INTERNATIONAL CONFERENCE ON HARMONIZATION RECOMMENDED FORCED DEGRADATION STUDIES AND DEVELOPMENT OF A NEW VALIDATED ISOCRATIC REVERSE-PHASE ULTRA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN AND AMLODIPINE IN BULK DRUG AND MARKETED FORMULATION

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INTRODUCTION

Telmisartan (TEL), chemically described as 2-(4-[(4-methyl-6-[(1-methyl-1H-1,3-benzoimidazol-2-yl)-2-propyl-1H-1,3-benzoimidazole-1-yl] methyl] phenyl) benzoic acid, molecular formula C_{30}H_{33}N_3O_5 and molecular weight 514.62. Amlodipine (AMD) is chemically described as 6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, molecular formula C_{20}H_{20} GN_3O_8 and molecular weight 408.88 [1-3] Figs. 1 and 2.

TEL is an angiotensin II receptor antagonist which helps to lower arterial hypertension by inhibiting the angiotensin-converting enzyme that converts angiotensin I into its active form angiotensin II causes vasoconstriction. AMD in contrast is a dihydropyridine-class calcium channel blocker. AMD acts by blocking transmembrane calcium influx through the calcium channel, resulting in the relaxation of the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure. Combining the angiotensin II receptor antagonist TEL with the calcium channel blocker AMD has the added benefit of reducing cardiovascular mortality and morbidity over other dual therapies while providing equivalent blood pressure control. Each antihypertensive drug has been combined with multiple other antihypertensive medications into a single pill, but this combination is unique, due to the complementary mechanisms of its components appear to enhance the effectiveness beyond that provided by each drug alone [4-8].

To establish inherent stability characteristics of a drug, International Conference on Harmonization (ICH) stability testing guideline Q1A (R2) [9] suggests that stress studies should be carried out, leading to the identification of likely degradation products. For stability samples, it also requires that analytical test procedures should be stability indicating and they should be fully validated. The literature survey revealed that several analytical methods have been reported for the quantitative estimation of TEL alone and in combination with other drugs. Several reverse-phase high-performance liquid chromatography (RP-HPLC), high-performance thin-layer chromatography methods [10-19], ultraviolet (UV) spectroscopy method [20-23] were reported for estimation of TEL and AMD, but very few research papers have reported their degradation profile [24-27]. However, in the reported methods we found some drawbacks which are listed below.

- In one research paper forced degradation studies mentioned in the title, but in the entire research paper, no forced degradation methods and chromatograms were mentioned.
- The buffer solution pH was adjusted to 3.6 mentioned in the research paper. The strong acidic condition may cause larger retention time (RT) for TEL as it remained fully undissociated, which results in strong hydrophobic attraction with silica bed.

Hence, it is thought of interest to develop new sensitive and accurate stability indicating reverse-phase ultra high-performance liquid chromatography (RP UHPLC) methods for effective quantitative estimation of TEL and AMD in the bulk and tablet dosage form. First pKa values of both drugs were investigated and pKa of TEL and AMD was found to be 4.45 and 9.45, respectively. As per thumb rule, the mobile phase pH is selected 2 units above or below the pKa value of the drug.

Results:

Major degradation of TEL and AMD was observed under acidic, alkaline, hydrolytic and oxidation conditions. The described method was validated as per the ICH guideline and validation parameters such as system suitability, linearity, accuracy, precision, specificity, and robustness results were within acceptable limits.

Conclusion:

The method was found to be simple and reproducible for quantitative estimation and stability study of title drugs in pharmaceutical preparations.

Keywords: Telmisartan, Amlodipine, Reverse-Phase ultra-high-performance liquid chromatography, Forced degradation, Method validation.

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If we consider pKa of AMD, then we cannot choose the pH above 9.45, which cause hydrolysis of silica column bed. Therefore, the choice of pH is two units below the pKa of AMD. Again, with respect to TEL, we could choose the pH of mobile phase two units below of its pKa (4.45), but at strong acidic pH, TEL remains fully unionized, which results in strong hydrophobic attraction with silica bed that causes a longer RT of this drug. Therefore, we tried with a buffer like a phosphate buffer having the pH of around 7.0 with acetonitrile, which will be about two units far from the pKa of both drugs, and at this pH, both drugs will remain ionized, which makes the RT much shorter in short column length. Thus, we tried with different buffers having a pH between 4.5 and 6 with different ratios of acetonitrile in isocratic condition, and finally, ammonium acetate buffer pH of 4.5 with acetonitrile in the ratio (55:45 v/v) was chosen so as to get sufficient resolution between the peaks.

**MATERIALS AND METHODS**

**Chemicals and reagents**

TEL and AMD standard drugs were obtained as a gift sample from Micro Laboratories Limited, Hosur, India. The solvent methanol, acetonitrile, water and chemicals triethylamine, acetic acid and orthophosphoric acid used were of HPLC grade (Spectrochem, India). Sodium dihydrogen orthophosphate dehydrates, ammonium acetate (Merck India) sodium hydroxide, hydrochloric acid, hydrogen peroxide, used to be of AR grade (Fisher Scientific, India). The solvents and buffers for UHPLC used were filtered through Millipore nylon membrane filter (0.45 µm) and sonicated before use. The sample solutions for UHPLC were filtered through a 0.45 µm syringe filter before injections.

**Instrumentation**

Agilent 1260 UHPLC System consists of 1260 quaternary pump, standard autosampler, Poroshell 120EC-C<sub>18</sub> column (4.6 × 50 mm, 2.7 µm), PDA detector with Chemstation Software, Shimadzu AUX220 Weighing Balance, Elico India LI 127 pH meter and Grant Sub-aqua 12 Water bath, Shimadzu UV 1800 spectrophotometer, and ultrasonicator were used in the analysis.

**Preparation of standard stock solutions**

**Preparation of diluents**

Diluents used for the standards and sample solution preparations were as follows:

- Diluent A composed of methanol and acetonitrile in the ratio of 50:50 (v/v).
- Diluent B composed of acetonitrile and water in the ratio of 40:60 (v/v).
- Standard stock solutions were prepared by dissolving the drug in diluent A.

**Preparation of stock solution of telmisartan**

An accurately weighed quantity of 25 mg of standard TEL was transferred into a 25 ml volumetric flask. Dissolved and diluted to 25 ml with diluents A to obtain the concentration of 1000 µg/ml.

**Preparation of stock solution of amlodipine**

An accurately weighed quantity of 25 mg of standard AMD was transferred into a 25 ml volumetric flask. Dissolved and diluted to 25 ml with diluent A to obtain the concentration of 1000 µg/ml.

**Preparation of mixed standard solution**

A binary mixture standard solution was prepared by pipetting out 4 ml of TEL and 0.5 ml of AMD from stock solution (1000 µg/ml), transferred into a 10 ml volumetric flask and the volume was made up to 10 ml using diluent B. This solution contained 400 µg/ml of TEL and 50 µg/ml of AMD.

**Preparation of calibration curve standard solutions**

A series of five different concentrations of calibration curve binary mixture standard solutions of TEL and AMD were prepared from the stock solution which is in the range from 380 to 420 µg/ml for TEL and 30 to 70 µg/ml for AMD.

**Preparation of sample solution**

Twenty tablets of the commercial sample (Newtel AM, 40 mg and 5 mg) were weighed accurately and crushed to a fine powder. The tablet, powder equivalent 40 mg of TEL and 5 mg of AMD was weighed and transferred into a 100 volumetric flask. To this flask, 50 ml of diluents A was added, and the solution was sonicated for 30 min. The solution was cooled to ambient temperature. Then, the volume was made up to 100 ml with diluents B, filtered through Whatman filter paper and further filtered through 0.45 µm membrane filter. The prepared solution contains 400 µg/ml of TEL and 50 µg/ml of AMD.

**Determination of detection wavelength**

For the development of the method, UV spectrum of TEL and AMD was obtained separately by scanning the analytes at concentration levels 1000 µg/ml.

![Fig. 1: Structure of telmisartan](image1.png)

![Fig. 2: Structure of amlodipine](image2.png)

![Fig. 3: Ultraviolet spectrum of telmisartan and amlodipine](image3.png)
of 10 µg/ml in the range from 400 nm to 200 nm against blank as methanol. After a thorough examination of the spectra, the wavelength of 245 nm was selected as symmetric peaks were obtained at this wavelength which is shown in Fig. 3.

**Chromatographic conditions**

The chromatographic separation was performed on Poroshell 120EC-C<sub>18</sub> column (4.6 mm × 50, 2.7 µm). The mobile phase was composed of acetonitrile and buffer in the ratio of (45:55 v/v). The buffer used in the mobile phase contains 50 mM ammonium acetate in Mill Q water and pH was adjusted to 4.5 with acetic acid, filtered under vacuum through a 0.45 µm nylon filter and degassed in an ultrasonic bath before use. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.5 ml/min and the column temperature was maintained at 25°C. The injection volume was 10 µl and the elute was monitored at a wavelength of 245 nm using photodiode array detector.

**Procedure for forced degradation studies of standard drugs**

Forced degradation studies of standard drugs and tablet formulation were carried out under thermolytic, photolytic, acid, base, and neutral hydrolytic and oxidative stress conditions [28, 29].

**Acid degradation**

Pipetting out 4 ml of TEL and 0.5 ml of AMD standard solutions from stock solutions (1000 µg/ml), transferred to a 10 ml volumetric flask, added 2 ml of 0.1N HCl and diluted up to 10 ml with diluent B. The solution was heated on a water bath at 60°C for 4 h. The solution was allowed to ambient temperature repeated the same with 1N HCl.

**Alkali degradation**

Pipetting out 4 ml of TEL and 0.5 ml of AMD standard solutions from stock solutions (1000 µg/ml), transferred to a 10 ml volumetric flask, added 2 ml of 0.1N NaOH, and diluted up to 10 ml with diluent B. The solution was heated on a water bath at 60°C for 4 h. The solution was allowed to ambient temperature repeated the same with 1N NaOH.

**Degradation under the neutral hydrolytic condition**

Pipetting out 4 ml of TEL and 0.5 ml of AMD standard solutions from stock solutions (1000 µg/ml), transferred to a 10 ml volumetric flask, added 2 ml of distilled water and diluted up to 10 ml with diluent B. The solution was heated on a water bath at 60°C for 8 h. The solution was allowed to ambient temperature.

**Degradation under oxidative condition**

Pipetting out 4 ml of TEL and 0.5 ml of AMD solutions from stock solutions (1000 µg/ml), transferred to a 10 ml volumetric flask, added 2 ml of 3% v/v H<sub>2</sub>O<sub>2</sub> and diluted up to 10 ml with diluent B. The solution was heated on a water bath at 60°C for 1 h.

**Degradation under dry heat**

Dry heat study was performed by keeping drug sample on a Petri dish (about 100 mg) in an oven at 80°C for 24 h. Cooled, samples were withdrawn, dissolved in diluent A to prepare a sample solution to get concentrations of 1000 µg/ml. Pipetting out 4 ml of TEL and 0.5 ml of AMD standard solution of the stock solution (1000 µg/ml), transferred to a 10 ml volumetric flask, and diluted up to 10 ml with diluent B.

<table>
<thead>
<tr>
<th>Conditions (stress induced)</th>
<th>P (TEL)</th>
<th>TEL</th>
<th>AMD</th>
<th>P (AMD)</th>
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<tr>
<td></td>
<td>Percentage degradation</td>
<td>RT of degradants (min)</td>
<td>Percentage degradation</td>
<td>RT of degradants (min)</td>
</tr>
<tr>
<td>Acidic</td>
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<td>8.8 (0.1 N HCl)</td>
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<td>4.9</td>
</tr>
<tr>
<td></td>
<td>998.13</td>
<td>7.7 (1 N HCl)</td>
<td>-</td>
<td>5.4</td>
</tr>
<tr>
<td>Alkali</td>
<td>998.68</td>
<td>2.7 (0.1 NaOH)</td>
<td>-</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>996.87</td>
<td>2.9 (1 N NaOH)</td>
<td>-</td>
<td>7.3</td>
</tr>
<tr>
<td>Hydrolytic</td>
<td>993.42</td>
<td>8.2</td>
<td>--</td>
<td>7.28</td>
</tr>
<tr>
<td>Oxidative</td>
<td>997.46</td>
<td>21.9</td>
<td>4.193</td>
<td>11</td>
</tr>
<tr>
<td>Dry heat</td>
<td>999.45</td>
<td>0.4</td>
<td>-</td>
<td>0.6</td>
</tr>
<tr>
<td>UV light</td>
<td>999.72</td>
<td>100</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Sun light</td>
<td>999.53</td>
<td>0.5</td>
<td>-</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Table 1: Results of forced degradation studies**

TEL: Telmisartan, AMD: Amlodipine, RT: Retention time, P: Peak purity, UV: Ultraviolet

**Fig. 4:** (a) Chromatogram of standard amlodipine and telmisartan, (b) chromatogram of amlodipine and telmisartan in the formulation
Fig. 5: (a) Ultra high-performance liquid chromatogram of 0.1N HCl degradation. (b) Ultra high-performance liquid chromatogram of 1N HCl degradation. (c) Ultra high-performance liquid chromatogram of 0.1N NaOH degradation. (d) Ultra high-performance liquid chromatogram of 1N NaOH degradation. (e) Ultra high-performance liquid chromatogram of neutral hydrolytic degradation. (f) Ultra high-performance liquid chromatogram of oxidative degradation. (g) Ultra high-performance liquid chromatogram of dry heat degradation. (h) Ultra high-performance liquid chromatogram of direct sunlight degradation. (i) Ultra high-performance liquid chromatogram of photolytic degradation.
Sunlight degradation studies
Sunlight study was performed by exposing the drug samples in a Petri dish (about 100 mg) directly to sunlight for 8 h for 7 days. Samples were withdrawn, dissolved in diluent A to prepare sample solutions to get concentrations of 1000 µg/ml. Pipetting out 4 ml of TEL and 0.5 ml of AMD sample solutions and transferred the solution into a 10 ml volumetric flask and diluted up to 10 ml with diluent B.

Photo degradation studies
Photolytic studies were carried out by exposing the drugs in a Petri dish (about 100 mg) to UV short 254 nm and UV long light 366 nm for 24 h. Samples were withdrawn, dissolved in diluent A to prepare sample solutions to get concentrations of 1000 µg/ml. Pipetting out 4 ml of TEL and 0.5 ml of AMD sample solutions and transferred the solution into a 10 ml volumetric flask and diluted up to 10 ml with diluent B.

Procedure for forced degradation studies of drug products
A forced degradation studies of the table formulation in acidic, basic, water hydrolysis, and oxidative conditions were carried out using filtered solution (as described in sample preparation) to achieve 400 µg/ml of TEL and 50 µg/ml of AMD. For thermolytic and photolytic degradation, a quantity of powder equivalent to one tablet containing 40 mg of TEL and 5 mg of AMD was exposed. Then, the solutions were prepared as described in the preparation of the sample solution.

RESULTS
Optimization of the chromatographic conditions
Chromatographic conditions were optimized with a view to developing a stability-indicating method for the simultaneous quantitative estimation of TEL and AMD. To achieve the objective, different chromatographic options such as the selection of the mobile phase and stationary phase were evaluated to obtain a better resolution, less run time, high sensitivity, and symmetric peak in the method development. To optimize mobile phase under the isocratic condition, initially different mobile phases containing mixtures of commonly used solvents, namely methanol, acetonitrile with or without different buffers such as ammonium acetate and phosphate with different volume were tested at a flow rate of 1.0 or 0.5 ml/min. Different ratios of acetonitrile and ammonium acetate buffer were tested at a flow rate of 1.0 or 0.5 ml/min. The final mobile phase containing a mixture of 50 mM ammonium acetate in Mill Q water, pH adjusted to 4.5 with acetic acid and acetonitrile in the ratio of 55:45 was selected at a flow rate of 0.5 ml/min. A nonpolar Poroshell 120EC-C18 column was chosen as the stationary phase for this study. The column was maintained at room temperature. The injection volume was 10 µl. A study baseline was recorded with optimized chromatographic conditions at a wavelength of 245 nm and stabilized for about 30 min. Adequate separation of both drugs with good peak shape and less tailing was obtained with these optimized chromatographic conditions which also separates the degradants from standard drugs. Under the above-optimized conditions, the RT of 1.768 and 3.831 min was obtained for AMD and TEL which is shown in Fig. 4a and b.

Table 2: System suitability study results of telmisartan and amlodipine

<table>
<thead>
<tr>
<th>System suitability parameters</th>
<th>TEL</th>
<th>AMD</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tailing factor</td>
<td>1.43</td>
<td>0.75</td>
<td>NMT 2.0%</td>
</tr>
<tr>
<td>Theoretical plate count</td>
<td>4892</td>
<td>4287</td>
<td>NLT 2000</td>
</tr>
<tr>
<td>The percentage RSD for the areas of five replicate injections of the peak</td>
<td>0.57</td>
<td>0.32</td>
<td>NMT 2.0%</td>
</tr>
</tbody>
</table>

RSD: Relative standard deviation, NMT: Not more than, TEL: Telmisartan, AMD: Amlodipine, NLT: Not less than

Table 3: Linearity study results of telmisartan and amlodipine

<table>
<thead>
<tr>
<th>S. No</th>
<th>TEL</th>
<th>Area response</th>
<th>AMD</th>
<th>Area response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (µg/ml)</td>
<td></td>
<td>Concentration (µg/ml)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>380</td>
<td>21,987.07</td>
<td>30</td>
<td>1525.7</td>
</tr>
<tr>
<td>2</td>
<td>390</td>
<td>22,434.07</td>
<td>40</td>
<td>1525.7</td>
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<tr>
<td>3</td>
<td>400</td>
<td>22,816.13</td>
<td>50</td>
<td>1983.7</td>
</tr>
<tr>
<td>4</td>
<td>410</td>
<td>23,176.70</td>
<td>60</td>
<td>2385.2</td>
</tr>
<tr>
<td>5</td>
<td>420</td>
<td>23,605.71</td>
<td>70</td>
<td>2795.6</td>
</tr>
<tr>
<td>Smale</td>
<td>39.799</td>
<td></td>
<td>30</td>
<td>1525.7</td>
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<tr>
<td>Intercept</td>
<td>6848.4</td>
<td></td>
<td>40</td>
<td>1983.7</td>
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<tr>
<td>Regression coefficient</td>
<td>0.9987</td>
<td></td>
<td>50</td>
<td>2385.2</td>
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<tr>
<td>Correlation coefficient</td>
<td>0.9993</td>
<td></td>
<td>60</td>
<td>2795.6</td>
</tr>
<tr>
<td>LOD µg/ml</td>
<td>0.2927</td>
<td></td>
<td>70</td>
<td>3140.4</td>
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<tr>
<td>LOQ µg/ml</td>
<td>0.8893</td>
<td></td>
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</table>

TEL: Telmisartan, AMD: Amlodipine, LOD: Limit of detection, LOQ: Limit of quantification
Degradation observed
The chromatograms of the TEL and AMD standards and tablet formulation showed well-separated peaks of pure TEL and AMD as well as some additional degradants when they were subjected to various stress conditions such as acid, alkali, neutral, hydrogen peroxide, dry heat, sunlight, and UV light. The peaks of the degraded products were well resolved from the TEL and AMD drug's peak. The identification of the degradants was carried out by comparing the chromatograms of "stressed samples" with that of the "standard solution." In stress testing, the current regulatory guidelines do not provide sufficient information about degradation conditions. However, Blessy and Ruchi [30] in their article on stress testing suggested that a target degradation of 5–20%
has been accepted as reasonable for validation of chromatography assay. Similarly, Singh and Bakshi [31] in their article on stress testing suggested a target degradation by 20–80% for establishing stability-indicating studies and also intermediate degradation products should not interfere with any stage of drug analysis. In this study, conditions used for forced degradation were attenuated to achieve degradation in the range of 5%–80% for TEL and AMD drug substances. The numbers of degradation products with their RT and percentage degradation of TEL and AMD are listed in Table 1 and shown in Fig. 5a-i.

**Method validation**

The developed method has been validated according to the ICH guideline [32]. The validation parameters such as system suitability, linearity, precision/reproducibility, accuracy, specificity, and robustness were considered for the newly developed method.

**System suitability**

The system suitability of the method was tested by injecting one blank injection, five injections of TEL and AMD mixed standard solution of concentration 400 µg/ml, 50 µg/ml. System suitability parameters such as theoretical plates, tailing factor, and areas percentage relative standard deviation (R. S. D) were studied and found that all the system suitability parameters are within acceptance criteria. Results of system suitability studies are shown in Table 2.

**Linearity**

The linearity of the method was tested by preparing five different mixed standard solutions from 50% to 150% of TEL and AMD and injected in triplicate for each concentration. The mixed standard solutions contain the concentration ranges from 380 to 420 µg/ml for TEL and 30 to 70 µg/ml for AMD. From the chromatograms, linearity plots were drawn by taking concentrations on X-axis and area of peaks on Y-axis. The regression equations obtained for TEL and AMD were 39.799x + 6884.4 and 40.053x + 356.27 which is shown in Fig. 6a and b. The linear regression coefficient and correlation coefficient values for TEL and AMD were found to be 0.9987, 0.9993, and 0.9983, 0.9991 respectively, indicating a high degree of linearity which is shown in Table 3.

**Precision (repeatability)**

To demonstrate the system and method precision of the analytical method, a homogeneous standard solution of TEL and AMD having concentration 400 µg/ml and 50 µg/ml was analyzed for 6 times and RT and areas were measured and percentage R. S. D was calculated. Similarly, the intermediate precision of the method was determined by analyzing RT and areas of the mixed homogeneous standard solution having concentration 400 µg/ml and 50 µg/ml for 6 times on different days, by different analysts. The precision study results were considered for the newly developed method.

### Table 4: Precision study results of telmisartan and amlodipine

| Six injections | TEL | | | AMD | | |
|---|---|---|---|---|---|
| Results of system precision | Area response | RT (min) | | Area response | RT (min) |
| Mean±SD | 22.84±34.26 | 3.92±0.0090 | | 23.75±2.45 | 1.78±0.0043 |
| Percentage RSD | 0.04 | 0.25 | | 0.23 | 0.25 |
| Results of method precision | | | | | |
| Mean±SD | 22.82±34.26 | - | | 23.70±2.43 | - |
| Percentage RSD | 0.20 | - | | 0.40 | - |
| Results of intermediate precision | | | | | |
| Mean±SD | 22.73±2.49 | - | | 23.16±2.79 | - |
| Percentage RSD | 0.13 | - | | 0.34 | - |

SD: Standard deviation, RSD: Relative standard deviation, TEL: Telmisartan, AMD: Amlodipine, RT: Retention time

### Table 5: Results of recovery study of telmisartan

<table>
<thead>
<tr>
<th>Level of recovery (%)</th>
<th>Concentration taken (µg/ml)</th>
<th>Amount added (µg/ml)</th>
<th>Amount recovered (µg/ml)</th>
<th>Percentage recovery</th>
<th>SD</th>
<th>Percentage RSD</th>
<th>SEM</th>
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<tbody>
<tr>
<td>50</td>
<td>40</td>
<td>340</td>
<td>376.8</td>
<td>99.2</td>
<td>0.1527</td>
<td>0.04</td>
<td>0.0881</td>
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<tr>
<td>50</td>
<td>40</td>
<td>340</td>
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<td>99.1</td>
<td>0.1000</td>
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<td>99.8</td>
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<td>100</td>
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<td>340</td>
<td>399.6</td>
<td>99.9</td>
<td>0.2645</td>
<td>0.06</td>
<td>0.1527</td>
</tr>
<tr>
<td>100</td>
<td>40</td>
<td>340</td>
<td>399.5</td>
<td>99.9</td>
<td>0.2645</td>
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<td>0.1527</td>
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<td>150</td>
<td>40</td>
<td>380</td>
<td>425.8</td>
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<td>380</td>
<td>425.4</td>
<td>101.3</td>
<td>0.2645</td>
<td>0.06</td>
<td>0.1527</td>
</tr>
</tbody>
</table>

SD: Standard deviation, RSD: Relative standard deviation, SEM: Standard error of mean

### Table 6: Results of recovery study of amlodipine

<table>
<thead>
<tr>
<th>Level of recovery (%)</th>
<th>Concentration taken (µg/ml)</th>
<th>Amount added (µg/ml)</th>
<th>Amount recovered (µg/ml)</th>
<th>Percentage recovery</th>
<th>SD</th>
<th>Percentage RSD</th>
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<tr>
<td>50</td>
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<td>25</td>
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SD: Standard deviation, RSD: Relative standard deviation, SEM: Standard error of mean
illustrate that the method is precise (R. S. D % <2) which is shown in Table 4.

Accuracy (recovery test)
The accuracy of the method was demonstrated by recovery studies. Recovery study was carried out in three different levels, with each level in triplicate for standard drugs (nine determinations). The known concentration of TEL and AMD standard drugs was spiked at 50%, 100%, and 150% levels into the tablet sample solutions containing 40 µg/ml of TEL and 5 µg/ml of AMD. The percentage recoveries were calculated by analyzing the prepared solutions which are shown in Tables 5 and 6. The average recovery of three levels (9 determinations) for TEL and AMD was 100% and 98.3%, respectively. The results of the accuracy studies express that recovery is well within the limit. Hence, the developed method is accurate.

Specificity
Different forced degradation studies were carried out for specificity study. Tablet samples were stressed with different conditions (similar to standard drug degradation studies) and injected into the UHPLC system. Photodiode array detection was used as evidence of the specificity of the method and to evaluate the homogeneity of the drug peak. The peak purity values of TEL and AMD were 99.956 and 99.948, respectively, which are more than 997 for tablet samples at 245 nm which shows that the peaks of analyte were pure and also the formulation excipients and degradants were not interfering with the analyte peaks which are shown in Fig. 7a-i.

Robustness
The robustness of the method was evaluated after introducing small deliberate changes in experimental conditions in the analysis of TEL and AMD standard solution at the concentration of 400 µg/ml and 50 µg/ml and chromatograms were studied. In all conditions areas percentage R. S. D and tailing factors were within acceptance criteria. Hence, it is concluded that the analytical procedure is robust which are shown in Table 7.

Assay
The prepared sample solution of tablet formulation having a concentration of 400 µg/ml and 50 µg/ml for TEL and AMD was analyzed by the newly developed method. The standard and tablet sample solutions peak areas were compared to calculate the content of TEL and AMD which is shown in Table 8.
AUTHORS' CONTRIBUTIONS

Biswa Ranjan Patra has carried out a review of literature and experimental work in the department of pharmaceutical analysis laboratory, PES College of Pharmacy, Bengaluru. Dr. Nagaraj Gowda drafted the manuscript. The final draft of the manuscript was reviewed and edited under the guidance of Dr. Mohan S.

CONFLICTS OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of the research paper.

REFERENCES