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Development And Validation Of A Stability-Indicating Hplc Method For The Determination Of Related Substances Of Telmisartan, Amlodipine, And Chlorthalidone In A Fixed-Dose Combination Tablet Formulation

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ABSTRACT:

Background: Telmisartan, Amlodipine, and Chlorthalidone have a widespread use in their combination in management of hypertension and cardiovascular risk. Concomitant presence of pharmacologically distinct drugs in a unit dosage form presents unique analytical problems, in particular in respect to detection and quantification of the corresponding substances, such as impurities in the process and degradation products.

Objectives: This study aimed to develop and validate a gradient High Performance Liquid Chromatography (HPLC) method for the determination of related substances of telmisartan, amlodipine, and chlorthalidone in a fixed-dose combination tablet formulation, with specific focus on the known impurity Amlodipine Impurity-D.

Methods: Chromatographic separation was carried out using an Inertsil ODS-2 (250 × 4.6 mm, 5 µm) column using a gradient mobile phase system comprising a phosphate buffer (pH 5.0) with 1-octane sulfonic acid sodium salt and a solvent mixture of acetonitrile and methanol, detected at 235 nm. The method was validated guidelines for specificity, linearity, accuracy, precision, LOD, LOQ, solution stability, and ruggedness as per in ICH Q2 (R1).

Results: The technique was shown to be very linear ($r \geq 0.9995$) with all analytes with the available concentration ranges. The precision in the form of the percent relative standard deviation (%RSD) was less than 2.00% on system suitability and less than 1.97% on method precision. Impurity-D mean recovery was 105.50% and unknown impurities (Telmisartan, Chlorthalidone and Amlodipine) were recovered with an acceptance criteria of 80.0–120.0%. LOD and LOQ values confirmed adequate sensitivity at sub-µg/mL levels. Forced degradation experiments were shown to be specific and capable of stability, with purity angles always lower than purity limits under all circumstances.

Conclusion: The HPLC technique that has been validated is specific, precise, accurate, linear, rugged and stability revealing. It has been used in normal analysis of related

substances in telmisartan, amlodipine, and chlorthalidone fixed-dose combination tablets in terms of quality control analysis.

INTRODUCTION

Hypertension is one of the most common risk factors of cardiovascular diseases in the world, and its successful treatment often requires combination pharmacotherapy of several physiological pathways. Telmisartan- angiotensin II receptor blocker (ARB); amlodipine- dihydropyridine calcium channel blocker; chlorthalidone- thiazide-like diuretic; are three different complementary antihypertensive mechanisms. This combination of three agents into a single fixed-dose combination (FDC) tablet has great benefits in terms of adherence and convenience of the therapy to the patient [1,2].

The existence of two or more APIs in the same formulation with different structural and physicochemical characteristics will require the design of effective selective analysis techniques with the ability to simultaneously detect the parent compounds and their known related substances as well as the possible degradation products under stressful conditions [3]. Telmisartan is a benzimidazole derivative, which is prone to hydrolytic and oxidative degradation, whereas amlodipine is a 1,4-dihydropyridine that is prone to photosensitivity and base-catalyzed degradation [4,5]. The sulfonamide diuretic chlorthalidone is also likely to undergo hydrolytic fracture in both acidic and alkaline environments [6]. Along with the associated substances of amlodipine, one of the most significant process-related impurities is Impurity-D (also known as Amlodipine Related Compound-A, USP), which must be quantitatively controlled as required by the pharmacopoeial- and regulatory-based requirements [3].

A number of HPLC procedures have been reported to date to analyze these components individually or in pairs, but no stability-indicating procedure has been described in the published literature to the determination of related substances in the telmisartan-amlodipine-chlorthalidone combination tablet all at the same time. The challenge of creating such a method should counteract the difficulty of obtaining sufficient chromatographic resolution of analytes with very different polarity and detector response, and run time that is practically feasible.

The aim of the present studies was to development and validation of a gradient reversed-phase HPLC method for the determination of related substances of telmisartan, amlodipine, and chlorthalidone in their fixed-dose combination tablet formulation. Validation was carried out in accordance with the (ICH) Q2 (R1) guidelines, encompassing specificity, linearity and range, accuracy, precision, LOD, LOQ, solution stability, and ruggedness.

MATERIALS AND METHODS

Materials and chemicals

Potassium dihydrogen orthophosphate (AR grade), 1-octane sulfonic acid sodium salt (AR grade), orthophosphoric acid (HPLC grade), and triethylamine (HPLC grade) were used for mobile phase preparation. Tetrahydrofuran (THF, HPLC grade, without stabilizer), acetonitrile (gradient grade), methanol (gradient grade), and HPLC-grade water were employed as solvents. Amlodipine Impurity-D working reference standard (Amlodipine Related Compound-A, USP) was used for the quantitation of the known impurity. Telmisartan, Chlorthalidone, and Amlodipine working reference standards were used for unknown impurity and linearity assessments. Telmisartan, Amlodipine, and Chlorthalidone Tablets (Label Claim 40, 5, 12.5 mg respectively) served as the test sample throughout the validation experiments.

Instrumentations

Chromatographic analysis was carried out using a Waters Separation Module HPLC system equipped with ultraviolet (UV) and photodiode array (PDA) detectors. An Inertsil ODS-2 (250 × 4.6 mm, 5 µm) column (G.L. Sciences, Japan) was employed as the stationary phase for all experiments.

Chromatographic conditions

UV detection was performed at 235 nm lambda max with an injection volume of 10 µL and a flow rate of 1.0 mL/min at 45°C of column temperature and5°C of sample temperature. A total run time of 60 minutes was employed with gradient programme (Table 1).

Table 1: Gradient programme for the HPLC method

Time (Min)	% of Mobile phase A	% of Mobile phase B
0	65	35
10	65	35
17	45	55
24	55	45
30	25	75
38	35	65
52	65	35
60	65	35

Mobile Phase and Diluent Preparation

Mobile Phase A was prepared by mixing a phosphate buffer and a solvent mixture (acetonitrile:methanol, 100:80, v/v) in the ratio of 70:30 (v/v). Mobile Phase B was prepared by mixing the same buffer and solvent mixture in the ratio of 30:70 (v/v). The phosphate buffer was prepared by dissolving 6.8 g of potassium dihydrogen phosphate and 0.5 g of 1-octane sulfonic acid sodium salt in 1000 mL of water, with 2 mL of triethylamine added and the pH adjusted to 5.0 ± 0.05 using orthophosphoric acid.

Diluent-1 was prepared by mixing methanol, acetonitrile, and water in the ratio of 60:30:10 (v/v/v). Diluent-2 was prepared by mixing a second phosphate buffer (pH 2.4 ± 0.05, prepared identically to the above buffer but pH-adjusted with orthophosphoric acid) and the solvent mixture in the ratio of 65:35 (v/v).

Standard preparation of Amlodipine Impurity D

For the known impurity standard, approximately 2.6 mg of Amlodipine Impurity-D working reference standard was accurately weighed and transferred into a 250 mL volumetric flask. Approximately 12.5 mL of THF was added and mixed, followed by approximately 200 mL of Diluent-1 and sonication to dissolve. The volume was made up with Diluent-1. 5 mL of this solution was then diluted to 200 mL with Diluent-2 to yield the working standard preparation.

Sample preparation

At least 20 tablets were weighted separately and the average weight of the tablets was determined. Precisely, 20 mg of amlodipine (when compared to 160 mg of telmisartan and 50 mg of chlorthalidone) was placed in a 100 mL volumetric flask as tablet powder. The solution was sonicated (5 minutes) and 5 mL of tetrahydrofuran was added to ensure that the solution dissolved at the beginning. Then, about 70 mL of Diluent-1 was added and further sonication carried out in 30 minutes with occasional shaking to extract all of it. Diluent-1 was added to the volume to 100 mL. This suspension was centrifuged and the clear supernatant taken. A stock solution 5 mL of this solution was diluted by 25 mL of Diluent-2 and injected into the chromatographic system.

Method Validation

The analytical method was validated as per ICH Q2 (R1) guidelines [7]. The following validation parameters were assessed: system precision, specificity (including forced degradation under acid, alkali, peroxide, thermal, and UV stress conditions), linearity and range, limits of detection (LOD) and quantitation (LOQ), accuracy (recovery), method precision, intermediate precision, ruggedness, and solution stability.

System suitability test

For the suitability test of method, six replicate injections of mixture of Amlodipine impurity D, Telmisartan, Amlodipine and Chlorthalidone having concentration 0.2, 0.2, 1.6 and 0.5 μg/mL were injected. It was passes if RSD of area counts of six replicate injections, signal to noise ratio (S/N) and tailing factor were found ≤ 10%, ≥ 10 and ≤ 2, respectively [7,8].

Specificity

Specificity of the method is confirmed by measuring the analyte response in the presence of its potential impurities and degradation products. Selectivity is confirmed by separation of all the possible impurities for each other along with analyte peak. The specificity of the developed method were checked in the presence of its impurities and forced degradation products [9].

Forced degradation studies

Forced degradation studies were conducted to establish the specificity and degradation separation capability of the developed related substances method. Stress conditions were selected to generate representative degradation products while avoiding excessive secondary degradation that could interfere with interpretation of degradation pathways. The samples were subjected to acid hydrolysis (0.1 N methanolic HCl), alkali hydrolysis (0.1 N methanolic NaOH), oxidative degradation (30% H_2O_2), thermal degradation (60°C), and photolytic degradation conditions. Photolytic studies were performed using exposure conditions not less than 1.2 million lux hours and integrated near-ultraviolet energy not less than 200 Wh/m² in accordance with ICH Q1B recommendations. The stability-indicating capability of the method was established through adequate separation of degradation products from the analyte peaks and confirmation of peak purity using PDA detection.

Since the present analytical procedure was developed specifically for related substances determination rather than quantitative assay evaluation, specificity was established through separation of degradation products and PDA-based peak purity assessment. Formal assay-based mass balance determination was therefore outside the predefined scope of this study.

Acid degradation

Transferred accurately weighed tablet powder equivalent to 20 mg of Amlodipine into 100 ml volumetric flask. Added 5 ml of THF and sonicate for 5 min, further added about 70 ml of diluent-1 and sonicated for 30 min with intermittent shaking. Added 5 ml of 0.1N Methanolic HCl and heated in a water bath at 60°C for 30 minutes. Cooled and neutralized with 5 ml of 0.1N Methanolic NaOH. Made up the volume with diluent-1 and mixed well. Centrifuged to get clear supernatant. Diluted 5 m1 this solution to 25 m1 with diluent-2. Injected this solution in to the HPLC system.

Alkali degradation

Transferred accurately weighed tablet powder equivalent to 20 mg of Amlodipine into 100 ml volumetric flask. Added 5 ml of THF and sonicate for 5 min, further added about 70 ml of diluent-1 and sonicated for 30 min with intermittent shaking. Added 2 ml of 0.1N Methanolic NaOH and heated in a water bath at 60°C for 30 minutes. Cooled and neutralized with 5 ml of 0.1N Methanolic HCl. Made up the volume with diluent-1 and mixed well. Centrifuged to get clear supernatant. Diluted 2 m1 this solution to 25 m1 with diluent-2. Injected this solution in to the HPLC system.

2.5.3.3 Peroxide degradation

Transferred accurately weighed tablet powder equivalent to 20 mg of Amlodipine into 100 ml volumetric flask. Added 5 ml of THF and sonicate for 5 min, further added about 70 ml of diluent-1 and sonicated for 30 min with intermittent shaking. Added 1 ml of 30 % H2O2 and heated in a water bath at 60°C for 30 minutes. Cooled and made up the volume with diluent-1 and mixed well. Diluted 5 m1 this solution to 25 m1 with diluent-2. Injected this solution in to the HPLC system.

Thermal degradation

Transferred accurately weighed tablet powder equivalent to 20 mg of Amlodipine into 100 ml volumetric flask. Added 5 ml of THF and sonicate for 5 min, further added about 70 ml of diluent-1 and sonicated for 30 min with intermittent shaking and heated in a water bath at 60°C for 30 minutes. Cooled and made up the volume with diluent-1 and mixed well. Diluted 5 m1 this solution to 25 m1 with diluent-2. Injected this solution in to the HPLC system.

Photolytic degradation

Transferred accurately weighed tablet powder equivalent to 20 mg of Amlodipine (Previously kept in not less than 1.2 million lux hours and an integrated near-ultraviolet energy of not less than 200 Wh/m²) into 100 ml volumetric flask. Added 5 ml of THF and sonicate for 5 min, further added about 70 ml of diluent-1 and sonicated for 30 min with intermittent shaking. Made up the volume with diluent-1 and mixed well. Diluted 5 ml this solution to 25 m1 with diluent-2. Injected this solution in to the HPLC system.

Linearity and Range

Linearity was assessed for the known impurity (Impurity-D) and for all three APIs serving as references for unknown impurity quantitation, using five concentration levels spanning 50% to 150% of the limit concentration. For area normalisation purposes, linearity was additionally evaluated across a broader concentration range spanning impurity-limit levels through assay-level concentrations. The peak area of the standards was plotted against the corresponding concentrations to create the calibration curve. From the calibration curve, the value of the correlation coefficient and equation of regression was determined.

Limit of Detection and Quantification

The LOD and LOQ were determined experimentally by serial dilution of standard solutions followed by replicate injections into the HPLC system, in accordance with approaches described in ICH Q2(R1). The concentrations corresponding to the lowest detectable and quantifiable responses were established experimentally and subsequently confirmed by repeat injections at these levels. Signal-to-noise (S/N) ratios were additionally evaluated as supportive evidence, where an S/N ratio of approximately 3:1 was considered for LOD and approximately 10:1 for LOQ. Precision at the LOQ level was also assessed to confirm reproducibility.

Accuracy

Accuracy was evaluated by a spike-recovery approach at three concentration levels (50%, 100%, and 150% of the limit concentration), with three preparations at each level. For Impurity-D (known impurity), recovery was determined by spiking the known impurity standard into the sample matrix. Mean recovery across all nine determinations was fell within the 80.0-120.0% acceptance range and a %RSD was not more than 10%.

Method Precision

Method precision was evaluated by preparing and analysing six independent sample solutions. The highest unknown impurity and total impurities across all preparations, with a %RSD of not more than 10.0%.

Intermediate precision was assessed by analysing six sample preparations spiked with Impurity-D at the 100% limit concentration level. The mean recovery of Impurity-D was within %RSD of not more than 10.0%.

Ruggedness

Ruggedness was evaluated by comparing the performance of the method under normal analytical conditions against results obtained under deliberately altered conditions, including a different column, instrument and a different analyst.

Solution Stability

Solution stability was evaluated by monitoring area counts of the standard and sample solutions over a 46-hour period under chilled (5°C) conditions, with measurements taken at the initial time point, 16 hours, 31 hours, and 46 hours. For standard solutions, the percentage deviation from the initial mean area count was not more than ±10.0%.

Results of experiment

RP-HPLC method development and optimization

The main aim of developing the chromatographic method was to obtain sufficient resolution of telmisartan, amlodipine, chlothalidone and Amlodipine Impurity-D in the presence of formulation excipients and possible degradation products. Inertsil ODS-2 reversed-phase column was chosen as the stationary phase because it is known to be very effective in separating structurally diverse pharmaceutical molecules. A binary mobile phase system was used to design a gradient elution programme, in which Mobile Phase A (buffer-rich) and Mobile Phase B (solvent-rich) were used to give the varying eluting strength required

to separate early-eluting polar components (including chlorthalidone) and the more lipophilic telmisartan. The 235 nm reading provided an appropriate compromise in detecting all three APIs as well as the known impurity, considering the UV absorption properties of each of them [10].

During method development, several chromatographic conditions including variations in mobile phase composition, buffer pH, gradient program, and column selection were evaluated to achieve satisfactory resolution of analytes and degradation products. The optimized chromatographic conditions presented in this manuscript were selected based on overall specificity, peak shape, and resolution performance.

The optimised process allowed a retention time of approximately 4.4, 13.0, 20.9 and 30.8 minutes with chlothalidone, Impurity -D, amlodipine and telmisartan respectively and a total run time of 60 minutes. The ratio between the retention time of Impurity-D and amlo-dipine was 0.62. The chromatographic system showed sufficient separation between all the nearby peaks with USP resolution values of 23.2, 16.9, and 18.3 of the Impurity-D/amlodipine, amlodipine/chlorthalidone and amlodipine/telmisartan peaks respectively. The maximum trailing factors on all the peaks were below 1.3 (Figure 1).

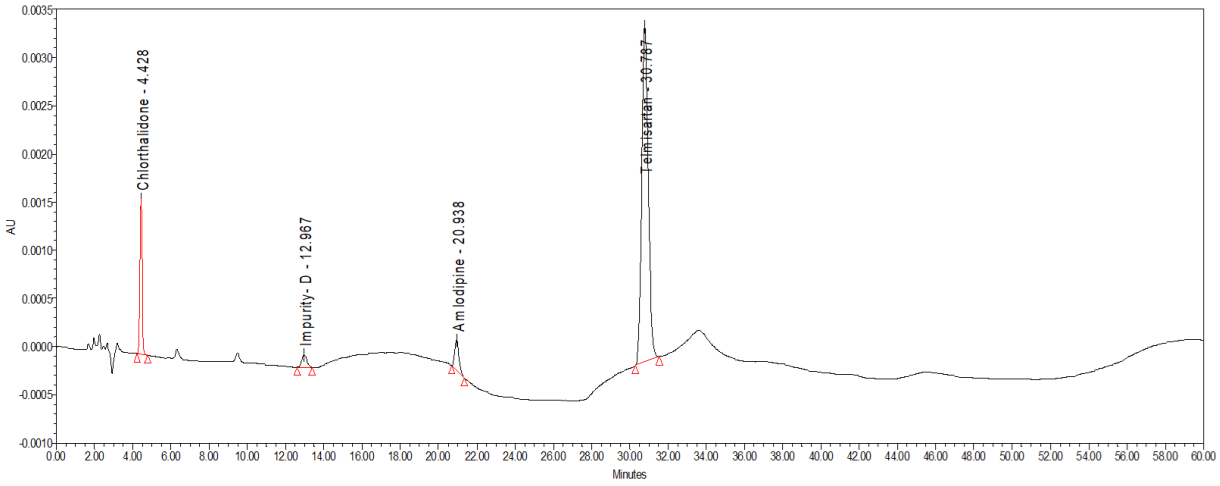


Figure 1. HPLC chromatogram of chlorthalidone, Impurity-D, amlodipine, and telmisartan

It is possible to explain the observed retention behavior using the pKa values and ionization state of the analytes in the applied chromatographic conditions [11]. Telmisartan (pKa 3.5) is mostly in the neutral state at moderately acidic pH and highly hydrophobic with the C18 stationary phase giving the longest retention time (approximately 30.8 min). Chlorthalidone (pKa 9.4) and amlodipine (pKa 8.6), on the other hand, are more polar and more ionized, and thus eluted sooner at 4.4 and 20.9 min, respectively. The gradient programme, having an increasing organic composition, ensures the further strengthening of the elution, which allows separating efficiently the earlier-eluting polar compound (chlorthalidone) and the more lipophilic telmisartan [11]. Therefore, a combination of the pH, gradient profile, and RP-C18 stationary phase can control ionization and hydrophobic interactions between the analyte and stationary phase, which result in the eventually observed retention order and chromatographic resolution.

Method validation results

System suitability test

System suitability was confirmed by performing six replicate injections of the standard preparation. The %RSD of area counts for Impurity-D, telmisartan, amlodipine, and chlorthalidone were 2.00%, 0.50%, 0.60%, and 2.40%, respectively, all well within the acceptance criterion of not more than (NMT) 10.0%. The tailing factor was NMT 2.0 for all peaks, confirming adequate column performance. The system suitability data are summarised in Table 2. The standard preparation chromatogram is presented in Figure 1.

Table 2. Results of system suitability test

Inj. No.	Impurity-D	Telmisartan	Amlodipine	Chlorthalidone	Limit

1	2415	83158	4908	13098	
2	2437	84022	5065	13315	
3	2510	83849	4889	13177	
4	2503	82835	5003	13120	
5	2544	83790	5064	13109	
6	2453	83596	4765	13097	
Mean	**2477**	**83542**	**4949**	**13153**	
± SD	**49.55**	**455.27**	**117.25**	**84.86**	
% RSD	**2.00**	**0.50**	**0.60**	**2.40**	**NMT 10.0 %**

Specificity

Excipient interference was assessed by preparing and injecting blank and placebo solutions. No peaks were observed at the retention times of telmisartan, amlodipine, chlorthalidone, or Impurity-D in either the blank or placebo chromatograms (Figure 2A and 2B), confirming that matrix components did not contribute to the analytical signal.

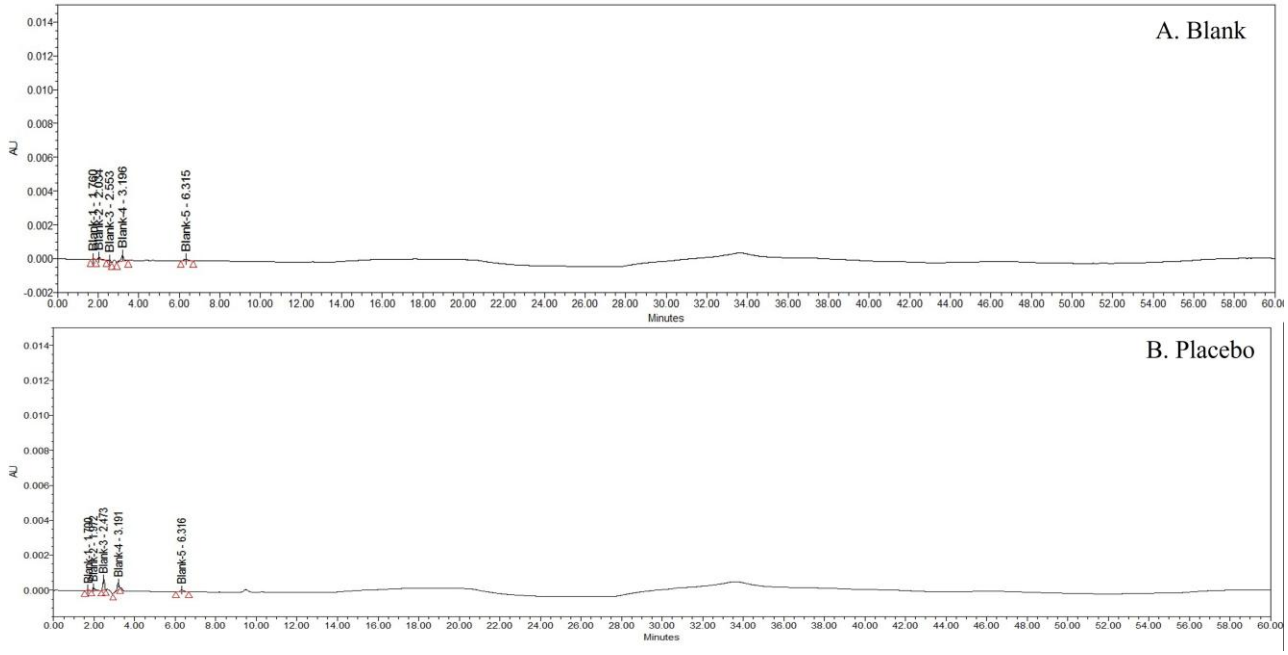


Figure 2. HPLC chromatograms of Blank and Placebo

Forced degradation

Forced degradation studies were conducted by subjecting the tablet preparation to the following stress conditions: acid degradation (0.1 N methanolic HCl, 60°C, 30 minutes), alkali degradation (0.1 N methanolic NaOH, 60°C, 30 minutes), peroxide degradation (30% H_2O_2, 60°C, 30 minutes), thermal degradation (water bath at 60°C, 30 minutes), and UV degradation (254 nm, 24 hours). Both samples were analyzed with the PDA detector, and the purity angle of both API peaks in each instance was determined to be smaller than the purity threshold in question, which verified the purity of the peak and the lack of degradation products that could co-elute with the API peak. Table 3 summarizes the degradation profiles and purity data of telmisartan, amlodipine and chlorthalidone in all the conditions of stress. Their representative chromatograms are shown in Supplementary Figure 1.

Telmisartan was the most stable chemical with the assay values between 98.08% (degraded by alkali) and 99.77% (as is sample). Chlorthalidone had the highest degradation rates in the presence of alkali and peroxide where it yielded an assay of 96.54% and 96.74 respectively. Amlodipine was also susceptible to oxidative stress with the minimal assay percentage of 88.69 per cent being taken up in peroxide degradation conditions. These observations validate the necessity of a stability-indicating technique and point to the specific vulnerability of amlodipine to oxidation degradation, which could be explained by the weakness of the dihydropyridine ring to oxidation. Amlodipine Impurity-D was evaluated as a specified known impurity using a qualified reference standard. Other impurities and degradation products observed during specificity and forced degradation studies were present below the identification threshold recommended by ICH Q3B(R2) for drug products with a maximum daily dose ≤ 2 g/day. Therefore additional structural characterization was considered outside the scope of the present validation study.

Table 3: Forced degradation results for Telmisartan, Chlorthalidone, Amlodipine (peak purity assessment using PDA showed purity angle less than purity threshold, indicating no evidence of co-eluting peaks)

Stress Condition	Telmisartan		Chlorthalidone		Amlodipine	
	% Assay	% Degradation	% Assay	% Degradation	% Assay	% Degradation
Sample (as is)	99.77	0.23	99.87	0.13	100.07	−0.07
Acid degradation (0.1 N MeOH–HCl, 60°C, 30 min)	99.55	0.45	99.10	0.90	98.07	1.93
Alkali degradation (0.1 N MeOH–NaOH, 60°C, 30 min)	98.08	1.92	96.54	3.46	96.68	3.32
Peroxide degradation (30% H_2O_2, 60°C, 30 min)	98.41	1.59	96.74	3.26	88.69	11.31
Thermal degradation (60°C, 30 min)	99.68	0.32	99.34	0.66	99.20	0.80
UV degradation (254 nm, 24 h)	99.40	0.60	99.28	0.72	99.39	0.61
% Degradation was calculated as (100 − % Assay). Corresponding impurity profiles were evaluated from chromatographic data to ensure no significant unaccounted peaks.						

The selected stress conditions generated measurable degradation and allowed adequate separation of degradation products from the analyte peaks without evidence of co-elution. Thermal degradation at 60°C for 30 min produced limited degradation, consistent with the relatively stable nature of the studied compounds under thermal conditions. The objective of forced degradation studies in analytical method development is to demonstrate specificity and degradation separation capability rather than to reproduce formal long-term or accelerated stability conditions.

Linearity and Range

For Impurity-D, linearity was established over the concentration range 0.100–0.301 µg/mL, yielding a slope of 13134.58, an intercept of -89.70, and a correlation coefficient (r^2) of 0.9996. Telmisartan demonstrated linearity from 0.803 to 2.409 µg/mL (r^2 = 0.9995), chlorthalidone from 0.252 to 0.756 µg/mL (r^2 = 0.9999), and amlodipine from 0.101 to 0.303 µg/mL (r^2 = 0.9997). All correlation coefficients exceeded the acceptance criterion of not less than (NLT) 0.99. For area normalization, linearity was confirmed over substantially wider concentration ranges for all three APIs ($r \geq 0.9999$). These findings confirm that the method maintains a linear relationship between detector response and analyte concentration across all relevant working ranges.

Limit of detection and quantification

LOD and LOQ values were experimentally established by serial dilution and confirmed through replicate analysis at concentrations near the detection and quantitation limits. Signal-to-noise ratios were additionally evaluated to support the

experimentally determined values. The LOQ solutions demonstrated S/N ratios greater than 10, while LOD solutions showed S/N ratios greater than 3, confirming adequate sensitivity of the method for impurity determination.

Accuracy

For Impurity-D (known impurity), recovery was determined by spiking the known impurity standard into the sample matrix. Mean recovery across all nine determinations was 105.50%, with a %RSD of 7.00% (NMT 10.0%), and all individual recoveries fell within the 80.0–120.0% acceptance range. Although the 50% level showed slightly elevated recoveries, the overall mean and %RSD were within acceptable limits, indicating satisfactory accuracy of the quantitative method for Impurity-D.

For unknown impurities, recovery studies were performed by spiking telmisartan, chlorthalidone, and amlodipine working reference standards into placebo at 50%, 100%, and 150% of limit concentration. Mean recoveries were 97.78% (%RSD 0.61%) for telmisartan, 98.57% (%RSD 1.36%) for chlorthalidone, and 101.20% (%RSD 3.45%) for amlodipine. All mean recoveries and %RSD values met the pre-defined acceptance criteria, confirming the accuracy of the method across the full working range. The resulting values are summarised in Table 4. (Recovery accuracy data)

Table 4: Summary of recovery accuracy for Impurity-D and unknown impurities (telmisartan, chlorthalidone, and amlodipine) evaluated at 50%, 100%, and 150% of the limit concentration

Spiking Level	**Impurity-D (%Recovery)**	**Unknown (Telmisartan) (%Recovery)**	**Unknown (Chlorthalidone) (%Recovery)**	**Unknown (Amlodipine) (%Recovery)**
50%	110.37	97.93	100.06	97.67
100%	107.77	98.34	98.64	103.27
150%	98.40	97.07	97.01	102.65
Mean	**105.50**	**97.78**	**98.57**	**101.20**
Standard Deviation	**7.36**	**0.59**	**1.35**	**3.49**
%RSD	**7.00**	**0.61**	**1.36**	**3.45**
Acceptance criteria: Recovery 80.0–120.0%; %RSD NMT 10.0%.				

Method Precision

Impurity-D was not detected (ND) in any of the six preparations of method precision study, reflecting the low background impurity content of the test batch. The highest unknown impurity was consistently 0.026–0.027% across all preparations, with a %RSD of 1.96% (NMT 10.0%). Total impurities ranged from 0.131% to 0.139%, with a mean of 0.136% and a %RSD of 1.97%, as shown in Table 5. These results confirm that the method exhibits satisfactory repeatability for the determination of related substances in the test formulation.

Table 5. Summary of Method Precision and Intermediate Precision Results

Parameter	**Statistic**	**Impurity-D (% Recovery)**	**Highest Unknown Impurity (%)**	**Total Impurities (%)**
Method Precision (n = 6)	Mean	ND	0.026	0.136
	SD	NA	0.00	0.00
	%RSD	NA	1.96	1.97
	Mean	93.60	–	–

Intermediate Precision (n = 6)	SD	2.82	–	–
	%RSD	3.01	–	–
Acceptance criteria: %RSD **NMT 10.0%**; recovery for Impurity-D **80–120%**. **Abbreviations:** ND = Not detected; NA = Not applicable.				

The mean recovery of Impurity-D was found 93.60% during intermediate precision study, with a standard deviation of 2.82 and a %RSD of 3.01% (NMT 10.0%), as presented in Table 5. All individual recoveries were within the 80.0-120.0% acceptance range. These findings demonstrate that the method delivers consistent performance under altered operating conditions such as a different analyst, confirming adequate intermediate precision.

Ruggedness

During ruggedness study the highest single impurity values were found 0.026% (original) and 0.027% (changed conditions), and total impurity values were 0.136% and 0.145%, respectively. The close agreement between results under original and changed conditions confirms that the method is rugged and not susceptible to minor variations in analyst, instrument or column identity.

Solution stability

For standard solutions, the percentage deviation from the initial mean area count was within ±10.0% for Impurity-D (+4.5% maximum), telmisartan (+1.5% maximum), chlorthalidone (+3.4% maximum), and amlodipine (–8.6% maximum). For sample solutions, area count deviations from initial values were within ±3.0% for telmisartan (+0.9%), chlorthalidone (+0.8%), and amlodipine (+0.8%) [12].

These findings confirm that all analytes are stable in analytical solution for up to 46 hours under chilling conditions. It is noted that the amlodipine standard solution showed the greatest deviation among the standard solutions (–8.6%), which, while within the acceptance limit, is consistent with the known susceptibility of the dihydropyridine scaffold to slow oxidative processes in solution and warrants monitoring during routine analysis.

CONCLUSIONS

In this study, a gradient reversed-phase HPLC method was developed and validated for the simultaneous determination of related substances of telmisartan, amlodipine, and chlorthalidone in a fixed-dose combination tablet formulation, with specific quantitative capability for Amlodipine Impurity-D. The method was validated in accordance with ICH Q2 (R1) guidelines and demonstrated satisfactory performance across all evaluated parameters.

Key findings are summarised as follows. Specificity was confirmed by the absence of excipient interference and the demonstration of peak homogeneity across all five stress degradation conditions, with purity angles consistently below purity thresholds for all three APIs. Linearity was established over the range 50%-150% of limit concentration for each analyte ($r \geq 0.9995$), as well as over a broader range for area normalisation purposes ($r \geq 0.9999$). Precision was satisfactory, with %RSD below 2.00% for system precision and below 1.97% for method precision. Accuracy was demonstrated with mean recoveries of 105.50% for Impurity-D and 97.78%–101.20% for unknown impurity analytes, all within the 80.0-120.0% acceptance range. Sensitivity was adequate, with LOD and LOQ values confirming detection and quantitation at sub-0.1 µg/mL levels for amlodipine and telmisartan. Ruggedness was established across two analysts and two columns, with negligible impact on quantitative results. Solution stability was confirmed for up to 46 hours under chilled conditions.

The main advantages of the developed method include its ability to simultaneously detect and quantify a known process-related impurity (Impurity-D) alongside unknown related substances of three structurally diverse APIs within a single chromatographic run of 60 minutes, its compatibility with widely available reversed-phase column chemistries and UV detection infrastructure, and its demonstrated stability-indicating capability under a comprehensive range of forced degradation conditions. The method accommodates both external standard quantitation for the known impurity and area normalisation for unknown impurity determination, providing a comprehensive analytical framework for related substance control.

The validated HPLC method demonstrated satisfactory specificity, precision, accuracy, linearity, ruggedness, and degradation separation capability for related substances determination in telmisartan, amlodipine, and chlorthalidone fixed-dose combination tablets. The method may be applied for routine impurity monitoring and quality control applications.

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Data curation, Formal analysis, Writing original draft. Conceptualization, Visualization, Methodology, Supervision, Writing – review & editing: Data curation, Writing, Reviewing & Editing.

Declaration of Use of AI Tools

In the preparation of this manuscript, the authors used generative AI tools, specifically QuillBot and Grammarly, to assist in the paraphrasing and enhancement of the language and readability of the text. These AI tools were applied under human oversight to improve the clarity and flow of the manuscript. The authors have thoroughly reviewed and edited the output generated by these tools to ensure the accuracy and integrity of the content.

The use of these AI tools was limited to the writing process and did not influence the research or data analysis. The authors remain fully responsible and accountable for the content of the manuscript and its final presentation.

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