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# DEVELOPMENT AND VALIDATION OF A FORCED DEGRADATION UPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF EPLERENONE AND TORSEMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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**ABSTRACT**

**Aim:** To develop and validate a rapid, sensitive, and stability-indicating Ultra Performance Liquid Chromatographic (UPLC) method for simultaneous estimation of Torsemide (TOR) and Eplerenone (EPL) in combined pharmaceutical dosage forms.

**Methods:** Chromatographic separation was performed on a Waters ACQUITY UPLC system with a C-18 BEH column (1.7 µm, 2.1 x 50 mm) using an isocratic mobile phase of Phosphate Buffer (pH 4.0) and Acetonitrile in a ratio of 40:60 (v/v) at a flow rate of 0.8 ml/min. Detection was performed at 230 nm. The method was validated in accordance with the recommendations outlined in ICH Q2(R1) for specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantification (LOQ), and forced degradation studies.

**Results:** The retention times of Torsemide and Eplerenone were 1.267 and 2.133 minutes, respectively, with a total run time of 4 minutes. The developed method showed remarkable linearity for the range 2-16 µg/ml for Torsemide ($r^2$ = 0.9999) and 5-40 µg/ml for Eplerenone ($r^2$ = 0.9998). Recovery percentages validated the accuracy, with rates ranging from 99.30% to 99.84%. The method validation parameters, such as precision (%RSD < 0.21) and robustness, also established that the method is reliable. Under basic and oxidative forced degradation conditions, 25.2% (for TOR) and 57.7% (for EPL) and 22.5% (for TOR) and 83.6% (for EPL) degradations were observed, respectively. At the same time, the two drugs remained stable under acidic, photolytic, and thermal stress.

**Conclusion:** The UPLC method developed was found to be accurate, precise, robust, and stability-indicating. The technique can be employed for routine QC and stability studies of these drugs, in bulk and combined dosage forms.

***Keywords:*** *UPLC, Torsemide, Eplerenone, Method Validation, Forced Degradation, Stability-Indicating Assay.*



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## 1. INTRODUCTION

Analytical method development and validation is one of the key steps in the pharmaceutical industry to ensure the identity, purity, potency, and quality of drug substances and products [1]. Modern analytical chemistry practice is all moving towards higher throughput, higher resolution and lower analysis time and cost [2]. Ultra-Performance liquid chromatography (UPLC) is an improvement of traditional High-Performance liquid chromatography (HPLC). UPLC has several advantages over conventional HPLC methods, such as greater chromatographic resolution, higher sensitivity, and significantly reduced analysis times and solvent consumption- due to the use of smaller particles (<2 µm) packed into columns and higher operating pressures [3, 4]. Torsemide (TOR) is a high-effect loop diuretic that is used for the treatment of edema due to congestive heart failure, renal disease, and hepatic cirrhosis [5]. Eplerenone (EPL) is a selective aldosterone receptor antagonist that is used for the treatment of hypertension and post-myocardial infarction congestive heart failure [6]. These two drugs work together synergistically to treat cardiovascular disease by targeting

fluid overload as well as the harmful effects of aldosterone. A robust analytical method is essential to ensure the quality and stability of such combination therapy.Forced degradation (stress testing) studies are a part of method development, in accordance with ICH guidelines Q1A(R2) and Q2(R1) [7, 8]. Stability studies enable demonstrating the analytical method's specificity and its ability to provide information on the degradation pathways of the drug substance, which is of paramount importance when formulating and designing shelf life [9].The literature survey demonstrates that many HPLC and UV methods have been developed for the individual estimation of TOR and EPL, and only a few methods describing simultaneous estimation of TOR and EPL have been reported [10-12]. Nevertheless, to the best of our knowledge, no stability-indicating, validated UPLC method has been reported for the simultaneous determination of TOR and EPL, along with an extensive forced degradation study. The purpose of the present study is to develop a rapid, robust, and stability-indicating UPLC method, validated in accordance with ICH guidelines, for the simultaneous determination of torsemide and Eplerenone in bulk and pharmaceutical dosage forms.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Reagents

Torsemide (99.8%) and Eplerenone (99.9%) reference standards were purchased from Medopharm and Apex Laboratories (Chennai, India). The commercial tablet preparation (Eptus T, labelled claim: 10 mg Torsemide and 25 mg Eplerenone) was obtained from the local market. Acetonitrile and Water (UPLC-grade) from MilliporeSigma were used. Analytical grade Ortho-Phosphoric Acid and Disodium Hydrogen Phosphate were procured from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, Missouri, USA).

### 2.2. Instrumentation and Chromatographic Conditions

Analysis was conducted on a Waters ACQUITY UPLC system consisting of a quaternary solvent manager, a sample manager, and a photodiode array (PDA) detector. For EMPOWER4, the output signal was converted and monitored using EMPOWER software. In the improved method, separation was performed on a Waters ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 × 50 mm). Isocratic mobile phase comprised of Phosphate Buffer (pH 4.0, adjusted with Ortho-Phosphoric Acid), Acetonitrile (40:60, v/v). The flow rate was 0.8 ml/min, the column was held at ambient conditions, the detection wavelength was 230 nm, and the injected volume was 2 µl.

### 2.3. Preparation of standard solutions & sample solutions.

Preparation of standard stock solution: 10 mg of TOR and 25 mg of EPL were weighed into a 50 ml volumetric flask. Around 20 mL of mobile phase was added, and the mixture was sonicated for 5 minutes to ensure complete dissolution. The solution was completed to the line with mobile phase to achieve final concentrations of TOR 200 µg/mL and EPL 500 µg/mL.

**Typical approach:** Twenty tablets were pulverized. An amount of the powder corresponding to 10 mg TOR and 25 mg EPL was weighed into a 100 ml volumetric flask. A mobile phase of ~50 ml was added, followed by sonication for 20 min. The volume was completed to the mark with mobile phase and filtered through a 0.45 µm membrane filter.

### 2.4. Method Validation

The developed UPLC method was validated in accordance with ICH Q2(R1) guidelines [8] for the following parameters.

**2.4.1. Specificity-** Specificity was assessed by comparing chromatograms of standard solution, sample solution, and placebo (if available) separately to look for interferences at the retention times of TOR and EPL.

**2.4.2. Linearity and Range:** Accurate weights of the standard stock solution were diluted with the mobile phase to have δ-values between 2 and 16 µg/ml for TOR and 5 and 40 µg/ml for EPL. Peak area vs Concentration data were treated by means of linear least-squares regression analysis.

**2.4.3. Intra-day precision** (or repeatability) was determined by performing six independent extractions at 100% the test concentration in one day. Inter-day precision (intermediate precision) was evaluated by a second analyst on another day.

**2.4.4. Accuracy (Recovery):** Accuracy was determined by the standard addition method at three concentration levels (50%, 100%, and 150% of the target concentration) in triplicate. The percentage recovery and %RSD were calculated.

**2.4.5. Robustness:** The robustness was investigated by deliberately changing the chromatographic conditions, such as flow rate (±0.2 ml/min) and detection wavelength (±2 nm). It was calculated as the %RSD of peak areas.

**2.4.6. LOD and LOQ:** The limit of detection (LOD) was calculated according to the signal-to-noise ratio (S/N) of 3:1, and the limit of quantification (LOQ) was calculated according to the S/N of 10:1.

### 2.5. Forced Degradation Studies

To determine themethod's stability-indicating capability, forced degradation studies were performed on the drug standard solution. Stress and the manner it was applied are shown below:

- **Acidic Degradation:** Exposed to 2N HCl at 75°C for half an hour.
- **Alkali Degradation:** Exposed to 2N NaOH at 75°C for 30 minutes.
- **Oxidative Degradation:** 20% $H_2O_2$ and 30 min at 75°C
- **Photolytic degradation:** Solid state exposed to UV light (1.2 million Lux hour).
- **Thermo-Gravimetric Degradation:** Solid samples were heated at 100 °C in an oven for 12 hours.

Immediately after stress, samples were diluted with the mobile phase to the desired concentration and subjected to the developed UPLC method.

## 3. RESULTS AND DISCUSSION

### 3.1. Method Development and Optimization

The main aim of the current work is to develop a rapid, specific, and simultaneous method for estimating TOR and EPL using UPLC. The isobestic point at 230 nm was selected for the validation set of compounds. Different mobile-phase compositions were screened, and Phosphate Buffer (pH 4.0): Acetonitrile 40:60 (v/v) was found to be the most suitable based on resolution, peak shape, and run time (4 minutes). Retention times for TOR and EPL were 1.267 min and 2.133 min, respectively (Fig. 01).

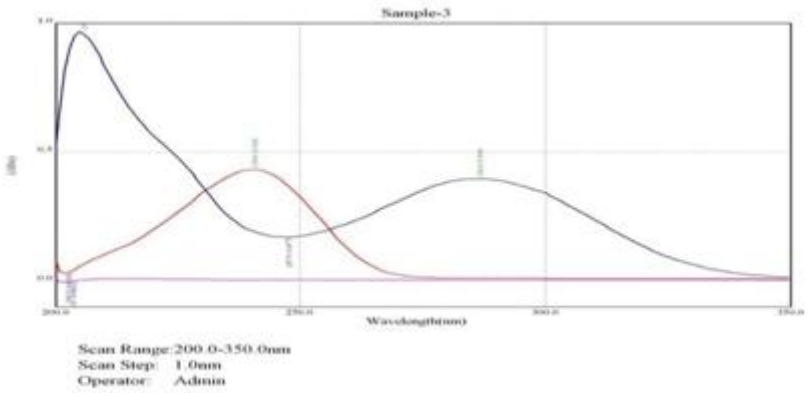



**Figure 01: Isosbestic Point of Torsemide and Eplerenone at 230 nm**

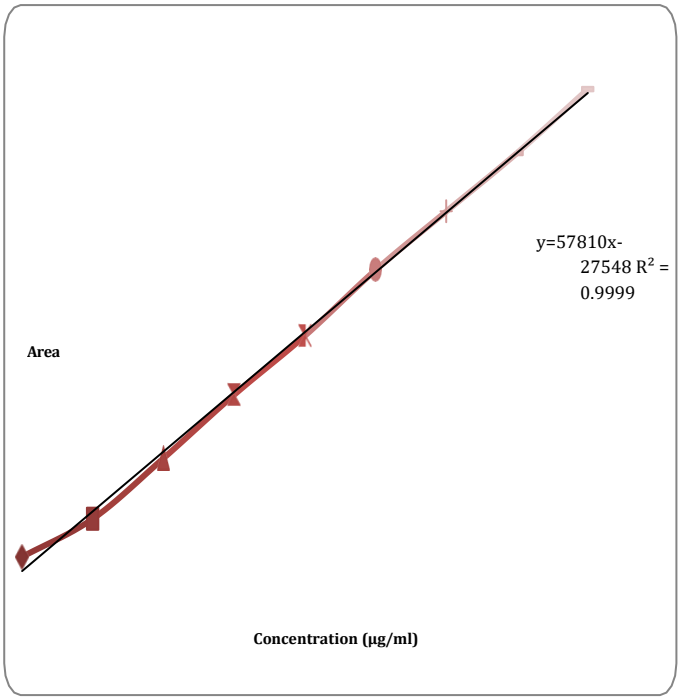
### 3.2. Method Validation

**3.2.1. Specificity:** The peaks of TOR and EPL on the chromatograms of standard and sample solutions were well separated without interference from the excipients or degradation products, which proved that the specificity of the method was achieved.

**3.2.2. Linearity:**The method was linear in terms of TOR and EPL concentration range between 2-16 µg/ml and 5-40 µg/ml, respectively. The regression equations were:

- TOR: 57810x - 27548 (r2 = 0.9999)
- EPL: y = 61219x - 309244 ($r^2$ = 0.9998)

The excellent linear relationship is indicated by the high correlation coefficients (Figures 2 and 3).



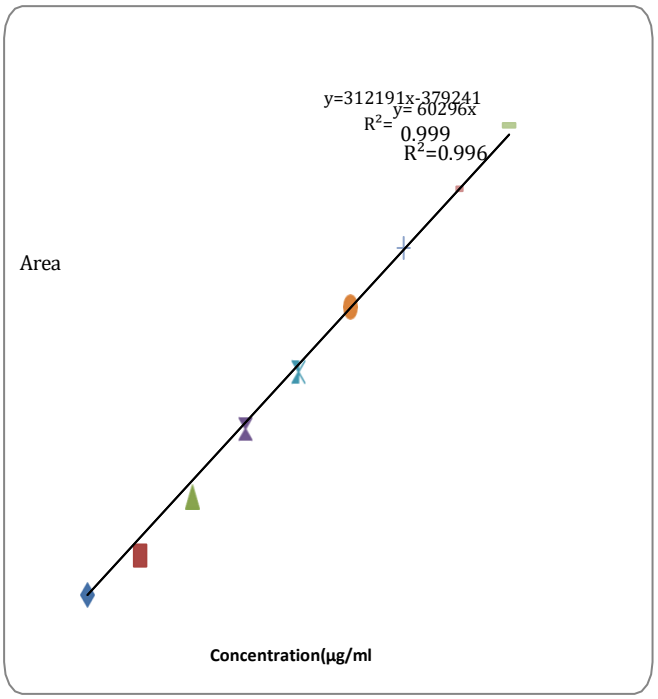




**Figure 02: Linearity of Torsemide** **Figure 03: Linearity of Eplerenone**

**3.2.3. Precision:** The results of the precision study are summarized in Table 1. The %RSD for both intra-day and inter-day precision was less than 0.21%, demonstrating the high precision of the method.

**Table 01. Results of Precision Studies**

| Drug | Intra-day Precision (%RSD, n=6) | Inter-day Precision (%RSD, n=6) |
|---|---|---|
| Torsemide | 0.15 | 0.19 |
| Eplerenone | 0.15 | 0.21 |

**3.2.4. Accuracy:** The recovery of TOR and EPL at 50%, 100%, and 150% levels ranged from 99.30% to 99.84%, with %RSD values less than 1.0%, confirming the high accuracy of the method (Table 02).

**Table 02. Results of Accuracy (Recovery) Studies**

| Spike Level | Torsemide (% Recovery) | Eplerenone (% Recovery) |
|---|---|---|
| 50% | 99.32 | 99.30 |
| 100% | 99.46 | 99.46 |
| 150% | 99.49 | 99.84 |

**3.2.5. Stability:** The robustness of the method was established by making deliberate changes to the flow rate (±0.2 ml/min) and wavelength (±2 nm) and comparing the system suitability parameters and assay results (%RSD <1.0).

**3.2.6. LOD and LOQ:** The limits of detection (LOD) and quantification (LOQ) were found to be 3.24 and 3.63 (S/N ratio) for TOR and EPL, respectively. Indeed, LOQ values were 11.66 and 10.74 (S/N ratio), respectively (high method sensitivity).

**3.3. Forced Degradation Studies**

The overview of the forced degradation results is presented in Table 03. The drugs were relatively stable under acid, thermal, and photolytic stress, whereas the degradation was substantial under both base and oxidative conditions. Chromatograms obtained from the degraded samples showed clear separation of the parent drugs and their degradation products, further confirming that the method was stability-indicating (Figures 04, 05, and 06).

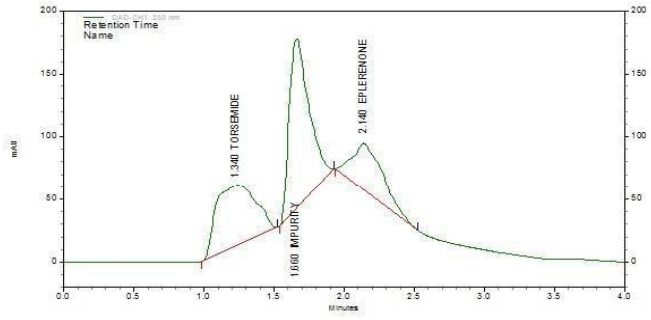



Figure 04: Base degradation chromatogram of Torsemide and Eplerenone

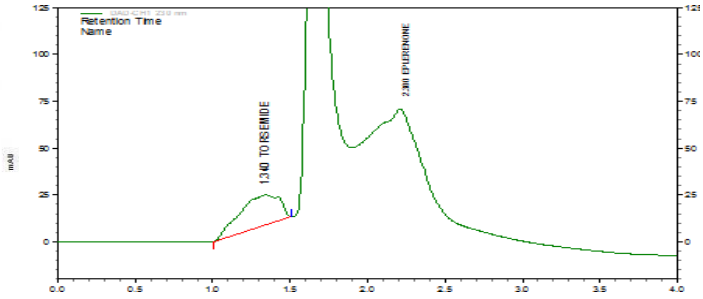



Figure 05: Oxidative degradation chromatogram of Torsemide and Eplerenone

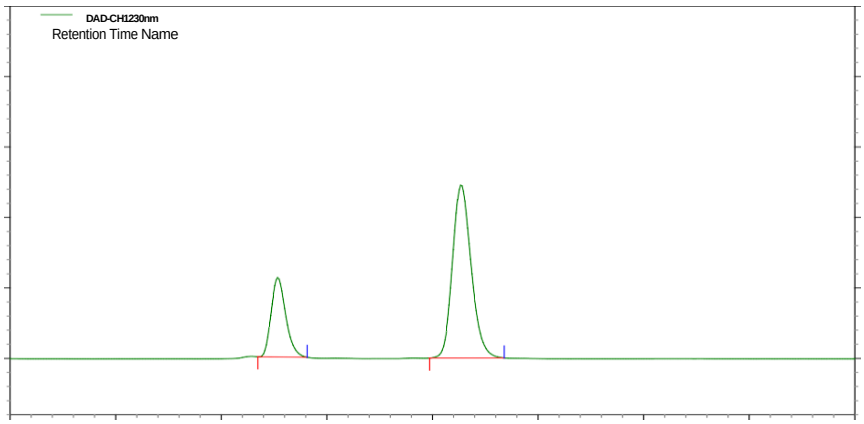



Figure 06: Photolytic Degradation Chromatogram of Torsemide and Eplerenone

**Table 03. Summary of Forced Degradation Studies**

| Stress Condition | % Drug Remaining (TOR) | % Degradation (TOR) | % Drug Remaining (EPL) | % Degradation (EPL) |
|---|---|---|---|---|
| Acid (2N HCl) | 97.5 | 1.8 | 94.2 | 5.6 |
| Base (2N NaOH) | 74.1 | 25.2 | 42.1 | 57.7 |
| Oxidation (20% $H_2O_2$) | 76.8 | 22.5 | 16.2 | 83.6 |

| Stress Condition | % Drug Remaining (TOR) | % Degradation (TOR) | % Drug Remaining (EPL) | % Degradation (EPL) |
|---|---|---|---|---|
| Photolysis | 98.2 | 1.1 | 94.7 | 5.1 |
| Thermal | 96.7 | 2.5 | 95.2 | 4.6 |

## 4. CONCLUSION

The developed UPLC method for the simultaneous determination of Torsemide (TOR) and Eplerenone (EPL) has proven to be a reliable and efficient analytical tool. The technique demonstrated excellent accuracy, precision, and robustness, meeting the ICH validation guidelines. The retention times for TOR and EPL were well-defined, and the method exhibited strong linearity within the specified concentration ranges. Additionally, the forced degradation studies revealed that both drugs are stable under acidic, photolytic, and thermal stress conditions, but are susceptible to degradation under basic and oxidative conditions, underscoring the importance of stability-indicating methodologies in pharmaceutical analysis. This UPLC method not only facilitates routine quality control but also supports stability studies, ensuring the integrity of these drugs in bulk and pharmaceutical dosage forms. Overall, the findings underscore the method's potential utility in the pharmaceutical industry, particularly for combination therapies targeting cardiovascular diseases.

## 5. ACKNOWLEDGMENTS

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## 6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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