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Development and Validation of a New Stability-Indicating Isocratic RP-UHPLC Method for Simultaneous Estimation of Telmisartan and Hydrochlorothiazide in Tablet Dosage Form

Biswa Ranjan Patra*, Mohan S, Nagaraj Gowda

Department of Pharmaceutical Analysis
PES College of Pharmacy
Hanumantha Nagar, B.S.K I Stage
Bengaluru, Pin: 560050, Karnataka, India

Abstract:

Aim: The aim of this research work is to develop a simple, rapid and precise stability-indicating ultra-high performance liquid chromatography (UHPLC) method for the quantitative estimation of telmisartan and hydrochlorothiazide in combined tablet dosage form.

Method: The method uses poroshell 120EC-C_{18} column (4.6 x 50mm,2.7µm) with mobile phase containing acetonitrile: 50mM ammonium acetate buffer in the ratio (45: 55 v/v), pH adjusted to 4.5 with acetic acid. Flow rate was 0.3ml min^{-1}, column temperature at 25 ^{0}C and detection was monitored by PDA detector at wavelength of 275 nm. ICH recommended stress degradation studies were performed on telmisartan, hydrochlorothiazide standards and tablets; further stressed samples were analyzed by the proposed method.

Results: Major degradation of telmisartan and hydrochlorothiazide were observed under acidic, alkali and oxidation conditions. The described method was validated as per ICH guideline and validation parameter such as system suitability, linearity, accuracy, precision, specificity and robustness results were within acceptable limits.

Conclusion: The method was found to be suitable and accurate for quantitative estimation and stability study of title drugs in pharmaceutical preparations.

Key words: Telmisartan, Hydrochlorothiazide, RP-Ultra High Performance Liquid Chromatography, Forced Degradation, Method Validation.

1. INTRODUCTION:

Telmisartan, chemically described as 2-(4-{[4-methyl-6-(1-methyl-1H-1, 3-benzoimidiazol-2-yl)-2-propyl-1H-1, 3-benzoimidazole-1-yl] methyl} phenyl) benzoic acid, molecular formula C_{33} H_{30} N_4 O_2 and molecular weight 514.62. Hydrochlorothiazide is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide, molecular formula C_7 H_8 $N_3O_4S_2Cl$ and molecular weight 297.74[1]. Telmisartan is an angiotensin II receptor blocker works by relaxing the blood vessels and hydrochlorothiazide helps the kidneys to eliminate fluid and sodium from the body. Combining the angiotensin II receptor antagonist telmisartan with the thiazide diuretic hydrochlorothiazide provides antihypertensive therapy with complementary mechanisms of action. The addition of hydrochlorothiazide to telmisartan achieved significant reductions in blood pressure in nonresponders to telmisartan monotherapy. The antihypertensive efficacy of telmisartan and hydrochlorothiazide was similar to or significantly greater than that of various comparator agents [2-8]. In order to establish inherent stability characteristics of a drug, ICH stability test guideline Q1A (R2) suggests that stress studies should be carried out, leading to identification of likely degradation products, and hence, supporting the suitability of the proposed analytical procedures. For stability samples, it also requires that analytical test procedures should be stability indicating and they should be fully validated. Literature survey revealed that several analytical methods have been reported for the quantitative estimation of telmisartan alone and in combination with other drugs. Several RP-HPLC and HPTLC methods [9-22], UV spectroscopy method [23-26] bioanalytical methods [27], were reported for estimation of telmisartan and hydrochlorothiazide but few research paper have reported their degradation profile[28-33].Hence, it is thought of interest to develop new sensitive and accurate stability indicating RP-UHPLC methods for an effective quantitative estimation of telmisartan and hydrochlorothiazide in tablet dosage form.

2. EXPERIMENTAL:

2.1. MATERIAL AND INSTRUMENTS USED:

2.1.1. Chemicals and Reagents:

Telmisartan & hydrochlorothiazide standard drugs were obtained as a gift sample from Micro Laboratories Limited, Husur, India, The solvents and reagents used in this method were HPLC and analytical grade. Mill Q HPLC water was used for all purposes in the analysis. Telmisartan and hydrochlorothiazide tablet (Newtel-H) label claim 40 mg and 12.5 mg from Systopic Laboratories Pvt Ltd, India was purchased from the local market.

2.1.2. Instrumentation:

UHPLC system(Agilent 1260 UHPLC System) consists of 1260 quaternary pump, standard auto sampler, poroshell 120EC-C_{18} column (4.6 x 50mm, 2.7µm), PDA detector with chemstation Software, ShimadzuAUX220 Weighing Balance, Elico India LI 127 pH meter and Grant Sub-aqua 12 Water bath were used in the analysis.

2.2. METHOD:

2.2.1. Preparation of Standard Stock solutions

Preparation of Diluents

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

Diluent A composed of methanol and acetonitrile in the ratio of 50:50 (v/v).

Diluent B composed of acetontile and water in the ratio of 40:60 (v/v)

Standard stock solutions were prepared by dissolving the drug in diluent A

Preparation of Stock Solution of Telmisartan:

An accurately weighed quantity of 25mg of standard telmisartan was transferred in to 25ml volumetric flask. Dissolved and diluted to 25 ml with diluent A to obtain the concentration of 1000 µg ml^{-1}.

Preparation of Stock Solution of Hydrochlorothiazide:

An accurately weighed quantity of 25mg of standard hydrochlorothiazide was transferred in to 25ml volumetric flask. Dissolved and diluted to 25 ml with diluent A to obtain the concentration of 1000 µg ml^{-1}.

2.2.2. Preparation of mixed Standard solution:

A binary mixture standard solution was prepared by pipetting out 2ml of telmisartan and 0.625 ml of hydrochlorothiazide from stock solution (µg ml^{-1}), transferred to 10ml volumetric flask and the volume was made up to 10ml by using diluent B. This solution contained 200 µg $ml^{-1}$of telmisartan and 62.5 µg $ml^{-1}$of hydrochlorothiazide.

2.2.3. Preparation of Calibration curve standard solutions:

A series of five different concentrations of calibration curve binary mixture standard solutions of telmisartan and hydrochlorothiazide were prepared from the stock solution which are in the range of 180 to 220 µg ml^{-1} for telmisartan and 42.5 to 82.5 µg ml^{-1} for hydrochlorothiazide.

2.2.4. Preparation of Sample solution:

20 tablets of the commercial sample (Newtel-H, 40 mg and 12.5 mg) were weighed accurately and crushed to fine powder. The tablets powder equivalent 40 mg of telmisartan and 12.5 mg of hydrochlorothiazide was weighed and transferred in to a 100 volumetric flask. To this flask, 50 ml of diluents A was added and solution was sonicated for 30 minutes. The solution was cooled to ambient temperature. Then the volume was made up to 100ml with diluents A, filtered through whatman filter paper and further filtered through 0.45µm membrane filter. Pipetting out 5 ml from the above prepared solution (400 µg ml^{-1}, 125 µg ml^{-1}), transferred to 10ml volumetric flask and the volume was made up to 10ml by using diluent B. The prepared solution contains 200 µg $ml^{-1}$of telmisartan and 62.5 µg $ml^{-1}$of hydrochlorothiazide.

2.2.5. Determination of Detection wavelength:

A double beam spectrophotometer having matched pair of quartz cuvettes were used for the scanning the analytes at concentration levels of 10 µg ml^{-1} in the range of 400 nm to 200 nm, to obtain overlain spectra. The spectra of the telmisartan and hydrochlorothiazide showed that a balanced wavelength was found to be 275 nm (iso-absorptive point of both the drugs) which was selected for the present work.

2.2.6. Chromatographic Conditions:

The chromatographic separation was performed on poroshell 120EC-C_{18} column (4.6mm x 50, 2.7µm).Mobile phase was composed of acetonitrile and buffer in the ratio of (45: 55 v/v). The buffer used in the mobile phase contains 50mM ammonium acetate in Mill Q water and pH was adjusted to 4.5 with acetic acid, filtered under vacuum through a 0.45µm nylon filter and degassed in an ultrasonic bath prior to use. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.3 ml $minute^{-1}$ and column temperature was maintained at 25 ^{0}C. The injection volume was 10µl and the elute was monitored at a wavelength of 275nm using photodiode array detector.

2.2.7. Procedure for Forced Degradation Studies of Standard Drugs:

Forced degradation studies of standard drugs and tablet formulation were carried out under thermolytic, photolytic, acid, base hydrolytic, water hydrolysis and oxidative stress conditions.

2.2.7.1. Acid Degradation:

Pipetting out 2 ml of telmisartan and 0.625 ml of hydrochlorothiazide standard solutions from stock solutions (1000 µg ml^{-1}), transferred to 10ml volumetric flask. In to it 2ml of 0.1N HCl was added and diluted up to 10ml with diluent B. The solution was heated on water bath at 60 ^{0}C for 4 hours. The solution was allowed to ambient temperature. Repeated the same with 1N HCl.

2.2.7.2. Alkali Degradation:

Pipetting out 2 ml of telmisartan and 0.625 ml of hydrochlorothiazide standard solutions from stock solutions (1000 µg ml^{-1}), transferred to 10ml volumetric flask. In to it 2ml of 0.1N NaOH was added and diluted up to 10ml with diluent B. The solution was heated on water bath at 60 ^{0}C for 4 hours. The solution was allowed to ambient temperature. Repeated the same with 1N NaOH.

2.2.7.3. Degradation under Neutral Hydrolytic Condition:

Pipetting out 2 ml of TEL and 0.625 ml of HCZ standard solutions from stock solutions (1000 µg ml^{-1}), transferred to 10ml volumetric flask, added 2ml of distilled water and diluted up to 10ml with diluent B. The solution was heated on water bath at 60 ^{0}C for 8 hours. The solution was allowed to ambient temperature.

2.2.7.4. Degradation under Oxidative Condition:

Pipetting out 2 ml of TEL and 0.625 ml of HCZ standard solutions from stock solutions (1000 µg ml^{-1}), transferred to 10ml volumetric flask, 2ml of 3% v/v H_2O_2 was added and diluted up to

10ml with diluent B. The solution was heated on water bath at 60 ^{0}C for 1 hour. The solution was allowed to ambient temperature.

2.2.7.5. Degradation under Dry Heat:

Dry heat study was performed by keeping drug samples (about 100mg) in an oven at 80 ^{0}C for 24 hours. Cooled, samples were withdrawn, dissolved in diluent A to prepare sample solution to get concentrations of 1000 µg ml^{-1}. Pipetting out 2 ml of telmisartan and 0.625 ml of hydrochlorothiazide sample solutions and transferred in to

a 10ml volumetric flask and diluted up to 10ml with diluent B.

2.2.7.6. Sunlight Degradation Studies:

Sunlight study was performed by exposing the drug samples in a petri dish (about 100mg) directly to sunlight for 8 hours for 7 days. Samples were withdrawn, dissolved in diluent A to prepare sample solutions to get concentrations of 1000 µg ml^{-1}. Pipetting out 2 ml of telmisartan and 0.625 ml of hydrochlorothiazide sample solutions and transferred the solution in to a 10ml volumetric flask and diluted up to 10ml with diluent B.

2.2.7.7. Photo Degradation Studies:

Photolytic studies were carried out by exposing the drugs in a petri dish (about 100mg) to UV short 254nm and UV long light 366nm for 24 hours. Samples were withdrawn, dissolved in diluent A to prepare sample solutions to get concentrations of 1000 µg ml^{-1}. Pipetting out 2 ml of telmisartan and 0.625 ml of hydrochlorothiazide sample solutions and transferred the solution in to a 10ml volumetric flask and diluted up to 10ml with diluent B.

2.3. Procedure for Forced Degradation studies of Drug products:

A forced degradation studies of tablet formulation in acidic, basic, water hydrolysis, oxidative conditions were carried out using filtered solution (as described in sample preparation) to achieve 200 µg $ml^{-1}$of telmisartan and 62.5 µg ml^{-1} of hydrochlorothiazide . For thermolytic and photolytic degradation, a quantity of powder equivalent to on tablet containing 40 mg of telmisartan and 12.5 mg of hydrochlorothiazide was exposed. Then the solutions were prepared as described in preparation of sample solution.

3. RESULTS:

3.1. Optimization of the Chromatographic Conditions:

UHPLC method was optimized with a view to develop a stability-indicating method. In order to develop stability indicating method, a study baseline was recorded with optimized chromatographic conditions set for telmisartan and hydrochlorothiazide and stabilized for about 30 minutes. A non-polar C_{18} column was choosen as the stationary phase for this study. To optimize mobile phase under isocratic conditions, different mobile phases containing mixtures of commonly used solvents with or without different buffers like ammonium acetate, phosphate with different volume were tested on C_{18} column as stanary phase . Mobile phase containing a mixture of 50mM ammonium acetate in Mill Q water, pH adjusted to 4.5 with acetic acid and acetonitrile in the ratio of 55:45 was found to be most suitable mobile phase composition for the desired study. The main objective of the chromatographic method development was to achieve a peak tailing factor less than 2, retention time in between 1 to 10 minutes due to short column length. The chromatographic separations for both the both drugs were achieved by using poroshell 120EC-C_{18} column (4.6 x 50mm, 2.7µm), mobile phase containing 50mM ammonium acetate in Mill Q water, pH adjusted to 4.5 with acetic acid and acetonitrile in the ratio of 55:45 (v/v) with flow rate of 0.3 ml min^{-1}, column temperature of 25 ^{0}C and detection wavelength of 275nm. In these selected optimized conditions, the drugs telmisartan and hydrochlorothiazide had adequate retention, good peak shape with less tailing and the chromatographic analysis was less than 10 minutes, which also separates the degradants from telmisartan and hydrochlorothiazide.Under the above optimized conditions a retention of 2.083 and 6.326 minutes were obtained for hydrochlorothiazide and telmisartan which is shown in Fig. 3.1(a) and 3.1(b).

3.2. Degradation Observed:

The chromatograms of the telmisartan and hydrochlorothiazide standards and tablet formulation showed well separated peaks of pure telmisartan and hydrochlorothiazide as well as some additional degradants when they were treated with various stress conditions like acid, alkali, neutral, hydrogen peroxide, dry heat, sunlight and UV light. The peaks of the degraded products were well resolved from the telmisartan and hydrochlorothiazide drugs peak. The degradants identification was based on the comparison of the chromatograms of "stressed samples" with that of the "standard solution". Blessy and Ruchi[34], in their article on stress testing suggested a target degradation of 5-20% has been accepted as reasonable for validation of chromatography assay. Similarly Singh and Bakshi[35], in their article on stress testing suggested a target degradation of 20-80% for establishing stability-indicating studies, and also intermediate degradation products should not interfere with any stage of drug analysis. In this study, conditions used for forced degradation were attenuated to achieve degradation in the range of 5-80% for telmisartan and hydrochlorothiazide drug substances. The numbers of degradation products with their retention time and percentage degradation of telmisartan and hydrochlorothiazide are listed in Table 3.2.and shown in Fig.3.2 (a) to 3.2 (i)

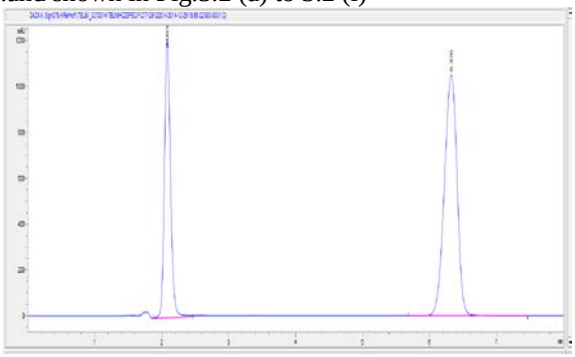


Fig.3.1(a) Chromatogram of hydrochlorothiazide (RT: 2.083 min) and telmisartan standard (RT: 6.326 minutes)

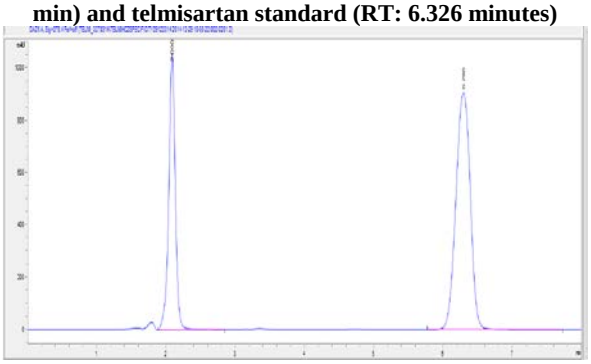


Fig.3.1(b) chromatogram of hydrochlorothiazide (RT: 2.083 min) and telmisartan (RT: 6.326 minutes) in Newtel-H tablet

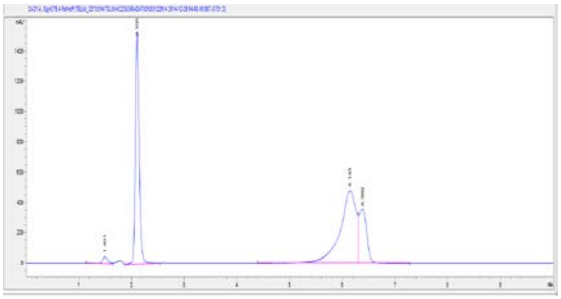


Fig.3.2 (a) Chromatogram of 0.1N HCl Degradation

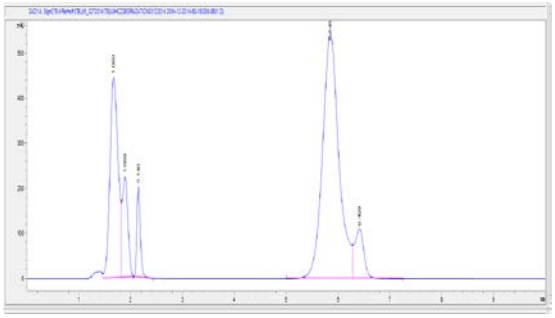


Fig.3.2 (b) Chromatogram of 1N HCl Degradation

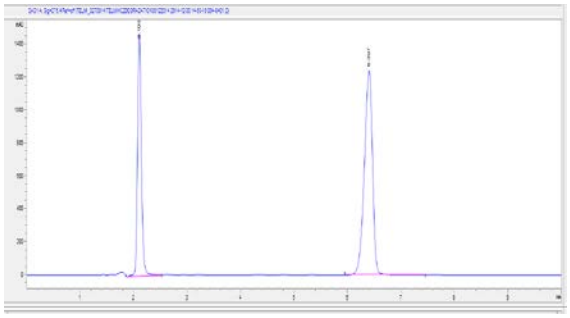


Fig.3.2 (c) Chromatogram of 0.1N NaOH Degradation

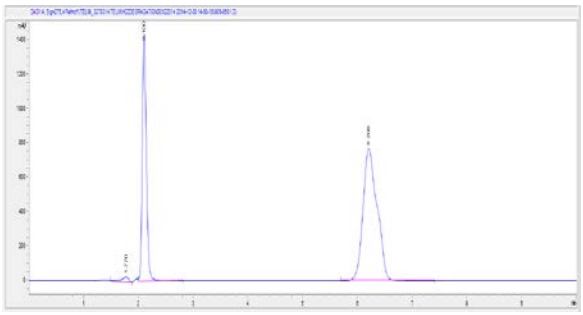


Fig.3.2 (d) Chromatogram of 1N NaOH Degradation

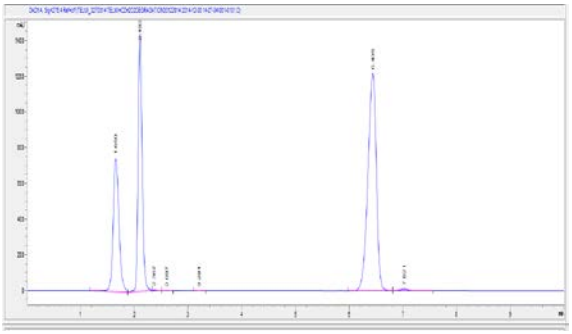


Fig.3.2 (e) Chromatogram of oxidation Degradation

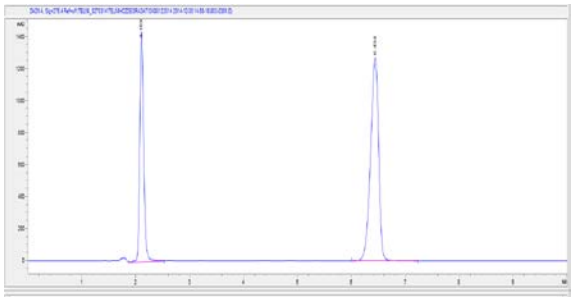


Fig.3.2 (f) Chromatogram of Neutral Hydrolytic Degradation

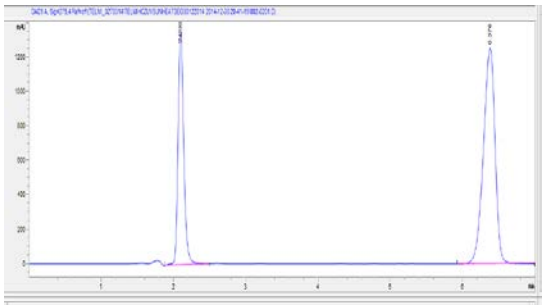


Fig.3.2 (g) Chromatogram of Dry Heat Degradation

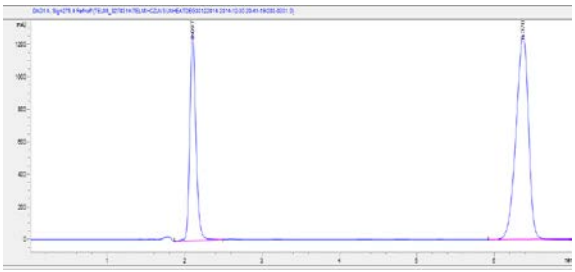


Fig.3.2 (h) Chromatogram of Direct Sunlight Degradation

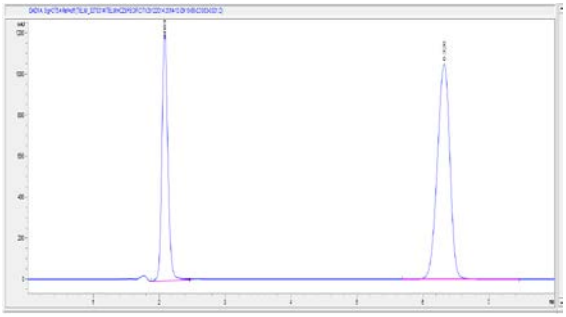


Fig.3.2 (i) Chromatogram of Photolytic Degradation

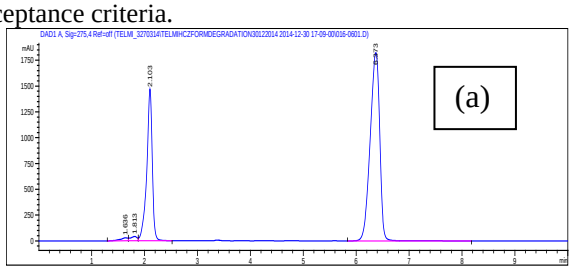
3.3. Method Validation:

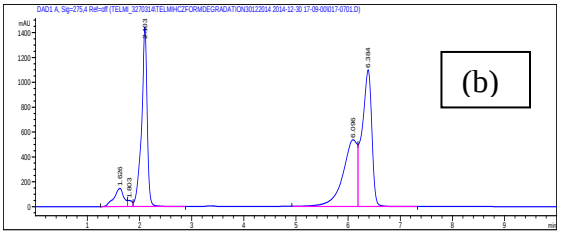
The validation of the current method has been performed according to the ICH guideline[36]. The following validation parameters were considered for the newly developed method such as system suitability, linearity, precision/reproducibility, accuracy, specificity, robustness, stability of analytical solution.

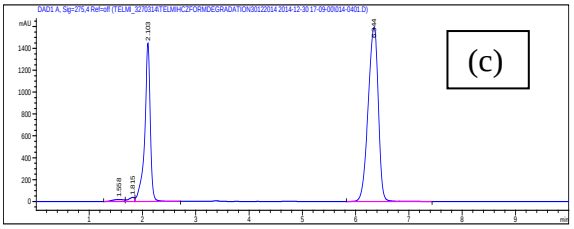
3.3.1. System Suitability:

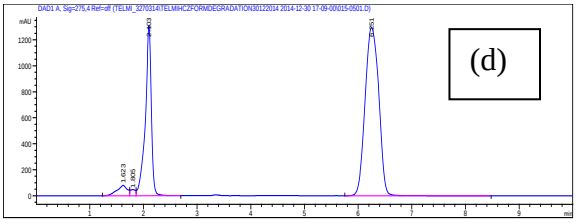
The system suitability of the method was tested by injecting one blank injection, five injections of telmisartan and hydrochlorothiazide mixed standard solution of concentration 200 µg ml^{-1},62.5 µg ml^{-1} . System suitability parameters like theoretical plates, tailing factor, areas % relative standard deviation (RSD) were studied and

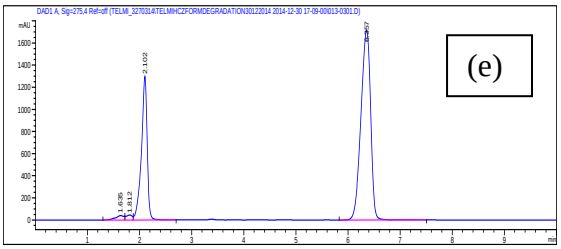
found that all the system suitability parameters are within acceptance criteria.

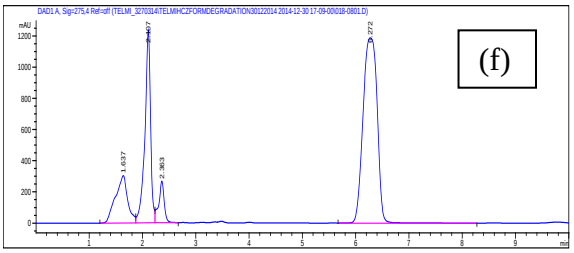


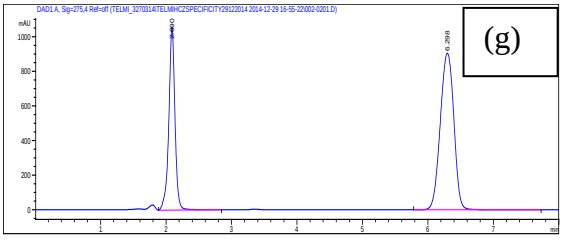


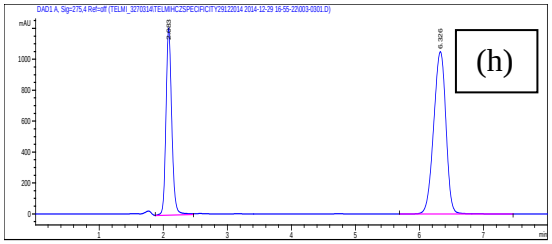












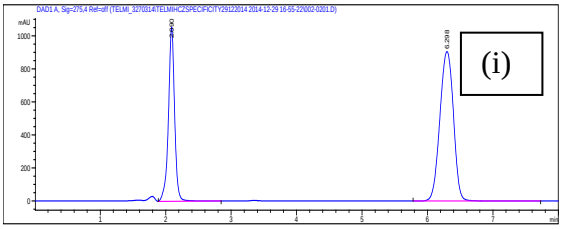


Fig. 3.3.5 - UHPLC Chromatograms of Specificity Study of Newtel-H **tablet in (a,b) 0.1&1N N HCl (4h), (c,d) 0.1&1 N NaOH (4h), (e) Neutral (8h),(f) 3% H_2O_2 (1h), (g,h) Sunlight (8 h) and UV (24 h) (i) Dry heat at 80^{0}C (24 h)**

3.3.2. Calibration & Linearity:

Linearity of the method was tested by preparing five different mixed standard solutions from 50% to 150% of telmisartan and hydrochlorothiazide and injected in triplicate for each concentration. The mixed standard solutions contain the concentration ranges from 180 to 220 µg ml^{-1} for telmisartan and 42.5 to 82.5 µg ml^{-1} for hydrochlorothiazide. From the chromatograms, linearity plots were drawn by taking concentration on X-axis and area of peaks on Y-axis. The regression equations obtained for telmisartan and hydrochlorothiazide were 71.513x+97.76 and 108.16x+825.44 which is shown in Table 3.3. The linear regression coefficient and correlation coefficients values for telmisartan and hydrochlorothiazide were found to be 0.9989, 0.9994 and 0.9986, 0.9992 respectively indicating a high degree of linearity.

3.3.3. Precision (Repeatability):

The system precision of the analytical method was determined by measuring retention time and areas of mixed homogeneous standard solution of telmisartan and hydrochlorothiazide having concentration 180.0 µg ml^{-1} , 42.5 µg ml^{-1} and % RSD was calculated which ensure that the analytical system is working properly. For method precision, retention time and areas of six determinations of mixed homogeneous standard solution of telmisartan and hydrochlorothiazide having concentration 200.0 µg ml^{-1}, 62.5µg ml^{-1} were measured and % RSD was calculated. Similarly intermediate precision of the method was determined by analyzing retention time and areas of the mixed homogeneous standard solution having concentration 200.0 µg ml^{-1}, 62.5µg ml^{-1} for six times on different days, by different analysts. The results of the precision study indicate that the method is precise. (RSD % <2)

3.3.4. Accuracy (Recovery Test):

Accuracy of the method was studied by recovery studies. To check the recovery of the proposed method, recovery studies were carried out in three different levels, with each level in triplicate for standard drugs (nine determinations). Spiked known quantity of telmisartan and hydrochlorothiazide standard drugs at 50%, 100% and 150% levels in to the

tablet sample solutions containing 40 µg ml^{-1} of telmisartan and 12.5 µg ml^{-1} of hydrochlorothiazide. The prepared solutions were then analyzed and percentage recoveries were calculated which is shown in Table.3.3. The recovery value of Bosentan ranges from 99.22 to 99.55%. The average recovery of three levels (9 determinations) for telmisartan and hydrochlorothiazide were 97.4%. and 98.82% respectively.

3.3.5. Specificity:

The specificity study was carried out in terms of different forced degradation studies. Tablet samples were stressed with different conditions (similar to standard drug degradation studies) and injected in to UHPLC system. Photodiode array detection was used as an evidence of the specificity of the method and to evaluate the homogenicity of the drug peak. The peak purity values for telmisartan and hydrochlorothiazide were 998.39 and 999.18 respectively which are more than 997 for tablet samples at 275nm which shows that the peaks of analyte were pure and also the formulation excipients and degradants were not interfering with the analyte peaks which are shown in Fig.3.3.5 (a) to 3.3.5(i).

3.3.6. Robustness:

The robustness of an analytical method was measure of its capacity to remain unaffected by small but deliberate variations in method parameters. To determine robustness of the method, experimental conditions were purposely altered chromatograms of telmisartan and hydrochlorothiazide were studied. The flow rate of the mobile phase was 0.3ml/minute; it was changes to ±0.02 units from 0.3 to 0.28 ml/minute and 0.32 ml/minute. The column temperature was 25 ^{0}C, changes to ±5 ^{0}C from 25 ^{0}C to 20 ^{0}C and 30 ^{0}C while the other mobile phase components were held constant. Similarly change in mobile phase composition was studied at buffer: acetonitrile (57:43v/v) and 53:47 (v/v). At all conditions area % RSD were within acceptance criteria. Hence it is concluded that the method is robust.

3.3.7. Solution Stability:

Stability of standard and sample solutions of telmisartan and hydrochlorothiazide were evaluated injecting sample and standard at regular intervals up to 48 hours and calculated the area % difference value. The area % difference value indicate that the solutions were stable for 48 hours at ambient temperature as there is no formation of unknown peaks and solution remained stable.

Table 3.1- Summary of Proposed Forced Degradation Studies

Conditions (Stress induced)	Peak Purity P(TEL)	TEL		HCZ		Peak Purity P(HCZ)
		% TEL Degradation	Retention Time of Degradants in Minutes	% HCZ Degradation	Retention time of Degradants in Minutes	
Acidic	998.81	52.6(0.1NHCl)	6.143	12.6	1.491	998.89
	998.57	63.1(1N HCl)	5.846	48.7	1.626,1.813	999.91
Alkali	998.36	11.7(0.1NHCl)	----	4.5	----	999.35
	998.91	18.3 (1NHCl)	----	5.7	1.623,1.770	999.24
Hydrolytic	999.92	8.0	----	3.5	----	999.64
Oxidative	998.12	10.2	7.021	8.8	1.650,2.367, 2.607,3.201	999.28
Dry Heat	999.45	1.6	----	1.3	----	999.69
UV light	999.89	0.6	----	0.4	----	999.82
Sun light	999.78	0.5	----	0.2	----	999.93

Table 3.2.: Summary of the Proposed Method Validation

Validation parameters		Observed Value TEL	Observed Value HCZ	Acceptance Criteria
System Suitability	Tailing factor	1.17	0.81	RSD NMT 2.0%
	Theoretical Plate count	5174	2801	RSD NLT 2000
	The % RSD for the areas of 5 replicate injections of TEL and HCZ peak	0.11	0.08	RSD NMT 2.0%
Linearity	Regression coefficient	0.9989	0.9986	RSD NLT 0.998
	Correlation coefficient	0.9994	0.9992	RSD NLT 0.998
	Slope	71.513	108.16	----
	Intercept	97.76	825.44	-----
Precision	System precision	0.13	0.35	RSD NMT 2.0%
	Method precision	0.14	0.07	RSD NMT 2.0%
	Intermediate precision	0.3	1.52	RSD NMT 2.0%
Accuracy	50%, 100% and 150% Level	97.4%	98.82%	Mean Recovery 95% and 105%.
Specificity	Peak purity	998.39	999.18	More than 997
Robustness	Change in column temperature ±5 ^{0}C	0.15%(20^{0}C), 0.08%(30^{0}C)	0.14%(20^{0}C), 0.17%(30^{0}C)	RSD NMT 2.0%
	Change in flow rate ±0.2mL/min	0.09 (-0.2) 0.2 (+0.2)	0.1(-0.2) 0.3 (+0.2)	RSD NMT 2.0%
	Change in organic phase ratio ±2%	0.1(-2 %) 0.09 (+2 %)	0.6(-2 %) 0.1(+2 %)	RSD NMT 2.0%

TEL: Telmisartan and HCZ: Hydrochlorothiazide

4. DISCUSSION:

The peaks of the degraded products were well resolved from the telmisartan and hydrochlorothiazide drugs peak. The chromatograms of the acid degraded samples showed 03 additional peaks of retention time of 1.491, 1.626 and 1.813 minutes for hydrochlorothiazide and 2 additional peaks of retention time of 5.846 and 6.143 minutes for telmisartan. The chromatograms of the alkali degraded samples showed 02 additional peaks of retention time of 1.623 and 1.770 minutes for hydrochlorothiazide. The chromatogram of the oxidation degraded sample showed showed 04 additional peaks of retention time of 1.650, 2.367, 2.607and 3.201minutes respectively for hydrochlorothiazide and 1 additional peak of retention time of 7.021 minutes for telmisartan. No additional peaks were developed in water, dry heat, UV light and sunlight degradation studies. Major degradation of telmisartan and hydrochlorothiazide were observed under acidic, alkali and oxidation conditions. Very less degradation was observed under hydrolytic, dry heat and photolytic conditions.

The response for the drugs were found to be linear in the concentration range 180-220 µg ml^{-1} for telmisartan and 42.5-82.5 µg ml^{-1} for hydrochlorothiazide respectively with respect to the peak area. The % RSD values for precision studies were found to be less than 2% for telmisartan and hydrochlorothiazide, this conform that the method is precise. The accuracy of the method was determined and the mean recovery of telmisartan and hydrochlorothiazide were 97.4% and 98.82% respectively. The specificity of the method was ascertained by peak purity study and peak purity value greater than 990 indicates a homogenous peak. The peak purity values for analyte TEL and HCZ were in the range of 997-1000 for drug substance as well as tablet formulation, indicating the peaks were pure and also that formulation excipients and degradants were not interfering with analyte peaks, thus establishing the specificity of the method. The low values of % RSD obtained after introducing small deliberate changes in the developed UHPLC method indicating the robustness of the method.

5. CONCLUSIONS:

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

Conflict of interest statement:

We declare that we have no conflict of interest.

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