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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:

The chromatographic conditions were successfully developed for the separation of Hydrochlorothiazide and Telmisartan by using Acquity UPLC HSS T3 (100 mm x 2.1 mm), 1.8µ, flow rate was 1ml/min, mobile phase ratio was 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 and detection wavelength was 225 nm.The Spectroscopic method was done in solvent using methanol and the instrument lab india 3000+ with uv win software. The instrument used for HPLC , WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software-2. The % purity of Hydrochlorothiazide and Telmisartan was found to be 99.87% and 100.27% respectively. The system suitability parameters for Hydrochlorothiazide and Telmisartan such as theoretical plates and tailing factor were found to be 4260, 1.2 and 5085 and 1.2, the resolution was found to be 7.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)).

Keywords: Hydrochlorothiazide and Telmisartan; Impurity; PDA Detection; ICH guidelines.

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-1 Hbenzimidazol]-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 1.1: Structural formula of TL and HC

1.2. Literature survey:

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].

1.3. INSTRUMENTATION AND MATERIALS:

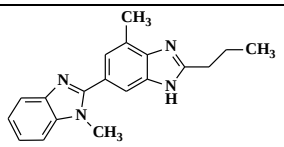
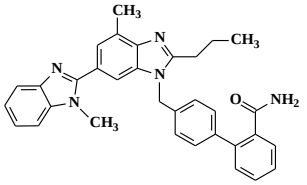
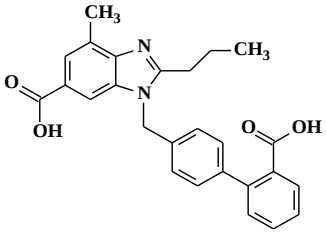
1.3.1. Instrumentation:

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.

1.3.2. Materials:

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. The impurity structures of TL and HC were tabulated in **Table no 1.1 &1.2.**

Table 1.1. TL-impurities

Name of the impurity	Chemical name	Chemical Structure
[Dibenzimidazole Derivative] [USP Telmisartan Related Compound A] TL IMP-1	4-Methyl-6-(1-Methyl-1*h*-Benzimidazol-2-Yl)-2-Propyl-1*h*-Benzimidazole	
Telmisartan Amide [USP Telmisartan Amide] TL IMP-2	4'-[[4-Methyl-6-(1-Methyl-1*h*-Benzimidazol-2-Yl)-2-Propyl-1*h*-Benzimidazol-1-Yl] Methyl] Biphenyl-2-Carboxamide	
[USP Telmisartan Diacid] TL IMP-3	4'-[[4-Methyl-6-(Carboxy)-2-Propyl-1*h*-Benzimidazol-1-YL]Methyl]Biphenyl-2-Carboxylic Acid	

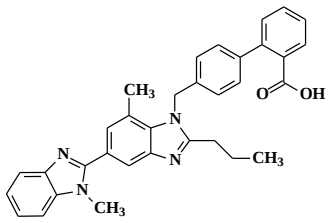
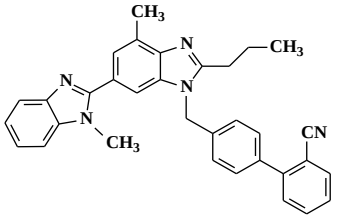
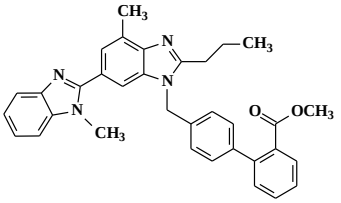
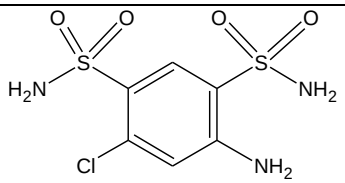
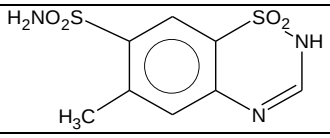
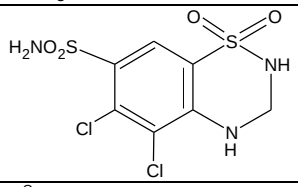
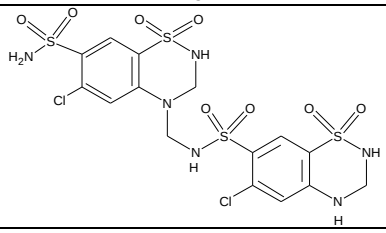
Telmisartan Isomer TL IMP-4	4'-[[7-Methyl-5-(1-Methyl-1*h*-Benzimidazol-2-Yl)-2-Propyl-1*h*-Benzimidazol-1-Yl]Methyl]Biphenyl-2-Carboxylic Acid	
Telmisartan Nitrile TL IMP-5	4'-[[4-Methyl-6-(1-Methyl-1*h*-Benzimidazol-2-Yl)-2-Propyl-1*h*-Benzimidazol-1-Yl]Methyl]Biphenyl-2-Carbonitrile	
Telmisartan Methyl Ester TL IMP-6	4'-[[4-Methyl-6-(1-Methyl-1h-Benzimidazol-2-Yl)-2-Propyl-1h-Benzimidazol-1-Yl]Methyl]Biphenyl-2-Carboxylic Acid, Methyl Ester	

Table 1.2. HC-impurities

Name of the impurity	Chemical name	Chemical Structure
(Benzothaidiazine related compound 'A') HC-Imp-1	4 – Amino – 6 – chloro – 1,3– benzenedisulphonamide	
(Chorothiazide) HC-Imp-2	6–CHLORO–2H–1,2,4, -BENZOTHIADIAZINE – 7 – SULFONAMIDE – 1,1 – DIOXIDE	
(5-Chloro hydrochlorothiazide) HC-Imp-3	5,6-Dichloro-3,4-Dihydro-2h-1,2,4-Benzothiadiazine-7-Sulfonamide 1,1-Dioxide	
*(*Hydrochlorothiazide dimer) HC-Imp-4	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	

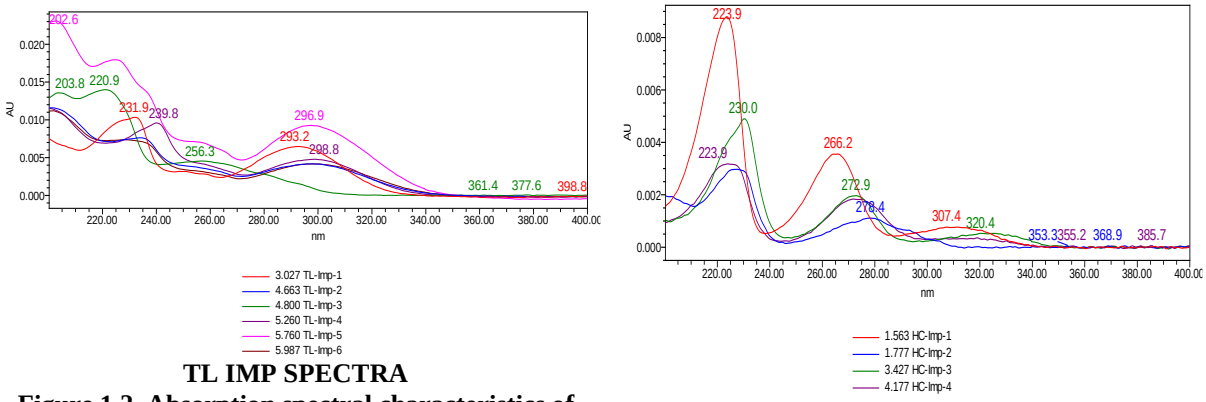
1.4. Method development and optimization for UPLC method:

Aim of this study 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 1.2**), 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. The absorption spectral characteristics of TL, HC and its impurities were presented in the **Fig 1.2.**



TL IMP SPECTRA

HC IMP SPECTRA

Figure 1.2. Absorption spectral characteristics of TL and HC impurities

1.5. Finalized chromatographic conditions for method validation

The finalized UPLC chromatographic conditions were summarized in the **Table 1.3.** The relative retention times of the impurities were presented in **Table 1.4.**

Table 1.3. Finalized chromatographic conditions

Parameter	Condition			
Column	Acquity UPLC HSS T3 (100 mm x 2.1 mm), 1.8µ			
Mobile phase A	1 mL of Ortho phosphoric acid is dissolved in 1000 mL of water adjusted the pH to 2.6 ± 0.05 with diluted sodium hydroxide solution. Filter through 0.22µ membrane filter.			
Mobile phase B	Acetonitrile			
Flow Rate	0.5 mL/min			
Column Temperature	45°C			
Wavelength	225 nm			
Injection Volume	2 µL			
Gradient Programme	**Time (min)**	**Flow rate ($mL.min^{-1}$)**	**Mobile phase- A (%)**	**Mobile phase - B (%)**
	0.0	0.50	90.0	10.0
	2.0	0.50	85.0	15.0
	5.0	0.50	50.0	50.0

	6.5	0.50	50.0	50.0
	7.3	0.50	40.0	60.0
	7.5	0.50	90.0	10.0
	10.0	0.50	90.0	10.0
Run time	10 minutes			
Sample concentration	400 µg/mL and 125 µg/mL for TL and HC respectively			
Retention times of TL and HC	2.05 and 5.40 minutes respectively			

Table 1.4. Relative retention times of TL and HC impurities

Name of the impurity	RRT
HC -Imp-1	0.29
HC -Imp-2	0.33
HC	0.38
TL-Imp-1	0.56
HC -Imp-3	0.64
HC -Imp-4	0.78
TL-Imp-2	0.87
TL-Imp-3	0.90
TL-Imp-4	0.98
TL	1.00
TL-Imp-5	1.07
TL-Imp-6	1.12

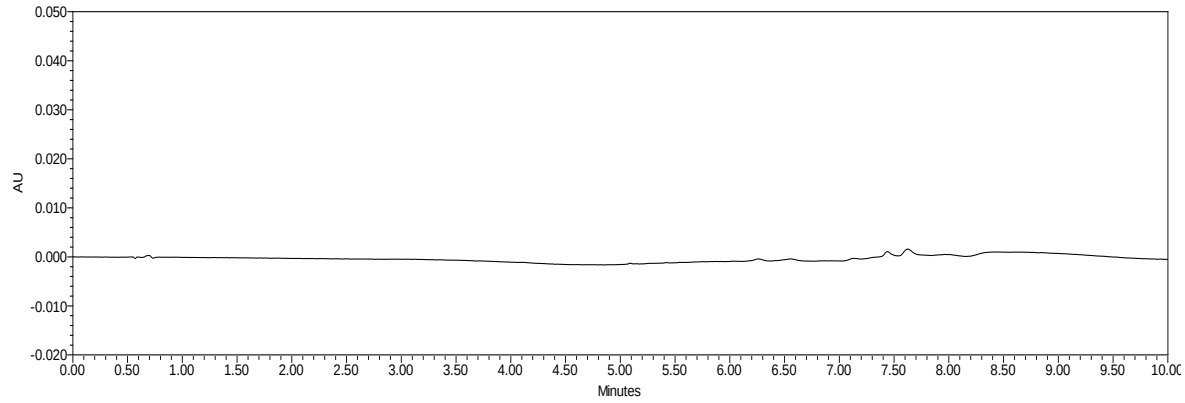


Fig.4.3.Diluent Chromato

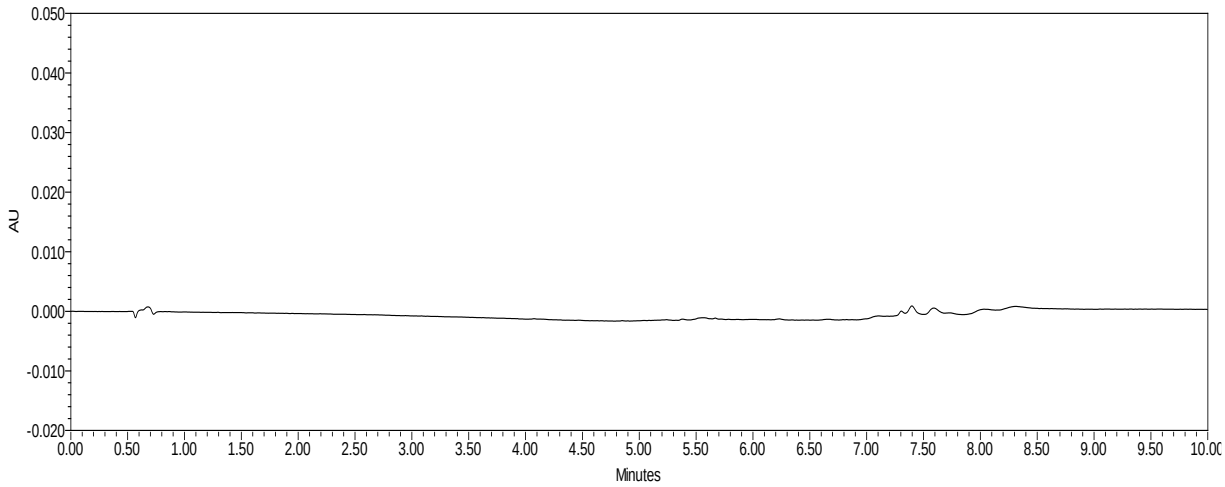


Fig.1.4.Placebo Chromatogram

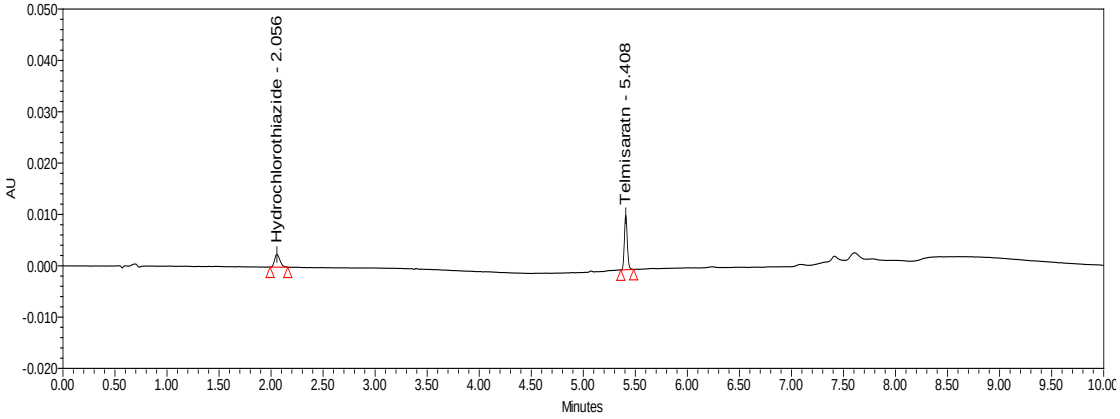


Fig.1.5.Diluted Standard Chromatogram

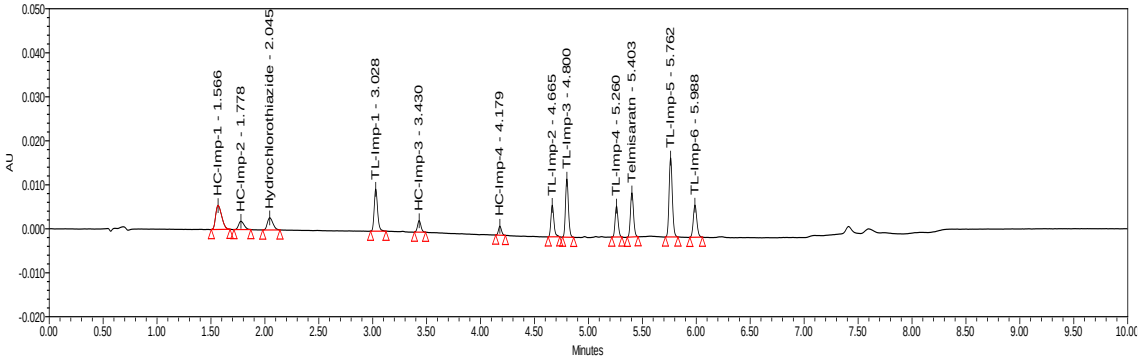


Fig.1.6.All Impurity Mix Chromatogram

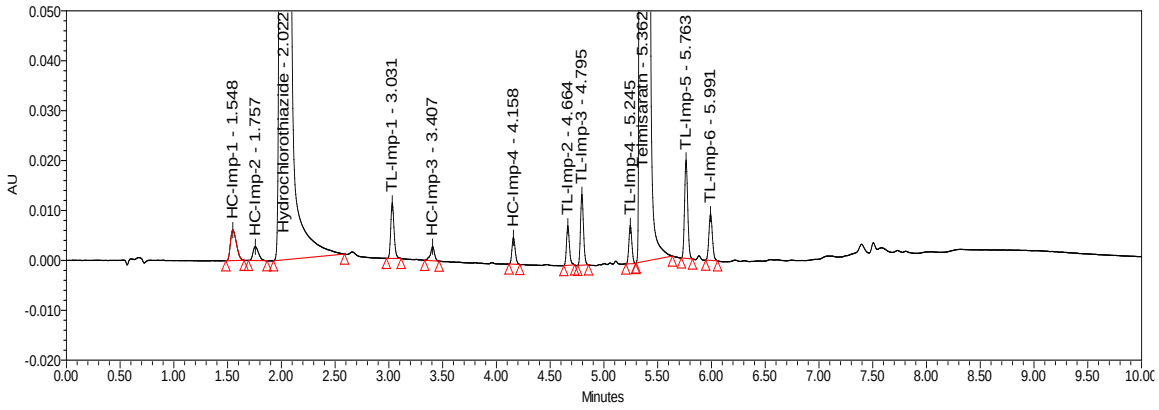


Fig.1.7. Sample Spiked With All Impurities

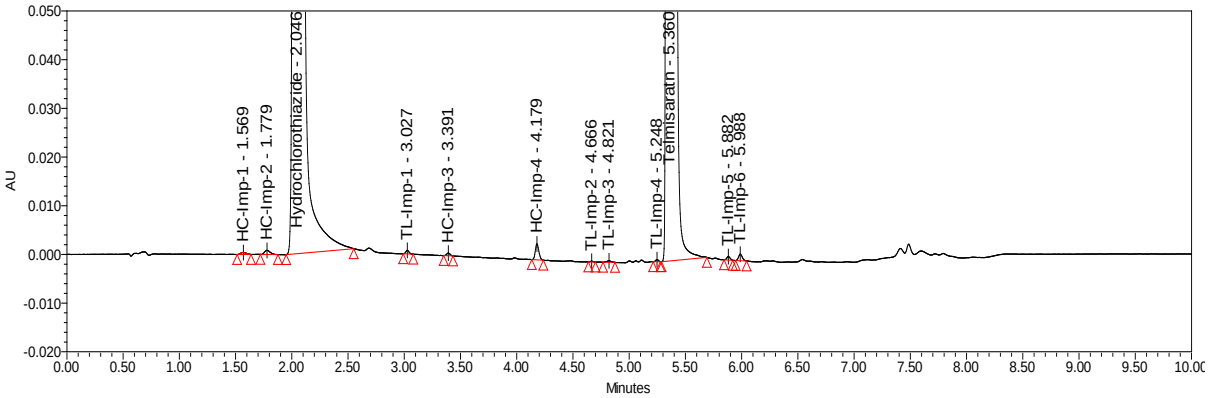


Fig.1.8. Control Sample

1.6. 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.

1.6.1. 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.

1.6.2. 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.

1.6.3. 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 1.5**.

Table 1.5. 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

Specificity

The developed method was checked for specificity with respect to diluent, placebo used in sample matrix and with ten potential impurities. No interference was observed at the retention time of main peaks and impurity peaks.

Forced degradation

Forced degradation studies were performed in different conditions like acid, alkali, oxidative, heat, humidity and photolytic degradations to know the stability indicating nature of the method.

Acid hydrolysis

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. Added 5 N HCl, 1 mL and heated the solution at 85°C for 60 minutes then neutralized with 5N NaoH solution 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.

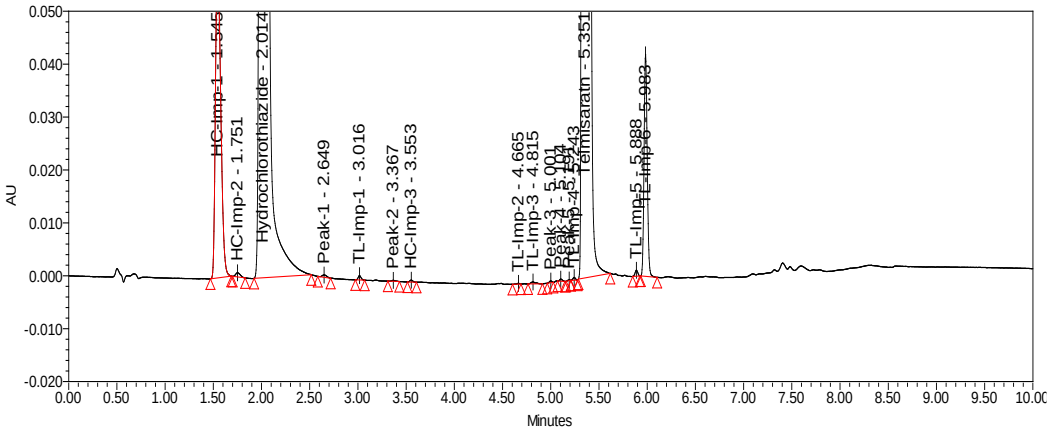


Fig.1.9.Chromatogram of Acid degradation sample:

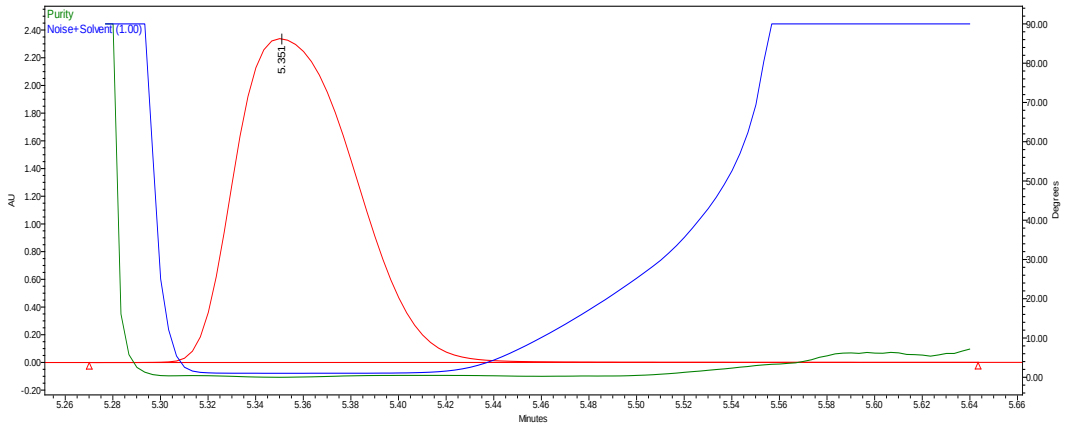


Fig. 1.10.Purity plot of TL in Acid degradation

Name	Retention Time	Purity1 Angle	Purity1 Threshold
Telmisartan	**5.351**	**0.210**	**1.044**

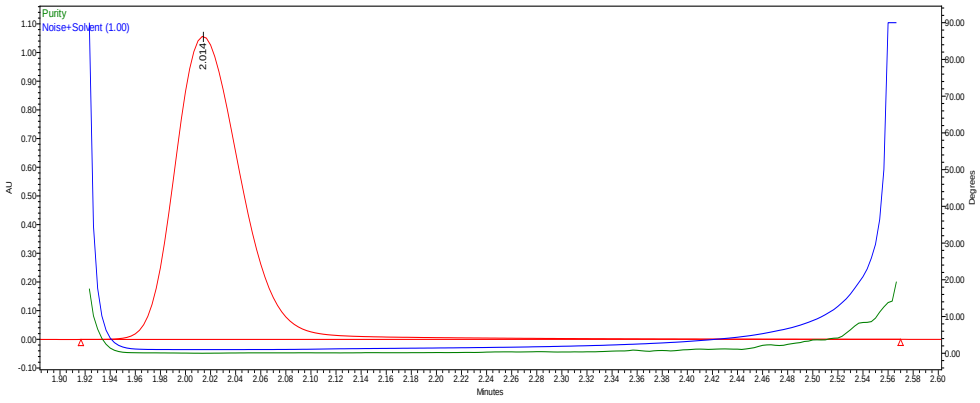


Fig. 1.11.Purity plot of HC in Acid degradation

Name	Retention Time	Purity1 Angle	Purity1 Threshold
Hydrochlorothiazide	2.014	0.051	1.008

Base hydrolysis

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. Added 5 N NaoH, 1 mL and heated the solution at 85°C for 60 minutes then neutralized with 5N HCl solution 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.

Chromatogram of Base degradation sample:

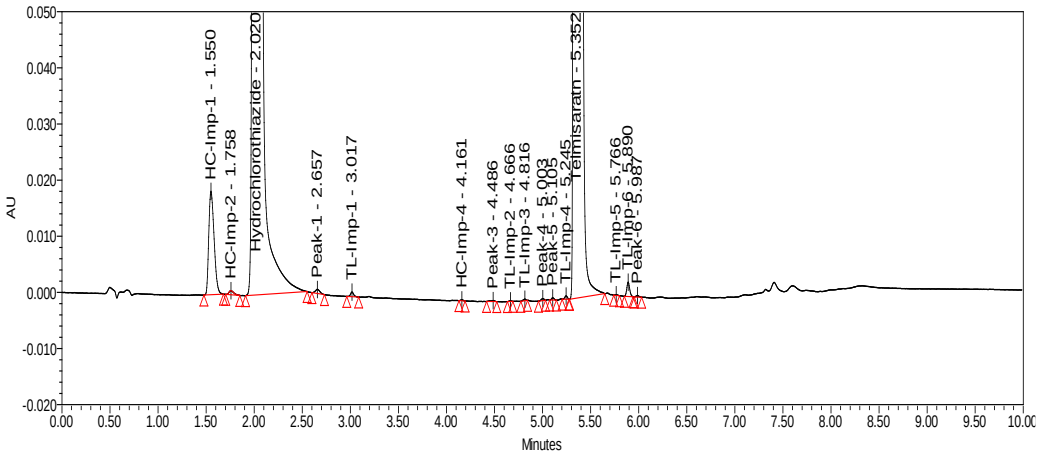


Fig 1.12.**Purity plot of TL in Base degradation**

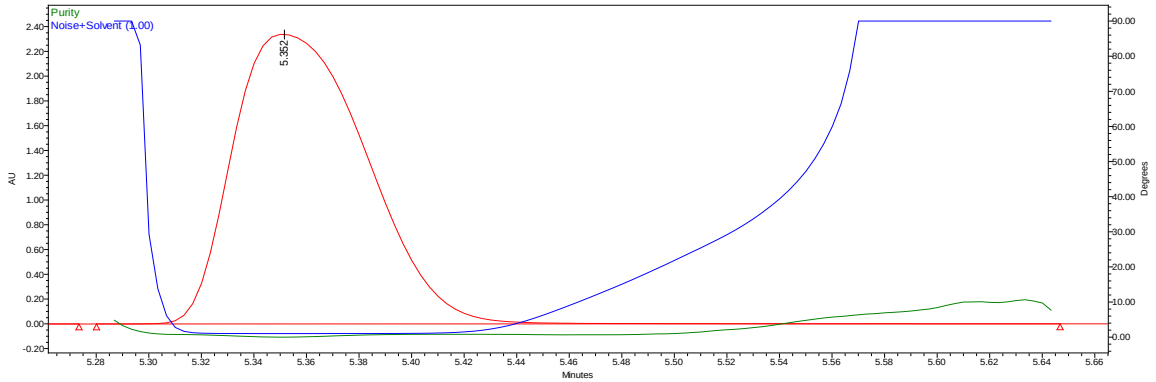
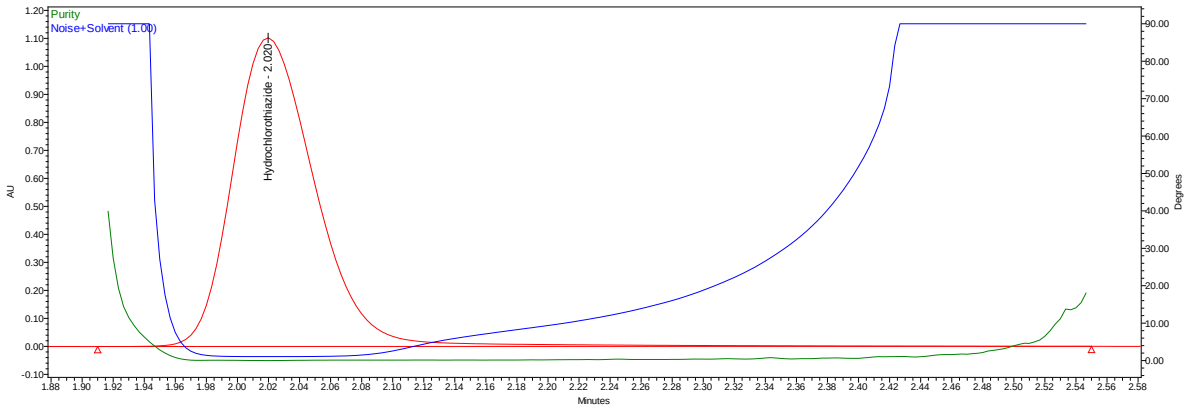


Fig.1.13.Purity plot of HC in Base degradation

Name	Retention Time	Purity1 Angle	Purity1 Threshold
Telmisartan	5.352	0.350	1.041

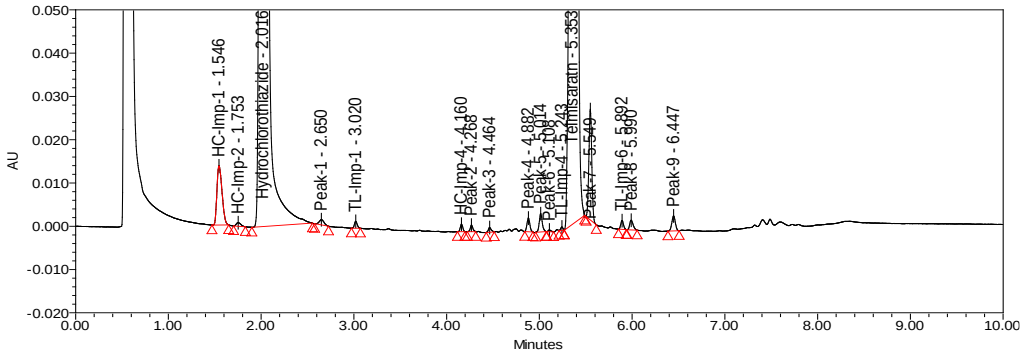


Name	Retention Time	Purity1 Angle	Purity1 Threshold
Hydrochlorothiazide	2.020	0.053	1.006

Peroxide degradation

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. Added 30% H_2O_2, 1 mL and heated the solution at 85°C for 60 minutes then 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.

Chromatogram of Peroxide degradation sample:



Purity plot of TL in peroxide degradation

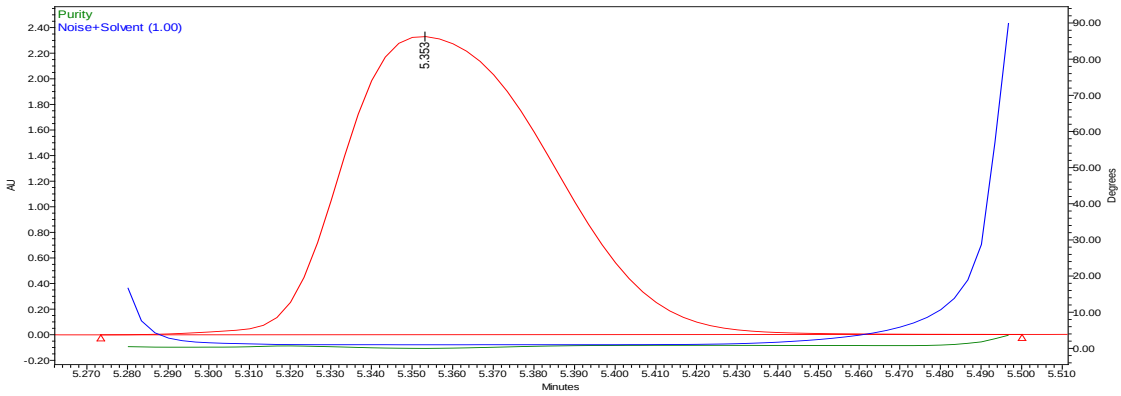


Fig.1.14. Purity plot of HC in peroxide degradation

Name	Retention Time	Purity1 Angle	Purity1 Threshold
Telmisartan	5.353	0.362	1.008

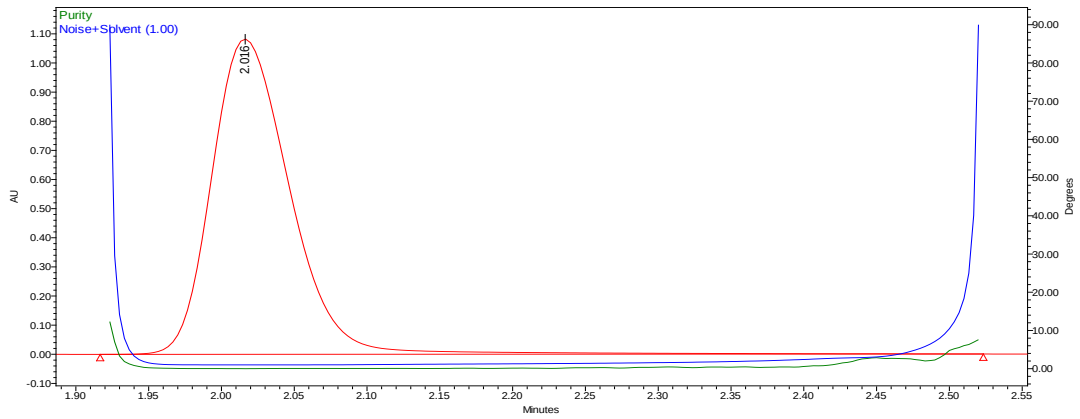
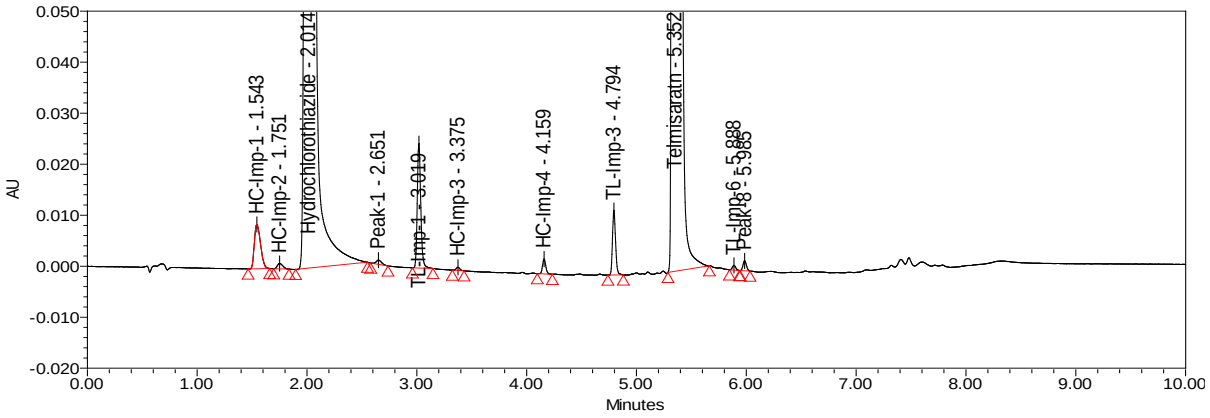


Fig.1.15.Thermal degradation

Name	Retention Time	Purity1 Angle	Purity1 Threshold
Hydrochlorothiazide	2.016	0.055	1.005

Thermal degradation

TL/HC tablets were exposed to heat at 105°C for 24 hrs and prepared the sample solution as per section 4.6.2 and injected into UPLC to find out the degradation products.



Purity plot of TL in Thermal degradation:

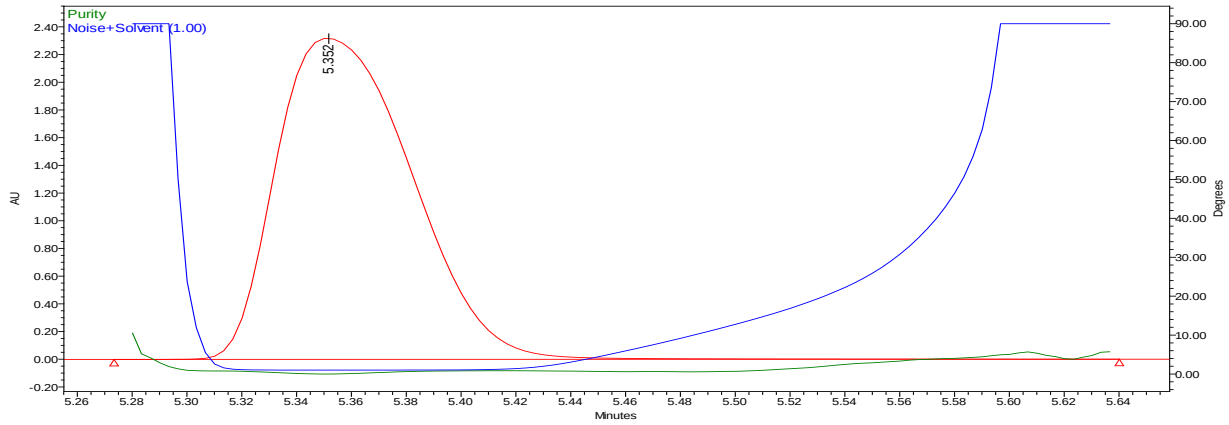


Fig.16.Chromatogram of Thermal degradation sample:

Name	Retention Time	Purity1 Angle	Purity1 Threshold
Telmisartan	5.352	0.372	1.033

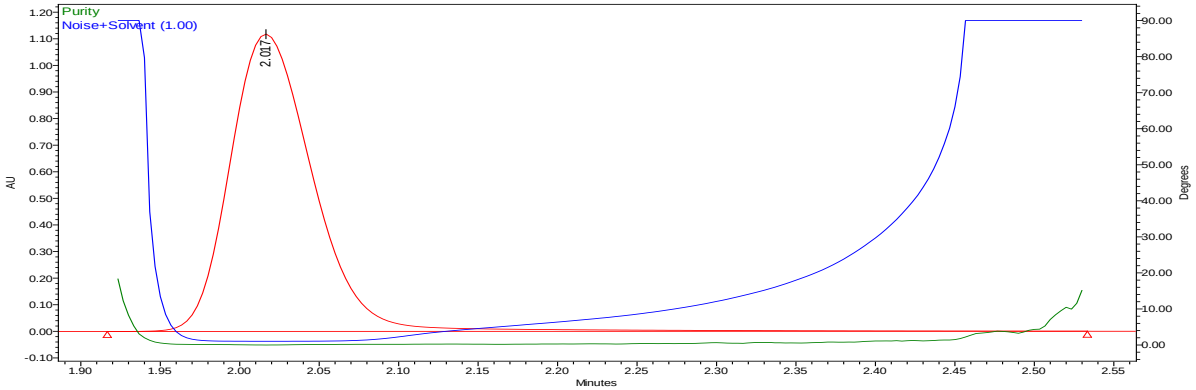
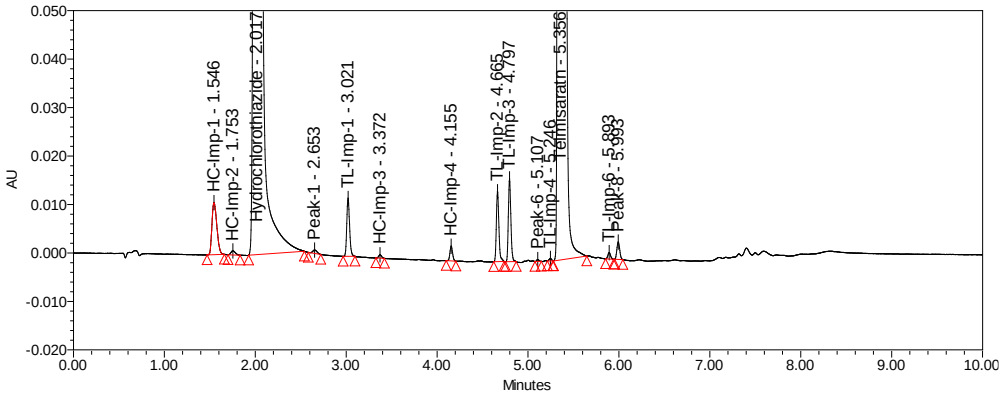


Fig.1.17.Chromatogram of Humidity degradation sample:

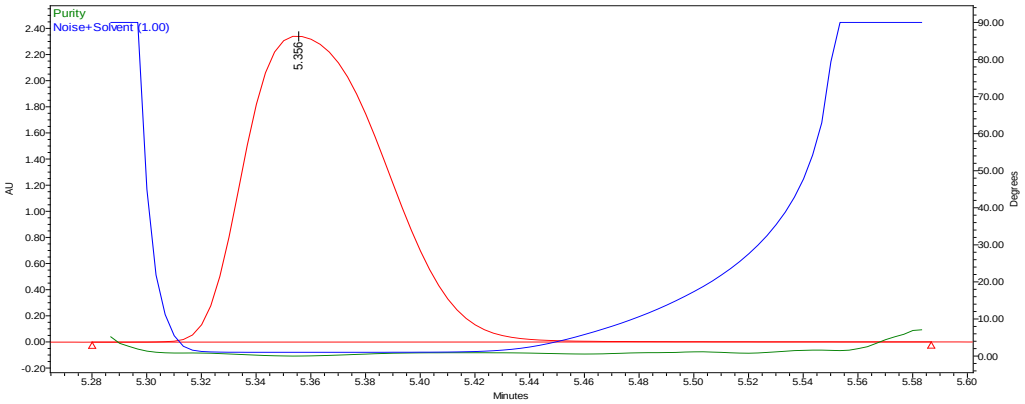
Name	Retention Time	Purity1 Angle	Purity1 Threshold
Hydrochlorothiazide	2.017	0.062	1.005

Humidity degradation

TL/HC tablets were exposed to 90%RH for 24 hrs and prepared the sample solution as per section 4.6.2 and injected UPLC to find out the degradation products.

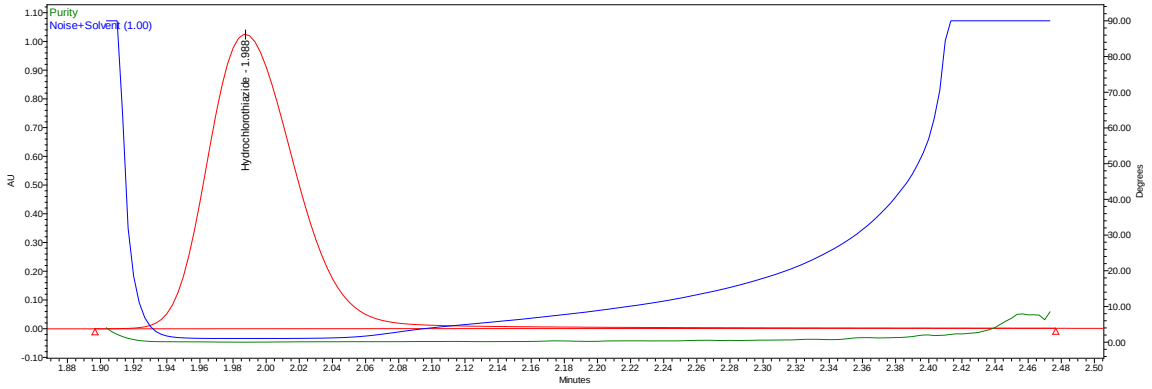


Purity plot of TL in Humidity degradation:



Name	Retention Time	Purity1 Angle	Purity1 Threshold
Telmisartan	5.356	0.388	1.026

Purity plot of HC in Humidity degradation:

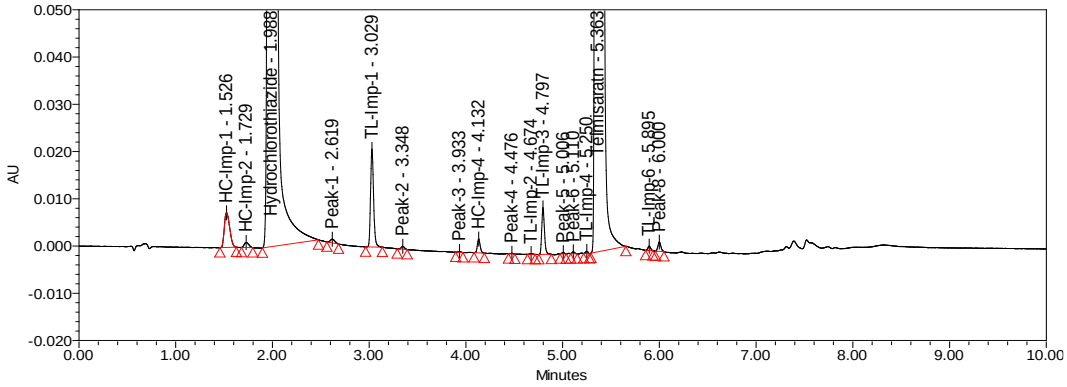


Name	Retention Time	Purity1 Angle	Purity1 Threshold
Hydrochlorothiazide	1.988	0.044	1.005

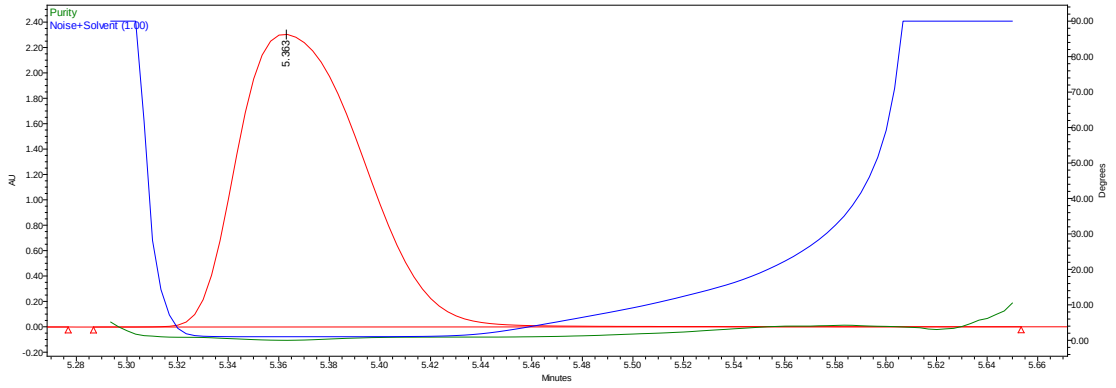
Photolytic degradation

TL/HC tablets were exposed to white fluorescent 1.2 million lux hours UV 200 watt hr/m^2 for 7 days and prepared the sample solution as per section 4.6.2 and injected UPLC to find out the degradation products.

Chromatogram of photolytic degradation sample:

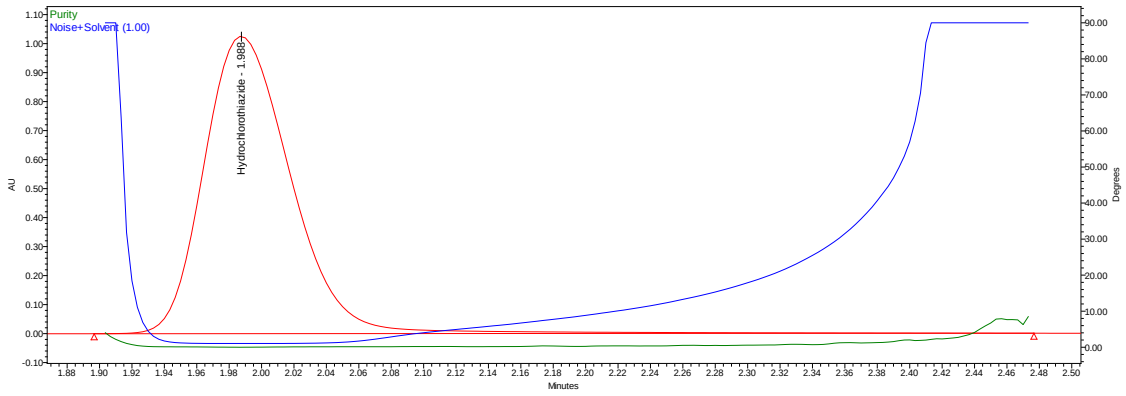


Purity plot of TL in Photolytic degradation:



Name	Retention Time	Purity1 Angle	Purity1 Threshold
Telmisartan	5.363	0.390	1.030

Purity plot of HC in Photolytic degradation:



Name	Retention Time	Purity1 Angle	Purity1 Threshold
Hydrochlorothiazide	1.988	0.044	1.005

Interpretation from the Forced degradation studies and mass balance

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 1.6.**)

Table 1.6.Mass balance:

Degradation condition	% Assay		% imps+ %Deg. products		Mass balance (%Assay+ %Imp+% Deg. products	
	TL	HC	TL	HC	TL	HC
Acid	98.3	93.8	1.264	7.20	99.56	101.0
Alkali	98.5	98.6	0.125	2.31	98.62	100.9
Oxidation	98.4	98.6	1.128	2.08	99.52	100.7
Thermal	99.0	98.9	0.791	1.70	99.79	100.6
Humidity	98.7	98.8	1.033	1.84	99.73	100.6
Photolytic	99.0	98.9	2.188	3.04	101.18	101.9

Method 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 results were tabulated in **table no 1.7.**

Table 1.7.Method precision:

Sample Name	Method precision		
	Avg	SD	%RSD
TL -Imp-1	0.198	0.002	1.0
TL -Imp-2	0.197	0.001	0.5
TL -Imp-3	0.199	0.001	0.5
TL -Imp-4	0.197	0.001	0.5
TL -Imp-5	0.197	0.000	0.0
TL -Imp-6	0.228	0.003	1.3
HC-Imp-1	0.565	0.004	0.7
HC-Imp-2	0.317	0.003	0.9
HC-Imp-3	0.246	0.003	1.2
HC-Imp-4	0.573	0.004	0.7

Intermediate precision:

The intermediate precision (Ruggedness) of the method was evaluated by different analyst using different column and different UPLC instrument on different day. The intermediate precision data was presented in **Table no 1.8.**

Table 1.8. Intermediate precision:

Sample Name	Intermediate precision		
	Avg	SD	%RSD
TL -Imp-1	0.199	0.002	1.0
TL -Imp-2	0.197	0.001	0.5
TL -Imp-3	0.201	0.002	1.0
TL -Imp-4	0.201	0.003	1.5
TL -Imp-5	0.199	0.001	0.5

TL -Imp-6	0.208	0.003	1.3
HC-Imp-1	0.565	0.006	1.1
HC-Imp-2	0.315	0.004	1.3
HC-Imp-3	0.243	0.002	0.8
HC-Imp-4	0.577	0.006	1.0

Limit of detection and Limit of Quantification

LOD and LOQ studies were carried out to evaluate the detection and quantization limits of the method to determine the presence of any impurities by using following equation:
LOD = 3.3 σ/S
LOQ = 10 σ/S
Where σ is the standard deviation and S is the slope of the curve. The determined LOD and LOQ values for TL & HC were given in **Table no**

Table no.1.9. LOD & LOQ

Sample Name	LOD	LOQ
TL	0.010	0.0212
TL -Imp-1	0.0090	00.018
TL -Imp-2	0.0090	0.0187
TL -Imp-3	0.0094	0.0188
TL -Imp-4	0.0095	0.0190
TL -Imp-5	0.0096	0.0193
TL -Imp-6	0.0097	0.0195
HC	0.0120	0.0204
HC-Imp-1	0.0248	0.0496
HC-Imp-2	0.0105	0.0211
HC-Imp-3	0.0097	0.019
HC-Imp-4	0.0099	0.0199

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.

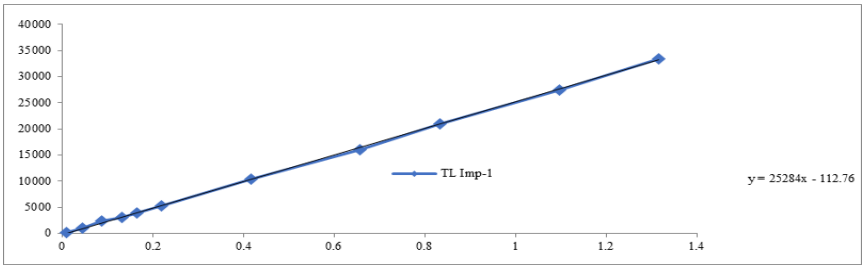


Fig.1.18.Linearity plot of TL-Imp-2

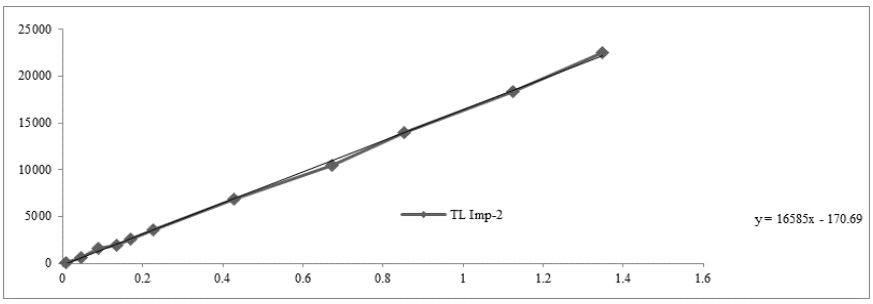


Fig.1.19.Linearity plot of TL-Imp-3

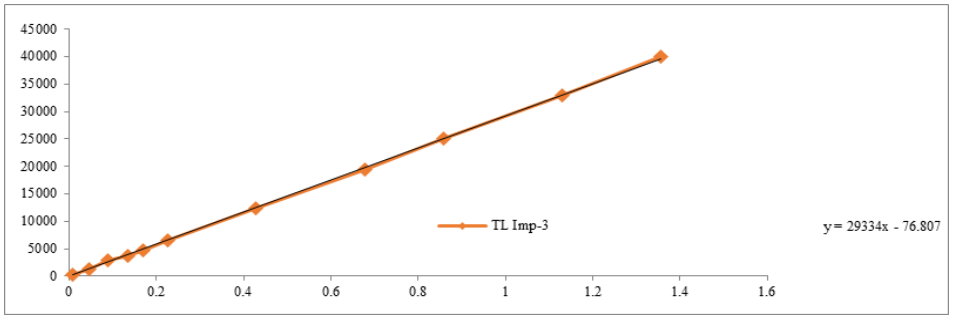


Fig.1.20.Linearity plot of TL-Imp-4

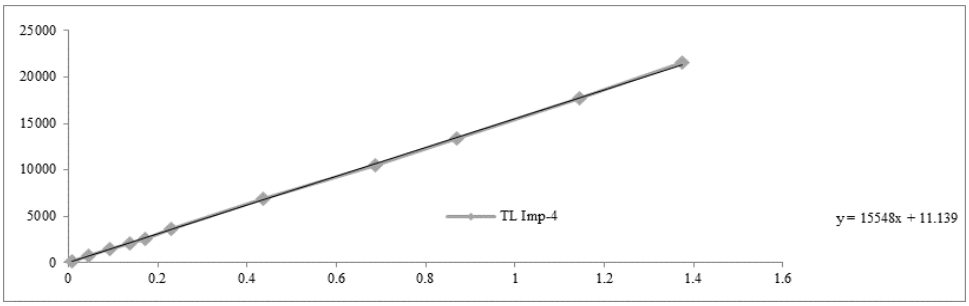


Fig 1.21.Linearity plot of TL-Imp-5

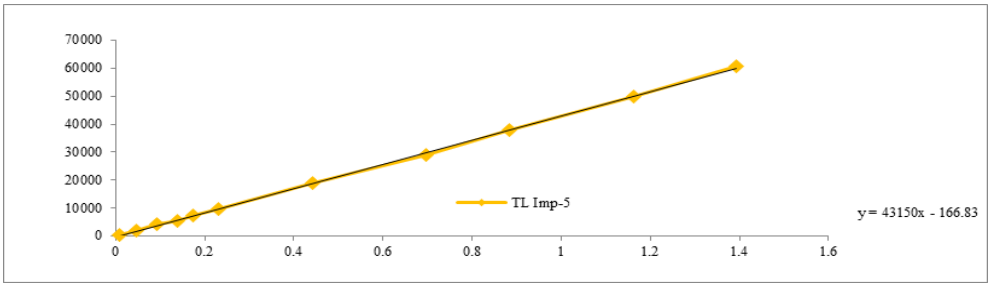


Fig.1.22.Linearity plot of TL-Imp-6

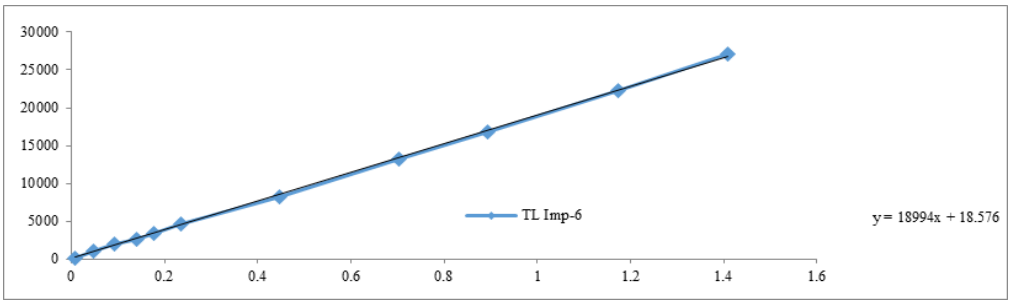


Fig.1.23.Linearity plot of TL

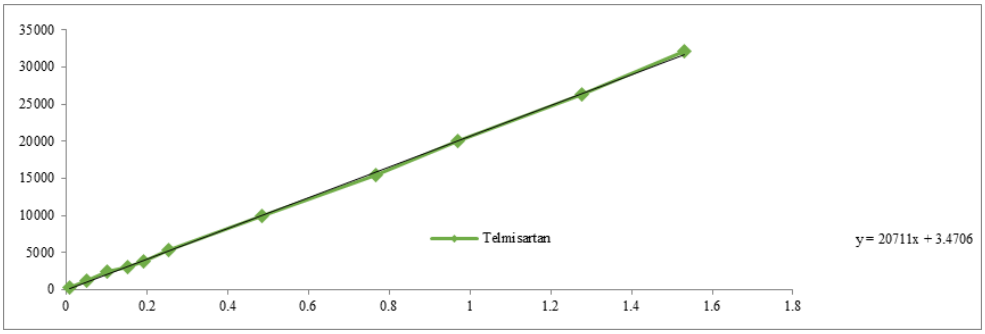


Fig.1.24.Linearity plot of HC-Imp-1

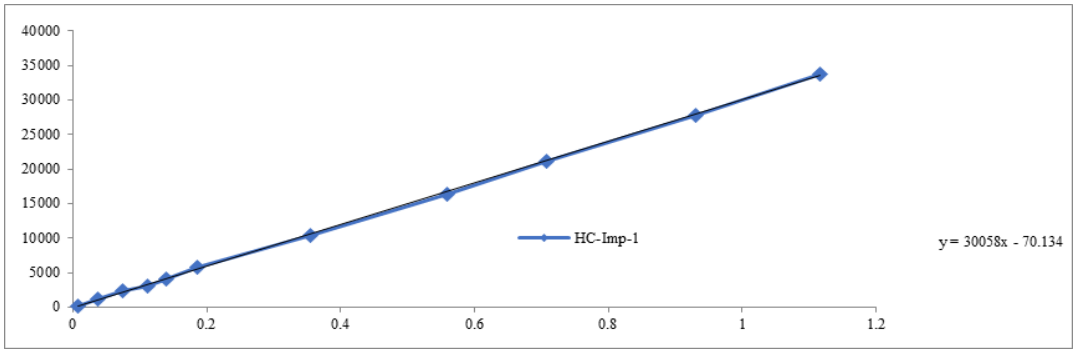


Fig.1.25.Linearity plot of HC-Imp-2

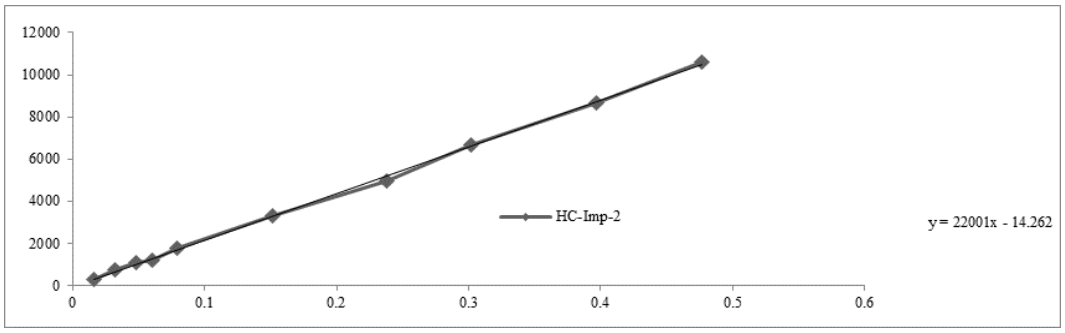


Fig.1.26..Linearity plot of HC-Imp-3

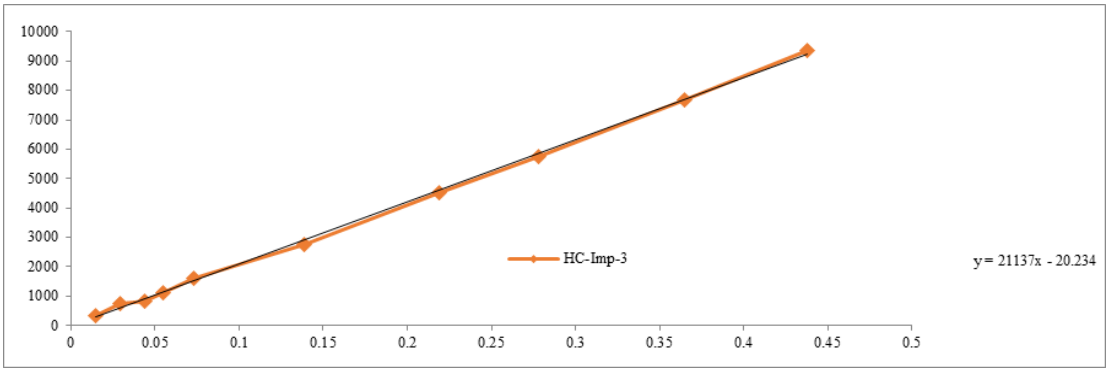


Fig.1.27.Linearity plot of HC-Imp-4

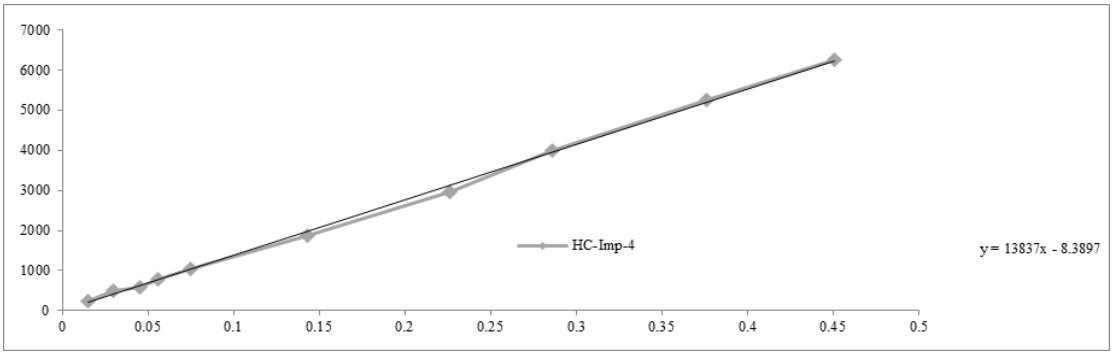


Fig.1.28.Linearity plot of HC

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.

Table No.1.10.Recovery results for TL & HC impurities

Impurity Name	Avg recovery & RSD in triplicate preparation							
	LOQ Level		50% Level		100% Level		150% Level	
	Avg Recovery	% RSD	Avg Recovery	% RSD	Avg Recovery	% RSD	Avg Recovery	% RSD
TL-Imp-1	102.6	1.8	98.1	1.7	97.0	0.3	96.6	0.7
TL -Imp-2	101.4	1.8	100.3	4.9	101.3	1.0	101.5	0.5
TL -Imp-3	100.0	1.8	96.3	1.8	94.3	0.3	94.4	0.4
TL -Imp-4	100.4	1.9	100.3	1.1	103.0	0.5	103.3	0.5
TL -Imp-5	93.8	0.6	100.0	0.9	101.2	0.3	101.3	0.8
TL -Imp-6	101.7	8.8	99.4	0.6	99.7	1.5	100.3	1.4
HC -Imp-1	94.8	1.7	97.5	1.5	103.6	1.6	103.8	1.0
HC -Imp-2	99.0	2.0	93.6	1.2	102.6	1.8	104.5	1.3
HC -Imp-3	100.0	1.0	94.0	2.1	102.4	1.2	101.3	0.3
HC -Imp-4	100.8	6.6	90.8	1.0	99.5	2.8	95.6	1.6

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. The RRT details for the OLM and HCT impurities were mentioned in table no.1.11.&1.12.

Table No.1.11. Robust data for TL impurities

Impurity Name	RRT's of the TL impurities										
	As per the method conditions	Flow rate		Column temperature		pH of the buffer		Gradient programme variation (±2% Absolute)		Wavelength (nm)	
		0.45 mL min^{-1}	0.55 mL min^{-1}	40°C	50°C	2.4	2.8	-2%	+2%	220 nm	230 nm
TL-Imp-1	0.56	0.59	0.54	0.56	0.56	0.57	0.56	0.61	0.48	0.56	0.56
TL-Imp-2	0.87	0.87	0.87	0.87	0.87	0.87	0.86	0.87	0.86	0.87	0.87
TL-Imp-3	0.90	0.90	0.89	0.90	0.89	0.92	0.90	0.90	0.89	0.90	0.90
TL-Imp-4	0.98	0.98	0.98	0.98	0.98	0.98	0.97	0.98	0.98	0.98	0.98
TL-Imp-5	1.07	1.08	1.08	1.08	1.07	1.07	1.05	1.08	1.08	1.07	1.07
TL-Imp-6	1.12	1.13	1.11	1.12	1.12	1.10	1.12	1.13	1.11	1.12	1.12

Table No.1.12.Robust data for HC impurities

Impurity Name	RRT's of the HC impurities										
	As per the method conditions	Flow rate		Column temperature		pH of the buffer		Gradient programme variation (±2% Absolute)		Wavelength (nm)	
		0.45 mL min^{-1}	0.55 mL min^{-1}	40°C	50°C	2.4	2.8	-2%	+2%	220 nm	230 nm
HC-Imp-1	0.29	0.34	0.26	0.30	0.28	0.30	0.29	0.33	0.26	0.29	0.29
HC-Imp-2	0.33	0.38	0.30	0.35	0.31	0.31	0.33	0.38	0.29	0.33	0.33
HC-Imp-3	0.64	0.67	0.61	0.65	0.62	0.64	0.65	0.67	0.60	0.64	0.64
HC-Imp-4	0.78	0.79	0.77	0.79	0.76	0.77	0.78	0.79	0.76	0.78	0.78

Conclusion

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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