76% | 90-110% |
| **Specificity** | Specific | Specific | No interference of any peak |
| **System precision %RSD** | 0.5 | 0.5 | NMT 2.0% |
| **Method precision %RSD** | 0.7 | 0.8 | NMT 2.0% |

| | | | | |
|---|---|---|---|---|
| **Accuracy %recovery** | | 99.59% | 99.55% | 98-102% |
| **LOD** | | 0.90 | 0.02 | NMT 3 |
| **LOQ** | | 2.73 | 0.07 | NMT 10 |
| **Robustness** | **FM** | 0.4 | 0.3 | %RSD NMT 2.0 |
| | **FP** | 1.1 | 0.8 | |
| | **MM** | 0.1 | 0.1 | |
| | **MP** | 0.3 | 0.5 | |
| | **TM** | 0.1 | 0.1 | |
| | **TP** | 0.3 | 0.3 | |

**Conclusion**

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Dapagliflozin in Tablet dosage form. Retention time of Metformin and Dapagliflozin were found to be 2.330min and 3.098 min. %RSD of the Metformin and Dapagliflozin were and found to be 0.7 and 0.8 respectively. % assay was obtained as 99.57% and 99.76% for Metformin and Dapagliflozin respectively. LOD, LOQ values are obtained from regression equations of Metformin and Dapagliflozin were 0.90ppm, 2.73ppm and 0.02ppm, 0.07ppm respectively. Regression equation of Metformin & Dapagliflozin is y = 2433.x + 15236 and y = 13061x + 672.2. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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