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ORIGINAL ARTICLE

Estimation of Telmisartan, Amlodipine and ChlorthalidoneIn Bulk And Fixed Dose Combination Using Stability Indicating Reverse Phase High Pressure Liquid Chromatography

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ABSTRACT
A simple, accurate and precise stability indicating reverse phase high pressure liquid chromatography was developed and validated for estimation of Telmisartan, Amlodipine and Chlorthalidone in bulk and pharmaceutical dosage form. Chromatographic separation was carried out on Intersil ODS 3V C_{18}(250 mm × 4.6 mm, 5 µm) with mobile phase of gradient system comprising of Phosphate Buffer pH 3.3 (A) and Acetonitrile (B) at detection wavelength 237 nm with flow rate 1.0 mL/min and column temperature of 40^0 C. The t_R value for Telmisartan, Amlodipine and Chlorthalidone were found to be 42.39 ± 0.02 min, 27.78 ± 0.03 min and 19.62 ± 0.01 min, respectively. The method was linear over the concentration ranges 160-480 µg/ mL for Telmisartan, 10-30 µg/ mL for Amlodipine and 25-75 µg/ mL for Chlorthalidone. The LOD was 8.73 µg/ mL for Telmisartan, 1.17 µg/ mL for Amlodipine and 1.84 µg/ mL for Chlorthalidone. The LOQ was 26.44 µg/ mL for Telmisartan, 3.54 µg/ mL for Amlodipine and 5.58 µg/ mL for Chlorthalidone. Under the forced degradation conditions, Telmisartan degraded significantly under acidic and oxidative stress conditions, degraded moderately under alkaline, thermal and photolytic stress conditions; and showed negligible degradation under neutral hydrolysis condition. Amlodipine degraded significantly under acidic, alkaline and oxidative stress conditions, degraded moderately under thermal stress condition and degraded the least under neutral and photolytic stress conditions. Chlorthalidone degraded extensively under acid and alkaline stress conditions, degraded moderately under oxidative, thermal and photolytic stress conditions and degraded the least under neutral hydrolysis condition.

KEYWORDS: Telmisartan (TEL), Amlodipine (AML), Chlorthalidone (CHLO), Stability Indicating Assay Method (SIAM), Reverse Phase High Pressure Liquid chromatography (RP-HPLC), Fixed Dose Combination (FDC), Validation (ICH Q2 R1)

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INTRODUCTION

A fixed dose combination(FDC) of Telmisartan (TEL), Amlodipine (AML) and Chlorthalidone(CHLO)(80 +5 + 12.5 mg)is a very effective three –in –one pill with minimum risk profile for cardiovascular events and longer duration of action for treatment of essential hypertension. This FDC offers several advantages and is available under several brand names in India. Telmisartan {4' – [[4-methyl – 6- (1-methyl – 1 H – benzimidazol -2-yl) – 2- propyl – 1 H- benzimidazol -1- yl] methyl] biphenyl -2- carboxylic acid} (Figure 1) is an angiotensin II receptor antagonist which is used in treatment of hypertension. Angiotensin II receptor blockers bind to angiotensin II type I receptors and inhibits its effect on vascular smooth muscle which cause reduction in arterial blood pressure [1]. It is official in BP [2], USP [3], EP [4] and IP [5]. A literature survey revealed that many methods are reported for determination of TEL, either alone or in combination by spectrophotometric[6], HPLC[7], LC-MS/MS[8, 9], and HPTLC[10].

Amlodipine {AML; 3- ethyl – 5-methyl (4RS)-2- [(2- aminoethoxy) methyl]-4- (2-chlorophenyl) -6- methyl -1, 4 - dihydropyridine -3,5 –dicarboxylatesulfonate}(Figure 2**)** is official in BP[11], USP[12], IP[13] and EP[14]. It is one of the calcium channel blockers which includes nitrous oxide release from coronary micro vessels through a kinin- dependant mechanism and contribute positively to therapeutic action of ACE inhibitors[15]. A literature survey revealed many methods for estimation of AML, either alone or in combination via spectrophotometry[16], HPLC[17], HPTLC[18], and LC-MS/MS[19, 20, 21].

Chlorthalidone is chemically a 2- chloro – 5- (1-hydroxy -3- oxo-1- isoindolinyl) benzene sulfonamide **(Figure 3)**and have pharmacological action like thiazide like diuretic. It inhibits Na^+ K^+ $2Cl^-$ co-transport in ascending loop of henle. It is used as antihypertensive and in treatment of other cardiovascular diseases[22]. It is official in BP[23], USP[24], IP[25] and EP[26]. Literature review reveals that many spectrophotometric[27], HPLC[28, 29], HPTLC [30]and LC/MS-MS[31]methods are reported for determination of CHLO, either alone or in combination.

The aim of the present work was to develop a stability indicating RP-HPLC method for simultaneous estimation of TEL, AML, and CHLO in bulk and fixed dose combination. It is pertinent to note that, all the published methods enabled estimation of drugs in combination products containing two drugs only like AML and TEL tablets or TEL and CHLO tablets. Hence, to achieve this aim, RP-HPLC chromatographic conditions were optimized, forced degradation conditions were applied and the method was validated to establish selectivity with respect to degradation products.

MATERIAL AND METHODS

Apparatus

Chromatographic separation of drugs was performed on a Shimadzu HPLC instrument (LC_2010 CHT) [software LC Solution, equipped with photo diode array detector (SPD-M20A), Auto-sampler]. The system contains a quaternary gradient pump, auto sampler, column oven and a PDA detector. Software said above was used to record and integrate the chromatograms. The remaining instruments like An analytical balance (Acculab ALC-210.4, Huntingdon Valley, PA), Photostability chamber (TH-90S, Thermolab, Mumbai, India) , Hot air oven (TO-90S, Thermolab), pH meter (Thermo Electron Crop., Pune, India), Sonicator (EN 30 US, Enertech Fast Clean, Mumbai, India) were also used at different stages of development of validation of stability indicating assay method.

Chemicals and Reagents

The API of Telmisartan, Amlodipine Besylate and Chlorthalidone were provided as gift samples from Torrent Pharmaceuticals, West Coast Pharmaceuticals and IPCA Laboratories Ltd respectively. Tablets TELISTA TRIO 80 (manufactured by Lupin Laboratories)was purchased from local chemist store. All the solvents like Methanol, Acetonitrile and Potassium Dihydrogen Phosphate buffer were of HPLC grade from Finar Chemicals Ltd, Ahmedabad, India. Water was also of HPLC grade from RFCL limited, New Delhi, India. AR grade ortho phosphoric acid (OPA) and triethyl amine (TEA) were from SD Fine Chemicals Pvt. Ltd., Ahmedabad, India. All the chemical reagents were of analytical grade.

Preparation of Standard Stock Solution

The standard solution was prepared by weighing accurately 80 mgTEL, 5 mg AML and 12.5 mg CHLO individually and transferred into clean and dry 25 mL volumetric flask. Initially about 5 mL methanol was added to the flask respectively and sonicated. The volume was made upto the mark with the diluent to achieve 3200 µg/mL TEL, 200 µg/mL AML and 500 µg/mL CHLO.

Preparation of test solution

Twenty TELISTA TRIO 80 tablets were accurately weighed, their average weight was calculated. Amount of finely powdered tablet equivalent to 80 mg TEL, 5 mg AML and 12.5 mg CHLO were weighed and transferred into a 25 mL volumetric flask and the volume was adjusted to mark with methanol. The contents of the flask were sonicated for 30 min to dissolve the active ingredients completely. The solution was filtered through a Whatmanfilter paper no. 41. From this 1.0 mL aliquot was transferred into a 10 mL volumetric flask and the volume was made up with diluent. This test solution containing working concentrations of 320 µg/mL TEL, 20 µg/mL AML and 50 µg/mL CHLO respectively, in mixture was analyzed for assay determination.

Preparation of Buffer

Dissolve 2.72 g of Potassium dihydrogen phosphate and 3.0 ml of TEA into 1000 ml of water. Adjust pH of this solution 3.3 with OPA.

Standardized Chromatographic conditions

The drug analytes and degradation products were well separated with Intersil ODS 3V C_{18} (250 mm × 4.6 mm, 5 µm) as a stationary phase and gradient system of Potassium Dihydrogen Phosphate Buffer with pH 3.3 (A) and Acetonitrile (B) in gradient ratio of (0 min 85:15 %V/V, 10 min 85:15 % V/V, 15 min 70:30 %

V/V, 20 min 70:30 % V/V, 25 min 60:40 % V/V, 30 min 60:40 % V/V, 35 min 55:45 % V/V, 40 min 50:50 % V/V, 45 min 45:55 % V/V, 50 min 40:60 % V/V, 55 min 85:15 % V/V, 60 min 85:15 % V/V) were used.. The flow rate was adjusted to 1 mL/min. For determination, 237 nm was the best detection wavelength selected. Column temperature was set to 40^0 C. Total run time was 60 min. Injection volume was 20 µL. Combination of water, Acetonitrile and Ortho phosphoric acid in ratio of 500:500:1 was used as a diluent.

METHOD VALIDATION

The proposed method was validated as per ICH guidelines Q2 R1.

System suitability test parameters

System suitability tests are used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters used in this test were the chromatographic peak resolution (>2), theoretical plate number (>2000) and tailing factor (<1.8). The repeatability of these parameters was checked by injecting six replicates of standard solution of TEL, AML and CHLO.

Linearity

From the standard stock solution containing 3200 µg/mL TEL, 200 µg/mL AML and 500 µg/mL CHLO, aliquots of 0.5 mL, 0.8 mL, 1 mL, 1.1 mL, 1.2 mL and 1.5 mL were transferred in cleaned and dried 10 mL volumetric flasks respectively. The volume was made up to the mark with diluent. This yielded solution of 160, 256, 320, 352, 384 and 480 µg/mL of TEL; 10, 16, 20, 22, 24 and 30 µg/mL AML; and 25, 40, 50, 55, 60 and 75 µg/mL of CHLO respectively. An injection volume of 20 µL of each solution was injected under operating chromatographic condition. Six replicates of each concentration were performed and then calibration plots were determined by linear least – squares regression.The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

Repeatability was determined by applying six replicates of test solution (320 µg/mL TEL,20 µg/mLAML and 50 µg/mL CHLO). The intraday and interday precisions were determined by responses of six replicates on same and different days for the test concentration. The results were reported in terms of % RSD.

Accuracy

Recovery study was carried out by standard addition method where known amount of standard concentration at 50%, 100% and 150% of the test solution were spiked in the test solution in triplicate. The amount of drugs was estimated by substituting values in regression equation. The % RSD of the recovery was calculated.

LOD and LOQ

The LOD and LOQ of the developed method were calculated from the calibration curve using equations, LOD= 3.3 ×ϭ/S and LOQ = 10 ×ϭ/S where ϭ is the standard deviation of y- intercept and S is the slope of the curve.

Robustness

By introducing small changes in the Flow rate (±0.1 mL), column temperature (± 5^0 C), wavelength (± 5 nm) and pH of buffer solution in mobile phase (± 0.2); the effects on the results were determined. One factor at a time was changed and the effect on peak area of the drug was studied. Robustness of the method was done on single level for six replicates and %RSD was calculated.

Specificity

The specificity of the method was checked by the purity of analyte peaks and also forced degradation studies.

FORCED DEGRADATION STUDIES

Force degradation studies was intended to ensure the effective separation of TEL, AML and CHLO from their degradation products which are generated under different stress conditions like acid and alkaline hydrolysis, neutral hydrolysis, oxidative degradation, thermal and photolytic degradation.

Acid Hydrolysis

Accurately weighed 80 mg TEL, 5 mg AML and 12.5 mg CHLO were transferred in a 10 mL volumetric flask individually and in combination. To this were added 5 mL methanol and 5 mL of 0.1 N HCl and kept at 80^0 C for 3 h. From that solution 0.4 mL was transferred into 10 mL volumetric flask, neutralized with 0.2 N NaOH and diluted to mark with diluent. This corresponds to 320 µg/ mL TEL, 20 µg/ mL AML and 50 µg/ mL CHLOwere injected under operating condition.

Alkaline Hydrolysis

Accurately weighed 80 mg TEL, 5 mg AML and 12.5 mg CHLO were transferred in 10 mL volumetric flask individually and in combination. To this were added 5 mL methanol and 5 mL of 0.1 N NaOH and it was kept at 80^0 C for 3 h. From that solution 0.4 mL was transferred into 10 mL volumetric flask, neutralized

with 0.1 N HCl and diluted to mark with diluent. This corresponds to 320 µg/ mL TEL, 20 µg/ mL AML and 50 µg/ mL CHLO were injected under operating condition.

Elevated Temperature and Humidity stress condition (Neutral Hydrolysis)

Accurately weighed 80 mg TEL, 5mg AML and 12.5 mg CHLO were transferred in 10 mL volumetric flask individually and in combination. To this were added 5 mL methanol and 5 mL of water and it was kept at 80^0 C for 3 h. From that solution 0.4 mL was transferred into 10 mL volumetric flask and diluted to mark with diluent. This corresponds to 320 µg/ mL TEL, 20 µg/ mL AML and 50 µg/ mL CHLO were injected under operating condition.

Oxidative Degradation

Accurately weighed 80 mg TEL, 5 mg AML and 12.5 mg CHLO were transferred in 10 mL volumetric flask individually and in combination. To this were added 5 mL methanol and 5 mL 3% H_2O_2 and it was kept at room temperature for 12 h. From that solution 0.4 mL was transferred into 10 mL volumetric flask and diluted to mark with diluent. This corresponds to 320 µg/ mL TEL, 20 µg/ mL AML and 50 µg/ mL CHLO were injected under operating condition.

Thermal Degradation

Accurately weighed quantity of 80 mg TEL, 5 mg AML and 12.5 mg CHLO were kept individually and in combination in petridish. Those were kept at 80^0 C for 6 h. After that those were dissolved in 10 mL methanol. From this solution 0.4 mL was transferred into 10 mL volumetric flask and diluted to mark with diluent. This corresponds to 320 µg/ mL TEL, 20 µg/ mL AML and 50 µg/ mL CHLO were injected under operating condition. Same condition was applied to formulation and solutions were prepared with the above mentioned concentrations according to the dilution scheme.

Photolytic Degradation

Accurately weighed quantity of 80 mg TEL, 5 mg AML and 12.5 mg CHLO were kept individually and in combination in petridish. It was exposed in photo stability chamber (TH-90S, Thermo lab, Mumbai, India) in UV light at 254 nm for 24 h to get 200 watt hours / m^2 intensity. After that those were dissolved in 10 mL methanol. This corresponds to 320 µg/ mL TEL, 20 µg/ mL AML and 50 µg/ mL CHLO were injected under operating condition.Same condition was applied to formulation and solutions were prepared with the above mentioned concentrations according to the dilution scheme.

RESULTS AND DISCUSSION

Optimized chromatographic condition

For separation of TEL, AML and CHLO from their potential degradation products, different mobile phases with different solvents in different ratio were tried like (1) Methanol : Buffer (pH 6.0)(60:40 %V/V) (2) Acetonitrile: Methanol: Buffer (pH 4.5)(40:40:20 % V/V/V) (3) Acetonitrile: Buffer (pH 3.5) (30:70 % V/V/V/V) (4) Buffer (pH 3.3) with TEA : Acetonitrile in a gradient run (0 min 90:10 %V/V, 50 min 40:60 % V/V, 60 min 40:60 % V/V, 62 min 90:10 % V/V, 80 min 90:10 % V/V). TEA was added to reduce the tailing observed due to interaction of basic function group with acidic silanol sites. TEL showed adequate retention in the column due to its hydrophobic nature and unionized mature of the acidic drug at pH 3.3 AML and CHLO showed moderate retention at pH 3.3. Finally, gradient system comprising Potassium Dihydrogen Phosphate Buffer with pH 3.3 (A) and Acetonitrile (B) in ratio of (0 min 85:15 %V/V, 10 min 85:15 % V/V, 15 min 70:30 % V/V , 20 min 70:30 % V/V, 25 min 60:40 % V/V, 30 min 60:40 % V/V, 35 min 55:45 % V/V, 40 min 50:50 % V/V, 45 min 45:55 % V/V, 50 min 40:60 % V/V, 55 min 85:15 % V/V, 60 min 85:15 % V/V)showed well resolved peaks. The drugs were resolved with $t_R$of 42.39 ± 0.02 min, 27.78 ± 0.03 min and 19.62 ± 0.01 min for TEL, AML and CHLO, respectively. Determination of all three drugs was done at wavelength 237 nm with column temperature 40^0 C and adjusted flow rate 1.0 mL/min. Injection Volume was set 20 µL for a good peak shape.

System Suitability Test Parameters

The result of system suitability test like number of theoretical plates, Tailing factor, resolution and %RSD of repeatability were found within the acceptable range which indicates that the system was suitable for the intended analysis. **(Table 1, Figure 4)**

Linearity

The method was found linear over the concentration range of 160-480 µg/ mL, 10-30 µg/ mL and 25-75 µg/ mL for TEL, AML and CHLO, respectively. The calibration curve obtained by the least square regression analysis between average peak area and concentration showed linear relationship with a correlation coefficient of 1.0, 0.9991 and 0.9991 for TEL, AML and CHLO respectively. The linear regression equation were y = 81,518.48x + 2237326.47, y = 41777x + 27473 and y = 52534x - 54129 for TEL, AML and CHLO respectively.**(Table 2, Figure 5, Figure 6, Figure 7)**

Precision

The repeatability was found to be satisfactory with % RSD of 1.35 for TEL, 0.82 for AML and 1.02 for CHLO. The Intraday Precision was found to be satisfactory with %RSD of 1.09 for TEL, 0.90 for AML and 0.85 for CHLO. The Interday precision was found to be satisfactory with %RSD 0.62 for TEL, 1.29 for AML and 0.89 for CHLO. Hence, confirming precision of the developed method.**(Table 3)**

Accuracy

The accuracy of the developed method was established by standard addition method by adding known standard concentration solutions to the pre- analyzed samples. Recoveries were in between 99.13 – 101.55 % for TEL,99.15 – 100.96 % for AML and 98.45 - 100.39% for CHLO which was in accordance with ICH guidelines which proves method to be accurate.**(Table 4)**

LOD and LOQ

The LOD calculated by formulae was found to be 8.73µg/ mL for TEL, 1.17 µg/ mL for AML and 1.84 µg/ mL for CHLO. The LOQ calculated by formulae was found to be 26.44 µg/ mL for TEL, 3.54 µg/ mL for AML and 5.58 µg/ mL for CHLO.

Robustness

Slight change in the chromatographic condition of the developed method like small changes in the Flow rate (±0.1 mL), column temperature (± 5^0 C), wavelength (± 5 nm) and pH of buffer solution in mobile phase (± 0.2) didn't affect the result significantly. The % RSD values were found below 2 indicated the method to be robust. **(Table 5)**

Analysis of the marketed formulation

The developed method was applied to marketed tablet preparation. The Assay results of TEL, AML and CHLO were found to be99.94 ± 0.53, 100.61 ± 1.69 and 101.26 ± 1.54, respectively of the labeled amount. **(Table 6, Figure 8)**

Specificity

The method was found to be specific with respect to excipients in a test sample as no interfering peaks were found. The specificity with respect to potential degradation products was established by performing force degradation and analyzing the samples. The specificity was further confirmed by peak purity (match factor) of 1000, 1000 and 1000 for TEL, AML and CHLO respectively for analyte peaks in test sample and stress samples.

Forced degradation studies

The result of forced degradation studies are summarized in table. Under the optimized chromatographic conditions, the analyte drug peaks were well resolved from potential degradation products and the percent degradation was calculated by comparing peak area with standard preparation.

During stress degradation experiments, it was observed that AML degraded significantly under acidic, alkaline and oxidative stress conditions, degraded moderately under thermal stress condition and degraded the least under neutral and photolytic stress conditions. In all 9 degradation products were found in different stress conditions.From the literature review, two reliable published papers indicate formation of dehydro derivative (impurity D as per EP) under acid hydrolysis and oxidative stress conditions. The present study also found one degradation product corresponding to t_R of 21.127 min under acid hydrolysis and oxidative stress conditions. This can therefore be attributed to dehydro derivative. The referred article also reported formation of one degradation product due to acetyl group under alkaline stress conditions. The present study also found other two degradation products corresponding to t_R 4.25 min and 6.27 min, one of which could be due to acetyl group containing degradation product. Another article of LC/MS-MS revealed some common degradation products under acidic and alkaline hydrolysis like AM1, AM6 and AM9. The present study also found one degradation product common for acid and alkali hydrolysis corresponding to t_R24.93 min which can be attributed to one of the above.

Chlorthalidone degraded extensively under acid and alkaline stress conditions, degraded moderately under oxidative, thermal and photolytic stress conditions and degraded the least under neutral hydrolysis condition.In all four degradation products were formed in different forced degradation conditions. One research paper reported two hydrolysis product of CHLO as (4' – chloro -3' – sulfamoyl – 2-benzophenone carboxylic acid) and (2- chloro-5 – (1- methoxy -3- oxo-1-isoindolinyl)benzenesulfonamide). The present study found two degradation products under acidic hydrolysis and alkaline hydrolysis corresponding to t_R of 23.78 min and 30.89 min which could be attributed to two hydrolysis products referred above.

Telmisartan degraded significantly under acidic and oxidative stress conditions, degraded moderately under alkaline, thermal and photolytic stress conditions; and showed negligible degradation under neutral hydrolysis condition. In allfour degradation products were formed under different degradation

conditions. One UHPLC-MS/MS study has reported two acid hydrolysis degradation products under stress conditions corresponding to m/z ration 529 (methyl ester) and 487 (with cleavage of propyl side chain). The present study also revealed formation of two degradation products corresponding to t_R of 34.063 min and 36.015 min and this could be attributed to be two hydrolysis products reported above. The drug is known to be stable under alkaline stress condition and it gives high dissolution under alkaline condition. No degradation product has been reported in the reference cited above and likewise no degradation product was found in our study. In the same way, no degradation product has been reported in the thermal stress condition and no degradation product was found in our study. The published paper has reported three degradation products corresponding to m/z ratio of 547, 531 and 429 under oxidative stress condition. The present study revealed two degradation products under oxidative conditions which compare well with the reported state.

The method is also deemed to be specific with respect to potential degradation products as all the observed degradation products were adequately resolved from all three analyte peaks. The impurities were found under acidic degradation, alkaline hydrolysis, oxidative degradation and thermal stress conditions. The sample tablet was exposed to thermal and photolytic degradation conditions as per ICH Q1A R2 guidelines and the results were comparable to standard mixture. The method was therefore considered to be stability indicating for tablet solid dosage form. **(Table 7, Table 8, Figure 9-14)**

It can be concluded that the proposed RP-HPLC method is precise, specific, linear and accurate for the estimation of TEL, AML and CHLO in pharmaceutical dosage form without interference from the excipients and potential degradation products in various stress conditions like acid hydrolysis, alkaline hydrolysis, neutral hydrolysis, oxidative, thermal and photolytic stress conditions. All the three drugs showed significant degradation under acidic, alkaline and oxidative stress condition with the exception of TEL under alkaline condition. All the three drugs showed moderate degradation under thermal and photolytic stress conditions and least degradation under neutral hydrolysis (elevated temperature and humidity). The results of stress testing were critically analyzed to establish correlation with degradation products reported in published literature. The developed method is validated as per ICH guidelines. The results showed the suitability of developed method for degradation kinetic studies and stability studies of the fixed dose combination. A method can also be suitably applied for estimation of FDC containing two drugs like TEL and CHLO; and AML with CHLO. The use of the method can be extended for estimation of one or more degradation products as all the degradation products were found adequately separated from one another and also from drug peaks.

Table 1: System Suitability Parameters for TEL, AML and CHLO

SR. No	Parameter	TEL	AML	CHLO
1	Retention time	42.39 ± 0.02 min	27.78 ± 0.03 min	19.62 ± 0.01 min
2	Theoretical factor	131703.33 ± 980.29	296445.17 ± 2825.33	131703 ± 980.286
3	Tailing Factor	0.90 ± 0.01	1.215 ± 0.01	1.135 ± 0.01
4	Resolution	43.898 ± 0.53	39.88 ± 0.35	--

Table 2: Linearity Data of TEL, AML and CHLO

S. No	TEL			AML			CHLO		
	Conc (µg/ mL)	Peak Area*± SD	%RSD	Conc (µg/ mL)	Peak Area* ± SD	%RSD	Conc (µg/ mL)	Peak Area* ± SD	%RSD
1	160	15096883 ± 168848.40	1.12	10	440061 ± 5762.73	1.31	25	1278338 ± 13464.24	1.05
2	256	22995286 ± 233103	1.01	16	690544.2 ± 10446.18	1.51	40	2035802 ± 24080.18	1.18
3	320	28591332 ± 310423.7	1.09	20	874816.80 ± 7467.402	0.85	50	2536975 ± 21485.50	0.85
4	352	31197231 ± 257201.7	0.82	22	956252.30 ± 4905.68	0.51	55	2867396 ± 20350.90	0.71
5	384	33609543 ± 181670.2	0.54	24	1030264 ± 15773.65	1.53	60	3066233 ± 55213.32	1.80
6	480	41057761 ± 227683.3	0.55	30	1266715 ± 11895.93	0.94	75	3906713 ± 44603.79	1.14

***** Average of six determinations

Table 3: Repeatability, Intraday and Interday Precision of TEL, AML and CHLO

Parameter	TEL			AML			CHLO		
	Conc (µg/ mL)	Peak Area* ± SD	%RSD	Conc (µg/ mL)	Peak Area* ± SD	%RSD	Conc (µg/ mL)	Peak Area* ± SD	%RSD
Repeatability	320	28242708.17 ± 380692.24	1.35	20	881503 ± 7241.75	0.82	50	2567877.167 ± 26091.36	1.02
Intraday Precision	320	28591332.33 ± 310423.67	1.09	20	866816.83 ± 7820.82	0.90	50	2536975.17 ± 21485.50	0.85
Interday Precision	320	28512663 ± 175614.73	0.62	20	857230.67 ± 11034.60	1.29	50	2560855.83 ± 22837.43	0.89

*Average of six determinations

Table 4: Recovery Study data of TEL, AML and CHLO

Drug	Amount of Test Solution (µg/mL)	Amount of Std added (µg/mL)	Peak area* ± SD	Total Amount Found (µg/mL)	Amount Recovered (µg/mL)	% Recovery	% RSD
TEL	160	0	15166706.33 ± 133894.96	158.607	0	--	--
	160	80	21902915 ± 112516.51	241.241	81.24	101.55	1.70
	160	160	28495635.67 ± 250105.47	322.12	162.12	101.322	1.89
	160	240	35043361.67 ± 277958.40	402.44	242.44	101.02	1.41
AML	10	0	445352 ± 3315.83	10	0	--	--
	10	5	652362 ± 3022.34	14.96	4.96	99.15	1.46
	10	10	862175 ± 7252.79	19.98	9.98	99.8	1.74
	10	15	1077938 ± 11614.10	25.14	15.14	100.96	1.84
CHLO	25	0	1264394.67 ± 15122.12	25.1	0	--	--
	25	12.5	2013998.67 ± 4565.91	37.31	12.31	98.45	0.70
	25	25	2678203.33 ± 21190.17	49.95	24.95	99.8	1.62
	25	37.5	3322595 ± 21194.39	62.22	37.22	99.24	1.08

*Average of three determinations

Table 5: Robustness Study of TEL, AML and CHLO

Condition	Variation	%Assay* ± SD (n=6)			% RSD#		
		TEL	AML	CHLO	TEL	AML	CHLO
Normal Condition	NA	99.94 ± 0.53	100.61 ± 1.69	101.26 ± 1.54	1.14	1.22	1.24
Flow rate (± 0.1 mL/min)	1.1 mL/min	98.96 ± 0.93	99.78 ± 1.52	100.84 ± 1.45			
	0.9 mL/min	99.69 ± 1.46	101.11 ± 0.90	99.82 ± 1.00			
Column temperature (± 5^0 C)	45^0 C	101.03 ± 1.18	100.45 ± 0.93	98.64 ± 0.83			
	50^0 C	100.73 ± 0.67	99.31 ± 1.33	99.55 ± 0.87			
Wavelength (± 5 nm)	242 nm	100.25 ± 1.45	98.12 ± 1.31	100.45 ± 1.12			
	232 nm	99.95 ± 1.23	101.25 ± 0.89	101.64 ± 1.49			
pH (±0.2)	3.5	101.95 ± 1.26	100.91 ± 1.20	99.81 ± 1.37			
	3.1	98.65 ± 1.34	99.82 ± 1.47	100.35 ± 1.75			

*Average of six determinations
#RSD of original and modified conditions

Table 6: Analysis of Marketed Formulation of TEL, AML and CHLO

Drug	Amount of Drug (mg)		%Label claimed* ± SD	% RSD
	Labelled	Estimated		
TEL	80	79.95	99.94 ± 0.53	0.53
AML	5	5.03	100.61 ± 1.69	1.69
CHLO	12.5	12.66	101.26 ± 1.54	1.52

* Average of six determinations

Table 7: Summary of Forced Degradation Study of TEL, AML and CHLO in Mixture

Degradation condition	t_R of Analyte (min)			Match Factor*			No. of degradation peaks	t_R of Degradation peak (min)	%Degradation		
	TEL	AML	CHLO	TEL	AML	CHLO			TEL	AML	CHLO
0.1 N HCl at 80^0 C, 3 h	42.403	27.870	19.587	993	1000	1000	9	19.20, 21.20, 23.78, 24.93, 25.10, 30.89, 34.06, 36.06, 37.61	19.50	24.36	23.54
0.1 N NaOH at 80^0 C, 3 h	41.707	27.873	19.593	998	1000	1000	5	4.25, 6.27, 23.78, 24.48, 30.89	10.11	24.86	25.25
Water, 80^0 C, 3h	42.397	27.843	19.540	1000	1000	1000	2	31.423, 34.063	12.64	3.34	4.67
3% H_2O_2, RT, 12 h	42.380	27.840	19.537	1000	1000	1000	5	15.25, 20.94, 21.97, 34.063, 45.24	19.38	14.89	29.80
Thermal at 80^0 C, 6 h	42.397	27.850	19.547	1000	1000	1000	2	7.077, 11.593	7.49	12.46	8.90
UV light, 254 nm, 24 h	42.387	27.847	19.547	1000	1000	1000	--	--	7.85	10.35	6.39

*Match factor between 990 to 1000 shows identical peaks

Table 8: Summary of Forced Degradation Study in Pharmaceutical Dosage Form

Degradation condition	t_R of Analyte (min)			Match Factor*			No. of degradation peaks	t_R of Degradation Peak (min)	%Degradation		
	TEL	AML	CHLO	TEL	AML	CHLO			TEL	AML	CHLO
Thermal at 80^0 C, 6 h	42.393	27.843	19.543	1000	1000	1000	2	7.102, 11.612	8.42	11.29	9.31
UV light, 254 nm, 24 h	42.390	27.847	19.543	1000	1000	1000	--	--	6.38	9.24	5.42

*Match factor between 990 to 1000 shows identical peaks

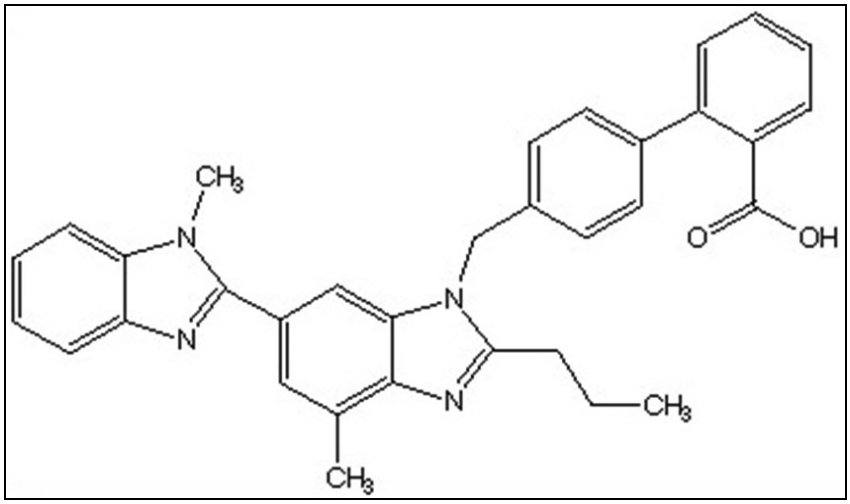

Figure 1: Structure of Telmisartan

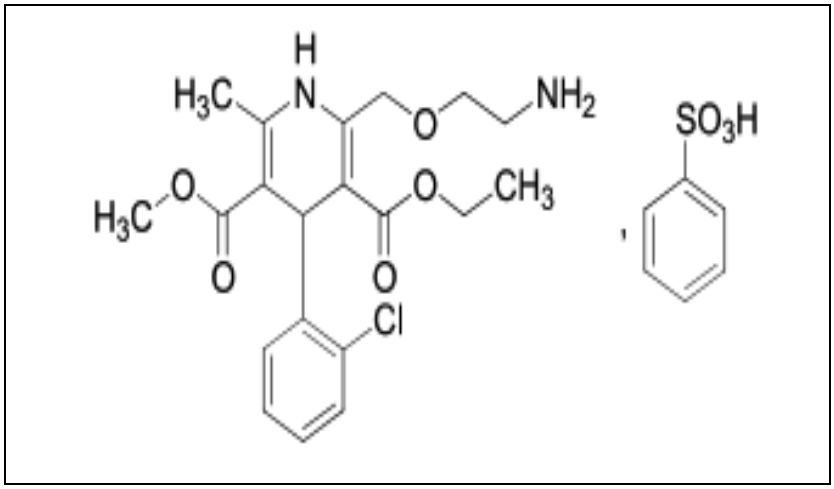

Figure 2: Structure of Amlodipine Besylate

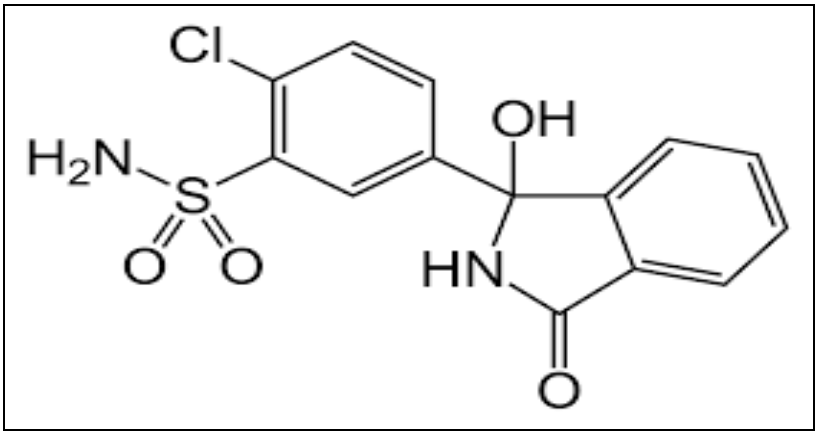

Figure 3: Structure of Chlorthalidone

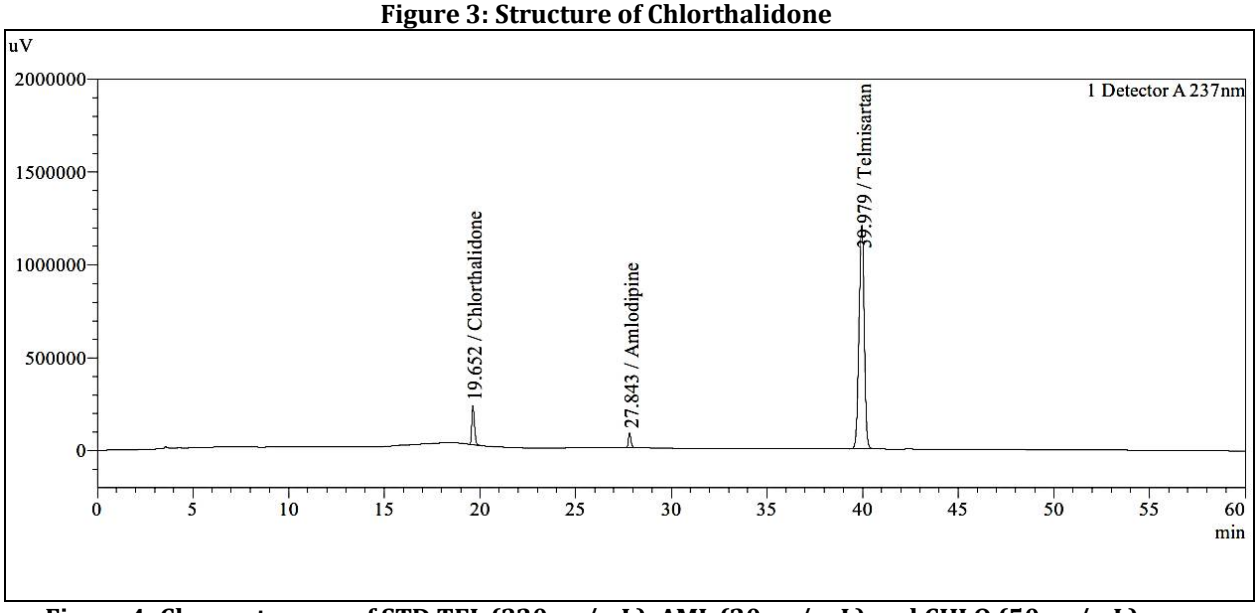

Figure 4: Chromatogram of STD TEL (320 µg/mL), AML (20 µg/ mL) and CHLO (50 µg/mL)

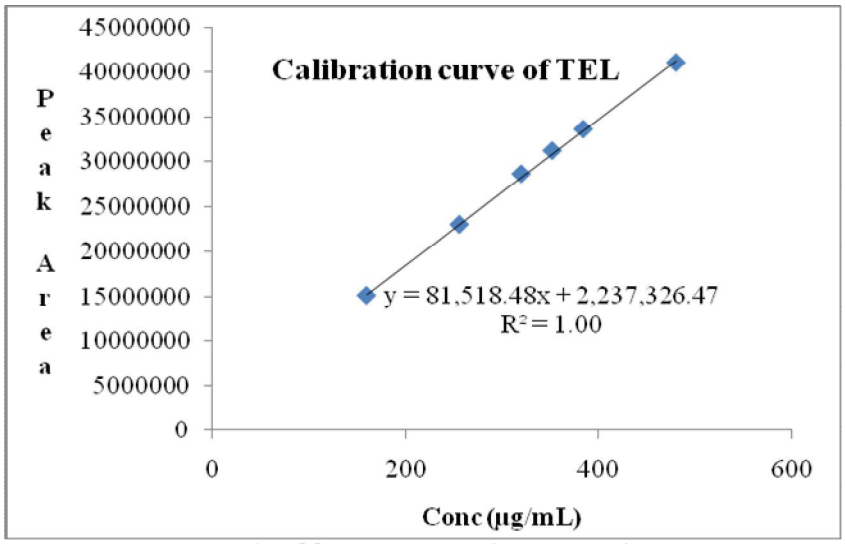

Figure 5: Calibration Curve of Linearity of TEL

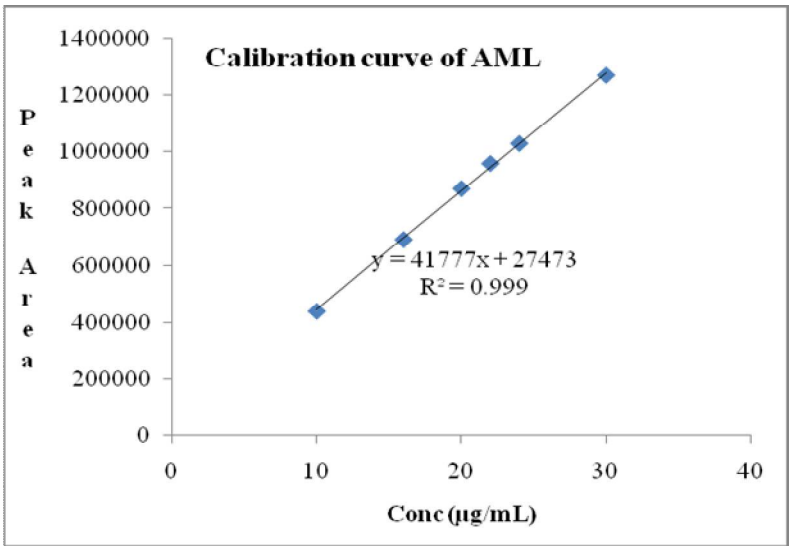

Figure 6: Calibration Curve of Linearity of AML

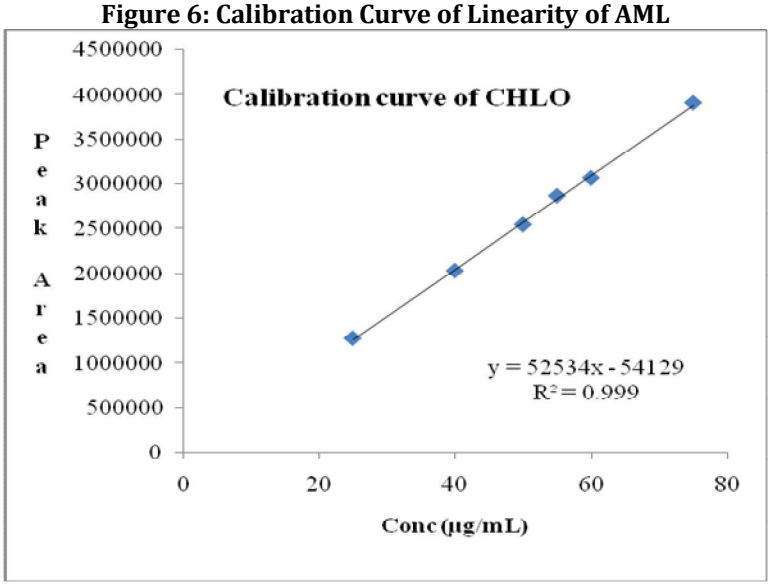

Figure 7: Calibration Curve of Linearity of CHLO

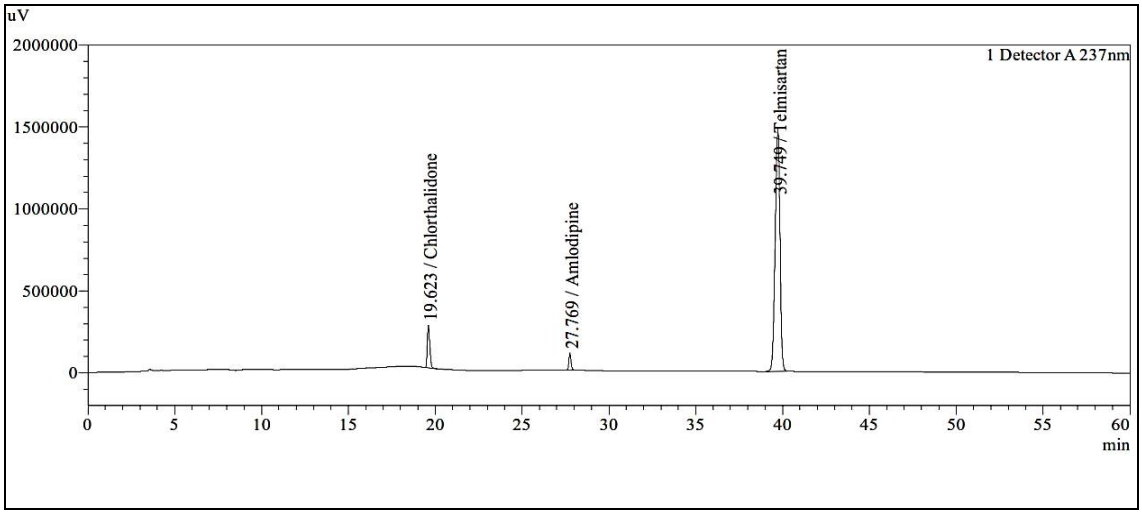

Figure 8: Chromatogram of Test Solution containing TEL (320 µg/ mL) , AML (20 µg/ mL) and CHLO (50 µg/ mL)

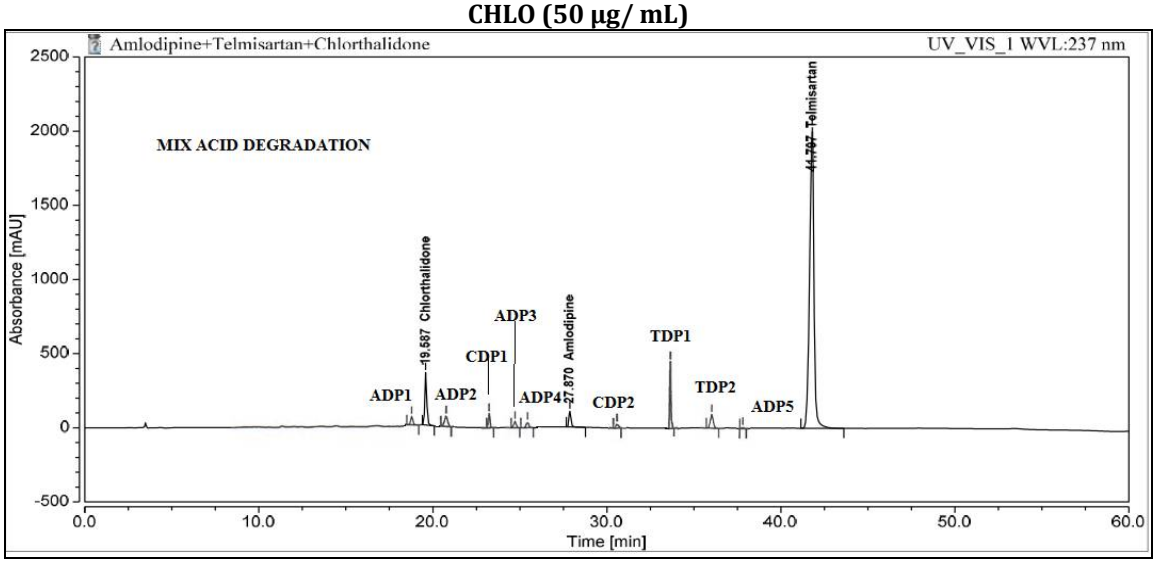

Figure 9: Chromatogram of acid hydrolysis of mixture of TEL, AML and CHLO

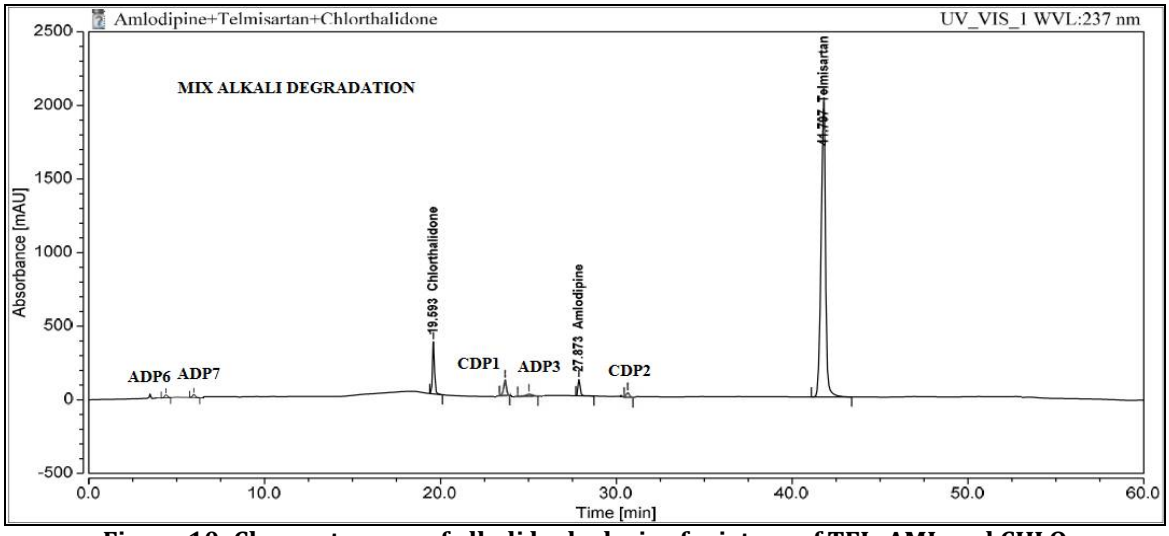

Figure 10: Chromatogram of alkali hydrolysis of mixture of TEL, AML and CHLO

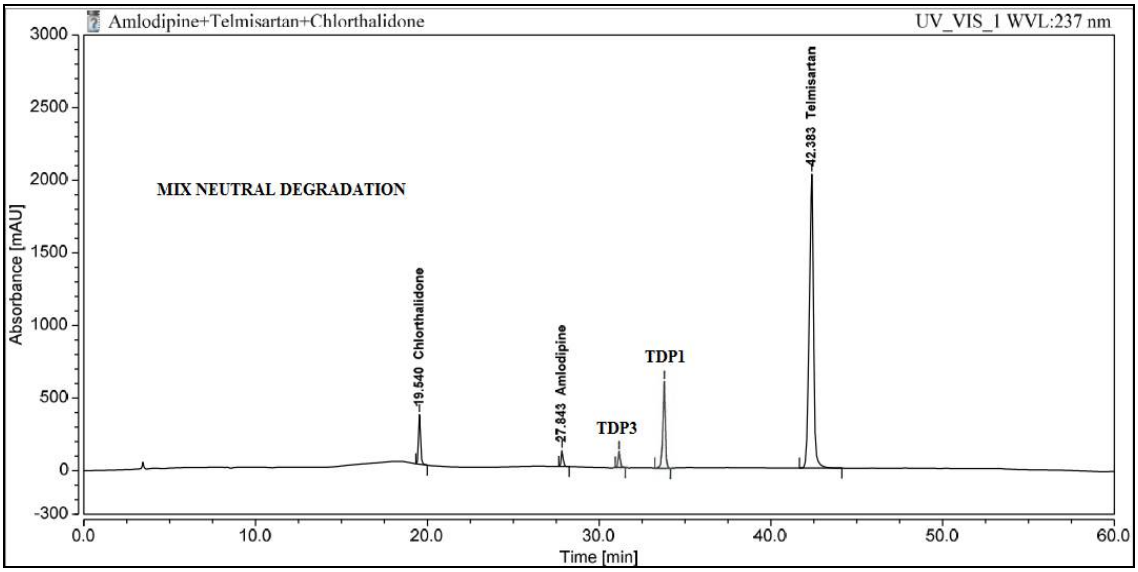

Figure 11: Chromatogram of neutral hydrolysis of mixture of TEL, AML and CHLO

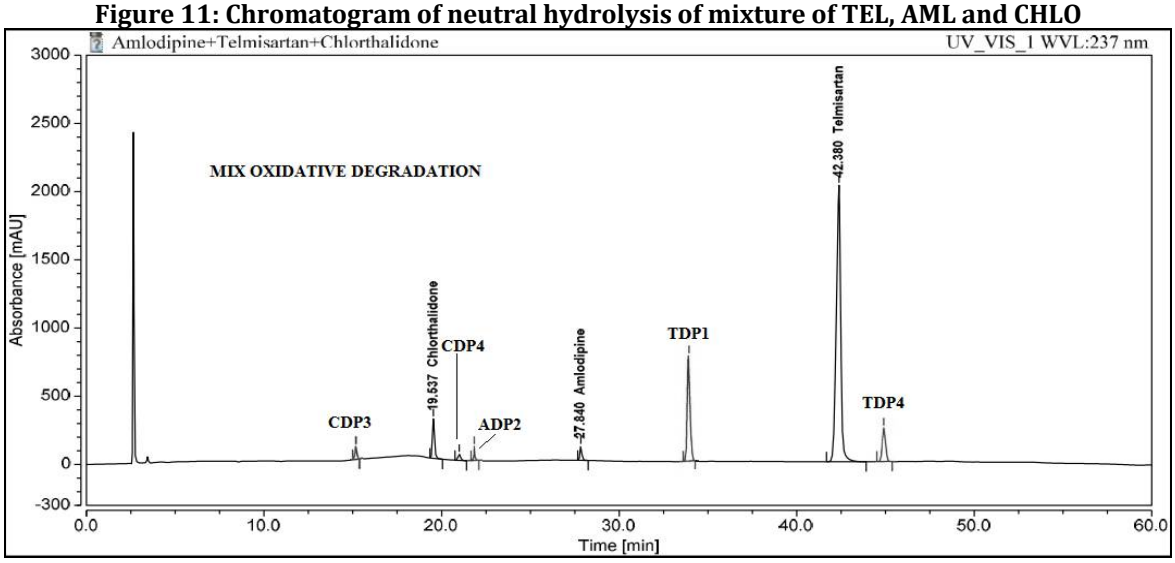

Figure 12: Chromatogram of oxidative degradation of mixture of TEL, AML and CHLO

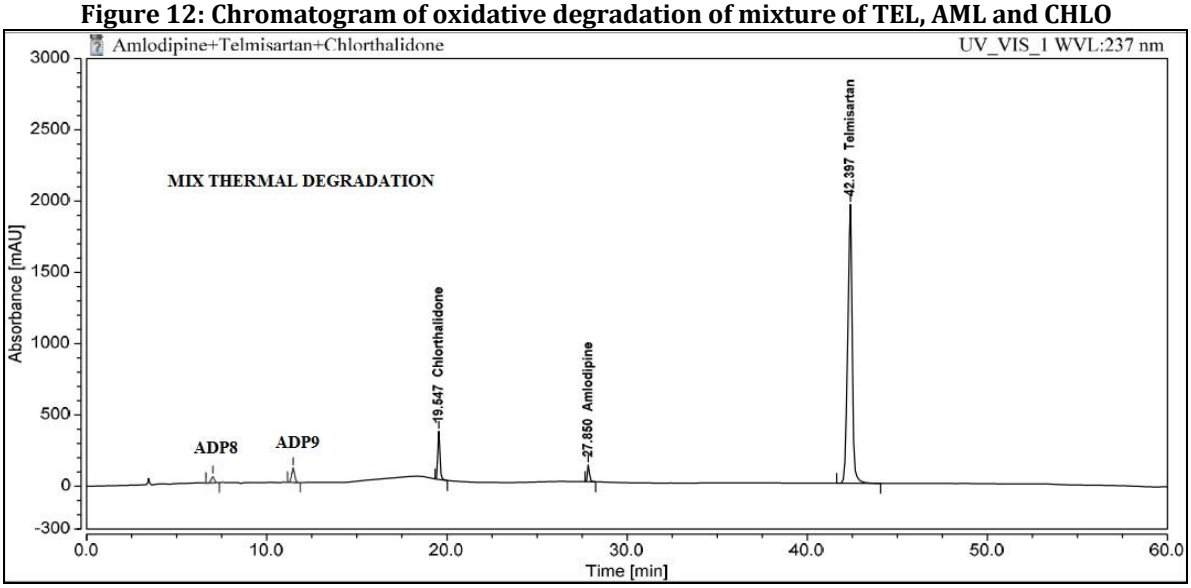

Figure 13: Chromatogram of thermal degradation of mixture of TEL, AML and CHLO

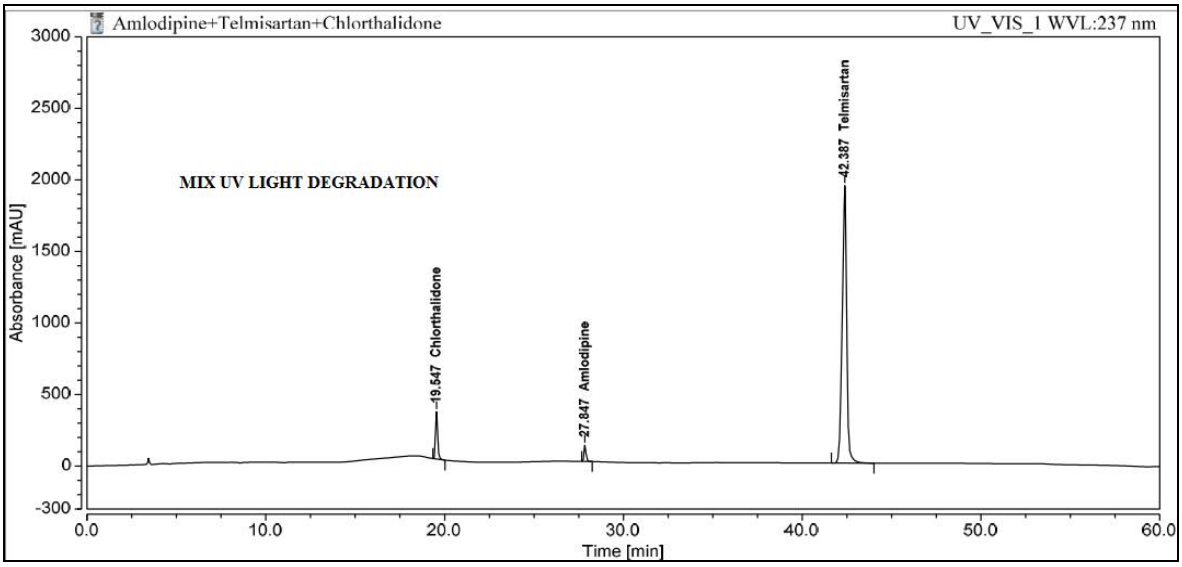

Figure 14: Chromatogram of Photolytic degradation (UVLight) of mixture of TEL, AML and CHLO

CONCLUSION

The proposed RP-HPLC method is precise, specific, linear and accurate for the estimation of TEL, AML and CHLO in pharmaceutical dosage form without interference from the excipients and potential degradation products in various stress conditions like acid hydrolysis, alkaline hydrolysis, neutral hydrolysis, oxidative, thermal and photolytic stress conditions. All the three drugs showed significant degradation under acidic, alkaline and oxidative stress condition with the exception of TEL under alkaline condition. All the three drugs showed moderate degradation under thermal and photolytic stress conditions and least degradation under neutral hydrolysis (elevated temperature and humidity). The results of stress testing were critically analyzed to establish correlation with degradation products reported in published literature. The developed method is validated as per ICH guidelines. The results showed the suitability of developed method for degradation kinetic studies and stability studies of the fixed dose combination. A method can also be suitably applied for estimation of FDC containing two drugs like TEL and CHLO; and AML with CHLO. The use of the method can be extended for estimation of one or more degradation products as all the degradation products were found adequately separated from one another and also from drug peaks.

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COMPETING INTERESTS

The authors have declared that no competing interest exists.

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