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Method Development and Validation for the determination of potential impurities present in Telmisartan and Hydrochlorothiazide in fixed dose combination drug product by using Reverse Phase - Ultra Performance Liquid Chromatography coupled with Diode-Array Detector

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ABSTRACT

A new selective, sensitive and Rapid Reverse phase-UPLC method was developed and validated to determine the known potential impurities present in Telmisartan (TL) and Hydrochlorothiazide (HC) in fixed dose combination drug product. The quantification was carried out by using Acquity UPLC, HSS T3 (100 × 2.1) mm, 1.8µ column, with a flow rate of 0.5mL/min at 225 nm .The mobile phase consists of 0.1% ortho phosphoric acid pH adjusted to 2.6 with diluted sodium hydroxide as Mobile phase A and acetonitrile as Mobile phase B. Separation of the impurities was achieved within 10.0 minutes of run time. Typical retention times of TL and HC were found to be about 5.4 and 2.0 minutes respectively. The product was subjected to various degradation conditions and validated in terms of linearity, precision, accuracy, LOD, LOQ and robustness in accordance with ICH guidelines. The known impurities quantified in this study were HC imp-1 to 4 for Hydrochlorothiazide and TL imp-1 to 6 for Telmisartan. Recovery was established for all the impurities with respective to LOQ to 150%. The data supports that the newly developed method is capable to determine all the potential impurities of TL and HC.

Keywords: Telmisartan, Hydrochlorothiazide, Forced Degradation, RP-UPLC and Stability Indicating

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INTRODUCTION

Telmisartan (TL) and Hydrochlorothiazide (HC) tablets are fixed dose combination (FDC) product available with brand name Micardis HCT tablets in US market. Telmisartan is an orally active angiotensin II antagonist acting on the AT_1 receptor subtype and hydrochlorothiazide, a diuretic[1-2] Micardis HCT tablets are formulated for oral administration in three combinations like 40 mg/12.5 mg, 80 mg/12.5 mg, and 80 mg/25 mg Telmisartan and Hydrochlorothiazide, respectively.

TL is a white to slightly yellowish solid. It is chemically described as 4'-[(1,4'-dimethyl-2'-propyl [2,6'-bi-1Hbenzimidazol]-1'-yl) methyl]-[1,1'-biphenyl]-2-carboxylic acid. Its empirical formula is $C_{33}H_{30}N_4O_2$, with a molecular weight of 514.63, It is practically insoluble in water and in a pH range of 3 to 9, sparingly soluble in strong acids (insoluble in hydrochloric acid), and soluble in strong bases. HC is a white or partially white, crystalline powder. It is chemically described as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7sulfonamide 1,1-dioxide. Its empirical formula is $C_7H_8ClN_3O_4S_2$ with a molecular weight of 297.74. It is slightly soluble in water and freely soluble in sodium hydroxide solution. TL and HC structural formula are illustrated in Figure 1.

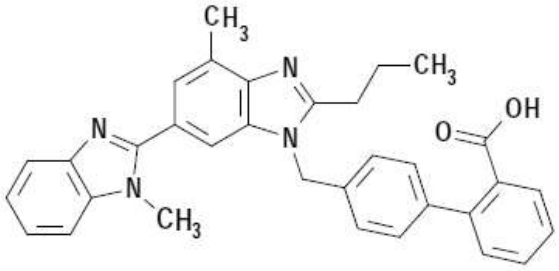


Figure 1: Structural formula of TL and HC

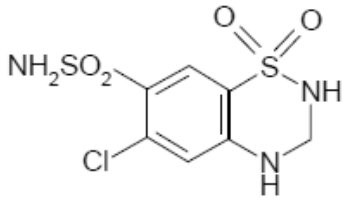


Figure 2: Chemical structures of potential Impurities of TL

An extensive literature survey revealed that few analytical methods were available using spectrophotometric technique for the determination of TL/HC in FDC tablets [3-6]. Some of the other spectrophotometric methods were also reported for the determination of TL/HC along with Amlodipine or Ramipril [7-8]. HPTLC and mass spectroscopic methods are also available for the determination of TL/HC or Ramipril/TL/HC in FDC tablets [9-12]. HPLC and UPLC methods are available for simultaneous quantification of TL/HC along with amlodipine or Ramipril in FDC tablets[13-19]. HPLC methods available for quantification of TL/HC in FDC tablets [20-26]. HPLC

methods are also available for determination of impurities present TL/HC in FDC tablets [27-28]. However there are no stability-indicating methods were available with shorter run time using UPLC for quantification of impurities present in sample matrix of TL/HC tablets. UPLC is selected as analytical tool, since, it is having multiple advantages in terms of better sensitivity, selectivity, reproducibility, ecofriendly, fast analytical capability. So, RP-UPLC technique was selected for separation and quantification of potential impurities present in TL/HC tablets.

The aim of the study was to develop a simple, precise, economic and accurate RP-UPLC method for the estimation of ten potential impurities present in TL and HC tablets as per International Conference on Harmonization (ICH) recommendation. The developed RP-UPLC method consumes less solvent consumption, shorter run time, having better selectivity, better sensitivity and yields very sharp and symmetrical peak shapes. Forced Degradation study was conducted on finished dosage form to identify degradant impurities. Forced degradation or stress studies are a part of the analytical development strategy and are also an integral component of validating analytical method that symbolizes the stability indicating nature of the method and also detecting capability of impurities. The analytical method should be stability-indicating and fully validated as per USP and ICH guideline recommendation [29-34].

MATERIALS AND METHOD

Instrumentation:

The UPLC system, used for method development and method validation was Waters-Acquity UPLC equipped with separation module consisting of Binary gradient pump, thermostatic column compartment, Photo diode array detector, Auto sampler, Computer with windows based Empower-3 Method validation manager software. The output signal was monitored and processed using Empower-3 software. Column used for chromatography was Acquity UPLC HSS T3 (100 mm × 2.1 mm), 1.8µ particle size.

Reagents:

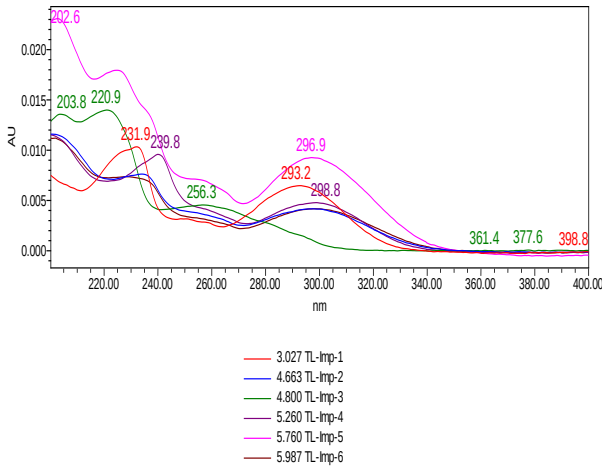
TL and HC drug substances, impurities of TL and HC, Micardis HCT (TL/HC) tablets were generously sponsored by Aurobindo pharma limited. Acetonitrile of gradient grade, ortho phosphoric acid and sodium hydroxide of AR grade were procured from Merck chemicals. Ultrapure water is prepared by using Millipore Milli-Q plus water purification system. All chemicals and reagents were used as such without further purification.

Method development and optimization for UPLC method:

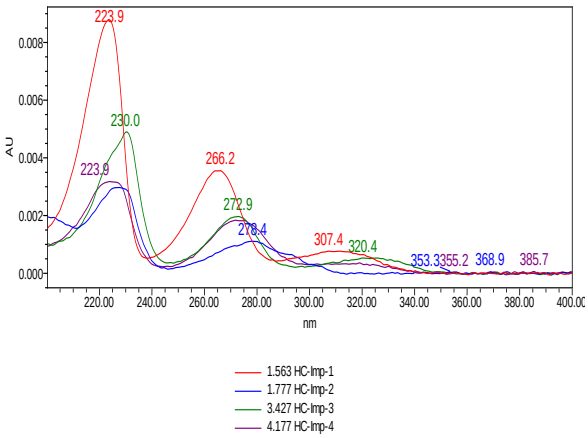
Aim of this paper was to develop very simple, sensitive, robust, precise and stability indicating chromatographic method which can separate TL and HC from its potential impurities with reduced run time. pKa of TL is about 3.65 & 6.13 and for HC about 9.09. Column life will be dependent on selection of buffer used in UPLC chromatographic condition due to its extremely lower ID with very less micron size. High viscous buffers shall be avoided to enhance the column life due to chocking of buffers either in column or tubing used in UPLC system. Keeping in view of this issue, trials were initiated using low viscous buffers like ortho phosphoric acid (OPA) in water as Mobile phase-A. Acetonitrile was used as Mobile phase-B. For initial trial purpose, effect of pH was studied in the range between pH 2.2 to 3.0. Trials using 0.1% OPA buffer pH adjusted to 2.6±0.05 with diluted sodium hydroxide solution is found to be suitable for separation of impurities present in TL/HC tablets with gradient elution mode.

Specificity of the method is dependent on appropriate column selection. Upon using different column chemistries available for UPLC method, Acquity UPLC HSS T3 (100 mm × 2.1 mm), 1.8µ particle size column is found to be suitable for separation of critical pair of peaks between TL and its impurities TL imp-4 & TL imp-5. This column is designed for superior polar compound retention, having wide usable pH range and more compatible for aqueous mobile phases. Since HC and its corresponding impurities are polar in nature, it is preferred to use more aqueous phase for retaining them in column. Hence the same column was preferred for method development and validation purpose. Effect of column oven temperature is established between 40°C to 50°C. In all temperatures method is found to be robust. In order to have optimum chromatographic condition, temperature at 45°C column oven temperature was selected during the development.

To know the elution pattern of these impurities, TL/HC tablets spiked with 0.5 % level for HC-Imp-1, 0.2% for HC-Imp-2, HC-Imp-3 and HC-Imp-4; 0.2 % level for TL-Imp-1, TL-Imp-2, TL-Imp-3, TL-Imp-4, TL-Imp-5 and TL-Imp-6 impurities against sample test concentration of 400 µg/mL and 125 µg/mL for TL and HC respectively. As most of the drug components are soluble in organic solvents during method development a degassed mixture of methanol: pH 2.6 buffer in the ratio of 60:40 v/v was used. In this diluent, solubility and stability for impurities of TL and HC are found to be satisfactory. Hence the same diluent was used for impurity, standard and sample preparations for the entire method development and validation activity. Spectral data for majority of impurities of TL and HC has shown wavelength maxima at about 225nm (Figure 4), the same maxima of 225nm has been chosen for quantification of impurities. For initial trial purpose 2µL injection volume has been chosen and found precise area counts for impurities as well as main drug. Hence the same was fixed for final method.



TL IMP SPECTRA



HC IMP SPECTRA

Figure 4 Absorption spectral characteristics of TL and HC impurities

The possible potential impurities that may arise from TL are mentioned below. Chemical structures of TL are given in **Figure 2**

4-Methyl-6-(1-Methyl-1*h*-Benzimidazol-2-Yl)-2-Propyl-1*h*-Benzimidazole **[Dibenzimidazole Derivative] [USP Telmisartan Related Compound A]** ***TL-Imp-1***

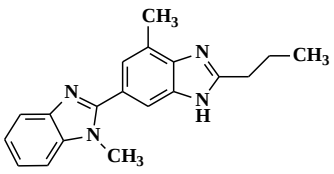
Telmisartan Amide: 4'-[[4-Methyl-6-(1-Methyl-1*h*-Benzimidazol-2-Yl)-2-Propyl-1*h*-Benzimidazol-1-Yl] Methyl] Biphenyl-2-Carboxamide **[USP Telmisartan Amide]** ***TL-Imp-2***

Telmisartan Diacid: 4'-[[4-Methyl-6-(Carboxy)-2-Propyl-1*h*-Benzimidazol-1-YL]Methyl]Biphenyl-2-Carboxylic Acid **[USP Telmisartan Diacid]** ***TL-Imp-3***

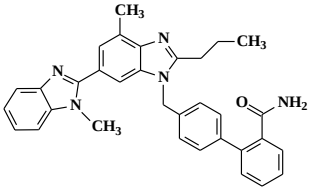
4'-[[7-Methyl-5-(1-Methyl-1*h*-Benzimidazol-2-Yl)-2-Propyl-1*h*-Benzimidazol-1-Yl]Methyl]Biphenyl-2-Carboxylic Acid **[Telmisartan Isomer]** ***TL-Imp-4***

4'-[[4-Methyl-6-(1-Methyl-1*h*-Benzimidazol-2-Yl)-2-Propyl-1*h*-Benzimidazol-1-Yl]Methyl]Biphenyl-2-Carbonitrile **[Telmisartan Nitrile]** ***TL-Imp-5***

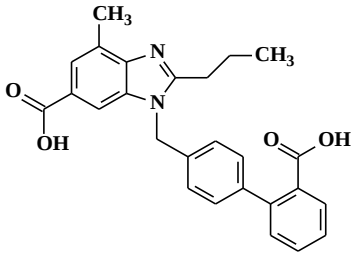
4'-[[4-Methyl-6-(1-Methyl-1h-Benzimidazol-2-Yl)-2-Propyl-1h-Benzimidazol-1-Yl] Methyl]Biphenyl-2-Carboxylic Acid, Methyl Ester **[Telmisartan Methyl Ester]** ***TL-Imp-6***



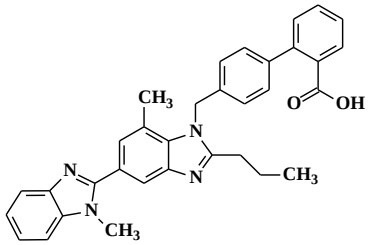
[Dibenzimidazole Derivative] [USP Telmisartan Related Compound A]



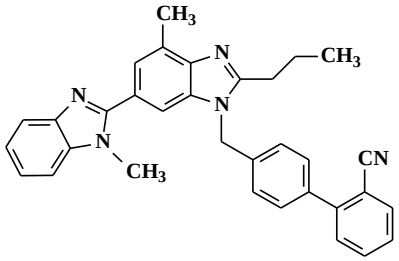
Telmisartan Amide [USP Telmisartan Amide]



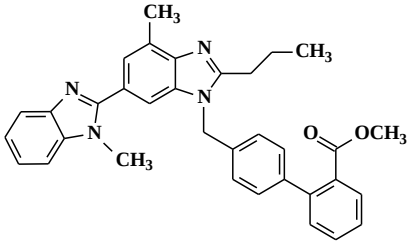
[Telmisartan Diacid [USP Telmisartan Diacid]



[Telmisartan Isomer]



[Telmisartan Nitrile]



[Telmisartan Methyl Ester]

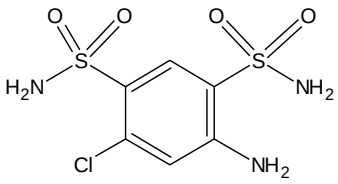
The possible potential impurities that may arise from HC are mentioned below. Chemical structures of HC are mentioned in **Figure 3**

4 – Amino – 6 – chloro – 1,3 – benzene disulphonamide ***(Benzothaidiazine related compound 'a')- HC-Imp-1***

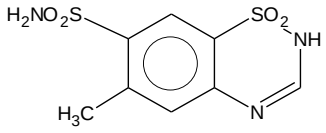
6–CHLORO–2H–1,2,4, -BENZOTHIADIAZINE – 7 – SULFONAMIDE – 1,1 – DIOXIDE ***(Chorothiazide)- HC-Imp-2***

5,6-Dichloro-3,4-Dihydro-2h-1,2,4-Benzothiadiazine-7-Sulfonamide 1,1-Dioxide (***5-Chloro hydrochlorothiazide*)- *HC-Imp-3***

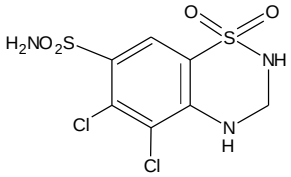
6-Chloro-N-[(6-Chloro-7-Sulfamoyl-2,3-Dihydro-4h-1,2,4-Benzothiadiazin-4-Yl1,1-Dioxide)Methyl]-3,4-Dihydro-2h-1,2,4-Benzothiadiazine-7-Sulfonamide1,1-Doxide ***(Hydrochlorothiazide dimer)- HC-Imp-4***



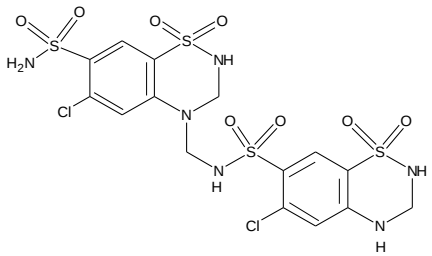
(Benzothaidiazine related compound 'A'



(Chorothiazide)



(5-chlorohydrochlorothiazide)



(*Hydrochlorothiazide dimer*)

Figure 3: Chemical structures of potential impurities of HC

Finalized chromatographic conditions for method validation

Transferred 1mL of Orthophosphoric acid in 1000mL of Milli-Q water and mix. Adjusted the pH to 2.60±0.05 with dilute sodium hydroxide solution. Filtered the solution with 0.22µ membrane filter (Millipore PVDF).This was used as mobile phase-A and acetonitrile was used as mobile phase-B. Gradient mode was chosen with gradient elution technique (Table I). Column used for chromatographic separation was Acquity UPLC HSS T3 (100 mm × 2.1 mm), 1.8µ particle size. Quantification wavelength was fixed at 225nm with an injection volume of 2µL. column oven temperature was kept at 45°C. Diluent consists of degassed mixture of methanol: pH 2.6 buffer in the ratio of 60:40 v/v.

Table I. Gradient program

Time (min)	Flow rate ($mL.min^{-1}$)	Mobile phase- A (%)	Mobile phase - B (%)
$T_{0.01}$	0.50	90	10
$T_{2.0}$	0.50	85	15
$T_{5.0}$	0.50	50	50
$T_{6.5}$	0.50	50	50
$T_{7.3}$	0.50	40	60
$T_{7.5}$	0.50	90	10
$T_{10.0}$	0.50	90	10

In the finalized chromatographic conditions, typical relative retention times of HC-Imp-1, HC-Imp-2, HC, TL-Imp-1, HC-Imp-3, HC-Imp-4, TL-Imp-2, TL-Imp-3, TL-Imp-4,TL, TL-Imp-5, TL-Imp-6, are about 0.29, 0.33, 0.38, 0.56, 0.64,0.78, 0.87, 0.90, 0.98, 1.00, 1.07, 1.12 respectively. For system suitability data, refer Table II.

Table II. General system suitability data observed in finalized chromatographic conditions

Name of the component	USP Theoretical plates	USP Tailing factor	USP Resolution
HC-Imp-1	3513	1.38	---
HC-Imp-2	5484	1.27	2.10
HC	7040	1.29	2.74
TL-Imp-1	45757	1.20	12.47

HC-Imp-3	47154	0.85	6.54
HC-Imp-4	88830	1.17	12.21
TL-Imp-2	134650	1.32	8.85
TL-Imp-3	139835	1.19	2.60
TL-Imp-4	159953	1.08	8.49
TL	59988	1.38	1.63
TL-Imp-5	173503	1.15	5.60
TL-Imp-6	149655	1.17	3.79

Preparation of Solutions

Preparation of standard solution

Initial Standard stock solution of TL and HC (0.005 mg/mL as HC and 0.016 mg/mL of TL) was prepared by dissolving in diluent. This stock solution was further diluted with diluent to obtain a concentration of 0.3 µg/mL for HC and 0.96 µg/mL for TL. All impurities were prepared by dissolving in an appropriate amount of methanol, followed by using diluent at desired concentration levels for validation purpose.

Preparation of sample solution

Weighed and crushed not less than 10 tablets. Transferred an accurately weighed portion of sample powder, equivalent to about 15 mg of Hydrochlorothiazide into a 100 ml clean, dry volumetric flask, added 60 ml methanol and sonicated for about 20 minutes with intermediate shaking. Allowed the solution to cool to room temperature and diluted to volume with pH 2.6 buffer and mixed. Filtered the sample solution through a 0.22 µ filter (Millipore PVDF/mdi Nylon) by discarding the first few milliliters of filtrate.

Chromatographic System suitability parameters

The column efficiency as determined from standard of TL and HC peaks are not less than 5000 USP plate count and the Symmetry factor for the same peak is not more than 2.0. RSD for the peak areas of the six replicate injections of Telmisartan and Hydrochlorothiazide peaks in the standard solution should **not be more than 5.0%.** For data refer **Table III**.

Table III. Chromatographic system suitability data

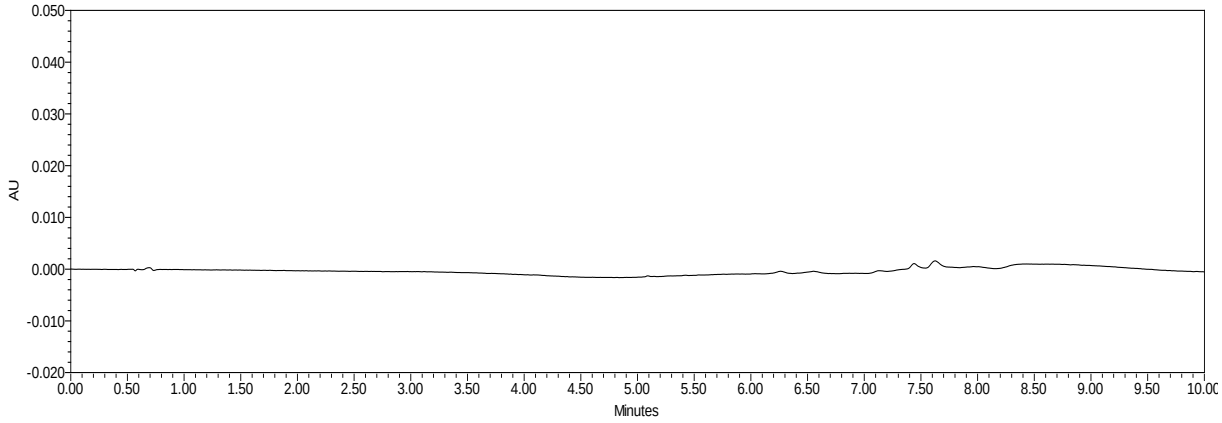
Name of the component	USP Theoretical plates	USP Tailing factor	% RSD
TL	175335	1.09	0.34
HC	6961	1.24	0.62

Identification of specified and unspecified impurities present in sample matrix

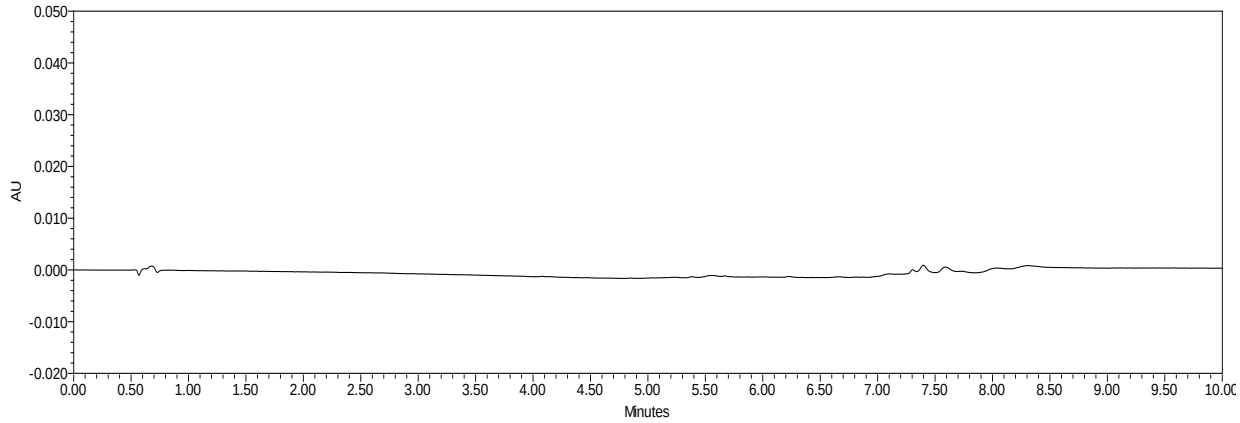
a) TL and HC specified impurities shall be identified and calculated using respective diluted standard solutions. Unspecified impurities of each drug component shall be identified using placebo degradation chromatograms of individual drug components in acid, alkali, oxidative,

thermal, Humidity and photolytic stress as mentioned in **Figure 5** and calculated using respective diluted standard solutions.

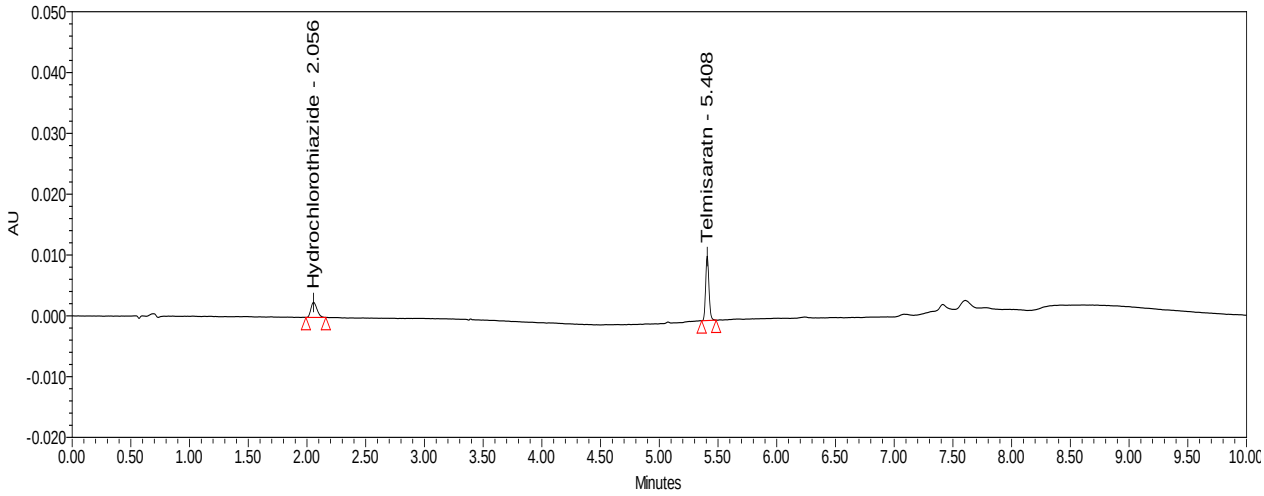
b) Any other unspecified impurity which does not matches with the retention time of degradation chromatogram of individual drug component shall be identified by checking the spectra using PDA (Photo diode array) detector and calculate based on spectral similarity with respective to its drug component. For unaccountable unspecified impurities, which may generate due to drug to drug interaction of TL and HC shall be calculated using diluted standard of drug component with lowest labeled amount i.e., HC.



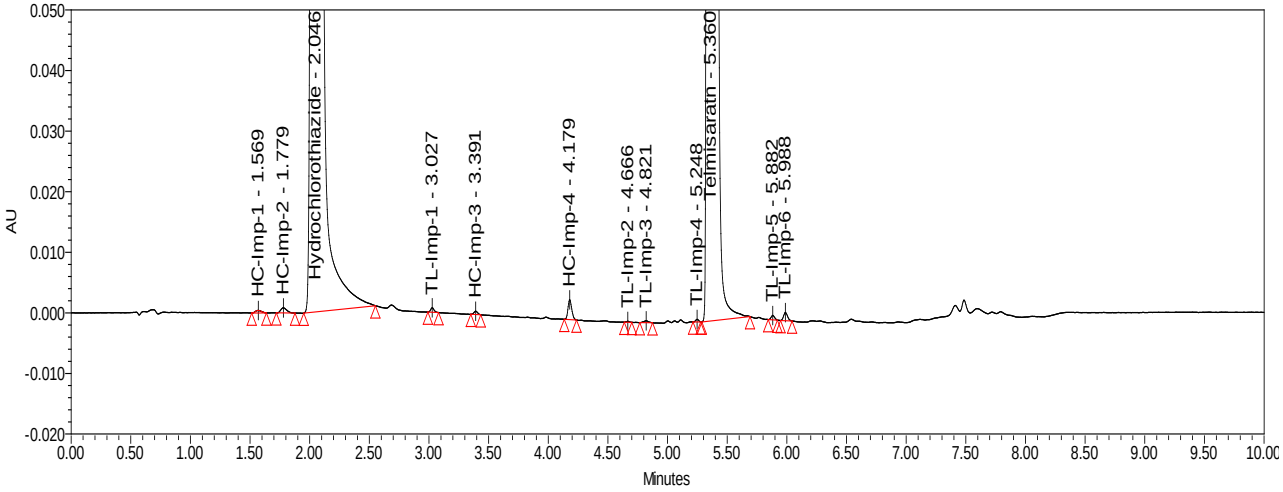
Diluent Chromatogram



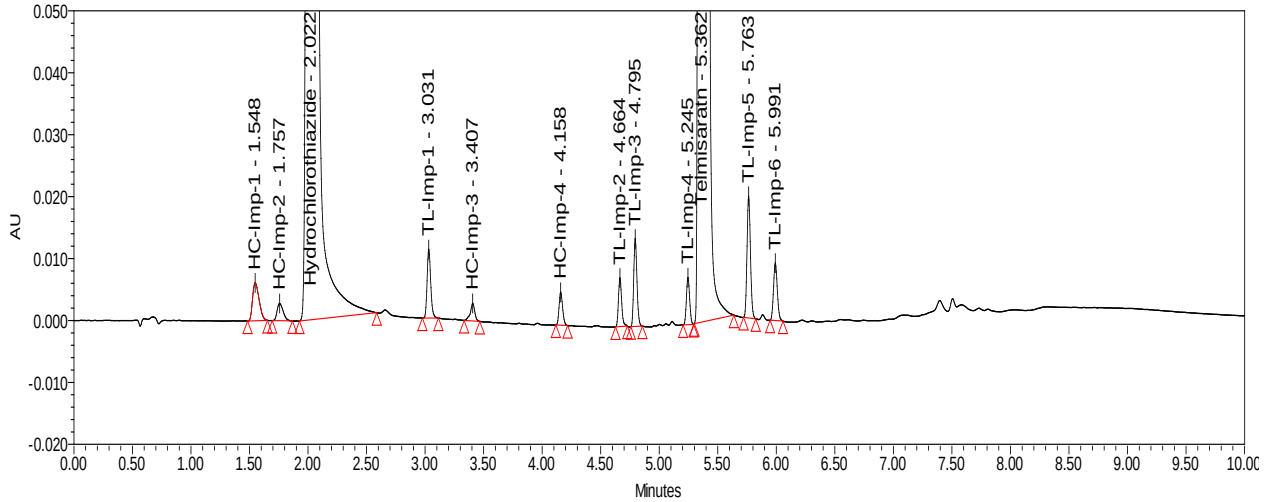
Common Placebo Chromatogram



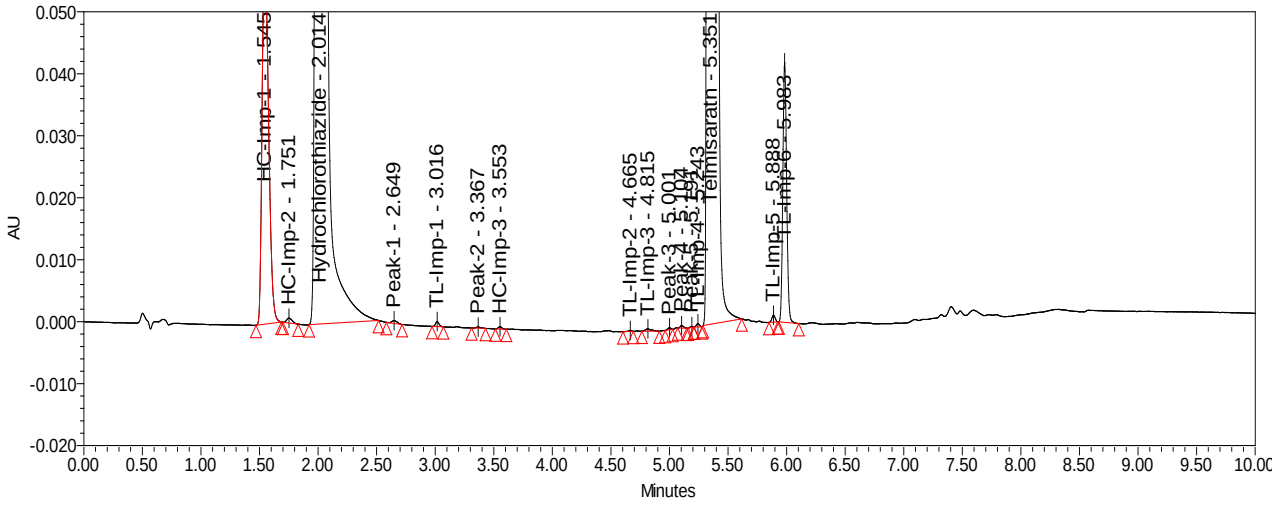
Diluted Standard Chromatogram



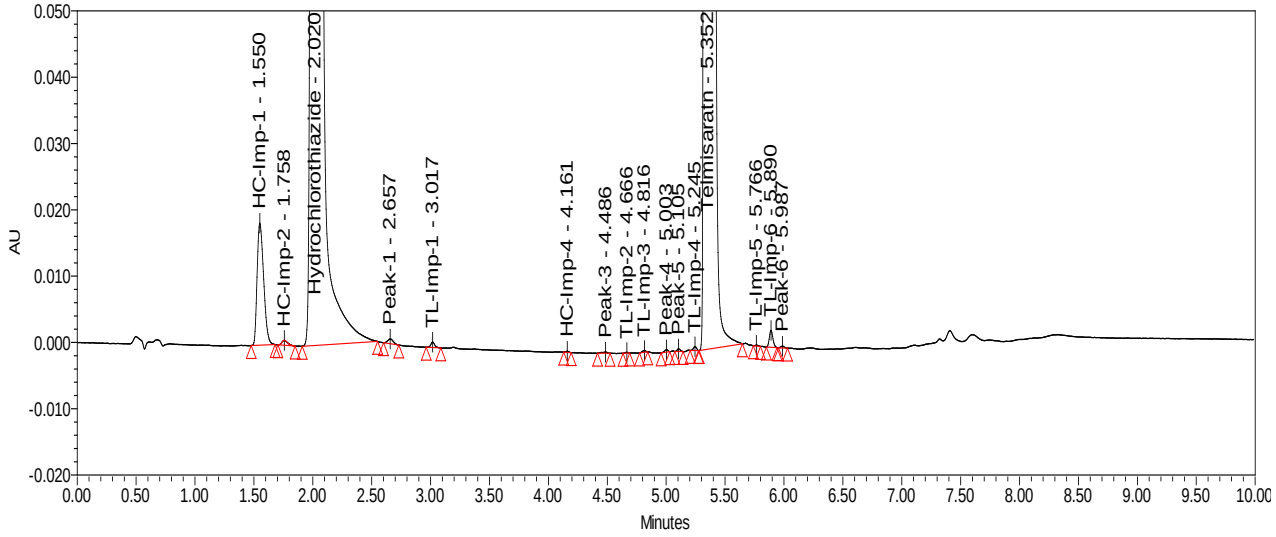
Control Sample Chromatogram



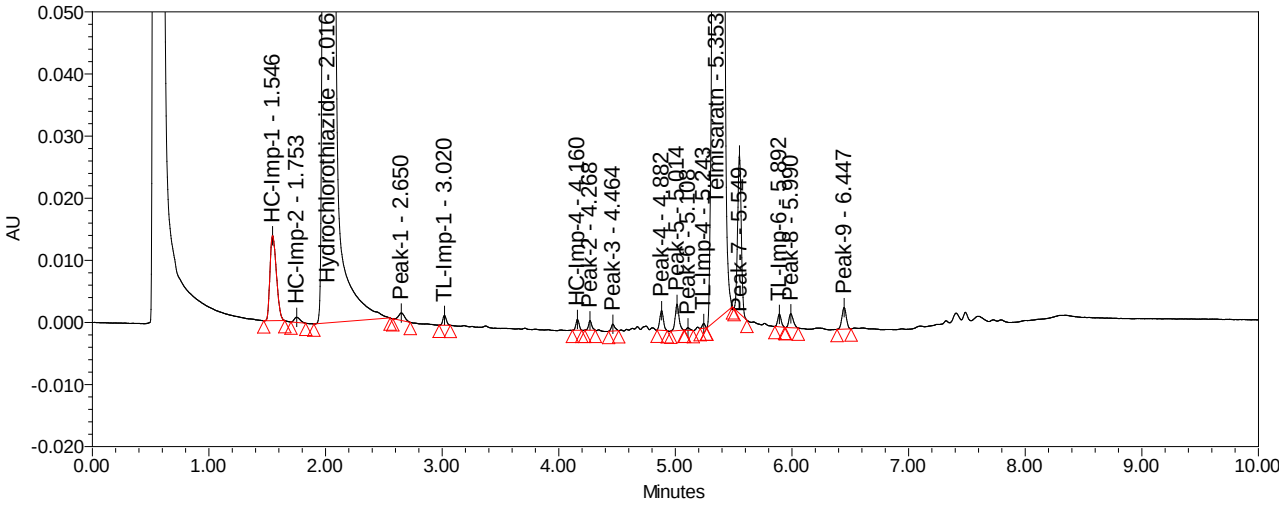
Spike Sample Chromatogram



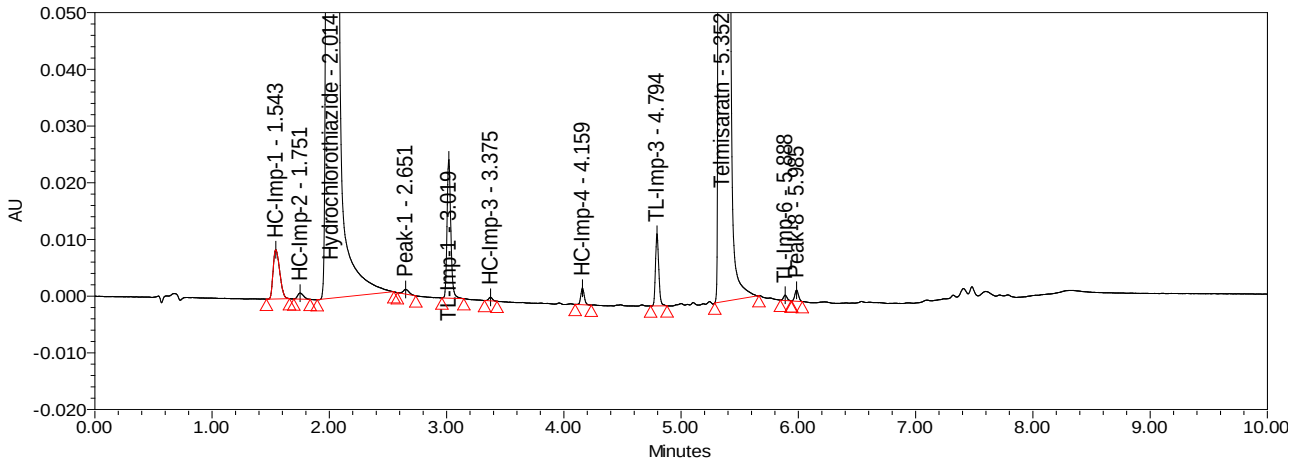
Acid Degradation Chromatogram



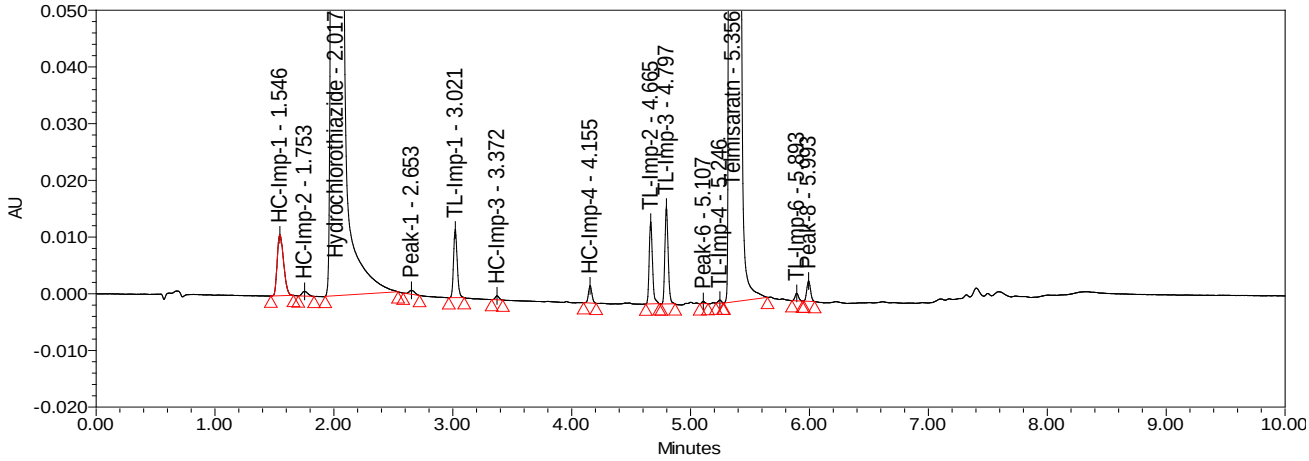
Base Degradation Chromatogram



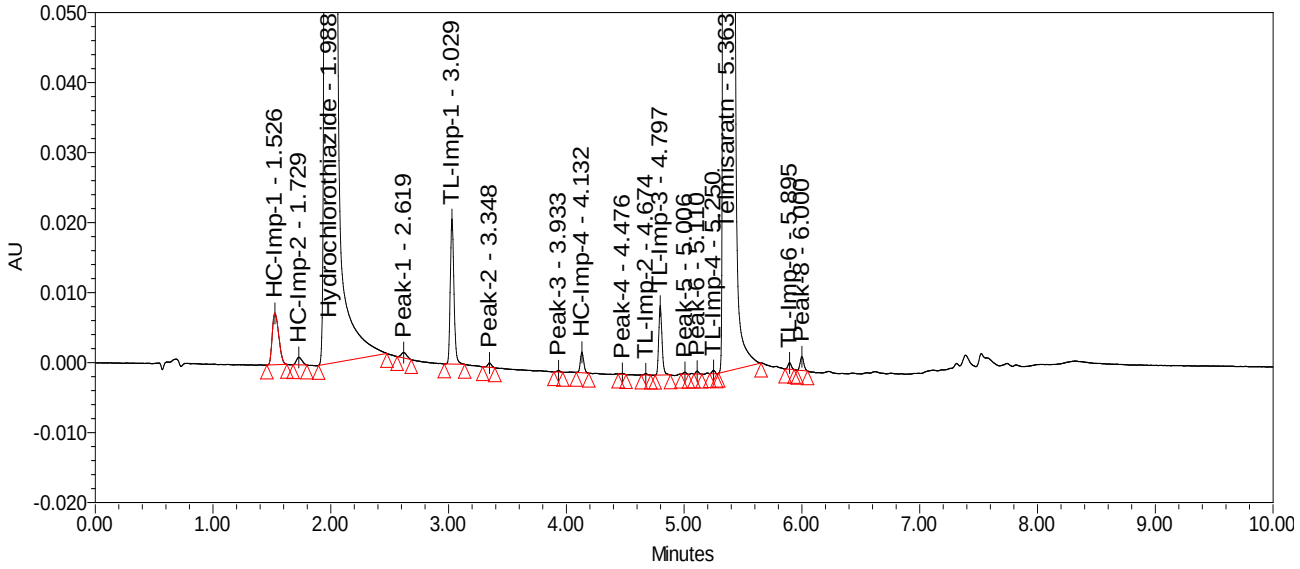
Peroxide Degradation Chromatogram



Thermal Degradation Chromatogram



Humidity Degradation Chromatogram



Photolytic Degradation Chromatogram

Figure 5: Typical chromatogram of Diluent, placebo, Diluted Standard solution, All impurity mixture at specification level, TL/HC tablets spiked with impurities, Stressed sample chromatograms

Analytical method validation

Micardis HC (TL/HC) tablets available in three strengths, namely 40 mg/12.5 mg, 80 mg/12.5 mg, and 80 mg/25 mg. For validation purpose Micardis HC (TL/HC) tablets 40mg/12.5mg has been chosen. The developed method was validated for Specificity, Forced degradation studies, Precision, Sensitivity (Limit of detection and Limit of Quantification), Linearity, Range, Accuracy, solution stability and Robustness as per ICH general recommendation.

Specificity and Stress studies

The developed method was checked for specificity with respect to diluent, placebo used in sample matrix and with Ten potential impurities .The stress conditions used for degradation study are Acid hydrolysis (5N HCl / 1mL/ 85°C / 60 mins), Base hydrolysis (5N NaOH / 1mL / 85°C / 60 mins), Oxidation (30% H_2O_2 / 1mL/ 85°C / 60 mins.), Thermal stress (105°C /24 hours), Humidity stress (95%RH / 24 Hours) and Photolytic stress (white fluorescent 1.2 million lux hours UV 200 watt hr/m^2 for 7 days).

Precision

The precision of the method was checked by injecting six individual preparations of TL/HC tablets spiked with 0.5 % level for HC-Imp-1, 0.2% for HC-Imp-2,HC-Imp-3, HC-Imp-4 0.2 %, for TL-Imp-1, TL-Imp-2, TL-Imp-3, TL-Imp-4, TL-Imp-5 and TL-Imp-6 impurities. The percentage RSD for % w/w of each impurity was calculated. The intermediate precision (Ruggedness) of the method was evaluated by different analyst using different columns and different UPLC instruments on different days.

Sensitivity (Limit of detection and Limit of Quantification)

LOD and LOQ studies were carried out to evaluate the detection and quantitation limits of the method to determine the presence of any impurities by using following equation:

$$LOD = 3.3\ \sigma/S$$

$$LOQ = 10\ \sigma/S$$

Where σ is the standard deviation and S is the slope of the curve.

Linearity and Range

Linearity curves were plotted from the finalized LOQ level to 150% of the impurity specification level. The correlation coefficient, slope and Y-intercept of the Linearity curve was calculated for each impurity.

Accuracy

To check the accuracy of the developed method, a known amount of the impurity stock solutions were spiked to the samples at LOQ concentration, 50%, 100% and 150% of the proposed specification level concentration. The % w/w of recoveries for all the impurities was calculated. Each concentration level is prepared for triplicate preparation.

Solution Stability

In order to demonstrate the stability of both reference and sample solutions, these solutions were injected immediately after preparation and at periodical intervals by maintaining at room temperature (~25°C) .

Robustness

To evaluate the robustness of the developed RP-UPLC method, small deliberate variations in optimized method parameters were done. The effect of change in flow rate, pH, wavelength variation, column oven temperature, gradient variation was studied.

RESULTS AND DISCUSSION

Specificity and Stress studies

The method was optimized to separate degradation products formed under different stress conditions. Diluent, placebo, placebo with individual drug components and sample solutions were prepared on different stress conditions viz., Acid hydrolysis (5N HCl / 1mL/ 85°C / 60mins), Base hydrolysis (5N NaOH / 1mL / 85°C / 60 mins), Oxidation (30% H_2O_2 / 1mL/ 85°C / 60 mins), Thermal stress (105°C /24 hours), Humidity stress (95%RH / 24 Hours) and Photolytic stress (white fluorescent 1.2 million lux hours UV 200 watt hr/m^2 for 7 days).

Placebo chromatograms were evaluated to check the level of interferences from excipients present in the tablet matrix. Placebo with individual drug components were evaluated to check the level of specified and unspecified impurities generated during stress conditions. Degradation behavior indicated that for Telmisartan, in acid, there was a slight degradation is observed for TL-Imp-6. In base and peroxide, there was slight degradation observed for TL-Imp-1 and TL-Imp-4. In thermal condition, there was a slight degradation was observed for TL-Imp-1, TL-Imp-3 and TL-Imp-4. In humidity condition, there was a slight degradation is observed for TL-Imp-1, TL-Imp-2 and TL-Imp-3. In Photolytic condition, there was a slight degradation observed for TL-Imp-1, TL-Imp-3 and TL-Imp-6.

When it comes to Hydrochlorothiazide, in acid condition, significant degradation was observed for HC -Imp-1and slight degradation observed for HC-Imp-2. In base condition, a slight degradation

was observed for HC -Imp-1 and HC-Imp-2.In peroxide, thermal, humidity and photolytic conditions, a slight degradation was observed for HC -Imp-1, HC-Imp-2 and HC-Imp-4. There was no considerable degradation is observed for unknown impurities of TL and HC. **(Table IVa and IVb**)

Table Iva: Summary of forced degradation study for TL

Stress condition and Time	% Assay of TL	% imps+ %Deg. products TL	mass balance (%Assay+ Imps+% Deg products)	Purity angle	Purity Threshold	Major Appeared impurities
Acid hydrolysis - Immediately	98.3	1.264	99.56	0.343	1.046	TL-Imp-6
Alkali hydrolysis- Immediately	98.5	0.125	98.62	0.350	1.040	No significant degradation observed
Oxidation – 60 minutes	98.4	1.128	99.52	0.371	1.001	Unspecified impurity at RRT 1.04.
Thermal deg – 24 hours	99.0	0.791	99.79	0.380	1.001	TL-Imp-1 and TL-Imp-3
Humidity deg - 24 hours	98.7	1.033	99.73	0.367	1.002	TL-Imp-1, TL-Imp-2 and TL-Imp-3
Photolytic deg - 7 days	99.0	2.188	101.18	0.400	1.001	TL-Imp-1 and TL-Imp-3

Note: The criteria for peak purity was purity angle is less than purity threshold

Table IVb: Summary of forced degradation study for HC

Stress condition and Time	% Assay of HC	% imps+ %Deg. products HC	mass balance (%Assay+ Imps+% Deg products)	Purity angle	Purity Threshold	Major Appeared impurities
Acid hydrolysis - Immediately	93.8	7.2	101.0	0.027	1.154	HC-Imp-1
Alkali hydrolysis- Immediately	98.6	2.312	100.91	0.059	1.170	HC-Imp -1
Oxidation – 60 minutes	98.6	2.079	100.67	0.032	1.005	HC-Imp -1 and HC-Imp-4
Thermal deg – 24 hours	98.9	1.698	100.59	0.029	1.004	HC-Imp -1 and HC-Imp -4
Humidity deg - 24 hours	98.8	1.84	100.64	0.036	1.005	HC-Imp -1 and HC-Imp -4
Photolytic deg - 7 days	98.9	3.036	101.93	0.028	1.004	HC-Imp -1, HC-Imp -2 and HC-Imp -4

Precision

The percentage RSD of %w/w for TL-Imp-1, TL-Imp-2, TL-Imp-3, TL-Imp-4, TL-Imp-5, TL-Imp-6, HC-Imp-1, HC-Imp-2, HC-Imp-3, HC-Imp-4, was 1.0,0.5,0.5,0.5,0.0,1.3,0.7, 0.9,1.2,0.7 respectively were obtained confirming the good precision of the developed method.

Intermediate Precision

The percentage RSD obtained in intermediate precision studied for TL-Imp-1, TL-Imp-2, TL-Imp-3, TL-Imp-4, TL-Imp-5, TL-Imp-6, HC-Imp-1, HC-Imp-2, HC-Imp-3, HC-Imp-4 was 1.0, 0.5. 1.0, 1.5, 0.5, 1.3, 1.1, 1.3, 0.8, 1.0 respectively confirm the intermediate precision. The overall percentage RSD of % w/w was found to be less than 15.0% between method precision and intermediate precision data.

Sensitivity (Limit of detection and Limit of Quantification)

The Limit of detection of %w/w for TL, TL-Imp-1, TL-Imp-2, TL-Imp-3, TL-Imp-4, TL-Imp-5, TL-Imp-6,HC,HC-Imp-1,HC-Imp-2,HC-Imp-3,HC-Imp-4 was 0.010, 0.0090, 0.0090, 0.0094, 0.0095, 0.0096, 0.0097, 0.0102, 0.0248, 0.0105, 0.0097and 0.0099 respectively. The Limit of quantification of %w/w for TL, TL-Imp-1, TL-Imp-2, TL-Imp-3, TL-Imp-4, TL-Imp-5, TL-Imp-6,HC,HC-Imp-1, HC-Imp-2, HC-Imp-3 and HC-Imp-4 is 0.0212, 0.018, 0.0187, 0.0188, 0.0190, 0.0193, 0.0195,0.0204,0.0496,0.0211,0.0194 and 0.0199 respectively. Hence developed method found to be sensitive enough to detect impurities at lowest concentration levels.

Linearity and Range

Calibration curve obtained by the least square regression analysis between peak area and concentration showed a linear relationship with a correlation coefficient of greater than 0.995 over the calibration ranges tested. Linear calibration plot for the related substances method is obtained over the calibration range LOQ to 150%. The results show an excellent correlation obtained between peak area and concentration of Telmisartan and Hydrochlorothiazide and all the impurities. (**Table V**)

Table V. Linearity table

Name of the component	Trend line equation	Range	Correlation coefficient	Intercept	Residual sum of squares
HC-Imp-1	y = 30058x - 70	0.007-1.117	0.99987	-70	202
HC-Imp-2	y = 22001x - 14	0.003-0.477	0.99953	-14	118
HC	y = 34039x - 92	0.003-0.460	0.99983	-92	105
TL-Imp-1	y = 25284x – 113	0.009-1.317	0.99984	-113	220
HC-Imp-3	y = 21137x – 20	0.003-0.438	0.99941	-20	117
HC-Imp-4	y = 13837x – 8	0.003-0.451	0.99939	-8	80
TL-Imp-2	y = 16585x – 171	0.009-1.348	0.99959	-171	233

TL-Imp-3	y = 29334x – 77	0.009-1.356	0.99988	-77	224
TL-Imp-4	y = 15548x + 11	0.009-1.374	0.99985	11	134
TL	y = 20711x + 3	0.010-1.532	0.99981	3	225
TL-Imp-5	y = 43150x - 167	0.009-1.394	0.99987	-167	360
TL-Imp-6	y = 18991x + 21	0.009-1.408	0.99975	21	218

Accuracy

Accuracy was assessed from three replicate determinations of four different levels including LOQ, 50%, 100% and 150% of the specification level of the impurities. The observed recovery results were found in the range between 90 to 110% with the RSDs lower than 5.0% demonstrating that the method is accurate within the desired range. (Table VIa to VId).

Table Via. Table for Accuracy study for Telmisartan- TL- Imp-1, TL- Imp-2 and TL- Imp-3

Sample spiked at level	TL- Imp-1 Amount added (%w/w)	Amount recovered (%w/w)	% Recovery	TL- Imp-2 Amount added (% w/w)	Amount recovered (%w/w)	% Recovery	TL- Imp-3 Amount added (% w/w)	Amount recovered (%w/w)	% Recovery
LOQ sample -1	0.0223	0.0228	102.2	0.0196	0.0202	103.1	0.0203	0.0207	102.0
LOQ sample -2	0.0216	0.0226	104.6	0.0194	0.0197	101.5	0.0198	0.0195	98.5
LOQ sample -3	0.0221	0.0223	100.9	0.0196	0.0195	99.5	0.0203	0.0202	99.5
50% sample -1	0.106	0.105	99.1	0.100	0.106	106.0	0.111	0.105	94.6
50% sample -2	0.103	0.102	99.0	0.100	0.097	97.0	0.107	0.105	98.1
50% sample -3	0.105	0.101	96.2	0.101	0.099	98.0	0.107	0.103	96.3
100% sample -1	0.212	0.205	96.7	0.214	0.215	100.5	0.223	0.211	94.6
100% sample -2	0.214	0.208	97.2	0.213	0.215	100.9	0.225	0.212	94.2
100% sample -3	0.211	0.205	97.2	0.206	0.211	102.4	0.222	0.209	94.1
150% sample -1	0.320	0.311	97.2	0.318	0.324	101.9	0.338	0.320	94.7
150% sample -2	0.319	0.308	96.6	0.315	0.320	101.6	0.336	0.318	94.6
150% sample -3	0.318	0.305	95.9	0.314	0.317	101.0	0.335	0.315	94.0

Table VIb: Table for Accuracy study for Telmisartan- TL- Imp-4, TL- Imp-5 and TL- Imp-6

Sample spiked at level	TL- Imp-4 Amount added (%w/w)	Amount recovered (%w/w)	% Recovery	TL - Imp-5 Amount added (% w/w)	Amount recovered (%w/w)	% Recovery	TL - Imp-6 Amount added (% w/w)	Amount recovered (%w/w)	% Recovery
LOQ sample -1	0.0196	0.0201	102.6	0.0214	0.0202	94.4	0.0242	0.0257	106.2
LOQ sample -2	0.0194	0.0193	99.5	0.0212	0.0198	93.4	0.0239	0.0257	107.5
LOQ sample -3	0.0196	0.0194	99.0	0.0214	0.0200	93.5	0.0230	0.0210	91.3
50% sample -1	0.100	0.101	101.0	0.106	0.107	100.9	0.104	0.103	99.0
50% sample -2	0.100	0.100	101.0	0.106	0.106	100.0	0.106	0.105	99.1
50% sample -3	0.101	0.100	99.0	0.107	0.106	99.1	0.110	0.110	100.0
100% sample -1	0.199	0.205	103.0	0.212	0.215	101.4	0.218	0.221	101.4
100% sample -2	0.201	0.208	103.5	0.214	0.217	101.4	0.221	0.219	99.1
100% sample -3	0.198	0.203	102.5	0.211	0.213	100.9	0.217	0.214	98.6
150% sample -1	0.302	0.313	103.6	0.322	0.327	101.6	0.331	0.336	101.5
150% sample -2	0.300	0.311	103.7	0.319	0.325	101.9	0.329	0.331	100.6
150% sample -3	0.299	0.307	102.7	0.320	0.321	100.3	0.328	0.324	98.8

Table Vic: Table for Accuracy study for Hydrochlorothiazide- HC - Imp-1, HC - Imp-2 and HC - Imp-3

Sample spiked at level	HC - Imp-1 Amount added (%w/w)	Amount recovered (%w/w)	% Recovery	HC - Imp-2 Amount added (% w/w)	Amount recovered (%w/w)	% Recovery	HC- Imp-3 Amount added (% w/w)	Amount recovered (%w/w)	% Recovery
LOQ sample -1	0.0653	0.0631	96.6	0.0205	0.0207	101.0	0.0218	0.0219	100.5
LOQ sample -2	0.0645	0.0606	94.0	0.0203	0.0197	97.0	0.0216	0.0218	100.9
LOQ sample -3	0.0619	0.0580	93.7	0.0205	0.0203	99.0	0.0218	0.0215	98.6
50% sample -1	0.259	0.254	98.1	0.098	0.091	92.9	0.104	0.097	93.3
50% sample -2	0.259	0.255	98.5	0.099	0.094	94.9	0.104	0.100	96.2
50% sample -3	0.261	0.250	95.8	0.099	0.092	92.9	0.105	0.097	92.4
100% sample -1	0.527	0.543	103.0	0.206	0.214	103.9	0.208	0.212	101.9
100% sample -2	0.529	0.548	103.6	0.208	0.215	103.4	0.210	0.218	103.8
100% sample -3	0.514	0.536	104.3	0.205	0.206	100.5	0.207	0.210	101.4
150% sample -1	0.784	0.822	104.8	0.313	0.330	105.	0.316	0.321	101.6
150% sample -2	0.777	0.808	104.0	0.310	0.326	105.2	0.313	0.316	101.0
150% sample -3	0.775	0.796	102.7	0.309	0.318	102.9	0.312	0.316	101.3

Table Vid: Table for Accuracy study for Hydrochlorothiazide- HC - Imp-4

Sample spiked at level	HC - Imp-4 Amount added (%w/w)	Amount recovered (%w/w)	% Recovery
LOQ sample -1	0.0253	0.0273	107.9
LOQ sample -2	0.0228	0.0219	96.1
LOQ sample -3	0.0243	0.0239	98.4
50% sample -1	0.085	0.078	91.8
50% sample -2	0.085	0.077	90.6
50% sample -3	0.081	0.073	90.1
100% sample -1	0.212	0.205	96.7
100% sample -2	0.215	0.220	102.3
100% sample -3	0.211	0.210	99.5
150% sample -1	0.317	0.307	96.8
150% sample -2	0.314	0.302	96.2
150% sample -3	0.313	0.294	93.9

Solution Stability:

The solution stability experiment data confirms that solutions of standard and samples are stable up to 29 hours at room temperature.

Robustness

No significant changes observed in the relative retention times of the main analyte and their corresponding impurities illustrating the robustness of the developed method. (Table VIIa and VIIb)

Table VIIa: RRT data on different robustness conditions for Telmisartan

Parameter	Variation	RRT of TL-Imp-1	TL-Imp-2	TL-Imp-3	TL-Imp-4	TL-Imp-5	TL-Imp-6
STP	-	0.56	0.87	0.90	0.98	1.07	1.12
Flow	-10%	0.59	0.87	0.90	0.98	1.08	1.13
Rate	+10%	0.54	0.87	0.89	0.98	1.08	1.11
Wave	-5 nm	0.56	0.87	0.90	0.98	1.07	1.12
length	+5 nm	0.56	0.87	0.90	0.98	1.07	1.12
% Organic in Mobile	-2% absolute	0.61	0.87	0.90	0.98	1.08	1.13
phase	+2% absolute	0.48	0.86	0.89	0.98	1.08	1.11
Column Oven	- 5°C	0.56	0.87	0.9	0.98	1.08	1.12
Temperature	+ 5°C	0.56	0.87	0.89	0.98	1.07	1.12

Table VIIb: RRT data on different robustness conditions for Hydrochlorothiazide

Parameter	Variation	RRT of HC-Imp-1	HC-Imp-2	HC-Imp-3	HC-Imp-4
STP	-	0.29	0.33	0.64	0.78
Flow Rate	-10%	0.34	0.38	0.67	0.79
	+10%	0.26	0.30	0.61	0.77
Wave length	-5 nm	0.29	0.33	0.64	0.78
	+5 nm	0.29	0.33	0.64	0.78
% Organic in Mobile phase	-2% absolute	0.33	0.38	0.67	0.79
	+2% absolute	0.26	0.29	0.6	0.76
Column Oven Temperature	- 5°C	0.30	0.35	0.65	0.79
	+ 5°C	0.28	0.31	0.62	0.76

DISCUSSION

A sensitive, specific, accurate, robust and validated stability indicating UPLC method is described for the determination of degradation products and process related impurities in TL/HC tablets. The behavior of TL and HC under various stress conditions is studied. All degradation products and process impurities are well separated from each other and from TL and HC which indicates the stability-indicating method. The correlation coefficient values for all impurities are found to be more than 0.995 which indicates that the method is having good linearity. Recovery results for all impurities are found to be between 90-110% which shows good recovery of the validated method. The proposed RP-UPLC

method is fast, precise, accurate, sensitive and efficient for the determination of potential impurities present in TL/HC in FDC product using single chromatographic condition.

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