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# Development and Validation of Rp-Hplc Method For Simultaneous Estimation of Dapagliflozin and Metoprolol In Synthetic Mixtures: Stability Indicating Study

**Niranjan Babu Mudduluru[*1], Saiprudhvi Nimmagadda[2], Grecika S[3]**

[1]Department of Pharmacognosy, Seven Hills College of Pharmacy, Tirupati, A.P., India
[2,3]Department of Pharmaceutical Analysis, Seven Hills College of Pharmacy, Tirupati, A.P., India

**Corresponding Author**
**Dr. M. Niranjan Babu**
Professor, Department of Pharmacognosy, Seven Hills College of Pharmacy, Tirupati, A.P., India – 517561, Contact: 7702484513, Email: principal.cq@jntua.ac.in

## ABSTRACT

Background: The invention of a composition combining beta-blockers and an SGLT2 inhibitor aims to treat heart failure with reduced ejection fraction (HfrEF), whether accompanied by type-2 diabetes or not. Objective: This study aimed to develop a straightforward, precise, accurate, and reproducible stability assay method for quantifying dapagliflozin and metoprolol in a synthetic mixture using RP-HPLC. Method: Separation was achieved using a Waters HPLC system with an Ultrasphere C18 column (250×4.6 mm, 5 µm), employing a mobile phase of Methanol : ACN : Phosphate Buffer pH 3.0 (60:10:30) adjusted with orthophosphoric acid. The flow rate was set at 0.8 ml/min, and detection was at 223 nm. Result: The developed method was successfully applied for quantification and validation of the drugs according to ICH guidelines. The retention times for dapagliflozin and metoprolol were 7.012 and 3.392 minutes, respectively. The linearity ranges were 1-50 µg/mL for dapagliflozin and 5-250 µg/mL for metoprolol. Limits of detection (LOD) and quantification (LOQ) were found to be 0.15 and 0.46 µg/mL for dapagliflozin, and 0.13 and 0.40 µg/mL for metoprolol. The method exhibited % recoveries within acceptable limits across 50%, 100%, and 150% of the working concentration levels. Stability studies subjected the drug to conditions including acid, base, oxidation, thermal, and photolytic stress, per ICH guidelines. Conclusion: The results demonstrate that the developed analytical method is suitable for its intended purpose, meeting the criteria defined in ICH Q2R2 guidelines.

**KEYWORDS:** Dapagliflozin, Metoprolol, RPHPLC, Stability, Validation

## INTRODUCTION

The combination of Dapagliflozin and Metoprolol is formulated as a fixed dosage form containing SGLT2 inhibitors and beta-blockers, along with one or more pharmaceutically acceptable excipients. This composition is intended for the prevention or treatment of heart failure with reduced ejection fraction (HfrEF), regardless of whether the patient also has type-2 diabetes, angina, myocardial infarction, arteriosclerosis, diabetic nephropathy, diabetic cardiac myopathy, renal insufficiency, peripheral vascular disease, left ventricular hypertrophy, cognitive dysfunction, or chronic heart failure [1].

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This combination is currently under Clinical Trial Phase III and was approved by CDSCO on 9th February 2023. Dapagliflozin (chemical formula $C_{24}H_{25}ClO_5$) belongs to the class of oral hypoglycemic agents known as Sodium Glucose Co-Transporter 2 (SGLT2) inhibitors. It functions by reducing the amount of sugar present in the blood, making it effective in managing type 2 diabetes mellitus. Dapagliflozin has also been associated with lowering blood pressure in recent studies [2].

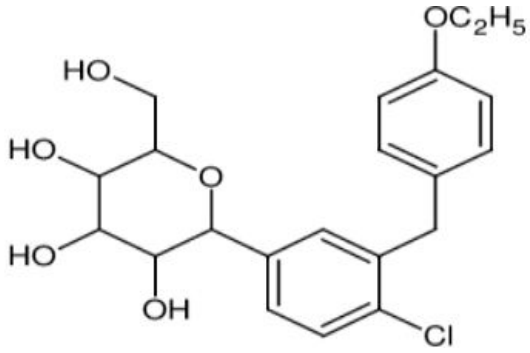


**Fig.1 Chemical structure of Dapagliflozin**

Metoprolol (chemical formula $C_{15}H_{25}NO_3$) is an anti-hypertensive drug that aims to prevent high blood pressure in the body. It is also used to reduce the risk of heart failure, stroke, myocardial infarction, and kidney failure. Hypertension occurs when there is persistent high pressure exerted by the blood against the walls of blood vessels, which exceeds normal levels. This condition can persist for many years or throughout a person's lifetime [3].

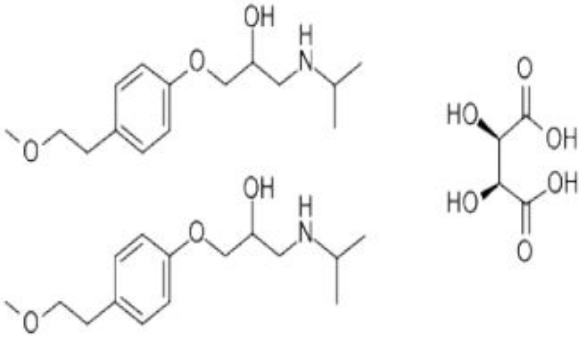


**Fig.2 Chemical structure of Metoprolol**

Forced degradation experiments are essential in pharmaceutical development to refine analytical methodologies, enhance the stability of active pharmaceutical ingredients (APIs) and drug products, and elucidate degradation pathways and by-products. Currently, there is a lack of literature detailing the degradation profiles of Dapagliflozin and Metoprolol according to ICH guidelines using analytical techniques[4]. This study employs high-performance liquid chromatography (RP-HPLC) to analyze Dapagliflozin and Metoprolol in pharmaceutical formulations. The paper presents a validated method that is accurate, specific, reproducible, and capable of detecting degradation products, as per the International Conference on Harmonization (ICH) guidelines.

## MATERIALS AND METHODS

Chemicals and Reagents All chemicals and solvents utilized were of analytical grade (RANKEM, INDIA). Solvents and solutions underwent filtration through a 0.45 µm pore size membrane filter and were degassed using sonication before application[5].

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Instrumentation The analysis was conducted using a Waters Alliance HPLC system equipped with a PDA detector. Signal outputs were monitored and processed using LC Solution software. The analytical column employed was Ultrasphere C18 (4.6 mm × 250 mm, 5 µm), and sample introduction was performed via an injection valve with a 20 µL sample loop.

Preparation of Solutions Preparation of Buffer Solution 2.04 gm of Potassium dihydrogen phosphate was accurately weighed and transferred into 1000 ml of water. The solution was thoroughly mixed, filtered, and adjusted to pH 3.0 using 1% Orthophosphoric acid. (Preparation of 1% Orthophosphoric acid: 1.66 ml of Orthophosphoric acid was diluted with 25 ml of water.)

Preparation of Mobile Phase The mobile phase was prepared as Methanol: ACN: Phosphate Buffer pH 3.0 (60:10:30% v/v/v).

Preparation of Standard Solutions Preparation of Standard Stock Solution of Dapagliflozin and Metoprolol: Dapagliflozin (10 mg) was accurately weighed and transferred into a 100 ml volumetric flask, sonicated to dissolve, and diluted to volume with diluent (resulting in Dapagliflozin 100 µg/ml). Metoprolol (50 mg) was accurately weighed and transferred into a 100 ml volumetric flask, diluted to volume with diluent (resulting in Metoprolol 500 µg/ml)[8].

Preparation of Working Standard of Dapagliflozin and Metoprolol: From the above stock solutions, 1 ml of Dapagliflozin stock solution and 1 ml of Metoprolol stock solution were transferred into separate 10 ml volumetric flasks and diluted to volume with diluent, resulting in solutions of Dapagliflozin 10 µg/ml and Metoprolol 50 µg/ml [6].

Preparation of Sample Solution of Dapagliflozin and Metoprolol: Preparation of Sample Stock Solution of Dapagliflozin and Metoprolol: (Label claim: Dapagliflozin-10 mg; Metoprolol-50 mg) A synthetic mixture equivalent to 10 mg of Dapagliflozin and 50 mg of Metoprolol was added to a 100 ml volumetric flask and diluted to volume with diluent [7].

**Table 1 Synthetic Mixture**

| **Ingredient** | **mg/tablet** |
|---|---|
| Dapagliflozin | 10 |
| Calcium hydrogen Phosphate anhydrrous | 76.3 |
| Lactose monohydrate | 97.84 |
| Low substituted hydroxy-propyl cellulose | 4.06 |
| Hydroxy propyl cellulose | 1.21 |
| Low substituted hydroxy-propyl cellulose | 1.626 |
| Sodium stearyl fumarate | 2.439 |
| Iron oxide red | 0.813 |

| **Ingredient** | **mg/tablet** |
|---|---|
| Metoprolol | 50 |
| Microcrystalline cellulose | 38.421 |
| Methyl cellulose | 1.8421 |
| Polyvinyl pyrolidine | 0.789 |

**Base Granule**

| | |
|---|---|
| MCC | 82.64 |
| Croscarmellose sodium | 5.43 |
| Polyvinylpyrrolidone | 7.242 |

**Lubrication**

| | |
|---|---|
| MCC | 27.03 |
| Purified talc | 2.84 |
| Silicone dioxide | 0.921 |
| Magnesium stearate | 0.710 |

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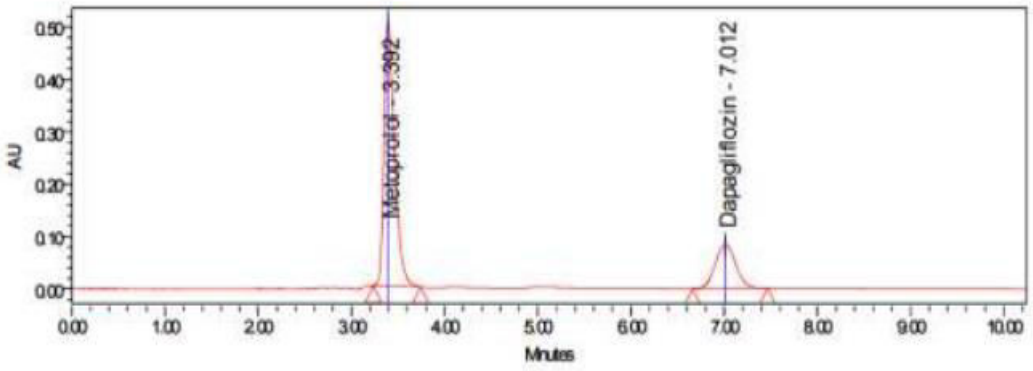
## RESULTS AND DISCUSSION

The developed method for simultaneous estimation of Dapagliflozin and Metoprolol proved to be accurate, precise, effective, and specific for their analysis. The use of Methanol: ACN: Phosphate buffer pH 3.0 in the ratio of (60:10:30% v/v/v) at a flow rate of 0.8 ml/min demonstrated satisfactory results with improved reproducibility and repeatability. UV detection at a wavelength of 223 nm enabled quantification, with retention times of 7.012 minutes for Dapagliflozin and 3.392 minutes for Metoprolol, respectively. The optimized method was validated according to ICH guidelines. The chromatogram depicting Dapagliflozin and Metoprolol is shown in Figure 1[9].




Peak Results

| | Name | RT | Area | Height | USP Tailing | USP Plate Count | Symmetry Factor | Purity1 Threshold | Purity1 Angle | Resolution |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Metoprolol | 3.392 | 391246 | 50999 | 1.55 | 4535 | 1.55 | 0.200 | 0.196 | |
| 2 | Dapagliflozin | 7.012 | 1277526 | 83666 | 1.07 | 4808 | 1.07 | 0.312 | 0.256 | 12.03 |

Fig. 1Chromatogram of Standard Metoprolol (50µg/ml) and Dapagliflozin(10µg/ml)

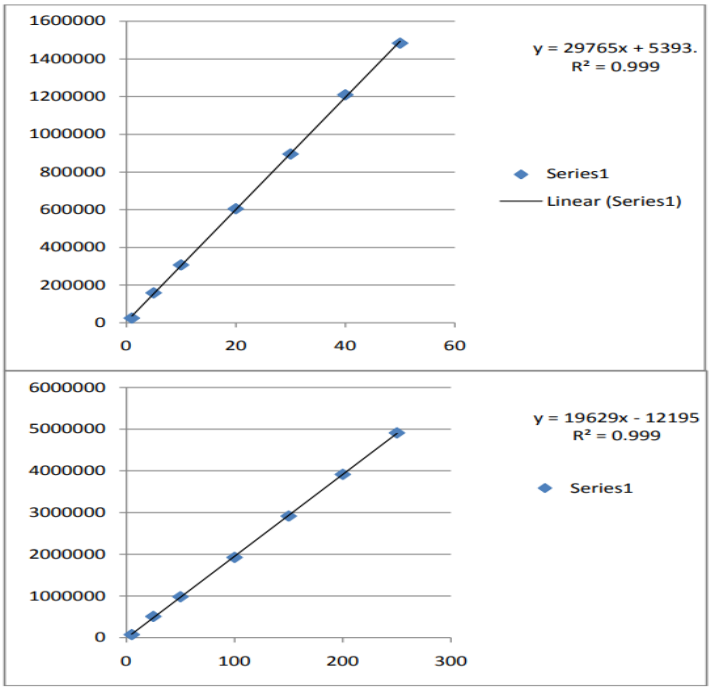



Fig. 2 and Fig. 3Calibration Curve of Dapagliflozin(1-50µg/ml)&Metoprolol (5-250µg/ml)

Table 2 Summary of Forced Degradation

| Sr. No. | Types of Degradation | Condition | Duration | Solution | Area | %Degradation |
|---|---|---|---|---|---|---|
| 1 | Acid Degradation | 0.1 N HCl | 1 Hour | Metoprolol | 947657 | 16.61 |
| | | | | Dapagliflozin | 293749 | 25.42 |
| 2 | Base Degradation | 0.1 N NaOH | 1 Hour | Metoprolol | 957093 | 15.78 |
| | | | | Dapagliflozin | 301472 | 23.46 |
| 3 | Oxidative Degradation | 1% $H_2O_2$ | 1 Hour | Metoprolol | 1006029 | 11.48 |
| | | | | Dapagliflozin | 331442 | 15.85 |
| 4 | Photo Degradation | UV chamber | 24 Hours | Metoprolol | 994476 | 12.49 |
| | | | | Dapagliflozin | 303973 | 22.86 |
| 5 | Thermal Degradation | 60°C | 2 Hours | Metoprolol | 963652 | 15.20 |
| | | | | Dapagliflozin | 296774 | 24.66 |

## CONCLUSION

Based on the above observations, it can be concluded that the developed stability-indicating method for the validation of Dapagliflozin and Metoprolol in synthetic mixture by RP-HPLC is specific, linear, accurate, precise, and robust. Therefore, the RP-HPLC method developed can be effectively applied for routine analysis.

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