

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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ABSTRACT

Objective: The objective of this research work is to develop a simple, rapid, cost-effective, accurate, precise, and robust stability-indicating reverse-phase ultra-high performance liquid chromatography method for the quantitative estimation of telmisartan (TEL) and amlodipine (AMD) in combined tablet dosage form.

Methods: The method uses Poroshell 120EC-C₁₈ column (4.6 mm × 50 mm, 2.7 μm) with an optimized mobile phase containing acetonitrile:50 mM ammonium acetate buffer in the ratio (45:55 v/v), pH adjusted to 4.5 with acetic acid. The flow rate was 0.5 ml/min, column temperature at 25°C and detection was monitored by a PDA detector at a wavelength of 245 nm. International Conference on Harmonization (ICH) recommended stress degradation studies were performed on TEL, AMD standard drugs, and tablet formulations; further stressed samples were analyzed by the proposed method.

Results: Major degradation of TEL and AMD was observed under acidic, alkali, 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 suitable and accurate 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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INTRODUCTION

Telmisartan (TEL), chemically described as 2-(4-[[4-methyl-6-(1-methyl-1H-1,3-benzimidazol-2-yl)-2-propyl-1H-1,3-benzimidazole-1-yl] methyl] phenyl) benzoic acid, molecular formula C₃₃H₃₀N₄O₂ and molecular weight 514.62. Amlodipine (AMD) is chemically described as 3-O-ethyl-5-O-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, molecular formula C₂₀H₂₅ClN₂O₅ 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.

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-C₁₈ column (4.6 \times 50 mm, 2.7 μm), PDA detector with Chemstation Software, ShimadzuAUX220 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 $\mu\text{g/ml}$.

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 $\mu\text{g/ml}$.

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 $\mu\text{g/ml}$), transferred into a 10 ml volumetric flask and the volume was made up to 10 ml using diluent B. This solution contained 400 $\mu\text{g/ml}$ of TEL and 50 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ for TEL and 30 to 70 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ of TEL and 50 $\mu\text{g/ml}$ 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

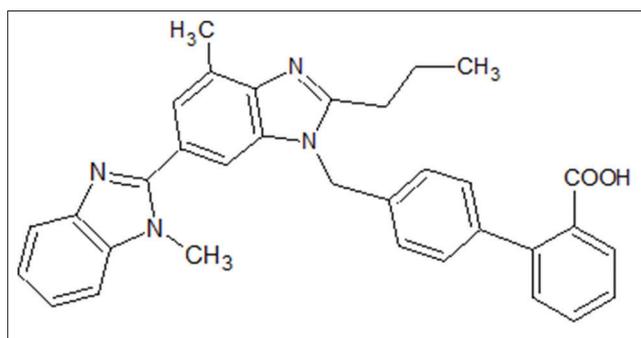


Fig. 1: Structure of telmisartan

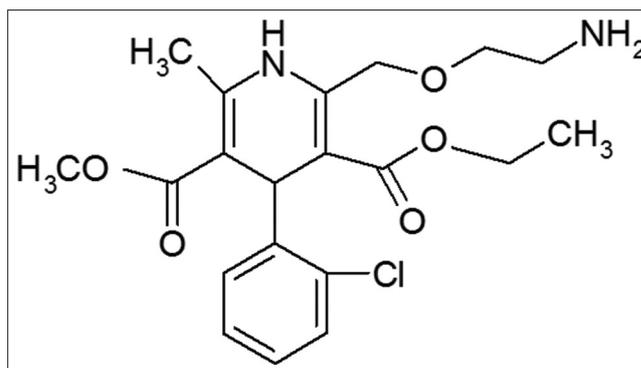


Fig. 2: Structure of amlodipine

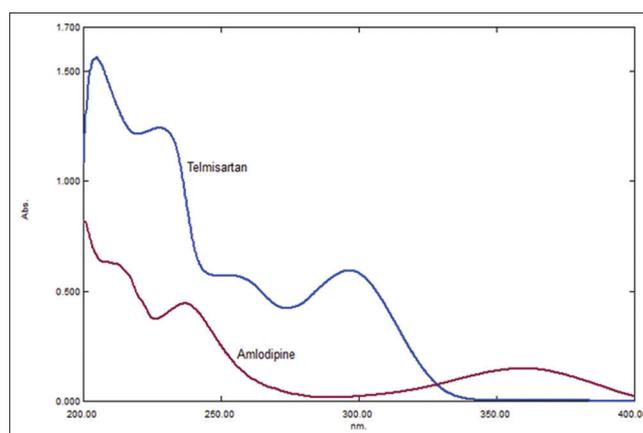


Fig. 3: Ultraviolet spectrum of telmisartan and amlodipine

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-C₁₈ 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 H₂O₂ 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 1: Results of forced degradation studies

Conditions (stress induced)	P (TEL)	TEL		AMD		P (AMD)
		Percentage degradation	RT of degradants (min)	Percentage degradation	RT of degradants (min)	
Acidic	999.24	8.8 (0.1 N HCl)	-	4.9	-	997.68
	998.13	7.7 (1 N HCl)	-	5.4	0.919	998.36
Alkali	998.68	2.7 (0.1 NaOH)	-	1.1	-	997.87
	996.87	2.9 (1 N NaOH)	-	7.3	0.918	997.38
Hydrolytic	993.42	8.2	--	72.8	-	999.77
Oxidative	997.46	21.9	4.193	11	1.027	999.62
Dry heat	999.45	0.4	-	0.6	-	999.96
UV light	999.72	100	-	100	-	999.39
Sun light	999.53	0.5	-	0.4	-	999.47

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

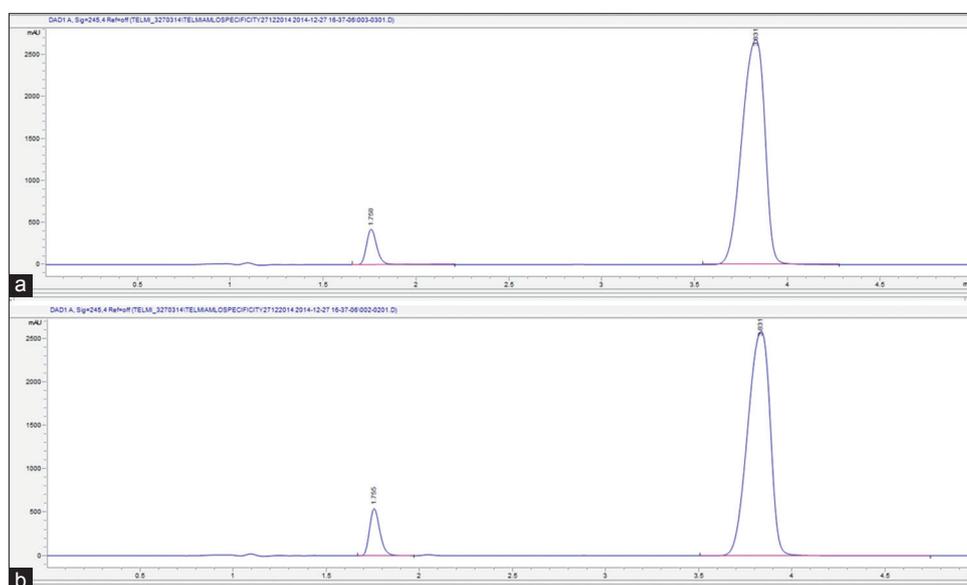


Fig. 4: (a) Chromatogram of standard amlodipine and telmisartan, (b) chromatogram of amlodipine and telmisartan in the formulation

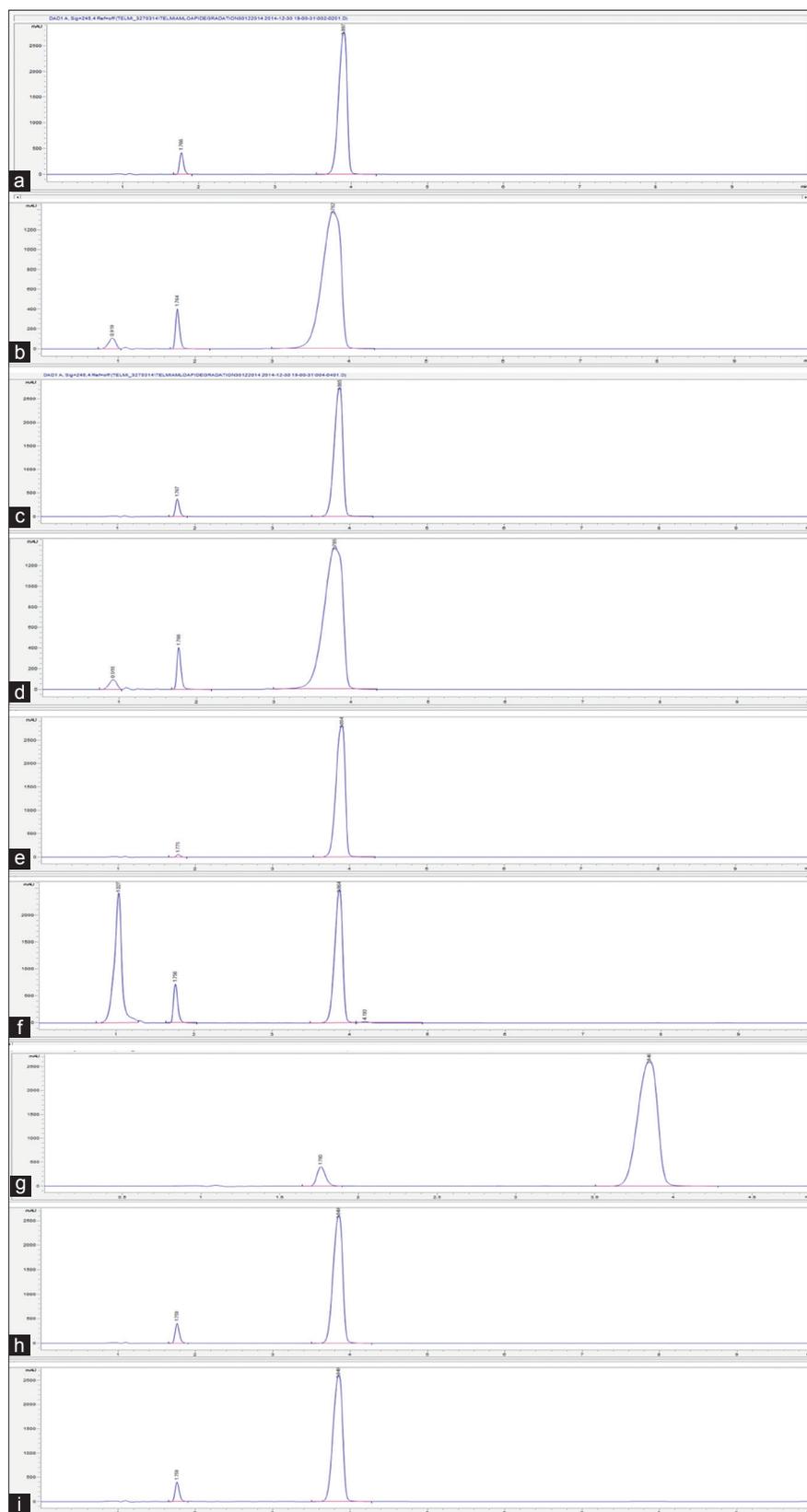


Fig. 5: (a) Ultra high-performance liquid chromatogram of 0.1N HCl degradation. (b) Ultra high-performance liquid chromatogram of 1N HCl degradation. (c) Ultra high-performance liquid chromatogram of 0.1N NaOH degradation. (d) Ultra high-performance liquid chromatogram of 1N NaOH degradation. (e) Ultra 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.

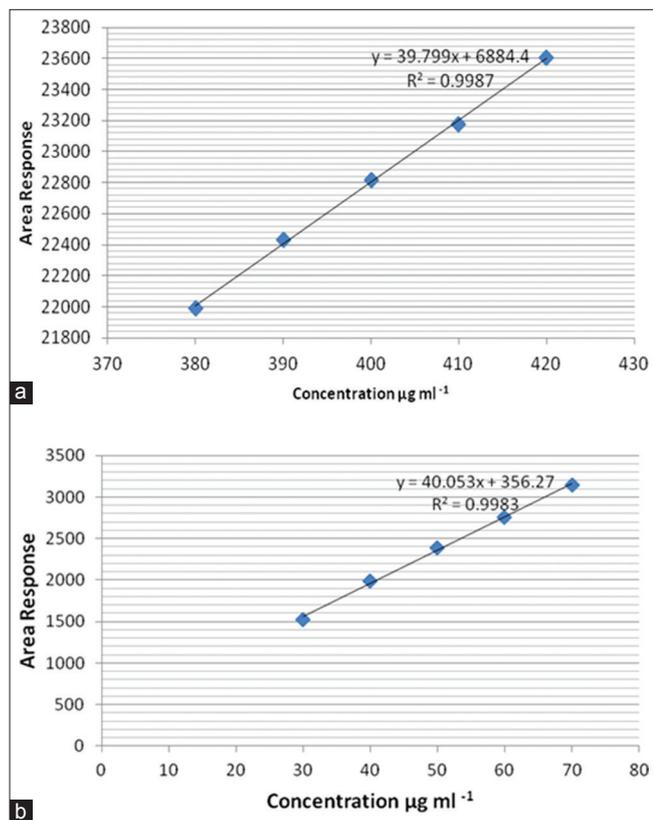


Fig. 6: (a) Linearity graph of telmisartan. (b) Linearity graph of amlodipine

Table 2: System suitability study results of telmisartan and amlodipine

System suitability parameters	TEL	AMD	Acceptance criteria
Tailing factor	1.43	0.75	NMT 2.0%
Theoretical plate count	4892	4287	NLT 2000
The percentage RSD for the areas of five replicate injections of the peak	0.57	0.32	NMT 2.0%

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

S. No	TEL		AMD	
	Concentration ($\mu\text{g/ml}$)	Area response	Concentration ($\mu\text{g/ml}$)	Area response
1	380	21,987.07	30	1525.7
2	390	22,434.07	40	1983.7
3	400	22,816.13	50	2385.2
4	410	23,176.70	60	2795.6
5	420	23,605.71	70	3140.4
Slope		39.799	-	40.053
Intercept		6884.3	-	356.27
Regression coefficient		0.9987	-	0.9983
Correlation coefficient		0.9993	-	0.9991
LOD $\mu\text{g/ml}$		0.2927	-	0.1371
LOQ $\mu\text{g/ml}$		0.8893	-	0.1125

TEL: Telmisartan, AMD: Amlodipine, LOD: Limit of detection, LOQ: Limit of quantification

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 tablet 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.

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%

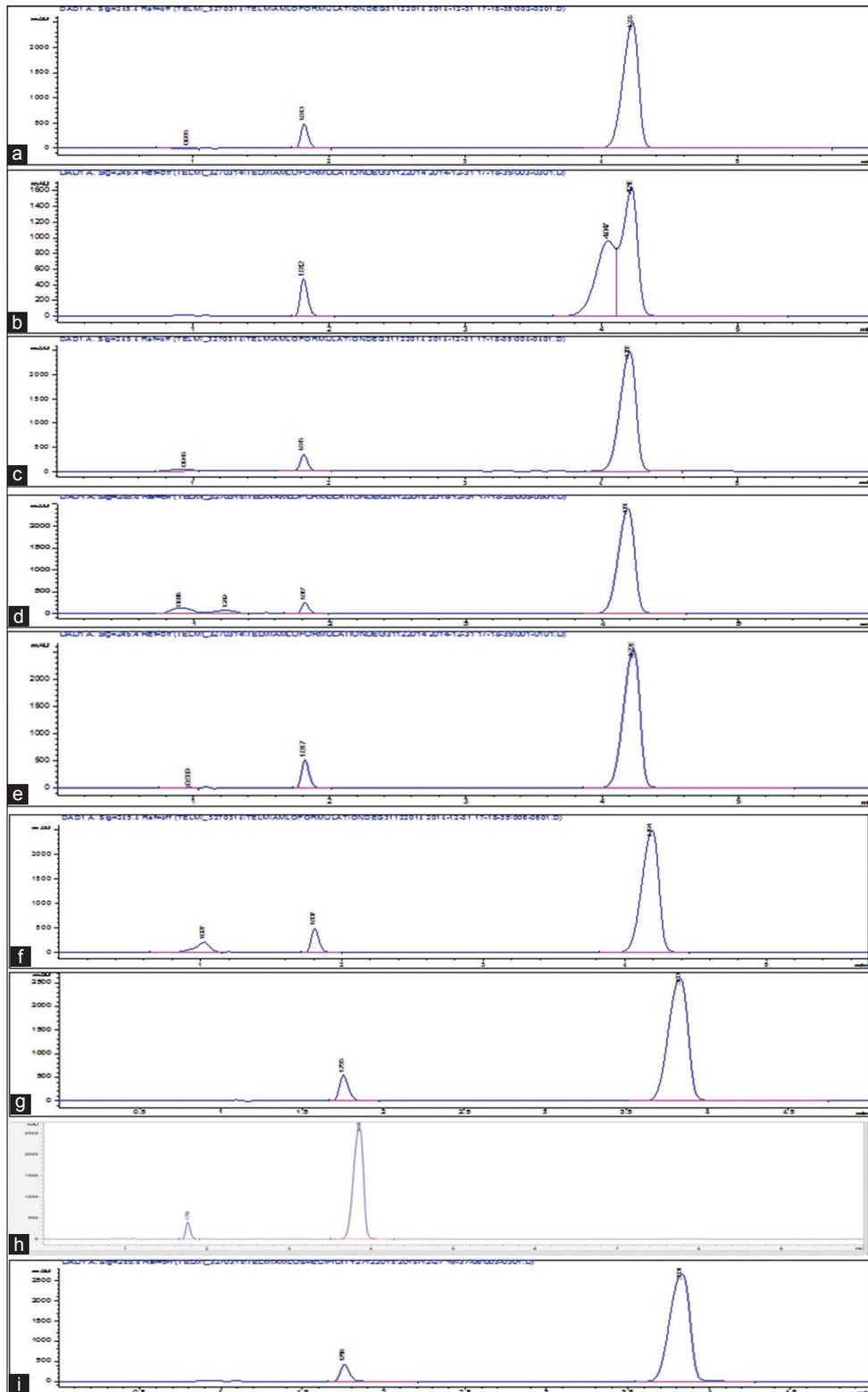


Fig. 7: Ultra high-performance liquid chromatograms of specificity study of telmisartan and amlodipine tablet (a and b) 0.1 and 1N N HCl (4 h), (c and d) 0.1 and 1 N NaOH (4 h), (e) neutral (8 h), (f) 3% H₂O₂ (1 h), (g and h) sunlight (8 h) and ultraviolet (24 h) (i) Dry heat at 80°C (24 h)

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

Table 4: Precision study results of telmisartan and amlodipine

Six injections	TEL		AMD	
	Area response	RT (min)	Area response	RT (min)
Results of system precision				
Mean±SD	22,844±98.3466	3.928±0.0090	2375.4±5.4543	1.786±0.0043
Percentage RSD	0.04	0.22	0.23	0.25
Results of method precision				
Mean±SD	22,820.8±34.266	-	2370.5±9.4238	-
Percentage RSD	0.20	-	0.40	-
Results of intermediate precision				
Mean±SD	22,738.9±21.2492	-	2316.3±7.7983	-
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

Level of recovery (%)	Concentration taken (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Percentage recovery	SD	Percentage RSD	SEM
50	40	340	376.8	99.2	0.1527	0.04	0.0881
50	40	340	376.6	99.1			
50	40	340	376.9	99.2			
100	40	360	399.4	99.8	0.100	0.03	0.0577
100	40	360	399.6	99.9			
100	40	360	399.5	99.9			
150	40	380	425.8	101.4	0.2645	0.06	0.1527
150	40	380	425.3	101.7			
150	40	380	425.4	101.3			

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

Table 6: Results of recovery study of amlodipine

Level of recovery (%)	Concentration taken (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Percentage recovery	SD	Percentage RSD	SEM
50	5	25	29.3	97.6	0.1527	0.51	0.0881
50	5	25	29.6	98.5			
50	5	25	29.4	98.1			
100	5	45	48.9	97.7	0.1154	0.23	0.0666
100	5	45	49.1	98.3			
100	5	45	49.1	98.2			
150	5	65	68.7	98.1	0.3214	0.46	0.1855
150	5	65	69.3	99.0			
150	5	65	68.8	98.2			

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

Table 7: Results of robustness for telmisartan and amlodipine

Parameters	TEL		AMD	
	Area percentage RSD	Tailing factor	Area percentage RSD	Tailing factor
Change in column temperature in ($\pm 5^\circ\text{C}$)				
20	0.5532	0.96	0.5162	0.84
25 (initial)	0.0679	1.11	0.2974	0.92
30	0.4235	1.02	0.1946	0.93
Change in mobile phase composition buffer: Acetonitrile (organic phase $\pm 2\%$)				
57:43	0.1348	1.06	0.3642	0.96
55:45 (initial)	0.1035	0.86	0.9149	1.14
53:47	0.1168	1.19	0.4746	0.93
Change in flow rate ± 0.2 ml/min				
0.48	0.1846	1.15	0.3842	0.97
0.5 (initial)	0.1261	1.24	0.9275	0.91
0.52	0.2666	1.03	0.4632	1.09

RSD: Relative standard deviation, TEL: Telmisartan, AMD: Amlodipine

Table 8: Assay results of telmisartan and amlodipine

Tablet formulation	Label claimed (mg/tablet)	Amount found (mg) (n=3)	Drug content (%)	SD	RSD	SEM
TEL	40	39.8867	99.71	0.6321	0.63	0.3649
AMD	5	5.0264	100.52	0.9764	0.97	0.5637

TEL: Telmisartan, AMD: Amlodipine, 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 $\mu\text{g/ml}$ of TEL and 5 $\mu\text{g/ml}$ 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 study 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 999.56 and 999.48, 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 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$ 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.

DISCUSSION

The peaks of the degradants were well resolved from the TEL and AMD drug's peak. The chromatogram of the acid degraded samples showed one additional peak of RT of 0.919 min for AMD. The chromatogram of the alkali degraded samples showed one additional peak of RT of 0.918 min for AMD. The chromatogram of the oxidation degraded samples showed one additional peak of RT of 1.027 min for AMD and one additional peak of RT of 4.193 min for TEL. No additional peaks were developed in water, dry heat, and UV light and sunlight degradation studies. Major degradation of TEL and AMD was observed under acidic, alkali, and hydrolytic and oxidation conditions. Very less degradation was observed under dry heat and photolytic conditions.

The response of the drugs was found to be linear in the concentration range of 380–420 $\mu\text{g/ml}$ for TEL and 30–70 $\mu\text{g/ml}$ for AMD, respectively, with respect to the peak areas. The percentage R. S. D values for precision studies were found to be <2%; this confirms that the method is precise. The accuracy of the method was determined and the mean recovery of TEL and AMD were 100.0% and 98.3%, respectively. The peak purity values for TEL and AMD were in the range of 997–1000 for drug substance as well as tablet formulation, indicating the peaks were pure and also that formulation excipients and degradants were not interfering with analyte peaks, thus establishing the specificity of the method. The low values of percentage R. S. D were obtained after introducing small deliberate changes in the developed UHPLC method, shows that the method is robust.

CONCLUSIONS

The developed isocratic RP-UHPLC method is highly sensitive, specific, accurate, and rapid with less run time and less consumption of solvents. The statistical analysis proved that the proposed method is reproducible, selective for the analysis of TEL and AMD in bulk and tablet formulations without any interference from common excipients. The proposed method separates the drug from its degradants. The developed method can be employed to isolate degradants and for routine quality control analysis of TEL and AMD tablets.

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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.

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