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Original Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF HYDROCHLOROTHIAZIDE, AMLODIPINE BESYLATE AND TELMISARTAN HYDROCHLORIDE IN MULTICOMPONENT TABLET DOSAGE FORM AND IN BIORELEVANT MEDIA (FASSIF) BY RP-HPLC TECHNIQUES

MADHUKAR A[a,b]*, N. KANNAPPAN[b], MAHENDRA KUMAR CB[c]

***[a]Bright Labs, Kothapet, Dilshuknagar, Hyderabad, Telangana, INDIA, [b]Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, INDIA, [c]Department of Pharmacy, St. Mary's College of Pharmacy, Secunderabad, Telangana, INDIA.**
Email: dr.amk2014@gmail.com

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ABSTRACT

Objective: A simple, rapid, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous analysis of Hydrochlorothiazide (HTZ), Amlodipine besylate (AML) and Telmisartan hydrochloride (TLM) in a tablet dosage form and in Biorelevant media has been developed and validated.

Methods: This method was performed with a thermosil C_{18} (4.6 × 100 mm i. d., 3.7 µm particle column with 40:60 (v/v) 20 mM potassium dihydrogen orthophosphate buffer: methanol as mobile phase at a flow rate of 1.0 ml/min. UV detection at 248 nm.

Results: By the method HTZ, AML and TLM were eluted with retention times of 1.823, 2.639, and 4.198 min, respectively. The method was continued and validated accordance with ICH guidelines. Validation revealed the method is rapid, specific, accurate, precise, reliable, and reproducible. Calibration curve plots were linear over the concentration ranges 6.25-100µg/mL for HTZ, 2.5-40µg/mL for AML, and 20-320µg/mL for TLM. Limits of detection (LOD) were 0.004, 0.0016, and 0.0128µg/ml and limits of quantification (LOQ) were 0.013, 0.0052, and 0.0416µg/mL for HTZ, AML and TLM respectively.

Conclusion: The statistical analysis was proves the method is suitable for the analysis of HTZ, AML and TLM as a bulk, in tablet dosage form and in biorelevant media without any interference from the excipients. It was also proved study for degradation kinetics of three drugs. It may be extended for its estimation in plasma and other biological fluids.

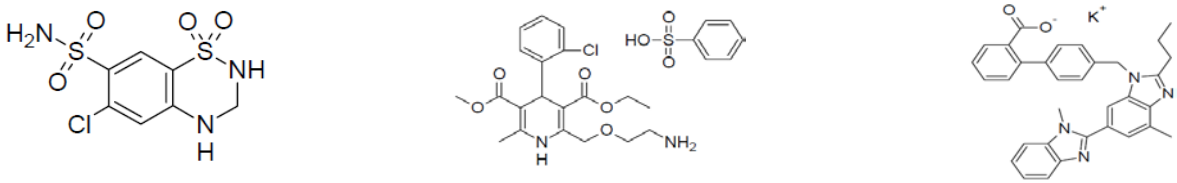
Keywords: Hydrochlorothiazide (HTZ), Amlodipine besylate (AML) and Telmisartan hydrochloride (TLM), RP-HPLC, Validation, FaSSIF.

INTRODUCTION

Hydrochlorothiazide (HTZ) chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide (Fig. 1). It is a diuretic drug and derivative of the thiazide class [1]. Amlodipine (AML) chemically (RS)-3-ethyl-5-methyle-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-di carboxylate (Fig. 1). It is a long-acting dihydropyridine-type (DHP) of calcium channel blocker [2]. Amlodipine is also acts as a functional inhibitor of acid sphingomyelinase [3]. Telmisartan (TLM) chemically 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yle)-2-propyle-1H-1,3-benzodiazol-1-yle]methyl}phenyl)benzoic acid (Fig. 1). It is an angiotensin II receptor blocking agent it shows high affinity to the angiotensin II receptor type-1 (AT1) [4].

In the scientific literature, analysis of HTZ, AML, and TLM has been reported as individual ingredients and in combination with other compounds. Analytical methods have included estimation of HTZ [5], AML [6], TLM [7, 8] individually. And in two component formulations of HTZ and AML have been analyzed in combination [9, 10]. Simultaneous HPLC analysis of HTZ and TLM [11] and in combinations of HTZ, AML and TLM analyzed in HPLC [12-14] and with other drugs had also been reported [15-17].

No other chromatographic methods are found for simultaneous analysis of HTZ, AML, and TLM in a combined dosage form and in biorelevant media. The method described is rapid, economical, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH guidelines [18-20].



HTZ **AML** **TLM**

Fig. 1: Chemical structures of Hydrochlorothiazide (HTZ), Amlodipine besylate (AML) and Telmisartan (TLM)

MATERIALS AND METHODS

Experimental

Materials and methods

Pharmaceutical grade working standards Hydrochlorothiazide (HTZ), Amlodipine besylate (AML), Telmisartan hydrochloride (TLM) were obtained from Hetero Labs, Jedcharla, India. All chemicals and reagents were HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The analysis was performed using Waters-2695 (Modal Alliance) High Performance liquid chromatography, analytical balance

(Mettler Toledo), PDA Detector (Standard cell) and data handling system (Empower 2), pH meter (lab India), Sonicator. The column used is Thermosil C_{18} (100×4.6 mm, packed with 3.7 µm) with the flow rate 1.0 ml/min (isocratic).

Preparation of blank fasted state simulated intestinal fluid (FaSSIF)

Accurately weighed 1.74g of Sodium hydroxide pellets, 19.77g of Sodium dihydrogen orthophosphate, and 30.93g of sodium chloride dissolved in 5 L of purified water and adjusted the pH to 6.5 exactly by used 1N Hydrochloric acid [27].

Preparation of FaSSIF

Accurately weighed 3.3g of sodium taurocholate dissolve in 500 mL blank FaSSIF solution, add 11.8 mL of a solution to 100mg/mL lecithin in methylene chloride, and forming an emulsion. The methylene chloride was eliminated under vacuum at 40°C. Then draw a vacuum for 15 minutes at 250mbar and also followed by 15 minutes at 100mbar. These results gave in a clear, micellar solution, having no perceptible odor for methylene chloride. After that, it was cool to room temperature and adjusts the volume upto 2L with blank FaSSIF [27].

Preparation of stock solution

Accurately weighed 10 mg of HTZ, AML, and TLM working standard and separately transferred into a 10 ml clean dry volumetric flasks, add about 7 ml of media (FaSSIF) to each volumetric flask and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Calibration standards at five levels were prepared by appropriately mixed and further diluted stock standard solutions in the concentration ranges from 6.25-100µg/ml for HTZ, 2.5-40µg/ml for AML, and 20-320µg/ml. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs.

Sample preparation

For the analysis of a tablet dosage form, 20 tablets were weighed individually and their average mass was determined. Then, the tablets were crushed to a fine powder. The powder equivalent to 12.5mg of HTZ, 5mg of AML and 40mg of TLM was transferred to a 10 mL volumetric flask and dissolved in 10 mL of biorelevant media, sonication was done for 15 min with swirling. After sonication, the solution was filtered through a membrane filter paper (#0.45µ). From the above stock solution 0.2 mL was transferred in to 10 mL volumetric flask and made volume upto the mark with diluent, the final concentrations were 25µg/ml, 10µg/ml and 80µg/ml of HTZ, AML, and TLM respectively, then injected into the chromatographic system, and analyzed quantitatively. The analysis was repeated six times and the possibility of excipient interference with the analysis was examined.

Optimization of HPLC method

The HPLC method was optimized and developed with a simultaneous assay method for HTZ, AML, and TLM respectively. The mixed standard stock solution (25µg/mL of HTZ, 10µg/mL of AML, 80µg/mL of TLM) injected in HPLC. Different ratios of methanol and potassium dihydrogen orthophosphate buffer at different pH and molarities were tried.

Method validation

The method validation was done according to the ICH guidelines. The following validation characteristic parameters are accuracy, precision, linearity, and specificity, LOD, LOQ and robustness.

Linearity and range

Linearity of the method was studied by the injecting the mixed standard solutions with the concentration ranges from 6.25-100µg/ml for HTZ, 2.5-40µg/ml for AML, and 20-320µg/ml for TLM levels of target concentrations were prepared and injected six times into the HPLC system keeping the constant injection volume. The peak areas were plotted against the concentrations to obtain the linearity graphs.

Precision

The precision of the optimized method was evaluated by carrying out six independent assays of test sample. %RSD of six assay values was calculated. Intermediate precision was carried out the samples by using another instrument and with the different analyst.

Limit of detection and quantification

The LOD and LOQ procedures were performed on samples contain very lower concentrations of analytes under the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analytes that can be detected and quantified, with acceptable accuracy and precision.

Robustness

Robustness was studied by evaluating the effect of small variations in the chromatographic conditions. The conditions studied were flow rate altered by ±0.1 ml/min, mobile phase composition with methanol ±5 ml. These chromatographic variations are evaluated for resolution between HTZ, AML, and TLM.

System suitability

The system suitability parameters with respect of tailing factor, theoretical plates, repeatability and resolution between HTZ, AML, and TLM peaks were defined.

Specificity

The specificity of the analytical method is the ability of the method to estimate the analyte response in the presence of additional components such as impurities, degradation products and matrix [21]. The peak purity of HTZ, AML, and TLM was assessed by comparing the retention time of standard HTZ, AML, and TLM good correlation was obtained between the Retention time of standard and sample of HTZ, AML, and TLM.

The specificity method was also evaluated to ensure that there were no interference products resulting from forced degradation studies.

Forced degradation study

Forced degradation or stress testing of a drug substance will help to identify the degradation products, which can help to establish the intrinsic stability of the molecule. All stress decomposition studies were performed at an initial drug concentration 25µg/mL of HTZ, 10µg/mL of AML, 80 µg/mL of TLM.

The degradation conditions are selected on the basis of literature survey [22-26]. The stability indicating study of HTZ, AML, and TLM were undergoes acid, alkali and oxidation degradation, photolysis and heat condition.

Placebo Interference

The placebo (in the present of excipients in tablet) sample was prepared as per the test method and analyzed in the HPLC. It expressed there is no additional peaks at the retention time of HTZ, AML, and TLM in the chromatograph it indicates that there is no placebo interference.

Acid degradation

Sample was treated with 3 ml of 1N hydrochloric acid and kept for 10 hrs. After 10 hrs the solution was neutralized with 3 ml of 1N sodium hydroxide, made the volume upto the mark with biorelevant media and analyzed using HPLC.

Alkali degradation

Sample was treated with 3 ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3 ml of 1N hydrochloric acid, made the volume upto the mark with biorelevant media and analyzed using HPLC.

Oxidative degradation

HTZ, AML, and TLM solutions of 25, 10 and 80µg/ml were mixed with 3 mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10 hrs. After 10 hrs made the volume upto the mark with biorelevant media and analyzed using HPLC.

Photolytic Degradation

The **s**amples were kept under UV light for different time intervals (15 mins – 7days) and made the volume upto the mark with biorelevant media and analyzed using HPLC.

Thermal Degradation

Samples were heated at 80^0 C for 15 mins - 60 mins and 220^0 C for 2-5 mins and analyzed.

Accuracy

Accuracy was carried out by applying the method to drug sample (HTZ, AML, and TLM combination of tablets) to which known amounts of HTZ, AML, and TLM standard powder corresponding to 50, 100 and 150% of label claim was added, mixed and the powder was extracted and determined by the system in optimized mobile phase. The experiment was performed in triplicate and percentage recovery, % RSD was calculated.

Analysis of marketed formulation

The marketed formulation was assayed by an above description. The peak areas were monitored at 248 nm, and determination of sample concentrations was using multilevel calibration developed on the same HPLC system under the same conditions using linear regression analyzed for HTZ, AML, and TLM in the same way as described above.

RESULTS

The simultaneous estimation of HTZ, AML, and TLM was done by RP-HPLC and in the optimized method the mobile phase consists of buffer (400 volumes of phosphate buffer and 600 volumes of Methanol and the pH was adjusted to be 3.0. Then finally filtered using 0.45µ membrane filter paper and degassed in sonicator for 15 minutes. The detection is carried out using PDA detector at 248 nm. The solutions are following at the constant flow rate of 1.0 ml/min.

The retention time for HTZ, AML, and TLM was 1.823, 2.639 & 4.198 minutes respectively. Linearity ranges for HTZ, AML, and TLM were 6.25-100µg/mL, 2.5-40µg/mL, and 20-320µg/mL respectively and the results were found for in the acceptable as (R^2) = 0.999 for HTZ, AML, and TLM also. LOD were 0.004, 0.0016, and 0.0128µg/ml and LOQ were 0.013, 0.0052, and 0.0416µg/mL for HTZ, AML and TLM respectively.

The all parameters value of RSD is less than 2.0% indicating the accuracy and precision of the method. The percentage recoveries were found 99.93-100.52%, 99.6-100.8% and 100.17-100.35% for HTZ, AML, and TLM respectively.

DISCUSSION

Method development and optimization

The HPLC procedure was optimized with a view to develop a suitable LC method for the analysis of HTZ, AML, and TLM in fixed dose for bulk and combined dosage form. It was found that 40:60 v/v (20 mM) potassium dihydrogen orthophosphate buffer: methanol gave acceptable retention time (1.823 min for HTZ, 2.639 min for AML and 4.198 min for TLM), plates, and good resolution for HTZ, AML, and TLM at the flow rate of 1.0 ml/min (Table 1; Fig. 2 & 3).

Table 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Thermosil C_{18} (100×4.6 mm, packed with 3.7 µm)
Mobile Phase	40:60v/v, (0.02M Phosphate Buffer: Methanol)
pH	3.0 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	8.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	15
Detection wavelength (nm)	248
Drugs RT (min)	1.823, 2.639 & 4.198

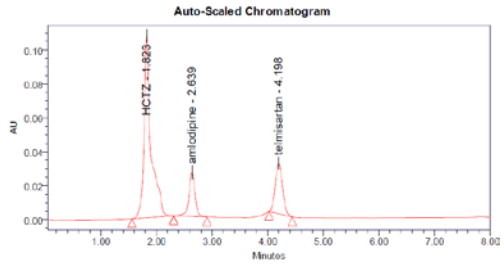


Fig. 2: Chromatogram of HTZ, AML, and TLM at 248 nm from bulk drug

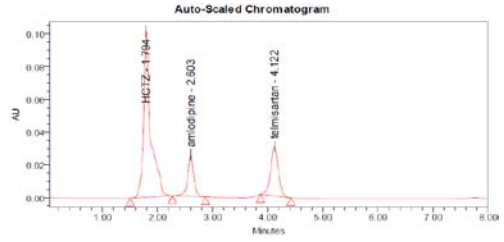


Fig. 3: Chromatogram of HTZ, AML, and TLM at 248 nm from pharmaceutical formulation (Telma AM H)

Validation of Developed method

Linearity

Linearity was evaluated by analysis of working standard solutions of HTZ, AML, and TLM of five different concentrations. The range of linearity ranges from 6.25-100µg/ml for HTZ, 2.5-40µg/ml for AML, and 20-320µg/ml for TLM (Table 2).

The result of correlation coefficients of HTZ, AML, and TLM (R^2) = 0.999, 0.999 & 0.999 respectively (Fig. 4-7). There was an excellent correlation between peak areas and concentrations of each drug.

Precision

The results of precision method were evaluated by carrying out six independent test samples of HTZ, AML and TLM. The percentage of RSD of six sample peak area values was calculated.

Different analyst from the same laboratory conditions analyzed the intermediate precision for the optimized method. The RSD values of intra-day and inter-day studies for HTZ, AML and TLM confirming good precision of the optimized method (Table 3).

LOD and LOQ

The LOD and LOQ values were found to be 0.004 and 0.013µg/mL for HTZ, 0.0016 and 0.0052µg/mL for AML and 0.0128 and 0.0416µg/mL for TLM (Table 5).

Specificity

Injected the extracted solutions commonly used excipients were performed to demonstrate for the absence of interaction with the drugs.

These results are expressed that there was no interference from the other excipients in the tablet formulation; therefore, confirm the method was specific.

System suitability

System suitability parameters such as the theoretical plates count, resolution, % RSD and peak tailing factors are determined (Table 5).

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Robustness

To ensure the insensitivity of the optimized RP-HPLC method to small alteration in the experimental conditions. The conditions studied were flow rate altered by ±0.1 ml/min, mobile phase composition with methanol ±5 ml. These chromatographic variations are evaluated for resolution between HTZ, AML, and TLM (Table 6).

Table 2: Data for linearity

Analyte	Concentration range (µg/mL)	Correlation Coefficient (R^2)	Slope	Intercept
HTZ	6.25-100	0.999	37791x	59447
AML	2.5-40	0.999	13125x	76149
TLM	20-320	0.999	1414. x	15105

Table 3: Intra-day and inter-day Precision results of HTZ, AML and TLM from tablets

No. of Preparation	HTZ		AML		TLM	
	Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
Pre-1	901825	897825	184724	189714	319784	325162
Pre-2	906134	895724	185617	187193	321874	320198
Pre-3	907721	890162	182816	188167	319264	322938
Pre-4	902835	891826	186272	189782	317916	321828
Pre-5	910026	889017	184726	186825	320163	323927
Mean	905708.2	892910.8	184831	188336.2	319800.2	322810.6
St. dev.	3398.9184	3740.2482	1301.6312	1379.1238	1437.9024	1909.3745
% RSD	0.3752	0.4188	0.7042	0.7322	0.4496	0.5914

Table 5: System suitability parameters for HTZ, AML, and TLM

System suitability parameters	HTZ	AML	TLM
Retention time (min)	1.823	2.639	4.198
Repeatability of retention time; %R. S. D (n=5)	0.163793	0.293152	0.240415
Repeatability of peak area; %R. S. D= (S. D./Mean)×100	0.57259161	0.482787475	0.82527181
Resolution (Rs)	-	4.53	7.09
Tailing factor (asymmetric factor)	1.45	0.96	1.12
USP plate count	7130	9456	12268
LOD (µg/mL)	0.004	0.0016	0.0128
LOQ (µg/mL)	0.013	0.0052	0.0416

Table 6: Robustness study for analytical method validation of HTZ, AML, and TLM tablets

Parameters		Adjusted to	Mean Area [a]	Mean RT	SD	% RSD
HTZ	Flow Rate ±0.1 ml/min	0.9 ml/min	1016052.2	2.03	1746.39	0.17
		1.1 ml/min	798422.00	1.63	1139.00	0.14
	Mobile Phase (40:60) (±5 ml)	35:65	898142.67	1.63	1309.34	0.15
		45:55	986892.83	1.80	1927.57	0.20
AML	Flow Rate ±0.1 ml/min	0.9 ml/min	216963.17	2.91	964.66	0.44
		1.1 ml/min	168430.5	2.32	1277.65	0.76
	Mobile Phase (40:60) (±5 ml)	35:65	187967.83	2.32	1328.81	0.71
		45:55	198902.67	3.11	619.49	0.31
	Flow Rate ±0.1 ml/min	0.9 ml/min	293213.17	4.65	2207.82	0.75
TLM		1.1 ml/min	257658.83	3.71	1185.00	0.46
	Mobile Phase (40:60) (±5 ml)	35:65	266109.33	3.71	623.10	0.23
		45:55	285970.83	5.23	950.86	0.33

[a]= 5 *Replicates*

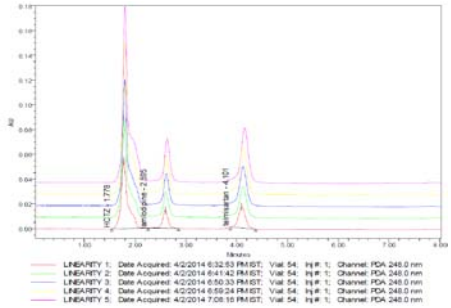


Fig. 4: Overlay linearity Chromatogram for HTZ, AML, and TLM

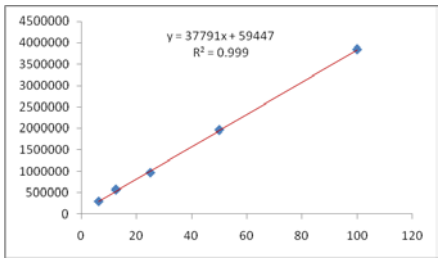


Fig. 5: Linearity Curve of Standard Hydrochlorothiazide (HTZ)

Solution stability studies

Three different concentrations of HTZ (25µg/mL), AML (10µg/mL) and TLM (80µg/mL) were prepared from the sample solution and stored at room temperature for 24 hrs. Then injected into the HPLC system and the additional peaks were not found in the chromatograms so, it was indicating the stability of HTZ, AML, and TLM tablet in the solution (Table 7)

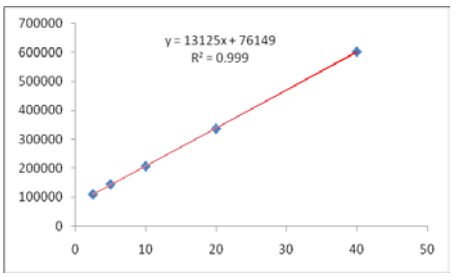


Fig. 6: Linearity Curve of Standard Amlodipine besylate (AML)

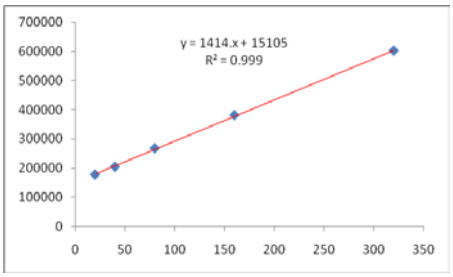


Fig. 7: Linearity Curve of Standard Telmisartan Hydrochloric acid (TLM)

Table 7: Solution stability study for analytical method validation of HTZ, AML, and TLM tablets

Name	Replicate (n = 5)	Initial	After 3 hrs	After 6 hrs	After 12 hrs	After 24 hrs
	Mean	896022.8	894439.4	893842	892867.6	889681.6
HTZ	SD	5069.394	3945.409	3430.281	3568.615	3792.128
	% RSD	0.565766	0.441104	0.383768	0.39968	0.426234
	Mean	188451.6	188439.4	188234.2	188052.8	187667.2
AML	SD	457.8638	422.4941	351.0131	399.2207	407.4907
	% RSD	0.242961	0.224207	0.186477	0.212292	0.217135
	Mean	316704.4	316684.2	316611	316318.6	315804.6
TLM	SD	1519.681	1501.023	1516.737	1460.5	1616.004
	% RSD	0.479842	0.473981	0.479054	0.461718	0.51171

Tablet 8: Accuracy Results of HTZ, AML and TLM from tablets

Brand Name	Analyte	Recovery levels	Actual Conc. (µg/mL)	Added Conc. (µg/mL)	Theoretical Conc. (µg/mL)	Found Conc. (µg/mL)	% Recovery	% RSD	% Error [a]
		50 %	12.5	6.25	18.75	18.78	100.16	0.183	0.16
Telma	**HTZ**	**100 %**	12.5	12.5	25	25.13	100.52	0.048	0.52
AM H		**150 %**	12.5	18.75	31.25	31.23	99.93	0.047	-0.06
		50 %	5	2.5	7.5	7.56	100.8	0.693	0.80
	AML	**100 %**	5	5	10	10.06	100.06	0.546	0.60
		150 %	5	7.5	12.5	12.45	99.6	0.314	-0.40
		50 %	40	20	60	60.21	100.35	0.391	0.35
	TLM	**100 %**	40	40	80	80.14	100.17	0.635	0.17
		150 %	40	60	100	100.27	100.27	0.315	0.27

[a][found conc. – theoretical conc./theoretical conc.] x 100.

Recovery studies

Good recoveries of the HTZ, AML, and TLM were obtained at different added concentrations for the tablets (Table 8).

Analysis of a commercial formulation

Experimentally the results for the amount of HTZ, AML, and TLM in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interaction from the excipients which are commonly present in formulation of tablets.

Degradation study

Acid degradation study

In acidic degradation study, sample was treated with 3 ml of 1N hydrochloric acid and kept for 10 hrs at 60ºC. After 10 hrs the solution was neutralized with 3 ml of 1N sodium hydroxide, made

the volume upto the mark with biorelevant media and analyzed using HPLC. The drug content was found to be degrading up to 5.608% in acidic condition (fig. 8 & 9, table 9 & 10).

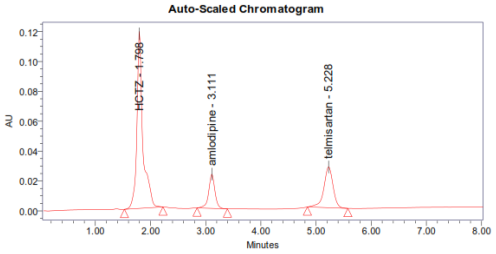
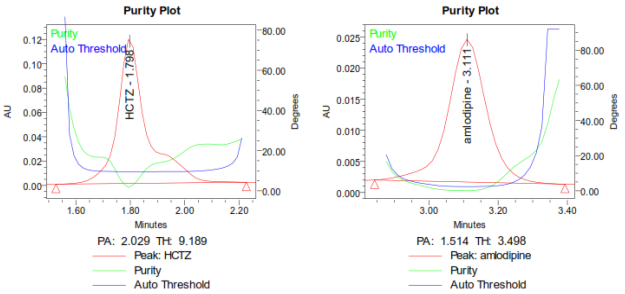


Fig. 8: Chromatogram of acidic forced degradation



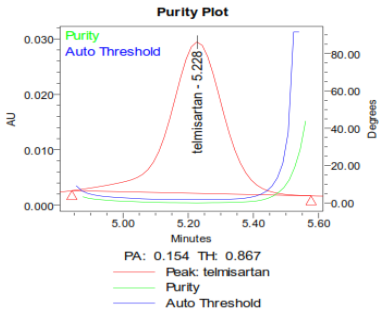


Fig. 9: Purity Plots for HTZ, AML and TLM in acidic forced degradation

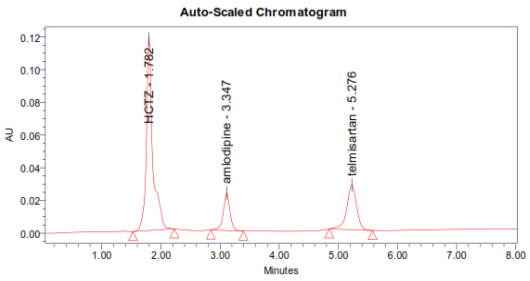
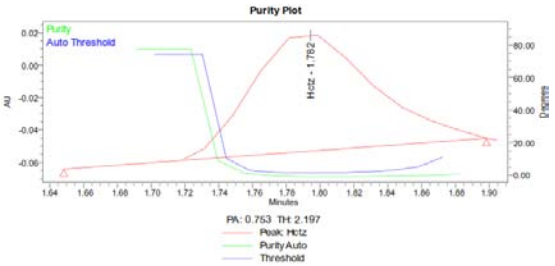


Fig. 10: Chromatogram of alkali forced degradation



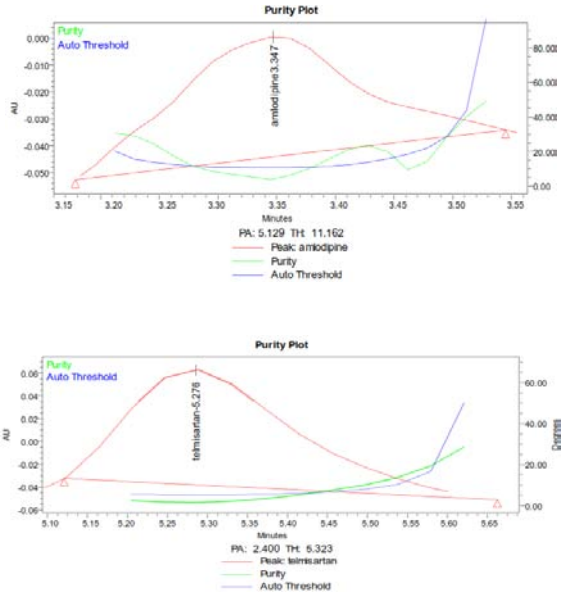


Fig. 11: Purity Plots for HTZ, AML and TLM in alkali forced degradation

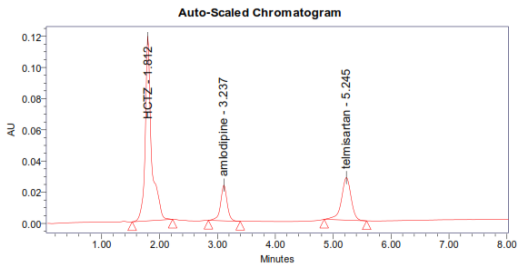
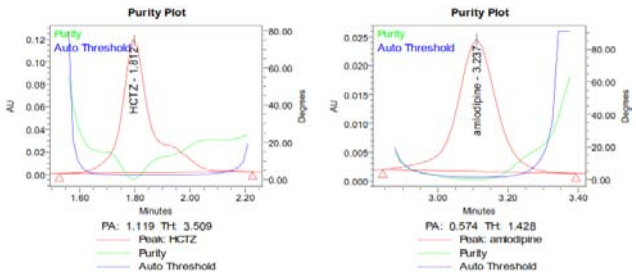


Fig. 12: Chromatogram of oxidative forced degradation



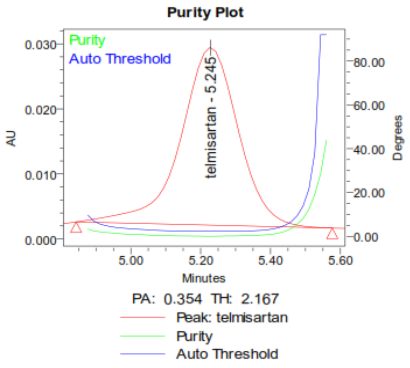


Fig. 13: Purity Plots for HTZ, AML and TLM in oxidative forced degradation

Alkaline degradation study

Alkaline degradation study was performed by the sample was treated with 3 ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3 ml of 1N hydrochloric acid, made the volume upto the mark with biorelevant media and analyzed using HPLC. In alkali degradation, it was found that around 13.858% of the drug degraded (fig. 10 & 11, table 9 & 10).

Oxidative degradation study

Oxidation degradation study was performed by the sample solutions were mixed with 3 mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10 hrs. After 10 hrs made the volume upto the mark with biorelevant media and analyzed using HPLC. In oxidative degradation, it was found that around 6.387% of the drug degraded (fig. 12 & 13, table 9 & 10).

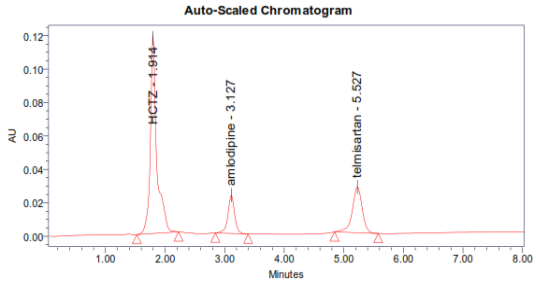


Fig. 14: Chromatogram of UV-light degradation

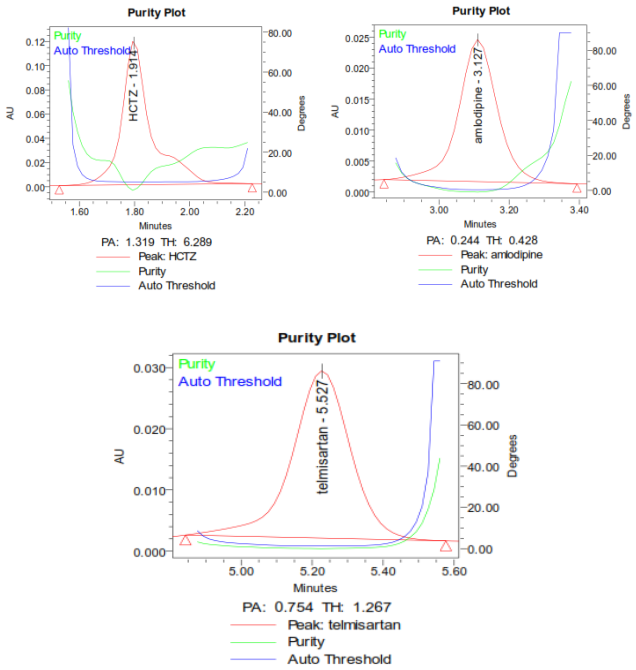


Fig. 15: Purity Plots for HTZ, AML and TLM in UV-light degradation

Thermal degradation study

Thermal degradation was performed by exposing solid drug at 80°C for 15 mins to 60 mins and at 220°C for 2-5 mins. Resultant chromatogram of thermal degradation study (fig. 16 & 17, table 9 & 10) indicates that drug is found to be slightly stable under thermal degradation condition. Only 5.742% drug content were degraded.

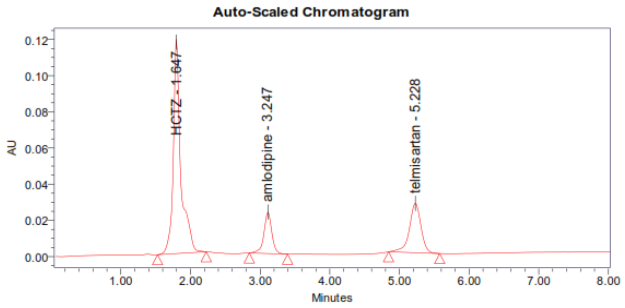


Fig. 16: Chromatogram of thermal degradation

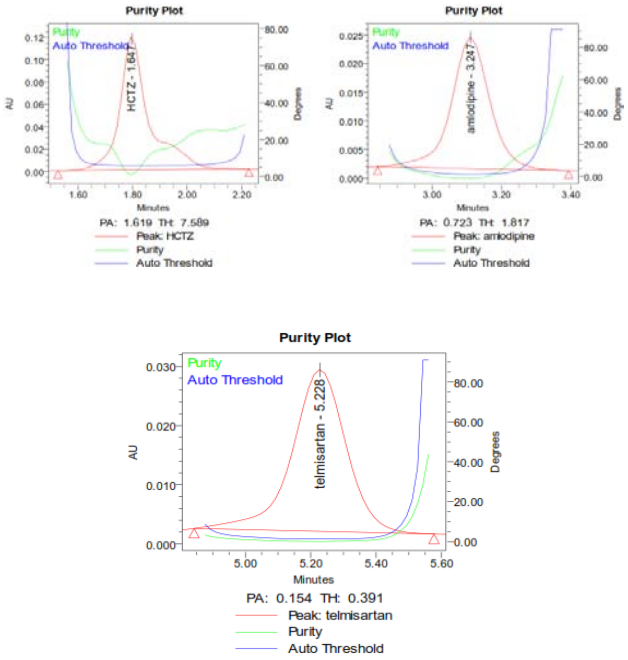


Fig. 17: Purity Plots for HTZ, AML and TLM in thermal degradation

Photolytic degradation study

Photolytic degradation study was performed by exposing the drug content in UV light for 15 mins to 7days. There is 5.347% degradation observed in above specific photolytic condition (fig. 14 & 15, table 9 & 10).

Table 9: Peak purity results of HTZ, AML and TLM

Stress	Purity Angle			Purity Threshold		
Condition	HTZ	AML	TLM	HTZ	AML	TLM
Acid Degradation	2.029	1.524	0.154	9.189	3.498	0.867
Alkali Degradation	0.753	5.129	2.400	2.197	11.162	5.323
Oxidative Degradation	1.119	0.574	0.354	3.509	1.428	2.167
Photolytic Degradation	1.319	0.244	0.754	6.289	0.428	1.267
Thermal Degradation	1.619	0.723	0.154	7.589	1.817	0.391

Table 10: Percentage of degradation of HTZ, AML and TLM

Drug Name		Acid	Alkali	Oxidative	Photolytic	Thermal
	Std Area	935905				
HTZ	Sample Area	909116	831257	882575	892547	876258
	% of Degradation	2.862	11.181	5.698	4.632	6.373
	Std Area	195063				
AML	Sample Area	179391	168274	180578	179587	185487
	% of Degradation	8.034	13.733	7.425	7.933	4.909
	Std Area	271001				
TLM	Sample Area	254934	225846	254635	261578	254892
	% of Degradation	5.928	16.662	6.039	3.477	5.944
Average of % Degradation		5.608	13.858	6.387	5.347	5.742

CONCLUSION

A new RP-HPLC method described in this manuscript provides a simple, convenient and reproducible approach for the simultaneous estimation and quantification of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan Hydrochloride in routine quality control analysis.

CONFLICT OF INTERESTS

Declared None

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