International Journal of Research and Development in Pharmacy & Life Sciences

Research Article Open Access

# Development and Validation of UV Spectroscopic and HPTLC Methods for Simultaneous Estimation of Dapagliflozin and Metoprolol in Synthetic Mixture

**Nidhi Tadvi*, Krishna Kalsara and Umesh Upadhyay**

*Department of Pharmaceutical Quality Assurance, Sigma Institute of Pharmacy, Gujarat, India*

## Abstract

A Specific, Precise, Accurate, Robust and cost-effective UV spectroscopic and HPTLC methods were developed for simultaneous determination of Dapagliflozin (DAPA) and Metoprolol (METO) in their synthetic mixture. The developed methods proved to be simpler in procedure and produced more accurate results. The result of analysis was validated according to ICH Guidelines. This simple and precise method can be used of both drug in quality control laboratories. For HPTLC the drugs were separated by Camag Linomate 5 sample applicator with a 100-µL applicator syringe. Chromatography was performed on 10 cm × 10 cm aluminium TLC plates precoated with silica gel 60-F254. Plates were developed in a mobile phase consisting of toluene/ chloroform/ methanol/ glacial acetic acid (4.5/2/3/0.5, v/v/v/v). Developed plate subjected to densitometric measurement in absorbance mode at wavelength 235 nm using Camag TLC scanner. The % RSD Value was found for the validation parameter that indicate the preciseness of the proposed method and is applicable for routine analysis for quantitative determination of Dapagliflozin (DAPA) and Metoprolol (METO) in bulk as well as synthetic mixture.

## Introduction to Disease

According to a 2023 paper, more than 64 million people worldwide have heart failure (HF), which is a life-threatening syndrome that causes poor quality of life, high costs, and significant morbidity and mortality. Heart failure is a prolonged, gradual disease categorized by failure of the heart muscles to supply enough blood to meet the nutritious and oxygen need of the body [1].

## Introduction to Drugs

### Dapagliflozin

Dapagliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor, and it was the first SGLT2 inhibitor to be approved. Dapagliflozin was approved by FDA in Jan 2014. indicated for managing diabetes mellitus Type-2. When combined with diet and exercise in adults, dapagliflozin helps to improve glycemic control by inhibiting glucose reabsorption in the proximal tubule of the nephron and causing glycosuria [2].

### Metoprolol

Metoprolol was developed since 1969 by US Pharmaceutical Holdings and FDA approved in 1978. Metoprolol is a selective beta-1 blocker commonly employed as the succinate and tartrate derivatives depending if the formulation is designed to be of immediate release or extended release. Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors. This inhibition decreases cardiac output by producing negative chronotropic and inotropic effects without presenting activity towards membrane stabilization nor intrinsic sympathomimetics [3].

## Introduction of UV VIS Spectroscopy

UV-Vi's spectroscopy, short for Ultraviolet-Visible spectroscopy, is a technique used to analyze the interaction of matter with light within the ultraviolet and visible regions of the electromagnetic spectrum. This analytical method is widely applied in various fields, including chemistry, biochemistry, pharmaceuticals, environmental science, and materials science, due to its versatility and sensitivity.

## Introduction of High-Performance Thin-Layer Chromatography

High-Performance Thin-Layer Chromatography (HPTLC) is a powerful chromatographic technique used for the separation, identification, and quantification of chemical compounds in complex mixtures. It's an advanced version of traditional thin-layer chromatography (TLC) that offers enhanced resolution, sensitivity, and reproducibility [4].

## Validation of Analytical Method

Validation is the process of establishing documentary evidence that a procedure or process is suitable for its intended use. It involves collecting and evaluating data generated from the process or method used in making a product. Method validation data provide information which enables the comparability of results from samples analyzed in different laboratories and using different methods to be assessed.

## Drug Profile

(Table 1, Table 2 and Table 3)

## Aim, Objective and Rational

The primary aim of this research is to develop and validate robust spectrophotometric and chromatographic methods for accurately estimating the concentrations of Dapagliflozin and Metoprolol in a

***Corresponding author:** Nidhi Tadvi, Department of Pharmaceutical Quality Assurance, Sigma Institute of Pharmacy, Gujarat, India, E-mail: nidhitadvi05288@gmail.com

**Received:** 10-July-2024, Manuscript No: ijrdpl-24-141301, **Editor Assigned:** 13-July-2024, pre QC No: ijrdpl-24-141301 (PQ), **Reviewed:** 27-July-2024, QC No: ijrdpl-24-141301, **Revised:** 30-July-2024, Manuscript No: ijrdpl-24-141301 (R), **Published:** 02-Aug-2024, DOI: 10.4172/2278-0238.1000222

**Citation:** Nidhi T (2024) Development and Validation of UV Spectroscopic and HPTLC Methods for Simultaneous Estimation of Dapagliflozin and Metoprolol in Synthetic Mixture. Int J Res Dev Pharm L Sci, 10: 222.

**Table 1:** Physiochemical properties of Dapagliflozin.

| Name | Dapagliflozin |
|---|---|
| IUPAC Name | (2S,3R,4R,5S,6R)-2-[4-Chloro-3-(4-ethoxybenzyl) phenyl]- 6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol |
| Class | Sodium glucose co-transporter 2 (SGLT2) inhibitor |
| CAS NO. | 461432-26-8 |
| Molecular Formula | C21H25ClO6 |
| Molecular weight | 408.88 g/mol |
| Official Status | - |
| Appearance | White to off-white crystalline solid |
| Physical state | Solid |
| Solubility | Soluble in Methanol ,Acetonitrile |
| pKa | 12.6 |
| Melting Point | 176- 178ºC |
| Partition coefficient (log P) | 2.52 |

**Table 2:** Physiochemical Properties of Metoprolol.

| Name | Metoprolol |
|---|---|
| IUPAC Name | 2-[(1R)-3-[bis(propan-2-yl) amino]-1phenylpropyl]-4-methylphenol |
| Class | Beta Blocker cardio selective |
| CAS NO. | 124937-51-5 |
| Molecular Formula | C15H25NO3 |
| Molecular Weight | 267.381 g·mol−1 |
| Official Status Official | in USP, BP, IP |
| Appearance | White crystalline powder |
| Physical state | Solid |
| Solubility | very soluble in water, and freely soluble in methanol, ethanol and in acetic acid |
| pKa | 9.7 |
| Melting Point | 120 -122 0C |
| Partition Co-efficient | 0.6 |

**Table 3:** Literature Summary.

| METHOD | DAPA | METO | DAPA+ METO |
|---|---|---|---|
| UV SPECTROPHOTOMETRY | √ | √ | - |
| HPLC | √ | √ | - |
| RP-HPLC | √ | √ | - |
| LC-MS/MS | √ | √ | - |
| HPTLC | √ | √ | - |
| STABILITY INDICATING HPLC METHOD | √ | √ | - |

synthetic mixture. Application of developed UV spectroscopic and HPTLC methods for the estimation of Dapagliflozin and Metoprolol in synthetic mixture [5].

The rational use of Dapagliflozin and Metoprolol in patients with heart failure is grounded in their distinct yet complementary mechanisms of action. Dapagliflozin is a sodium-glucose co-transporter-2 (SGLT2) inhibitor known for its ability to reduce heart failure hospitalizations and cardiovascular events, particularly in patients with heart failure with reduced ejection fraction (HFrEF). Metoprolol, on the other hand, is a beta-blocker that has been a cornerstone in the treatment of heart failure for years, helping to reduce heart rate and improve cardiac function [6].

The prescribed dosage involves Dapagliflozin and Metoprolol at 10 mg and 50 mg, respectively.

## Justification

Our comprehensive literature survey has revealed an existing gap - there are no reported spectroscopic and chromatographic methods available for the precise determination of Dapagliflozin and Metoprolol, especially when used together.Consequently, this research project holds significant promise at an industrial level, particularly when the formulation incorporating this drug combination enters the market. Dapagliflozin and Metoprolol combination drug currently in phase 3 [7].

## Experimental Work

## Identification of API

## Melting Point Determination

Melting point of Dapagliflozin and Metoprolol was carried out by melting point apparatus. 10 mg of powdered drug was filled in capillary that was attached with the tip of thermometer in melting point apparatus. Temperature at which the drug powder melted was noted down in melting point apparatus. It was performed in triplicate (Table 4 and Table 5).

## Solubility Study

Solubility of Dapagliflozin (DAPA) and Metoprolol (METO) was performed using various solvents like water, methanol, acetonitrile etc [8] (Table 6).

## IR Spectra

Drug Dapagliflozin (DAPA) and Metoprolol (METO) was placed in sample compartment of FT-IR instrument, where it was scanned in the range of 4000 - 650cm$^{-1}$ (Table 7, Table 8, Figure 1 and Figure 2).

## UV Absorption Study

Accurately weighed 10 mg of Dapagliflozin (DAPA) and Metoprolol (METO) were transferred separately in 10 ml volumetric flasks, dissolved in small volume of methanol and then volume was adjusted to the mark with methanol to obtain concentration of 1000 µg/ml. These solutions were further diluted to obtain concentration

**Table 4:** List of Instruments and Apparatus.

| Sr. No. | Instrument | Model No | Manufacturer |
|---|---|---|---|
| 1 | Ultra Sonicator | - | Trans-o-sonic |
| 2 | UV Visible Spectrophotometer | UV 1700 | Shimadzu |
| 3 | FT-IR | Alpha-II | Bruker |
| 4 | Analytical Weighing Balance | AUW 220D | Shimadzu |
| HPTLC | √ | √ | - |
| STABILITY INDICATING HPLC METHOD | √ | √ | - |

**Table 5:** Melting Point Study.

| Drugs | Reported Melting | Observed Melting |
|---|---|---|
| Dapagliflozin (DAPA) | 74-78 °C | 76-78°C |
| Metoprolol (METO) | 120°C | 120-122 °C |

**Table 6:** Solubility Study.

| Drugs | Dapagliflozin (DAPA) | Metoprolol (METO) |
|---|---|---|
| Water | Poorly soluble | Soluble |
| Methanol | Soluble | Soluble |
| Acetonitrile | Slightly soluble | Slightly soluble |

**Table 7:** IR value for Dapagliflozin.

| Sr. No. | Functional Group | Standard wavenumber | Observed wavenumber |
|---|---|---|---|
| 1 | . O-H | 3550-3200 | 3358.3, 3268.9 |
| 2. | C-H (Aliphatic) | 2960-2850 | 2862, 2907 |
| 3. | C=C | 1675-1600 | 1613 |
| 4. | C-O | 1300-1000 | 1271 |
| 5 | C-Cl | 850-550 | 823.7 |

**Table 8:** IR value for Metoprolol.

| Sr. No. | Functional Group | Reported Wavenumber | Observed Wavenumber |
|---|---|---|---|
| 1. | Alcohol OH Stretch | 3600-3400 | 3415.97 |
| 2. | Aromatic Ring | 2950-2850 | 2930.28, 639.53 |
| 3. | N-H Stretching | 1650-1550 | 1560.67 |
| 4. | C–O-C | 1400-1200 | 1383.42 |
| 5 | C-Cl | 850-550 | 823.7 |

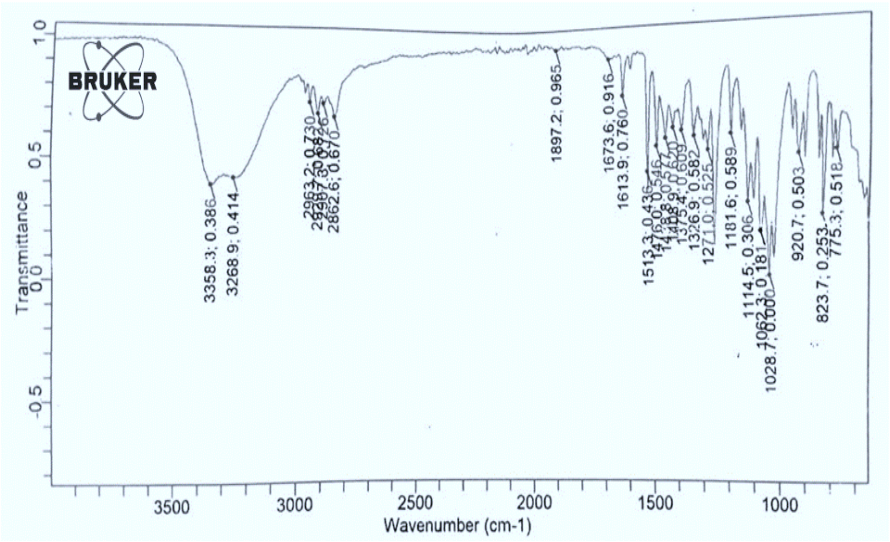


**Figure 1:** IR value for Dapagliflozin.

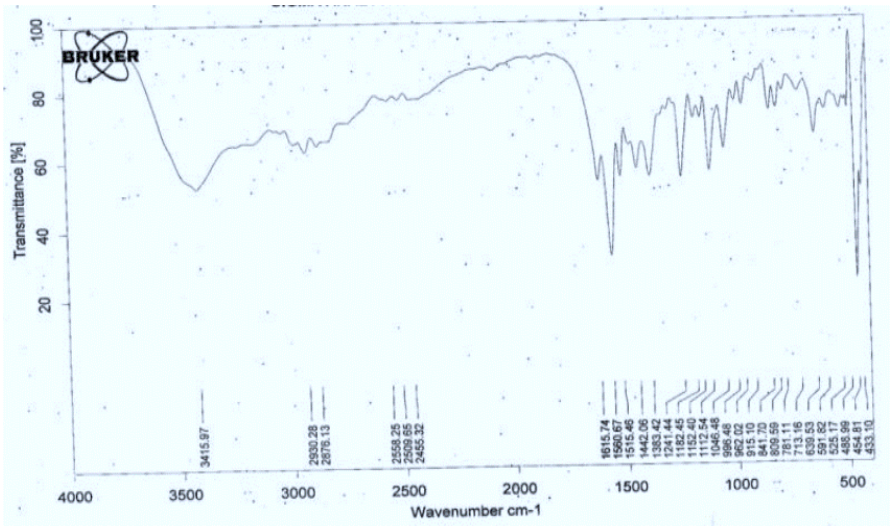


**Figure 2:** IR Value of Metoprolol.

of 10µg/ml. These standard solutions of Dapagliflozin (DAPA) and Metoprolol (METO) in methanol were scanned in UV range, 200- 400 nm in 1 cm cell using methanol as blank and maximum absorbance was measured for selection of λmax of DAPA and METO. Based on solubility, Dapagliflozin (DAPA) and Metoprolol (METO) was soluble in methanol. Hence, methanol was selected as diluent [9].

### Preparation of Stock Solution

Accurately weighed and transferred about 10 mg of Dapagliflozin (DAPA) and 50mg of Metoprolol (METO) in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with diluent. Concentration of Dapagliflozin (DAPA) is 100 µg/ml and Metoprolol (METO) 500µg/ml [10].

### Selection of Wavelength

In the present study drug solution of dapagliflozin (DAPA) is 10µg/ml and Metoprolol(METO) 50µg/ml solutions was prepared in methanol. The standard solution was then scanned in the UV region of 200-400 nm and the spectrum was taken. Wavelength at which the drug showed good absorbance was selected as a detection wavelength (235nm) (Figure 3).

An ideal wavelength is the one that gives Maximum response for the drugs that was to be detected [11].

## UV Absorption Study

### Q-Absorption Ratio Method

Let it be one drug X and Y According to Q-Absorption ratio method, use the ratio of absorption at two selected wavelengths. One is at iso -absorptive point and other being the λmax of one of the two components. Two equations were constructed as described below, using the relationship ax1=ay1 at λ1 and L=1. Cx= {(QM-Qy)/(Qx-Qy)}×( A1/ax1) .....(8) &

Cy= {(QM-Qx)/(Qy-Qx)}×( A2/ay1) .....(9)

Finally, equation 8 and equation 9 gives the absolute concentration value of drug X & Y (Beckett and Stenlake, 2005). where, A1 and A2 are the absorbance of mixture at 236 nm and 223.80 nm; ax1 and ay1 are absorptivity's of Dapagliflozin (DAPA) and Metoprolol (METO) at 236 nm and ax2 and ay2 are absorptivity's of Dapagliflozin (DAPA) and Metoprolol (METO) at 223.80 nm; QM = A2/A1, Qx = ax2/ ax1, Qy = ay2/ay1 (Figure 4, Figure 5, Figure 6, Figure 7 and Figure 8).

## UV Method

### Validation Parameters

**1.Linearity and range**- Representative calibration curve of Dapagliflozin (DAPA) and Metoprolol (METO) was obtained by plotting the mean absorbance of Dapagliflozin (DAPA) and Metoprolol (METO) against concentration over the range of 5-25 µg/ml and 25-125 µg/ml for DAPA and METO, respectively [12] (Figure 9 and Figure 10)

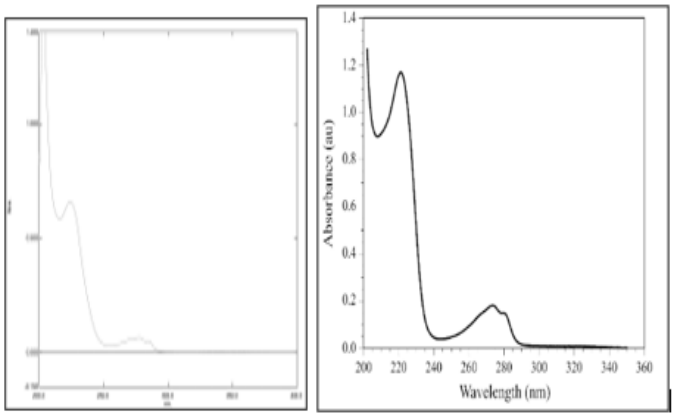


**Figure 3:** UV Spectrum of Dapagliflozin (DAPA) and Metoprolol (METO) in methanol.

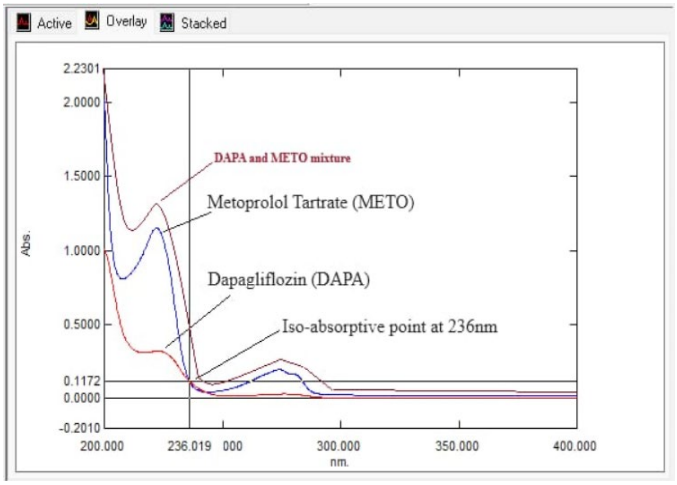


**Figure 4:** UV overlay graph of Dapagliflozin (DAPA) and Metoprolol (METO).

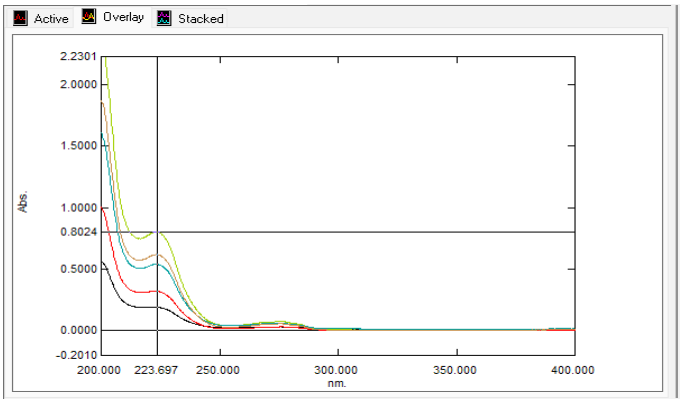


**Figure 5:** Zero-order spectra of 5-25 µg/ml of DAPA.

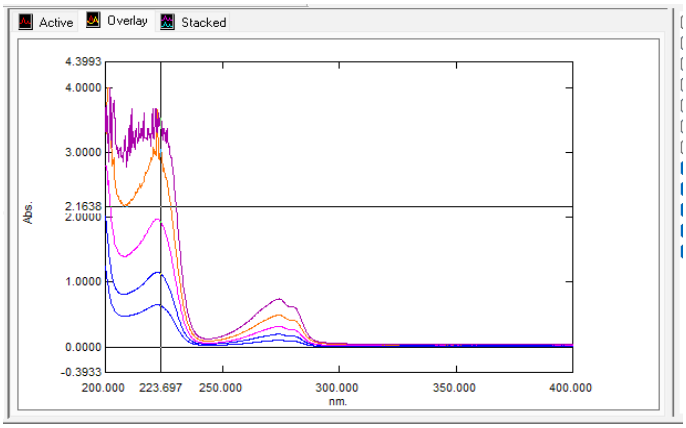


**Figure 6:** Zero-order spectra of 50-125 µg/ml of METO.

The overlay linearity UV spectrum of DAPA (5-25 µg/ml) and METO (25-125 µg/ml) at 223.80 nm and 236.00nm.

The calibration range was prepared in such a way that the ratio of combination was maintained throughout simultaneous estimation of both drugs in bulk and synthetic mixture [13] (Table 9, Table 10 and Table 11).

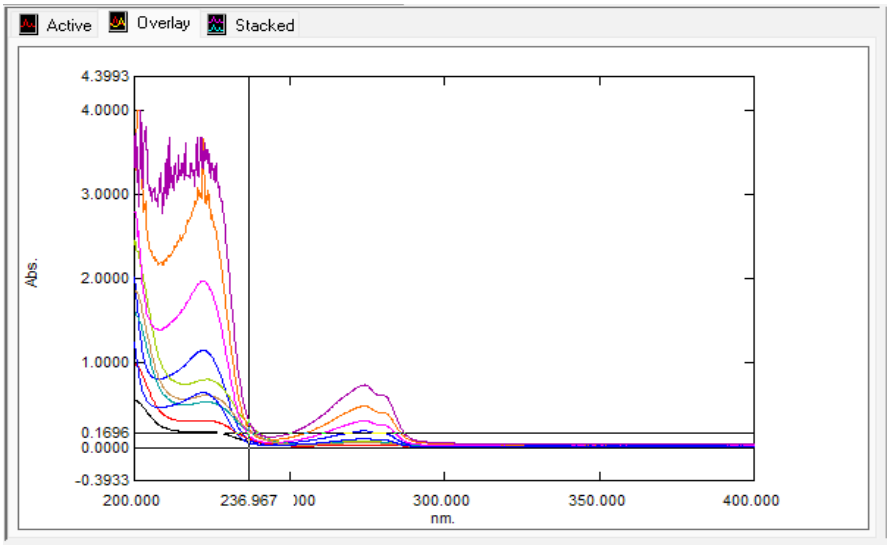


**Figure 7:** Overlain Zero-order spectra of 5-25 µg/ml of DAPA and 50-125 µg/ml of METO.

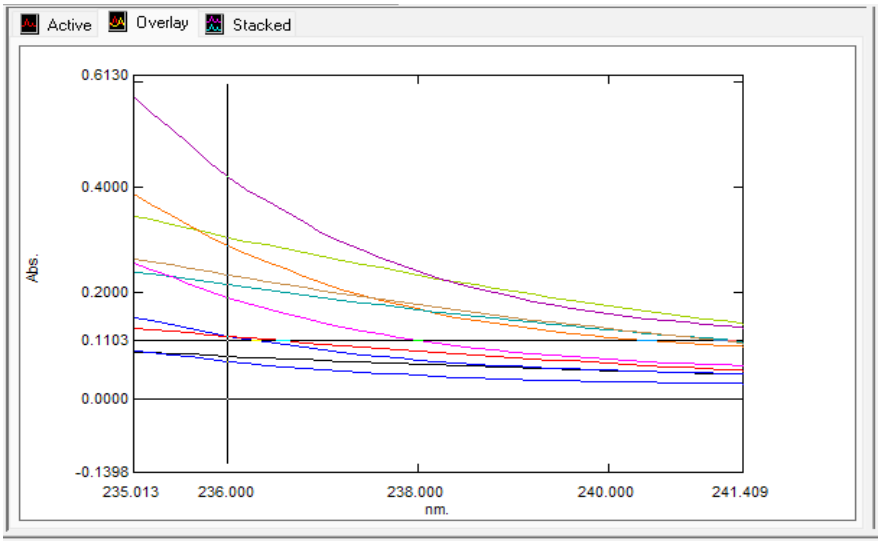


**Figure 8:** Iso-absorptive point of 5-25 µg/ml of DAPA and 50-125 µg/ml of METO.

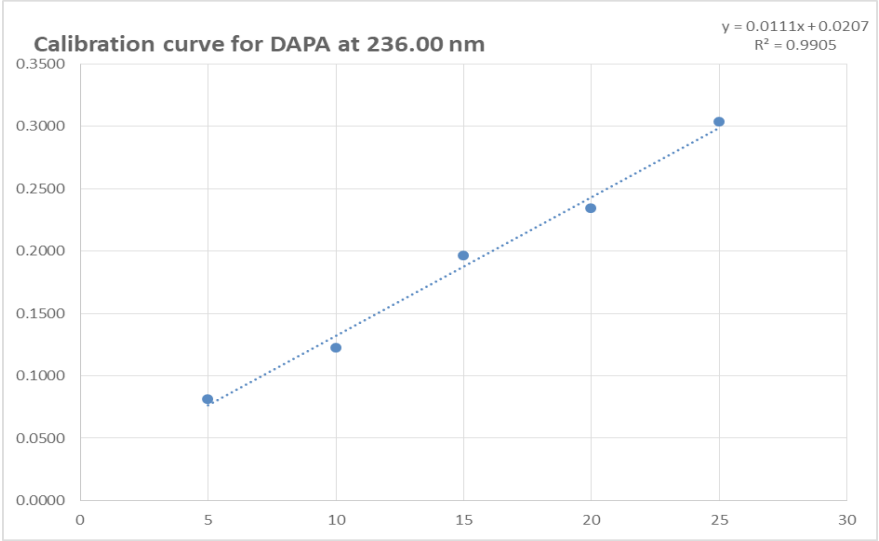


**Figure 9:** Calibration curve for DAPA at 236.00 nm.

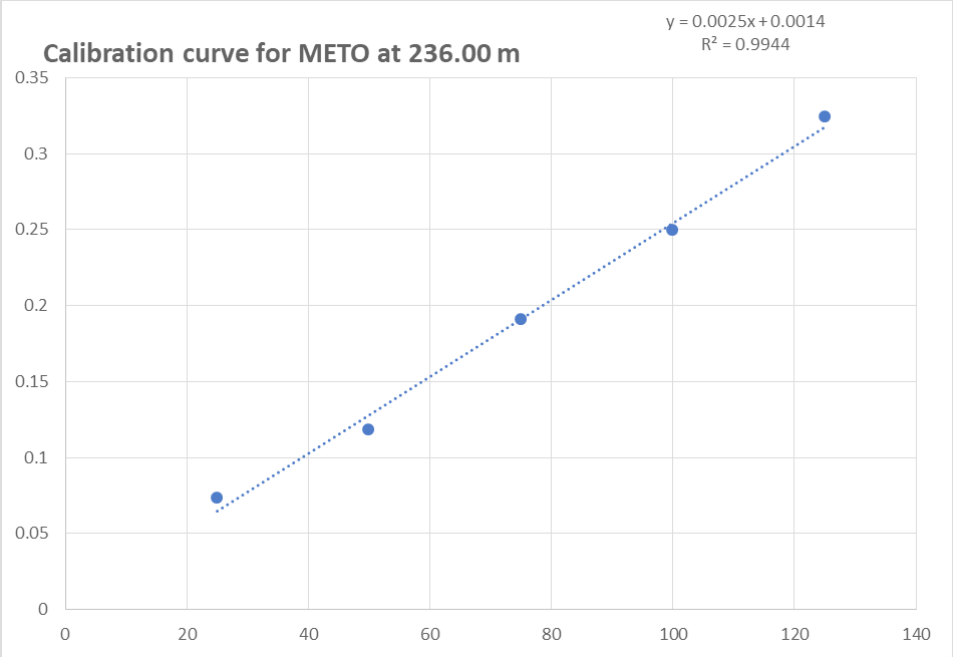


**Figure 10:** Calibration curve for METO at 236.00 nm.

**Table 9:** Calibration data of DAPA.

| Concentration (µg/ml) | At 236.00 nm | | At 223.80 nm | |
|---|---|---|---|---|
| | Absorbance Mean ± SD (n=6) | % RSD | Absorbance Mean ± SD (n=6) | % RSD |
| 5 | 0.0811 ± 0.0008 | 0.98 | 0.1939 ± 0.0020 | 1.03 |
| 10 | 0.1225 ± 0.0019 | 1.58 | 0.3220 ± 0.0032 | 1.00 |
| 15 | 0.1965 ± 0.0016 | 0.79 | 0.5072 ± 0.0031 | 0.61 |
| 20 | 0.2339 ± 0.0029 | 1.23 | 0.6143 ± 00036 | 0.59 |
| 25 | 0.3033 ± 0.0018 | 0.59 | 0.8040 ± 0.0034 | 0.42 |
| Regression Equation | y = 0.0111x + 0.0207 | | y = 0.0302x + 0.0346 | |
| Std. Dev of Intercept | 0.00137 | | 0.00119 | |
| Average of Slope | 0.01116 | | 0.03176 | |
| LOD | 0.412 µg/ml | | 0.125 µg/ml | |
| LOQ | 1.25 µg/ml | | 0.377 µg/ml | |

**Table 10:** Calibration data of METO.

| Concentration (µg/ml) | At 236.00 nm | | At 223.80 nm | |
|---|---|---|---|---|
| | Absorbance Mean ± SD (n=6) | % RSD | Absorbance Mean ± SD (n=6) | % RSD |
| 25 | 0.0731 ± 0.00110 | 1.51 | 0.5010 ± 0.00406 | 0.81 |
| 50 | 0.1184 ± 0.00233 | 1.97 | 1.1207 ± 0.00406 | 0.36 |
| 75 | 0.1910 ± 0.00233 | 1.22 | 1.9262 ± 0.00808 | 0.42 |
| 100 | 0.2494 ± 0.00261 | 1.05 | 2.7760 ± 0.03848 | 1.39 |
| 125 | 0.3238 ± 0.00450 | 1.39 | 3.4768 ± 0.05662 | 1.63 |
| Regression Equation | y = 0.0025x + 0.0014 | | y = 0.0304x - 0.3219 | |
| Std. Dev of Intercept | 0.0024 | | 0.0191 | |
| Average of Slope | 0.0127 | | 0.1597 | |
| LOD | 0.62 µg/ml | | 0.39 µg/ml | |
| LOQ | 1.88 µg/ml | | 1.19 µg/ml | |

**Table 11:** Absorptivity values of DAPA and METO.

| | | | | |
|---|---|---|---|---|
| ax1 | 0.0111 | ay1 | 0.0025 | 0.81 |
| ax2 | 0.0302 | ay2 | 0.0304 | 0.36 |

## Precision

### Repeatability

In UV spectroscopic method, repeatability has been carried out by analyzing the sample solution of DAPA and METO repeatability six times and absorbance was measured and % RSD [14] (Table 12).

### Intraday and Interday Precision

The precision of method was determined by carrying out Intraday and Interday precision. Intraday precision was determined by analysing sample solution of Dapagliflozin (DAPA) 5, 15, 25 µg/mL and Metoprolol (METO) concentration would be 25,75, 125 µg/mL which covers low, medium, and high concentrations of the calibration curve three times on the same day. Interday precision was determined by analysing sample solutions of Dapagliflozin (DAPA) 5, 15, 25 µg/mL and Metoprolol (METO) concentration would be 25,75, 125 µg/mL which covers low, medium, and high concentrations of the calibration curve on three consecutive days. The absorbance obtained were used to calculate mean and % RSD values shown in Table 13. The % RSD was found to be less than 2% which indicate method is precise [15].

(Table 13)

(Table 14)

### Accuracy

Accuracy of the method was confirmed by recovery study from synthetic mixture at three levels 50%, 100% and 150% of standard addition. The data shown in Table 15 indicate that the developed method is accurate. The % recovery of DAPA and METO was found to be in range of 98.00 – 102% (Table 15).

### LOD and LOQ

The LOD and LOQ were found for Dapagliflozin (DAPA) and Metoprolol (METO), shown in table respectively indicating high sensitivity of the method [16-19] (Table 16).

### Analysis of Synthetic Mixture

The developed and validated Q-Absorbance Ratio Spectrophotometric Method was applied for determination of Dapagliflozin (DAPA) and Metoprolol (METO) in synthetic mixture. The sample was analysed three times. The % assay was found to be 102.80 % and 100.67 % for Dapagliflozin (DAPA) and Metoprolol (METO), respectively [20] (Table 17),

## HPTLC

### List of Instrument and Apparatus

(Table 18)

### HPTLC Method Ddevelopment and Validation Instruments

The HPTLC instrument consisted of a CAMAG (Muttenz, Switzerland) Linomat V sample applicator with a 100-µL applicator syringe (Hamilton, Bonadauz, Switzerland). Chromatography was performed on 10 cm × 10 cm aluminium TLC plates precoated with silica gel 60-F254 (E. Merck, Darmstadt, Germany; supplied by Anchrom Technologists, Mumbai, India).

**Table 12:** Repeatability data of METO and DAPA.

| Sr. No. | DAPA (15 µg/ml) METO (75 µg/ml) | |
|---|---|---|
| | At 236.00nm | At 223.80nm |
| 1 | 0.2017 | 1.9291 |
| 2 | 0.2017 | 1.9231 |
| 3 | 0.2022 | 1.9179 |
| 4 | 0.2003 | 1.9257 |
| 5 | 0.2007 | 1.9288 |
| 6 | 0.2017 | 1.9325 |
| Mean | 0.2032 | 1.9271 |
| SD | 0.0020 | 0.0079 |
| % RSD | 0.98 | 0.41 |

**Table 13:** Precision data at 236.00nm.

| Drug | Conc. (µg/ml) | Intraday Precision | | Interday Precision | |
|---|---|---|---|---|---|
| | | Absorbance (Mean ± SD) (n=3) | % RSD | Absorbance (Mean ± SD) (n=3) | % RSD |
| DAPA | 5 | 0.0802 ± 0.0002 | 0.25 | 0.0813 ± 0.0009 | 1.11 |
| | 15 | 0.2007 ± 0.0009 | 0.45 | 0.2037 ± 0.0021 | 1.03 |
| | 25 | 0.3003 ± 0.0052 | 1.73 | 0.3041 ± 0.0018 | 0.59 |
| METO | 25 | 0.0728 ± 0.0010 | 1.37 | 0.0730 ± 0.0008 | 1.10 |
| | 75 | 0.1967 ± 0.0017 | 0.86 | 0.1937 ± 0.0020 | 1.03 |
| | 125 | 0.3261 ± 0.0045 | 1.37 | 0.3278 ± 0.0060 | 1.83 |

**Table 14:** Precision data at 223.80nm.

| Drug | Conc. (µg/ml) | Intraday Precision | | Interday Precision | |
|---|---|---|---|---|---|
| | | Absorbance (Mean ± SD) (n=3) | % RSD | Absorbance (Mean ± SD) (n=3) | % RSD |
| DAPA | 5 | 0.1951 ± 0.0012 | 0.61 | 0.1933 ± 0.0026 | 1.34 |
| | 15 | 0.5083 ± 0.0038 | 0.75 | 0.5146 ± 0.0066 | 1.28 |
| | 25 | 0.8058 ± 0.0041 | 0.51 | 0.8111 ± 0.0058 | 0.72 |
| METO | 25 | 0.5041 ± 0.0030 | 0.59 | 0.5010 ± 0.0073 | 1.46 |
| | 75 | 1.9273 ± 0.0053 | 0.27 | 1.9507 ± 0.0162 | 0.83 |
| | 125 | 3.4925 ± 0.0621 | 1.78 | 3.5157 ± 0.0540 | 1.54 |

**Table 15:** Accuracy data of DAPA and METO.

| Drug | % Level of spike | Amount of drug in sample (µg/ml) | Amount of std. added (µg/ml) | Total amount of drug (µg/ml) | Total amount of std. found (µg) Mean ± SD (n=3) | % Recovery |
|---|---|---|---|---|---|---|
| DAPA | 0 | 10 | 0 | 10 | 9.97 ± 0.125 | 99.7 |
| | 50 | 10 | 5 | 15 | 15.29 ± 0.21 | 101.93 |
| | 100 | 10 | 10 | 20 | 19.98 ± 0.24 | 99.9 |
| | 150 | 10 | 15 | 25 | 25.31 ± 0.84 | 101.24 |
| METO | 0 | 50 | 0 | 50 | 49.79 ± 3.60 | 99.58 |
| | 50 | 50 | 25 | 75 | 76.43 ± 1.03 | 101.91 |
| | 100 | 50 | 50 | 100 | 98.27 ± 1.07 | 98.27 |
| | 150 | 50 | 75 | 125 | 126.85 ± 1.00 | 101.48 |

**Table 16:** Calibration data of DAPA and METO.

| Particulars | At 236.00 nm | At 223.80 nm |
|---|---|---|
| Dapagliflozin (DAPA) | | |
| Std. Dev. of Intercept | 0.0014 | 0.0012 |
| LOD | 0.4125 µg/ml | 0.125 µg/ml |
| LOQ | 1.25 µg/ml | 0.377 µg/ml |
| Metoprolol (METO) | | |
| Std. Dev. Of Intercept | 0.0024 | 0.0191 |
| Average of Slope | 0.0127 | 0.1597 |
| LOD | 0.623 µg/ml | 0.39 µg/ml |
| LOQ | 1.88 µg/ml | 1.19 µg/ml |

**Table 17:** Data of determination of Dapagliflozin (DAPA) and Metoprolol (METO) in synthetic mixture.

| Drug | Conc. (µg/ml) | Amount found (µg/ml) | % Assay Mean ± SD (n=3) | % RSD |
|---|---|---|---|---|
| Dapagliflozin (DAPA) | 10 | 9.77 | 98.23 ± 1.81 | 1.84 |
| | | 10.02 | | |
| | | 9.67 | | |
| Metoprolol (METO) | 50 | 48.92 | 97.61 ± 0.96 | 0.98 |
| | | 48.28 | | |
| | | 49.23 | | |

**Table 18:** List of instruments and apparatus.

| Sr. No. | Instrument | Model No |
|---|---|---|
| 1 | High Performance Thin Layer Chromatography (HPTLC) | Make: Camag Linomate 5 (Semiautomatic Sampler applicator) Software: WinCATS 1.3.4 Mobile Phase Chamber: Camag Twin through glass chamber (10x10 & 20x10 cm) Syringe: Hamiltron Syringe (100 Micro liter) UV Cabinet: UV cabinet with dual Wavelength UV lamp (254 & 366 nm) Scanner: Camag TLC scanner 3 |
| 2 | UV-Visible Spectrophotometer | Make: Shimadzu Corporation, Japan<br>Model: UV 1700-Pharmaspec<br>Measurement Mode: ABS & % T (Transmittance) |
| 3 | pH meter | MAC/SR No. 1706 |
| 4 | Analytical Weighing Balance | AUW 220D Shimadzu |
| 5 | Ultra Sonicator | - |

A CAMAG TLC scanner 4 was used for densitometric scanning of the chromatogram. All drugs and chemicals were weighed on a Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

## Sample Application

Standards and synthetic mixture samples of DAPAGLIFLOZIN (DAPA) and Metoprolol (METO) were applied to the HPTLC plates in the form of narrow bands 6 mm in length applied 10 mm from the bottom and 15 mm from the left edge of the plate. Samples were applied under a continuous drying stream of nitrogen gas [21].

## Mobile Phase and Development

Plates were developed in a mobile phase consisting of toluene/ chloroform/ methanol/ glacial acetic acid (4.5/2/3/0.5, v/v/v/v). Linear ascending development was carried out in a twin-trough glass chamber equilibrated with the mobile phase vapours for 15 min. Ten millilitres of the mobile phase (5 mL in the trough containing the plate and 5 mL in the other trough) were used for each development and were allowed to migrate a distance of 80 mm. After development, the HPTLC plates were dried completely [22].

## Densitometric Analysis

Densitometric scanning was performed in the absorbance mode under control with CATS planar chromatography software (CAMAG, Muttenz, Switzerland). The source of radiation was a deuterium lamp, and bands were scanned at 235 nm. The slit dimensions were 5 mm in length and 0.45 mm in width, with a scanning rate of 20 mm/s. Concentrations of the compound were determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations from a linear regression equation [23].

## Selection of Diluent

Based on solubility, DAPAGLIFLOZIN (DAPA) and Metoprolol (METO) was soluble in methanol. Hence, methanol was selected as diluent [24].

## ValidationLinearity

To obtain calibration curve, aliquots of working standard solution of DAPAGLIFLOZIN (DAPA) (100µg/ml) and METOPROLOL (METO) 500 µg/ml ranging from 2, 4, 6, 8, and 10 µl were applied by Hamilton micro syringewith the help of Linomat V applicator on Aluminium plate pre-coated with silicagel G 60 F254 gave concentration of 400-1200 ng/band for DAPAGLIFLOZIN (DAPA) and 1000 – 6000 ng/band for METOPROLOL (METO) [25].

Plate was developed in previously saturated chamber (30 minutes) with mobilephasecontainingtoluene:chloroform:methanol:glacialacetic acid (4.5/2/3/0.5,v/v/v/v) and dried in air. Developed plate subjected to densitometric measurement in absorbance mode at wavelength 235 nm using Camag TLC scanner. Sample solution chromatographed six times and the mean peak area ofDAPAGLIFLOZIN (DAPA) and METOPROLOL (METO) was calculated [26].

## Accuracy

To ensure the reliability of the above method recovery studies were carried outby mixing standard quantity of standard drug with the pre-analyzed sample synthetic mixture and the contents were re-analyzed by the proposed method. Recovery studies were carried out at 50,100 and 150 % level [27].

The recovery study was performed three times at each level. Known amounts of DAPAGLIFLOZIN (DAPA) (0, 200, 400 and 600 ng per band) and METOPROLOL (METO) (0, 1000, 2000 and 3000 ng per band) were taken from the working standard solutions and were added to pre-quantified samples.The amounts of drug were estimated by measuring the areas and by fitting thesevalues to the straight-line equations of the calibration curves [28].

## Precision

The repeatability of measures of the peak area was determined by analysing DAPAGLIFLOZIN (DAPA) (600 ng per band) and METOPROLOL (METO) (3000 ng per band) seven times without changing the position of the plate. Therepeatability of injection was checked by applying seven tracks of DAPAGLIFLOZIN (DAPA) and METOPROLOL (METO) on the same plate. Peak area of same concentration was measured six times and % RSD was calculated [29].

Intra-day precision was determined by analysing sample solutions of DAPAGLIFLOZIN (DAPA) (200, 600 and 1200 ng per band) and METOPROLOL (METO) (1000, 3000 and 5000 ng per band) three times on the same day. Inter-day precision was determined by analysing sample solutions of DAPAGLIFLOZIN (DAPA) (200, 600 and 1000 ng per band) and METOPROLOL (METO) (1000, 3000 and 6000 ng per band) over 3 days. Thepeak areas obtained were used to calculate mean and relative standard deviation(% RSD) [30].

## Sensitivity

The limit of detection (LOD) is the lowest concentration of an analyte that canreliably be differentiated from background levels. The limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte thatcan be quantitatively determined with suitable precision and accuracy [31].

The LOD and LOQ were calculated from the following equations as per the ICHguidelines:

$LOD=3.3\times\sigma/S$

$LOQ=10\times\sigma/S$

where σ is the standard deviation of y intercepts of regression lines, and S is theslope of the calibration curve.

## Specificity

The specificity was estimated by comparing synthetic mixture to pure API. Thechromatogram was taken for DAPAGLIFLOZIN (DAPA) (600 ng per spot) and METOPROLOL (METO) (3000 ng per spot). Developed spot area and Rf value of DAPAGLIFLOZIN (DAPA) and METOPROLOL (METO) was determined [32].

## Robustness

The effects of small changes in the chamber saturation time and solvent migration distance were examined. The robustness of the method was determined in triplicate at concentrations of DAPAGLIFLOZIN (DAPA) (600 ng per spot) and METOPROLOL (METO) (3000 ng per spot) [33].

## Assay of Synthetic Mixture Synthetic

## Mixture Preparation

Synthetic mixture was prepared by mixing Dapagliflozin (DAPA) (10.0 mg), METOPROLOL (METO) (50 mg) with starch (140.0 mg), Hydroxy propyle methayl cellulose E5 (30.0 mg), Polly vinayl pyrrolidone (20.0mg) magnesiumstearate (2.5 mg) and talc (1.0mg), dissolved in 50.0 ml of distilled water and then diluted to the mark in a 100.0ml standard flask and sonicated for 5 min filtered and filtrate was used for validating the above-mentioned methods. Further diluted 1 ml of above solution to 10 ml volumetric flask and volume was make up to the mark with diluent. Further diluted 1 ml of above solution to10 ml volumetric flask and volume was make up to the mark with diluent [34].

Two microliters of these solutions were applied to HPTLC plates and analyzed for DAPA and METO content using the proposed method as described earlier. The possibility of interference from other components of the tablet formulationin the analysis was studied. Concentration of DAPAGLIFLOZIN (DAPA) (600 ng per spot) and METOPROLOL (METO) (3000 ng per spot). Peak area of above solution was measure using developed method [35].

## Optimization of the Mobile Phase

To make the HPTLC method suitable for estimating Dapagliflozin (DAPA) and

Metoprolol (METO) in combined dosage form, the mobile phase was selected on the basis of polarity to give a dense, compact band with an appropriate Rf value for the two drugs. Satisfactory resolution of the drugs was not achieved with mixtures of Ethyl Acetate: Chloroform (7:3), toluene/ acetonitrile (7/3, v/v), chloroform/ acetonitrile (6/4, v/v), chloroform/toluene/ acetonitrile (8/2, v/v), chloroform/ acetonitrile/ methanol (8/2,v/v), acetonitrile/ethyl acetate (8/2,v/v) and chloroform/ methanol/toluene (6/3/1, v/v). Toluene: chloroform: methanol: glacial acetic acid (4.5/2/3/0.5, v/v/v/v) was found to be a satisfactorymobile phase, giving good separation of Dapagliflozin (DAPA) and Metoprolol (METO) [36].

Chamber saturation time and solvent migration distance were

crucial to chromatographic separation. A chamber saturation time of less than 15 min and solvent migration distances greater than 80 mm resulted in diffusion of the analyte band. Therefore, Toluene: chloroform: methanol: glacial acetic acid (4.5/2/3/0.5, v/v/v/v) as the mobile phase, a chamber saturation time of 15 min under ambient conditions and a solvent migration distance of 80 mm were selected as the optimum conditions. These chromatographic conditions produced well-defined, compact bands of Dapagliflozin (DAPA) and Metoprolol (METO) with Rf 0.26 ± 0.02 and 0.74 ± 0.02, respectively [37].

(Table 19, Figure 11, Figure 12, Figure 13, Figure 14, Figure 15 and Figure 16

## Validation Parameters

### Linearity and Range

The linearity of an analytical method is its ability, within a given range, to provide results that are directly, or through a mathematical transformation, proportional to the concentration of the analyte. Representative calibration curve of Dapagliflozin (DAPA) and Metoprolol (METO) was obtained by plotting the mean peak area of Dapagliflozin (DAPA) and Metoprolol (METO)against concentration over the range of concentration of 400-1200 ng/band for Dapagliflozin (DAPA) and 1000 – 6000 ng/band for Metoprolol (METO), respectively [38].

**Table 19:** Trials for optimization of mobile phase.

| Sr. No. | Mobile Phase | Observation |
|---|---|---|
| 1 | Ethyl Acetate: Chloroform (7:3) | Both drug travel with solvent |
| 2 | toluene/ acetonitrile (7/3, v/v) | Both drug travel with solvent |
| 3 | Methanol: Chloroform (7:3) | Only one drug run, another drug spot not found |
| 4 | Methanol: Dichloromethane (6:4) | Both drugs not run on plate |
| 5 | Ethyl Acetate: Acetone: Methanol (8:1.6:0.4) | Both drugs travel very less |
| 6 | Chloroform/methanol/toluene (6/3/1, v/v). | Both drugs not run on plate |
| 7 | Toluene: chloroform: methanol: glacial acetic acid (4.5/2/3/0.5, v/v/v/v) | Both drugs separated well with good resolution and sharp peak |

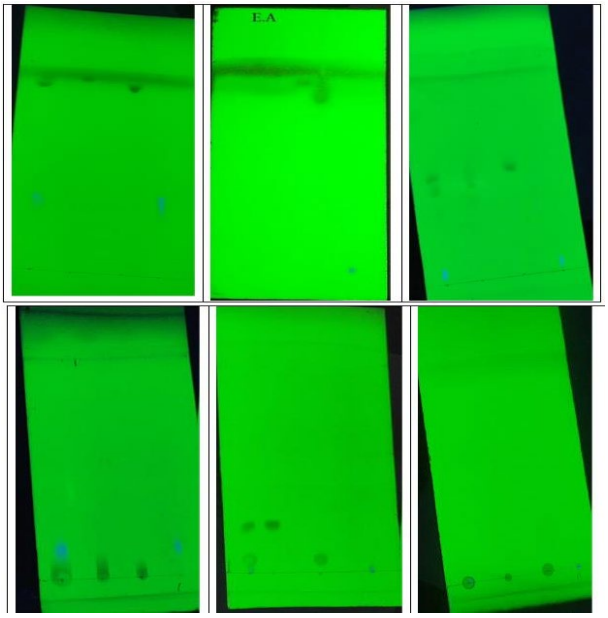


**Figure 11:** Photograph of developed HPTLC plate of Dapagliflozin (DAPA) and Metoprolol (METO) ,First DAPA and second line METO.

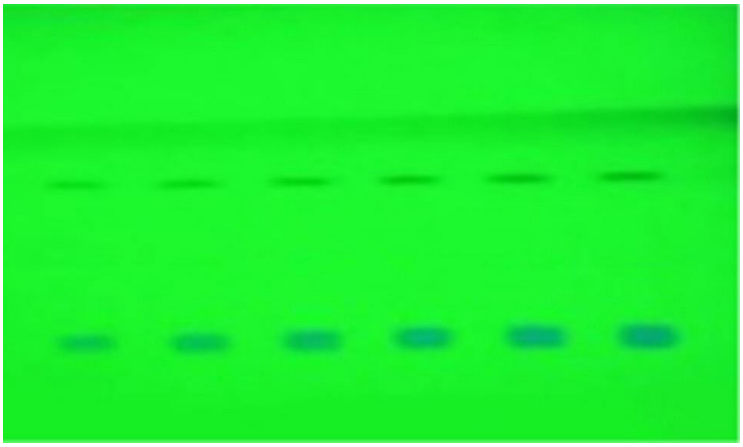

**Figure 12:** Densitograms of Dapagliflozin (DAPA) and Metoprolol (METO).

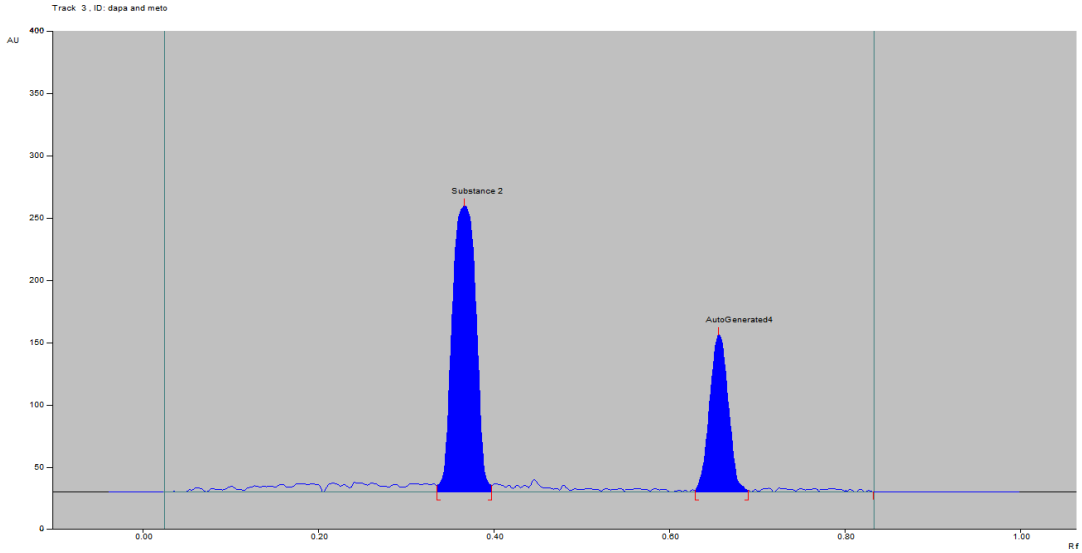


**Figure 13:** Densitograms of Dapagliflozin (DAPA) and Metoprolol (METO).

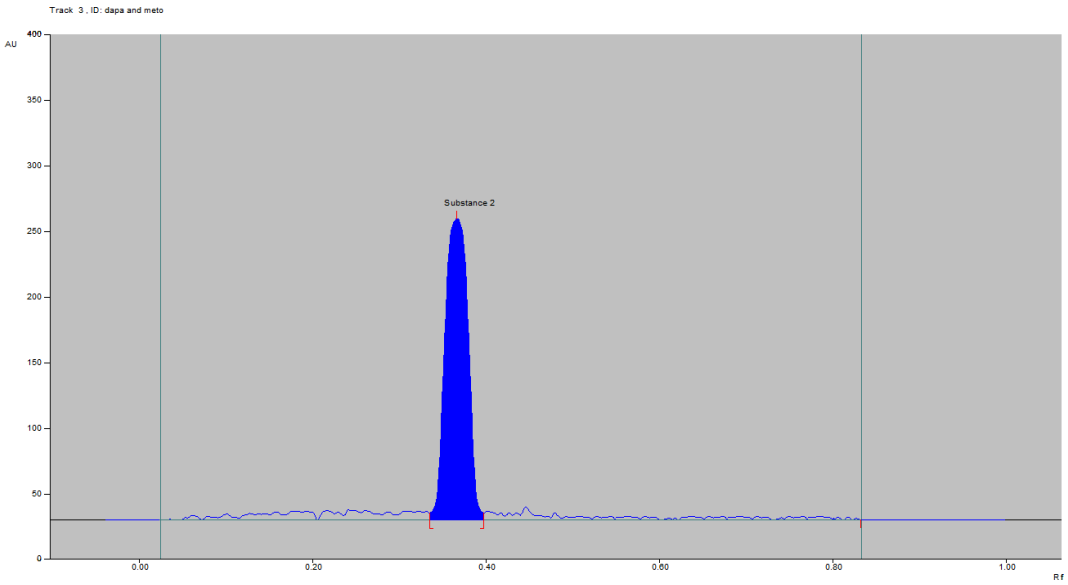


**Figure 14:** Densitogram of Dapagliflozin (DAPA).

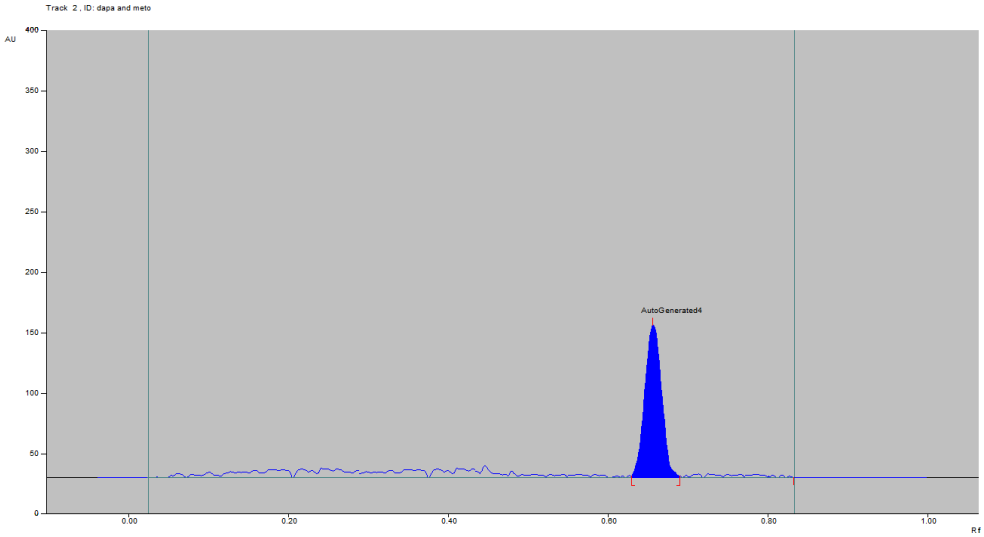


**Figure 15:** Densitogram of Metoprolol (METO).

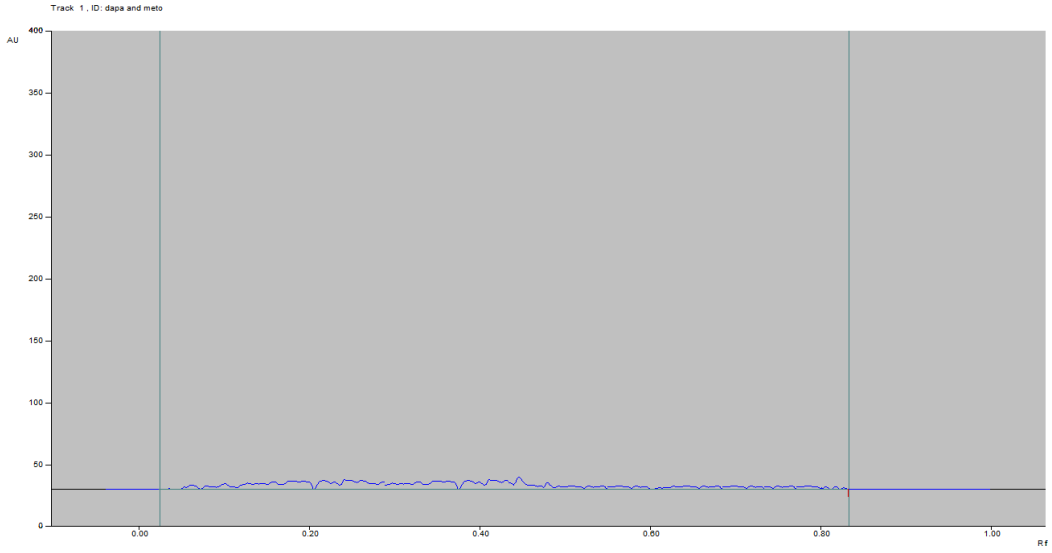


**Figure 16:** Densitograms of blank.

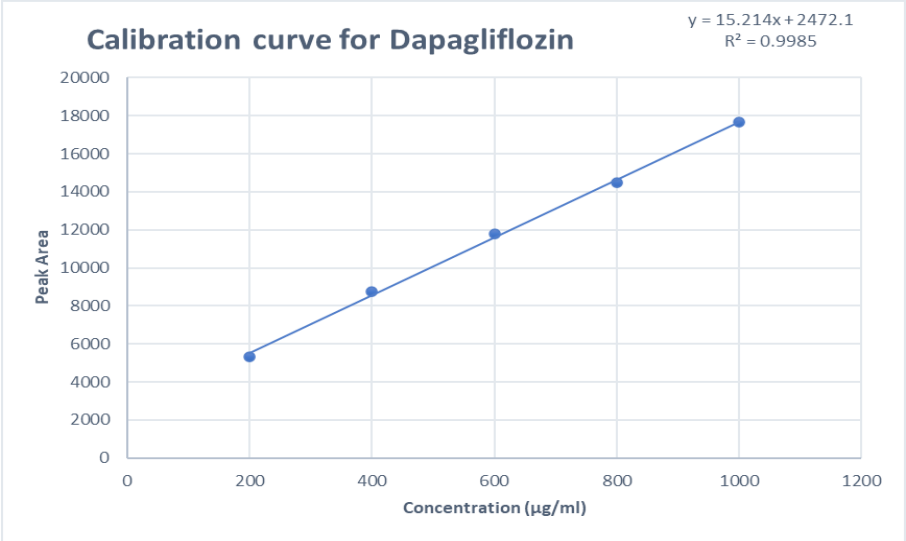


**Figure 17:** Calibration curve for 400-1200 ng/band for Dapagliflozin (DAPA).

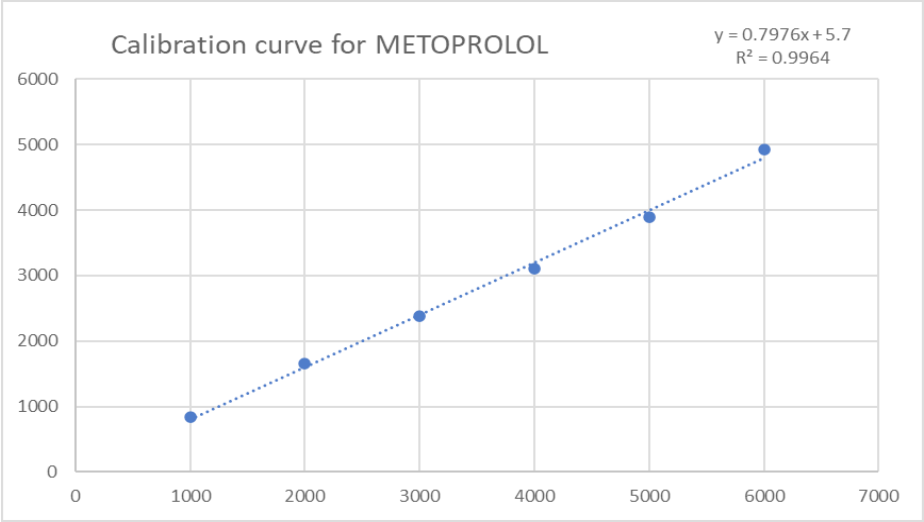


**Figure 18:** Calibration curve for 1000 – 6000 ng/band for Metoprolol (METO).

Responses were found to be linear in the above conc. range with correlation coefficients more than of 0.99 for both drugs [39].

Regression data showed a good linear relationship over the concentration range,demonstrating the suitability of the method for analysis Table 20. Fig. 19 shows a three-dimensional overlay of the HPTLC densitograms for Dapagliflozin (DAPA) and Metoprolol (METO), with calibration bands at 235nm (Figure 17, Figure 18, Table 20 and Figure 19) [40].

## Repeatability

Repeatability of the scanning device and injection was studied by applying and analysing Dapagliflozin (DAPA) (600 ng per band) and Metoprolol (METO) (3000 ng per band) six times (Table 21) [41].

## Intraday and Interday Precision

Intra-day precision is measured for an analytical procedure used within a laboratory over a short time by the same operator with the same equipment, whereas inter-day precision involves estimation of variations in analysis when the method is used on different days. The RSD values of the response were lessthan 2% and 3% for intra-day and inter-day precision, respectively. The % RSDwas found to be less than 2% which indicate method is precise (Table 22) [42].

## Accuracy

(Table 23)

Accuracy of the method was confirmed by recovery study from synthetic mixture at three levels 50%, 100% and 150% of standard

**Table 20:** Calibration data of Dapagliflozin (DAPA) and Metoprolol (METO).

| Concentratio n (ng/band) | Dapagliflozin (DAPA) | | Metoprolol (METO) | | |
|---|---|---|---|---|---|
| | Peak Area Mean ± SD (n=6) | % RSD | Concentratio n (ng/band) | Peak Area Mean ± SD (n=6) | % RSD |
| 200 | 5310 ± 73.19 | 1.38 | 1000 | 836 ± 16.23 | 1.94 |
| 400 | 8746 ± 169.75 | 1.94 | 2000 | 1649 ± 23.32 | 1.41 |
| 600 | 11799 ± 231.26 | 1.96 | 3000 | 2375 ± 42.48 | 1.79 |
| 800 | 14499 ± 242.90 | 1.67 | 4000 | 3101 ± 28.45 | 0.92 |
| 1000 | 17647 ± 314.69 | 1.78 | 5000 | 3897 ± 67.97 | 1.74 |
| 1200 | 21562.2 ± 261.13 | 1.21 | 6000 | 4925 ± 85.89 | 1.74 |
| Regression Equation | y = 15.809x + 2194.1 | | | y = 0.7976x + 5.7 | |
| LOD | 26.58 ng/band | | | 51.81 ng/band | |
| LOQ | 80.57 ng/band | | | 157.00 ng/band | |

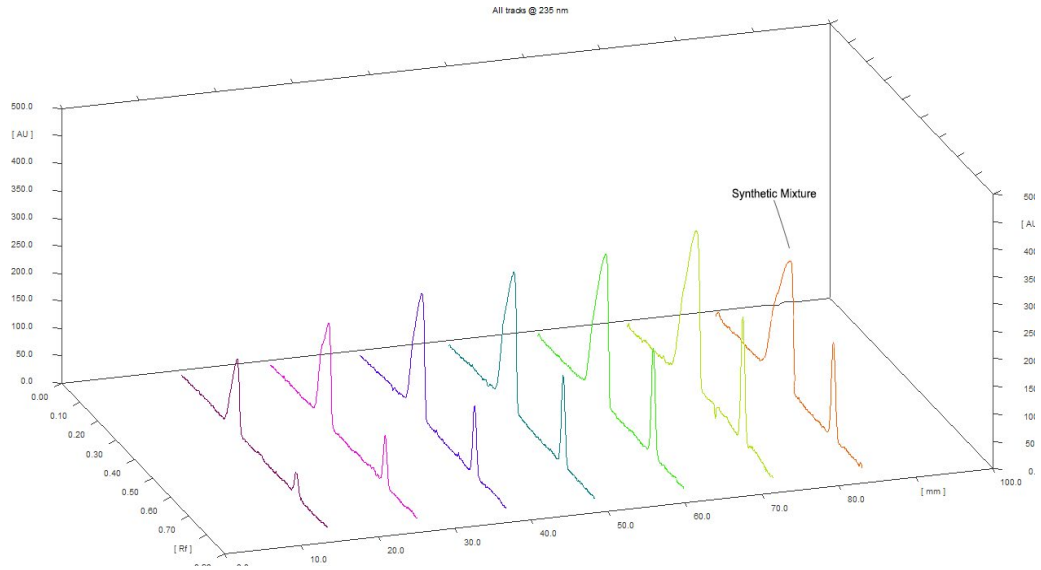


**Figure 19:** Shows a three-dimensional overlay of the HPTLC densitograms for Dapagliflozin (DAPA)and Metoprolol (METO), with calibration bands at 235 nm.

**Table 21:** Repeatability data of Dapagliflozin (DAPA) and Metoprolol (METO).

| Sr. No. | Dapagliflozin (DAPA) | Metoprolol (METO) |
|---|---|---|
| 1 | 11692.49 | 2399 |
| 2 | 11798.80 | 2374.90 |
| 3 | 11794.08 | 2398.17 |
| 4 | 11909.71 | 2372.76 |
| 5 | 11916.79 | 2351.15 |
| 6 | 11972.83 | 2398.65 |
| Mean | 11847 | 2382 |
| SD | 78.76 | 19.90 |
| % RSD | 0.66 | 0.83 |

**Table 22:** Precision data Dapagliflozin (DAPA) and Metoprolol (METO).

| Drug | Conc. (ng/band) | Intraday Precision | | Interday Precision | |
|---|---|---|---|---|---|
| | | Peak Area (Mean ± SD) (n=3) | % RSD | Peak Area (Mean ± SD) (n=3) | % RSD |
| DAPA | 200 | 5256.80 ± 53.10 | 1.01 | 5274.50 ± 81.11 | 1.54 |
| | 600 | 11794.87 ± 114.45 | 0.97 | 11936.85 ± 223.61 | 1.87 |
| | 1200 | 21346.58 ± 215.62 | 1.01 | 21275.42 ± 248.36 | 1.17 |
| METO | 1000 | 830.53 ± 4.83 | 0.58 | 847.94 ± 16.25 | 1.92 |
| | 3000 | 2378.30 ± 30.42 | 1.28 | 2382.82 ± 27.42 | 1.15 |
| | 6000 | 4883.76 ± 51.26 | 1.05 | 4851.09 ± 64.96 | 1.34 |

**Table 23:** Accuracy data of DAPA and METO.

| Drug | % Levelof spike | Amount ofdrug in sample (µg/ml) | Amount of std. added (µg/ml) | Total amount ofdrug (µg/ml) | Total amount of std. found (µg) Mean ± SD (n=3) | % Recovery |
|---|---|---|---|---|---|---|
| DAPA | 0 | 400 | 0 | 400 | 406.22 ± 0.64 | 101.55 |
| | 50 | 400 | 200 | 600 | 594.89 ± 22.03 | 99.15 |
| | 100 | 400 | 400 | 800 | 809.86 ± 17.55 | 101.23 |
| | 150 | 400 | 600 | 1000 | 994.30 ± 18.74 | 99.43 |
| METO | 0 | 2000 | 0 | 2000 | 2022.21 ± 25.86 | 101.11 |
| | 50 | 2000 | 1000 | 3000 | 2989.67 ± 46.46 | 99.66 |
| | 100 | 2000 | 2000 | 4000 | 3931.95 ± 45.46 | 98.30 |
| | 150 | 2000 | 3000 | 5000 | 4911.22 ± 57.28 | 98.22 |

**Table 24:** LOD and LOQ of DAPA and METO.

| Sr. No. | Dapagliflozin (DAPA) | Metoprolol (METO) |
|---|---|---|
| Std. Dev. Of Intercept | 128.91 | 62.80 |
| Average of Slope | 16 | 4 |
| LOD | 26.58 ng/band | 51.81 ng/band |
| LOQ | 80.57 ng/band | 157.00 ng/band |

**Table 25:** Robustness.

| Parameters | Change in condition | DAPA | | METO | |
|---|---|---|---|---|---|
| | | Peak Area | %RSD | Peak Area | %RSD |
| Migration distance (80mm) | 75 mm | 11765.89 ± 178.56 | 1.52 | 2480.48 ± 13.08 | 0.53 |
| | 85 mm | 11860.35 ± 161.34 | 1.36 | 2398.05 ± 31.16 | 1.30 |
| Chamber Saturation time (15 min) | 13 min | 11908.84 ± 171.63 | 1.44 | 2445.42 ± 41.26 | 1.69 |
| | 17 min | 11770.35 ± 218.93 | 1.86 | 2416.08 ± 43.44 | 1.80 |
| Mobile Phase Toluene: chloroform: methanol: glacial acetic acid | (4.0/2.5/3/0.5, v/v/v/v) | 11943.53 ± 160.96 | 1.34 | 2452.47 ± 33.00 | 1.34 |
| | (4.0/2/3.5/0.5, v/v/v/v) (4.5/2/3/0.5, v/v/v/v) | 11911.13 ± 162.16 | 1.36 | 2450.26 ± 18.89 | 0.77 |

addition [43]. The accuracy of an analytical method is the closeness of the results to the true value (100%). In recovery studies in which a known amount of standard was spiked into pre- analysed sample solutions the recovery was 99.15–101.55% for Dapagliflozin (DAPA) and 98.22–101.11% for Metoprolol (METO) (Table 23).

The values demonstrate that the method is accurate. The data shown in Table 23 indicate that the developed method is accurate [44-48]. The % recovery of DAPA and METO was found to be in range of 98.00 – 102%.

## LOD and LOQ

The LOD and LOQ were found for Dapagliflozin (DAPA) and Metoprolol (METO), shown in table respectively indicating high sensitivity of the method (Table 24) [49-51].

## Specificity

The specificity was estimated by comparing marketed formulation to pure API. The chromatogram was taken for Dapagliflozin (DAPA) (600 ng per spot) and Metoprolol (METO) (3000 ng per spot). Developed spot area and Rf value of Dapagliflozin (DAPA) and Metoprolol (METO) was determined and alsoshown peak purity data of both drug in Figure 20.

The low values of RSD (Table 7.7) obtained after introducing small, deliberate changes in the parameters of the developed HPTLC method confirmed its robustness (Table 25) [52-54].

## Analysis of Synthetic Mixture

The synthetic mixture was prepared from Dapagliflozin (DAPA) and Metoprolol (METO) and the excipients starch (140.0 mg), Hydroxy propyle methayl cellulose E5 (30.0 mg), Polly vinayl pyrrolidone (20.0mg) magnesium stearate (2.5 mg) and talc (1.0mg). Analysis of the synthetic mixture by the proposed method gave a recovery of 102.11% (±0.79%) for Dapagliflozin (DAPA) and 102.62% (±0.58%) for Metoprolol (METO). Single bands at Rf 0.26 ± 0.02 and 0.63 ± 0.02 were observed in the chromatograms for Dapagliflozin (DAPA) and Metoprolol (METO), and no interference from the excipients was observed. As the method can be successfully applied for analysisof the synthetic mixture, it could be used to analyse the pharmaceutical formulation (Table 26 and Figure 21) [55-59].

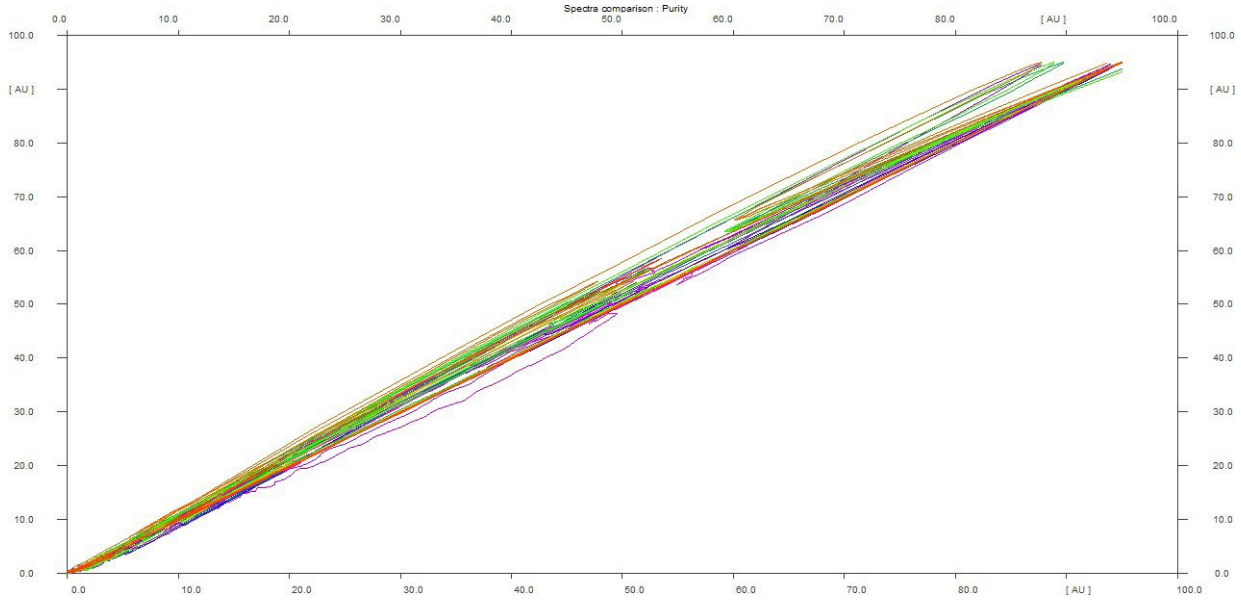


**Figure 20:** Purity spectra of both Dapagliflozin (DAPA) and Metoprolol (METO) Robustness.

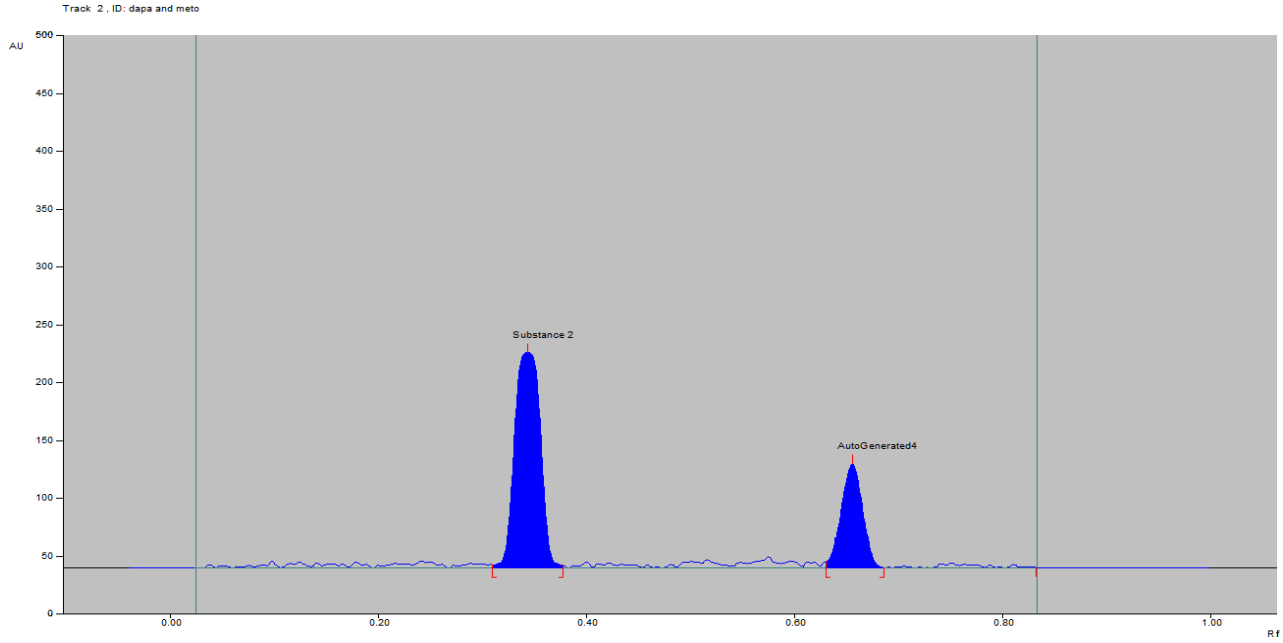


**Figure 21:** Densitogram of standard Dapagliflozin (DAPA) (400 ng per band) and Metoprolol (METO) (2000 ng per band) using optimized mobile phase.

**Table 26:** Data of determination of Dapagliflozin (DAPA) and Metoprolol (METO) in synthetic mixture.

| Drug | Conc. (ng/band) | Amount found (µg/ml) | % Assay Mean ± SD (n=3) |
|---|---|---|---|
| Dapagliflozin (DAPA) | 400 | 406.52 | 102.11 ± 0.79 |
| | | 406.64 | |
| | | 412.15 | |
| Metoprolol (METO) | 2000 | 2039.09 | 102.62 ± 0.58 |
| | | 2059.97 | |
| | | 2058.31 | |

**Table 27:** Summary of validation parameter for of Dapagliflozin (DAPA) and Metoprolol (METO**).**

| Sr. No. | Parameters | Dapagliflozin (DAPA) | Metoprolol (METO) |
|---|---|---|---|
| 1. | Concentration range (ng/band) | 200-1200 | 1000-6000 |
| 2. | Regression Equation | y = 15.809x + 2194.1 | y = 0.7976x + 5.7 |
| 3. | LOD | 26.58 ng/band | 51.81 ng/band |
| 4. | LOQ | 80.57 ng/band | 157.00 ng/band |
| 5. | Accuracy (% Recovery) (n=3) | 99.15 – 101.55 % | 98.22 – 101.11 % |
| 6 | Precision | | |
| | Repeatability (n=6) | 0.66 | 0.83 |
| | Intraday Precision (n=3) | 0.97 – 1.01 | 0.58 – 1.28 |
| | Interday Precision (n=3) | 1.17 – 1.87 | 1.15 – 1.92 |
| 7. | Assay | 102.11 ± 0.79 | 102.62 ± 0.58 |
| 8 | Robustness | Robust | |
| 9 | Specificity | Specific | |

**Table 28:** F Test Comparison of Hypothesis (Statistical method).

| Variable 1 DAPA Variable 2 | |
|---|---|
| F | 5.776666 |
| P(F<=f) one-tail | 0.0589 |
| F Critical one-tail | 6.388233 |
| Variable 1 METO Variable 2 | |
| F | 1.842424 |
| P(F<=f) one-tail | 0.284227 |

## Summary of Validation Parameters

The validation of the developed HPTLC method for determination of Dapagliflozin (DAPA) and Metoprolol (METO), indicates that the method is specific, linear, precise, and accurate. The summary of different validationparameters is shown in Table 27 and Table 28.

### References

1. Arati A, Ajinkya C (2016) "Heart Failure: Diagnosis, Management and Utilization." Journal of Clinical Medicine 5: 1-28.
2. Douglas L (2021) "Mechanisms and Models in Heart Failure: A Translational Approach." Circ Res 128: 1435-1450.
3. Sapna F (2023) "Advancements in Heart Failure Management: A Comprehensive Narrative Review of Emerging Therapies." Cureus 15: 1-13.
4. Paul MH, Andrew SF (2022) "Management of heart failure with reduced ejection fraction." Heart 108: 1571-1579.
5. Bhaumik M (2023) "Analysis of Different Brands of Dapagliflozin (10mg) Tablets Usinghigh Performance Liquid Chromatographic (HPLC) Method." Ijpra 8: 969-972.
6. Paul (1986) "An Updated Review of its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Efficacy, in Hypertension, Ischaemic Heart Disease and Related Cardiovascular Disorders." Drugs 31: 376-429.
7. Ganesh S (2020) "A Review on Advances in UV Spectroscopy." Research J Science and Tech 12: 47-51.
8. Attimared M (2011) "High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery." Pharmaceutical Method 2: 71-75.
9. Stanislava I (2022) "High-Performance Thin-Layer Chromatography (HPTLC) Method for Identification of Meloxicam and Piroxicam." Processes 10: 1-9.
10. Rashmi P (2012) "HPTLC Method Development and Validation: Strategy to Minimize Methodological Failures." Journal of Food and Drug Analysis 20: 794-804.
11. Drug bank DB06292 Dapagliflozin https://go.drugbank.com/drugs/DB06292
12. Drug bank DB00264 Metoprolol https://go.drugbank.com/drugs/DB00264
13. Gajanan VM, Krishna Radheshyam G (2017) "Estimation of Dapagliflozin from its Tablet Formulation by UV-Spectrophotometry." Pharm Methods 8: 102-107.
14. Vidhi SD, Dr. Paresh U (2020) "Development and validation of UV Spectroscopic method for Dapagliflozin in its API and its Tablet Formulation." aegaeum journal 8: 840-846.
15. Priya K, Maram (2015) "Unique UV spectrophotometric method for reckoning of dapagliflozin in bulk and pharmaceutical dosage forms." Journal of Chemical and Pharmaceutical Research 7: 45-49.
16. Bhagwat JB (2010) "Quantitative Estimation of Dapagliflozin in Blood Plasma by Using UV Spectroscopy." Pharmaceutica Analytica Acta 2: 1-3.
17. Bhavyasri K (2019) "Method Development and Validation For The Estimation Of Dapagliflozin In Bulk And Tablet Dosage Formby UV Visible Spectroscopy." International Journal of Recent Scientific Research 1: 34419-34422.
18. Jani BR, Shah K (2015) "Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture." Journal of Bioequivalence Studies 1: 1-8.
19. Sufiyan A, Rageeb MD (2021) "Development and Validation Of UV Spectrophotometric Method For Estimation Of Saxagliptin And Dapagliflozin In Bulk And Dosage Form." International Journal of Pharmaceutical Sciences and Research 12: 2185-2192.
20. Bhavya S, Surekha T (2020) "A Novel Method Development and Validation of Dapagliflozin and Metormin Hydrochloride using Simultaneous Equation Method by UV– Visible Spectroscopy in Bulk and Combined Pharmaceutical Formulation including Forced Degradation Studies." Journal of pharmaceutical science and research 12: 1100-1105.
21. Jani BR, Shah K, Kapupara PP (2015) "Development And Validation Of Uv Spectroscopic Method For Simultaneous Estimation Of Dapagliflozin And Metformin Hydrochloride In Synthetic Mixture." International Journal of Research and Development in Pharmacy and Life Sciences 4: 1569-1576.
22. Minal H (2019) "Ultraviolet-Spectrophotometric Method for Simultaneous Estimation of Dapagliflozin Propanediol and Metformin Hydrochloride." International Research Journal Of Pharmacy 10: 90-94.
23. Anokhi P (2022) "Analytical Method Development And Validation For Simultaneous Estimation Of Dapagliflozin And Teneligliptin Hydrobromide Hydrate From Synthetic Mixture By Three Different Uv Spectrophotometric Methods." World Journal of Pharmaceutical Research 11: 770-783.
24. Mante GV (2018) "RP-HPLC Method for Estimation of Dapagliflozin from its Tablet." International Journal of ChemTech Research 11: 242-248.
25. Shakir BS, Sravanthi P (2017) "Development and Validation of Dapagliflozin by Reversed-Phase High-Performance Liquid Chromatography Method and It's Forced Degradation Studies." Asian J Pharm Clin Res 10: 101-105.
26. Mitali VV (2017) "Development and Stability Indicating Hplc Method for Dapagliflozin in Api And Pharmaceutical Dosage Form." International Journal of Applied Pharmaceutics 9: 33-41.
27. Manasa S (2014) "Development and Validation of stability-Indicating RP-HPLC method for determination of Dapagliflozin." Journal of Advanced Pharmacy Education & Research 4: 350-353.
28. Vankalapati (2022) "Stability-indicating HPLC method development and validation for simultaneous estimation of metformin, dapagliflozin, and

saxagliptin in bulk drug and pharmaceutical dosage form." Biomedical chromatography 36: 7.

29. Suma BV, Deveswaran R (2019) "A New high-performance thin layer chromatographic method development and validation of dapagliflozin in bulk and tablet dosage form." Int J Pharm Pharm Sci 11: 58-63.
30. Abdelrahman (2020) "HPTLC Method for the Determination of Metformin Hydrochloride, Saxagliptin Hydrochloride, and Dapagliflozin in Pharmaceuticals." Current Analytical Chemistry 16: 609-619.
31. Saloni A (2022) "Development and validation of stability-indicating HPTLC method for simultaneous estimation of Metformin, Saxagliptin, and Dapagliflozin in their combined matrix using AQbD." Bepls 12: 32-42.
32. Soudamini A (2023) "Development and validation of hptlc technique for assessment of dapagliflozin and metformin hcl." Eur Chem Bull 12: 7676-7692.
33. Parixit P (2023) "Development and Validation of HPTLC Method for Simultaneous "Quantification of Dapagliflozin and Vildagliptin in Tablet Dosage Form." Jchr 13: 3643-3649
34. Goday, Swapna (2018) "Development and Validation of a LC-ESI-MS/MS Based Bioanalytical Method for Dapagliflozin and Saxagliptin in Human Plasma." Indian Journal of Pharmaceutical Education and Research 52: S277-S286.
35. Navneet V (2011) "UV- Spectrophotometric Determination of Metoprolol Succinate." Research J Pharm and Tech 4: 271-272.
36. Abhinaya K (2023) UV spectrophotometric method development and validation for the determination of metoprolol succinate in bulk and its pharmaceutical dosage form." Annals of Phytomedicine 12: 628-631.
37. Mustafa C (2011) "Spectrophotometric Determination of Metoprolol Tartrate in Pharmaceutical Dosage Forms on Complex Formation with Cu (II)." Pharmaceuticals 4: 964-975
38. Pagar SA (2013) "Development and validation of spectrophotometric method for determination of metoprolol succinate." IJPBS 3: 224-228.
39. Madhuri A (2015) "Development and validation of UV spectrophotometric method for simultaneous estimation of cilnidipine and metoprolol succinate in bulk drugs and combined dosage form." Der Pharmacia Lettre 7: 299-306
40. Bindi N (2012) "Development and validation of the simultaneous UV spectrophotometric method for estimation of metoprolol succinate and olmesartan medoxomil in the tablet dosage form." Pharmaceutical Methods 31: 44-47.
41. Lakavath S (2022) "Sensitive and Reproducible Study for UV-Spectrophotometric Method for Analysis of Clopidogrel and Metoprolol in a Combined Tablet Dosage Form." International Journal of Pharmaceutical Science Invention 11: 1-5.
42. Iram M, Rani S (2015) "Estimation of Metoprolol In Human Plasma By Hplc Method." Ijpps 7: 443-446.
43. Avjit C (2012) "Rp-Hplc method for the estimation of metoprolol succinate in bulk and in dosage forms." Int J Adv Pharm Biol Sci 2: 116-123.
44. Nirma M (2012) "Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in pharmaceutical dosage form." Pharmaceutical Method 3: 84-89.
45. Rawool ND, Venkatchalam A (2011) "Analytical Method for the Simultaneous Estimation of Hydrochlorothiazide and Metoprolol Tartrate using RP HPLC." Ijps 73: 219-223.
46. Brijesh S (2009) "Development of Reverse-Phase HPLC Method for Simultaneous Analysis of Metoprolol Succinate and Hydrochlorothiazide in a Tablet Formulation." Trop J Pharm Res 8: 539-543.
47. Vaijanath G (2007) "Simultaneous determination of metoprolol succinate and amlodipine besylate in pharmaceutical dosage form by HPLC." Jpba 46: 583-586.
48. Palani S, Kamarapu SK (2018) "Method Development And Validation For The Simultaneous Determination Of Metoprolol And Atorvastatin By Reversed-Phase High-Performance Liquid Chromatography In Its Bulk And Pharmaceutical Tablet Dosage Form Using Biorelevant Dissolution Media Fasted State Small Intestinal Fluid." Asian J Pharm Clin Res 11: 1-8.
49. Risk M (2023) "Comparative HPTLC study for simultaneous determination of ivabradine and metoprolol using UV and fluorescence detectors." Bmc Chemistry 17: 1-13
50. Pintu P (2021) "A robust high-performance thin-layer chromatography method for the simultaneous estimation of chlorthalidone and metoprolol succinate using quality risk assessment and design of experiments-based enhanced analytical quality by design approach." JPC 34: 229-242.
51. Jain PS (2012) "Development and Validation of HPTLC Method for Simultaneous Determination of Amlodipine Besylate and Metoprolol Succinate in Bulk and Tablets." Ijps 74: 152-156.
52. Rajendra K (2009) "High-Performance Thin-Layer Chromatographic Method for Simultaneous Analysis of Metoprolol Succinate and Amlodipine Besylate in Pharmaceutical Preparations." JPC 22: 115-119.
53. Kamini S (2019) "Validated HPTLC method for simultaneous estimation of metoprolol succinate and ramipril in bulk drug and marketed formulation." Clin Med Rep 2: 1-4.
54. Li S (2012) "Rapid and Sensitive LC-MS/MS Method for the Determination of Metoprolol in Beagle Dog Plasma with a Simple Protein Precipitation Treatment and Its Pharmacokinetic Applications." Molecules 17: 2663-2674.
55. Suresh B, Jayachandra, Devendra P (2016) Process for The Preparation of Dapagliflozin, USP US 2016/0214953 A1.
56. Vasireddi UMR (2018) Novel processes for preparation of dapagliflozin or its solvates or co - crystals thereof, WO2018/029611A1.
57. Janakraj KM, Jit C, Bimal K, Rajendra K, Prasant G (2005) Metoprolol manufacturing process, US 2005/0107635 A1.
58. Carmen AA, Jordi B (2013) synthesis and preparations of metoprololand its salts, US 2009/0247642 A1.
59. Ali RI, Sanayi VAS (2013) Tablet formulation comprising dapagliflozin and extended-release metformin, WO2013/137839 A1.