Application of developed and validated UHPLC-MS method for the forced degradation study of Telmisartan-an angiotensin II receptor blocker and Hydrochlorothiazide - A thiazide diuretic

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ABSTRACT

A novel high sensitive ultra high performance liquid chromatography mass spectrometry (UHPLC-MS) method was developed for the estimation of Telmisartan and Hydrochlorothiazide in combined tablet dosage form. The optimization of UHPLC method was performed using Hypersil Gold C_{18} (3μm, 150x3mm) column, isocratic elution of mobile phase, methanol and water with 0.1% formic acid (70:30, v/v) and flow rate of 0.5mL/min at ambient temperature. The target compounds were analyzed on an Orbitrap mass spectrometer (high resolution mass spectrometer) in positive electrospray ionization (ESI) mode. The retention times of Hydrochlorothiazide and Telmisartan were 1.60±0.01 and 2.27±0.05 respectively. The developed method was validated according to International Conference on Harmonization (ICH) guidelines. Then forced degradation study of Telmisartan and Hydrochlorothiazide was carried out in acid, alkali, oxidative and neutral media. The developed UHPLC-MS method was found to be precise, selective and rapid for the simultaneous determination of Telmisartan, Hydrochlorothiazide and their degradation products. During acid degradation of Telmisartan two degradation products (*m/z* 529 and 487) and oxidative degradation three degradation products (*m/z* 531, 547 and 429) were found, while no degradation was observed in other conditions. Among the identified degradants two degradation products of Telmisartan with *m/z* 531 and 547 are new and are not reported so far. In case of Hydrochlorothiazide only one degradant (*m/z* 286) was found during alkali degradation while no degradation was observed in other forced degradation conditions.

Keywords: Telmisartan, Hydrochlorothiazide, Validation, UHPLC-MS, Degradation products.

1. INTRODUCTION

Here we propose forced degradation study of Telmisartan and Hydrochlorothiazide with a developed and validated novel UHPLC-MS method by using Q Exactive orbitrap mass spectrometer. Telmisartan is an angiotensin II receptor blocker used in the treatment of hypertension. Angiotensin II receptor blockers bind to the angiotensin II type AT1 receptors with high affinity many times greater than that of AT2 receptors, causing inhibition of action of angiotensin II on vascular smooth muscle which leads to reduction in arterial blood pressure [1]. Telmisartan has longest half-life (24 h) and the largest volume of distribution among ARBs [2-4].

Telmisartan is chemically 4'-[(1, 4-'dimethyl-2'-propyl [2,6'-bi-1H-benzidazol]-1'-yl) methyl] [1, 1'-biphenyl]-2-carboxylic acid, Figure I(a). It is used in the treatment of hypertension. Hydrochlorothiazide is a diuretic drug of the thiazides class that acts by inhibiting the kidney's ability to retain water [5]; this reduces the volume of blood return to the heart and thus cardiac output [6] and in long term lowers peripheral vascular resistance [6]. Hydrochlorothiazide is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide, Figure I(b). It is frequently used in the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal
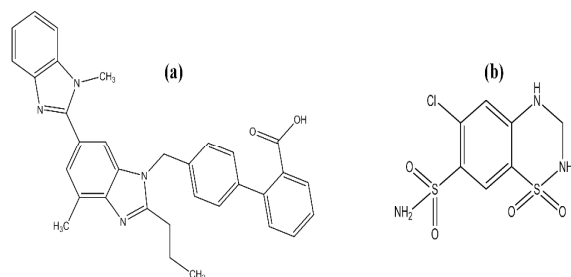
tubular acidosis and the prevention of kidney stones [5].

Figure - I: Structure of (a) Telmisartan and (b) Hydrochlorothiazide.

A detailed survey of literature of Telmisartan revealed, several methods based on different techniques were reported. HPLC [7-10] and electrochemical [11-12] methods for the determination of Telmisartan in plasma, urine and pharmaceutical formulation were developed and reported. Similarly, a survey of literature of hydrochlorothiazide revealed several methods based on HPLC coupled with UV/diode array, electrochemical detection, liquid chromatography/tandem mass spectrometry [13-15] were reported. Several analytical methods have been reported in combination of Telmisartan and Hydrochlorothiazide like, TLC densitometry [16], RP-HPLC [17-18], capillary electrophoresis [19], spectrophotometry [20] and liquid chromatographic-tandem mass spectrometry for the simultaneous quantitation of Telmisartan and Hydrochlorothiazide in human plasma [21]. Stability indicating RP-HPLC methods for the simultaneous determination of Telmisartan and Hydrochlorothiazide in pharmaceutical dosage form were reported [22-25]. Stability indicating Ultra Performance Liquid Chromatography methods for simultaneous determination of Telmisartan and Hydrochlorothiazide were also reported [26-27].

The literature survey revealed that no UHPLC-MS method for the simultaneous determination of Telmisartan, Hydrochlorothiazide and their degradation products in pure drugs and in pharmaceutical combined tablet dosage form was reported.

Therefore an UHPLC-MS method was developed and found to be simple, rapid, sensitive, selective, accurate, precise and robust for the determination of Telmisartan, Hydrochlorothiazide in combined tablet dosage form and their degradants in standard drugs. The Novelty of this method is low limit of quantification (LOQ), low limit of detection (LOD), highly sensitive and offers good separation with simple mobile phase and shorter analysis time.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

Telmisartan and Hydrochlorothiazide standards were purchased from Sigma-Aldrich. LC-MS grade solvents methanol and water were obtained from J. T. Beaker and formic acid from Fluka analytical. Telma-H tablets, containing Metformin Hydrochloride (40mg) and Hydrochlorothiazide (12.5mg) manufactured by Glenmark was purchased from local pharmacy.

2.2. Instrumental and Chromatographic conditions

Method development and validation was carried out on UHPLC system, consisting of a LC-pump (Accela), degasser and autosampler. Chromatographic separation was achieved using Hypersil Gold C_{18} (3μm, 150x3mm) column with a isocratic elution of the mobile phase system consisting of methanol (A) and water with 0.1% formic acid (B) in the ratio of 70:30 (v/v) at a constant flow rate of 500μL/min and ambient temperature. The autosampler was set to inject 5μL of sample with a chromatographic run time of 10min. Mass detection of the eluants was carried out in positive ionization mode on Q Exactive mass spectrometer (hybrid quadrupole orbitrap mass spectrometer) which is coupled with UHPLC system. Q Exactive orbitrap MS provides high resolution, high mass accuracy and good dynamic range. The set MS parameters were capillary temperature 320°C, spray voltage 3.60 kV, heater temperature 350°C, sheath gas flow rate 45, auxiliary gas flow rate 10 and sweep gas flow rate 2. High energy collision induced dissociation (HCID) was used for the MS/MS analysis. Data acquisition and processing were performed using Thermo Xcalibur Qual browser (Version 2.2).

2.3. Preparation of standard sample solution

Stock solutions of Telmisartan and Hydrochlorothiazide were prepared by dissolving 4mg and 1.25mg of Telmisartan and Hydrochlorothiazide in 4mL methanol. 50μL of standard stock solution was pipetted out to make up the volume up to 10mL with methanol.

2.4. Preparation of tablet sample solution

Twenty tablets of Telma-H each containing 40mg of Telmisartan and 12.5mg of Hydrochlorothiazide were accurately weighed and crushed into a fine powder. An amount of powder equivalent to 4mg of Telmisartan and 1.25mg of Hydrochlorothiazide was weighed dissolved in methanol completely then again methanol was added to make up the volume up to 4mL. 50μL of the sample stock solution was pipetted out and diluted with methanol to make up the volume up to 10mL.

2.5. Validation

Method validation was performed as per ICH guidelines for simultaneous estimation of Telmisartan and Hydrochlorothiazide in combined dosage form. The following validation was addressed: system suitability, specificity, linearity, accuracy, recovery and precision, limit of detection and limit of quantification.

2.5.1 System suitability

The system suitability was performed by analyzing five replicate injections of standard sample solution. Results of peak area of Telmisartan and Hydrochlorothiazide were noted and the acceptance criteria should not be more than 2.0% for the RSD for the peak areas of the both the drugs.

2.5.2. Specificity

The diluents solution chromatograms and sample chromatograms were compared for the interference of any extra peak at the same retention time of the sample. If there is no interference of any extra peak then this indicates that diluents solution used in sample preparation do not interfere in the estimation of Telmisartan and Hydrochlorothiazide.

2.5.3. Linearity

The calibration curve was established by plotting a graph of concentration versus area of Telmisartan and Hydrochlorothiazide standard and determining the correlation coefficient (R^2). A series of concentrations ranging from 2ng/mL to 6ng/mL of Telmisartan and 0.625 to 1.875ng/mL of Hydrochlorothiazide were prepared and analysed in triplicates (Correlation coefficient should be not less than 0.999).

2.5.4. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The percent recoveries were carried out using standards and tablet samples at 75%, 100% and 125% level, in triplicate at each level and analyzed by UHPLC-MS. The calculated values of percentage of recovery should be between 98.00% -102%.

2.5.5. Precision

The precision of the method was evaluated as intra-day and inter-day by carrying out six independent assays of test samples against a qualified reference standard and the %RSD of assay was calculated (%RSD should not be more than 2%).

2.5.6. Robustness

The robustness of a method is the ability of the method to remain unaffected by making slight deliberate changes in chromatographic conditions. The robustness of the method was studied by making slight changes in ratio of the mobile phase, flow rate and %RSD was calculated by 3 replicate injections.

2.5.7. Limit of Detection and Limit of Quantification

Limit of Detection and Limit of Quantification were calculated using formula $3.3\sigma/S$ and $10\sigma/S$ respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

2.6. Forced Degradation Study

Forced degradation study of standard drugs was carried out as per the ICH guidelines (ICH Q1A9R2). The forced degradation study of the standard drugs was performed under acid, alkali, oxidative and neutral stress conditions.

2.6.1. Standard stock solution preparation

Standard stock solutions of Telmisartan and Hydrochlorothiazide were prepared by dissolving 8mg of each drug in 8mL methanol.

2.6.2. Acid degradation sample preparation

2mL of stock solution was taken and diluted up to 4mL with 1N HCl. It was heated for 6hr at 80°C. Allowed the solution to achieve ambient temperature and then the solution was further diluted to achieve the concentration of 10ppm.

2.6.3. Alkali degradation sample preparation

2mL of stock solution was taken and diluted up to 4mL with 1N NaOH. It was heated for 6hr at 80°C. Allowed the solution to achieve ambient temperature and then the solution was further diluted to achieve the concentration of 10ppm.

2.6.4. Oxidative degradation sample preparation

2mL of stock solution was taken and diluted up to 4mL with 15% H_2O_2. It was heated for 6hr at 80°C. Allowed the solution to achieve ambient temperature and then the solution was further diluted to achieve the concentration of 10ppm.

2.8.5. Neutral degradation sample preparation

2mL of stock solution was taken and diluted up to 4mL with 2mL of water. It was heated for 6hr at 80°C. Allowed the solution to achieve ambient temperature and then the solution was further diluted to achieve the concentration of 10ppm.

3. RESULTS AND DISCUSSION

3.1. Method Development

A UHPLC-MS method was developed for the simultaneous estimation of Telmisartan and Hydrochlorothiazide in tablet dosage form, which can be conveniently employed for routine analysis. The chromatographic conditions were optimized in order to provide a good performance of the assay. The mobile phase for drugs was selected based on their polarity. Different trails were taken and finally the optimized mobile phase was methanol: water with 0.1% formic acid (70:30 v/v) with a flow rate of 500μL/min. The retention time of Hydrochlorothiazide is 1.60±0.01min and Telmisartan is 2.27±0.05min respectively. Figure II represents chromatogram and mass spectra of Telmisartan and Hydrochlorothiazide.
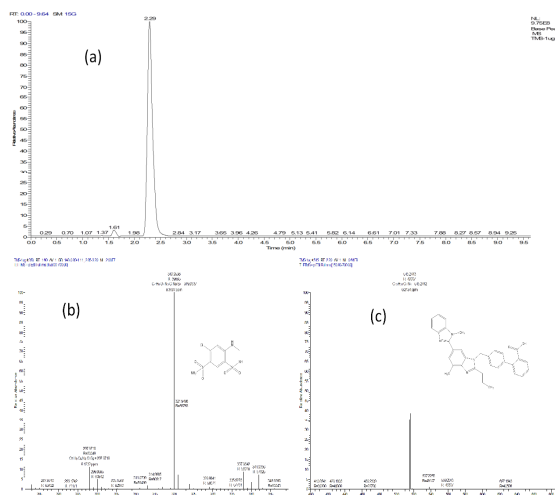

Figure - II: (a) Chromatogram and High resolution Mass Spectra of Telmisartan (b) and Hydrochlorothiazide(c).

Table 1: Summary of Validation Parameters of UHPLC-MS Method for simultaneous estimation of Telmisartan and Hydrochlorothiazide

Parameters		Telmisartan	Hydrochlorothiazide	Acceptance Criteria
Linearity Range (ng/mL)		2 to 6ng/mL	0.625 to 1.875ng/mL	Correlation Coefficient r2 > 0.999
Regression equation		y = 2E+08x + 2E+08	y =3E+06x - 183044	
Slope		200000000	3000000	
Intercept		200000000	-183044	
Correlation coefficient (r^2)		0.9996	0.9994	
LOD (ng/mL)		0.1422ng/mL	0.0376ng/mL	LOD = 3.3 X σ/S
LOQ(ng/mL)		0.4309ng/mL	0.114ng/mL	LOQ = 10 X σ/S
Accuracy(n=3)	Level 1	100.8	101.2	Recovery 98-102%
	Level 2	99.2	100.8	
	Level 3	100	100.6	
Intraday (%RSD), (n=6) Interday (%RSD), (n=6)		1.0	1.8	RSD < 2%
		1.8	1.2	
Specificity		No interference	No interference	No interference from blank with the main peak
Robustness		0.6-1.6	1.2-1.8	RSD< 2% in modified condition
System suitability studies		1.7	0.7	RSD<2%

*% RSD- Percent relative standard deviation, σ = The standard deviation of the response , S = Slope of the calibration curve, LOD- Lower limit of detection, LOQ- Lower limit of Quantification.

The method was validated as per ICH (Q2B) regulatory guidelines [28]. Summary of validation parameters of UHPLC-MS method for simultaneous estimation of Telmisartan and Hydrochlorothiazide is given in table 1.

3.2. Validation

3.2.1. System suitability

The system suitability was performed by analyzing five replicate injections and the %RSD for Telmisartan and Hydrochlorothiazide was

found to be 1.7 and 0.7 respectively, which is less than 2%.

3.2.2. Specificity

The optimized UHPLC-MS method was used for the analysis and identification of Telmisartan and Hydrochlorothiazide was shown to be specific, the retention time for Telmisartan and Hydrochlorothiazide were 1.60±0.01 and 2.27±0.05 minutes respectively. No interfering peaks were observed with the same retention time of the analytes.

3.2.3. Linearity

A linear response was observed for the intensity of peak area versus concentration over a working range of 2 to 6ng/mL for Telmisartan and 0.625 to 1.875ng/mL for Hydrochlorothiazide with a correlation coefficient of 0.9996 (Figure III) and 0.9994 (Figure IV).
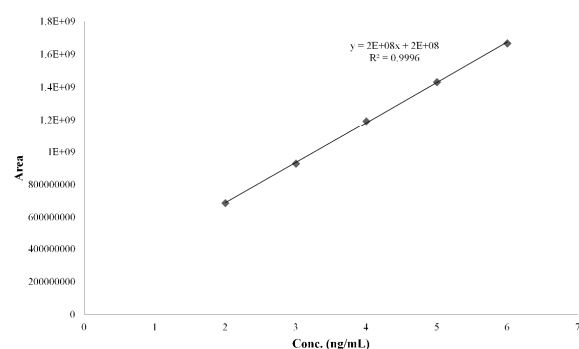

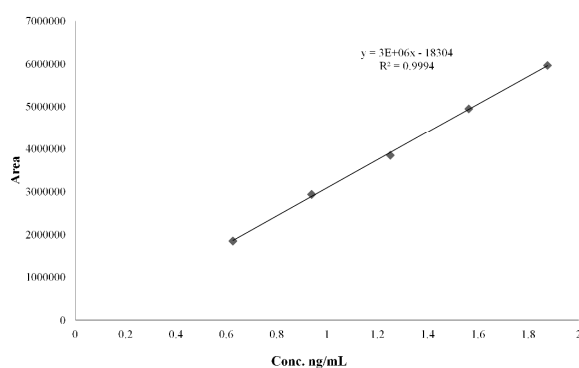**Figure – III: Calibration curve of Telmisartan.**

Figure – IV: Calibration cure of Hydrochlorothiazide.

3.2.4. Accuracy

Percent recovery of Telmisartan ranged from 99.2% to 100.8% and the percent recovery of Hydrochlorothiazide ranged from 100.6% to 101.2% showing the good accuracy of the method.

3.2.5. Precision

The precision of the analytical method was studied by determining the concentration of each drug in the tablet in six replicates. The results of the precision study indicate that the method is reliable and the %RSD for the precision study was 1.8% and 1.2% (inter-day precision), 1.0% and 1.8% (intra-day precision) for Telmisartan and Hydrochlorothiazide respectively.

3.2.6. Robustness

The result of the robustness study indicates that the method is robust and is unaffected by small variations in the chromatographic conditions. The %RSD was found to be less than 2.

3.2.7. Limit of Detection and Limit of Quantitation

Limit of detection of Telmisartan and Hydrochlorothiazide was found to be 0.1422ng/mL and 0.0376ng/mL respectively. Limit of Quantitation of Telmisartan and Hydrochlorothiazide was found to be 0.4309ng/mL and 0.114ng/mL respectively.

3.3. Degradation Studies

The identification of degradation products is very effective for knowing the pathways of degradation of drugs. Telmisartan and Hydrochlorothiazide were subjected to degradation for achieving maximum degradation. The drugs were subjected to acid, alkali, oxidative and neutral stress degradation. The degradation products were analyzed using UHPLC-MS and then they were fragmented by high energy collision induced dissociation in MS/MS mode to identify them. The drug Telmisartan was detected at m/z 515 (M+H)+ with fragments m/z 497, 469, 317, 305, 289, 263, 262, 248, 238, 221, 211and 167. The drug Hydrochlorothiazide was detected at m/z 297(M+H)+ /319 (M+Na)+ with fragments 268 and 204. The degradation behaviour of the drugs in individual stress condition is discussed below.

3.3.1. Acid Degradation

On acid degradation for 6hr at 80°C with 1N HCl, two degradation products of Telmisartan with m/z 529 and 487 were observed. On MS/MS analysis the degradation product with m/z 529 has given fragments at m/z 497, 317, 289, 211and 167. The other acid degradant m/z 487 has shown fragments at m/z 469 and 211. Figure V represents structures of Telmisartan degradation products and their fragments. For Hydrochlorothiazide no degradants were observed.

3.3.2 Alkali Degradation

On alkali degradation for 6hr at 80°C with 1N NaOH no degradation product was formed for Telmisartan. This show Telmisartan is stable under alkaline stress condition. Only one degradation product of Hydrochlorothiazide with

m/z 286 was observed, which gave a fragment ion at *m/z* 204 on MS/MS analysis. Figure VI represents structures of Hydrochlorothiazide degradation product and its fragment.
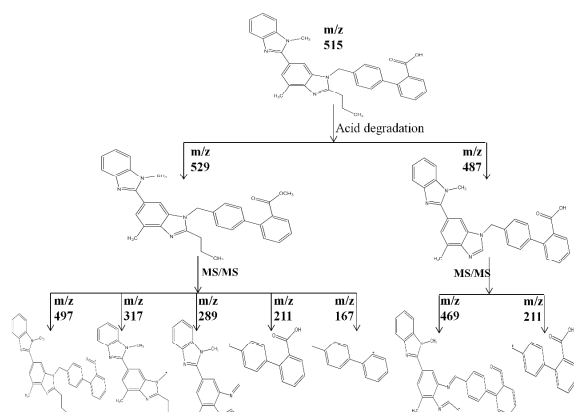

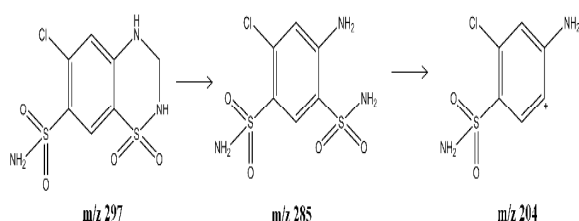**Figure - V: Acid degradation products of Telmisartan and their fragments from MS/MS.**

Figure - VI: Alkali degradation product of Hydrochlorothiazide (m/z 285) and its fragment (m/z 204) from MS/MS.

3.3.3. Neutral Degradation

On neutral degradation for 6hr at 80°C no degradation product was formed for both the drugs Telmisartan and Hydrochlorothiazide. This show Telmisartan and Hydrochlorothiazide are stable under neutral condition.
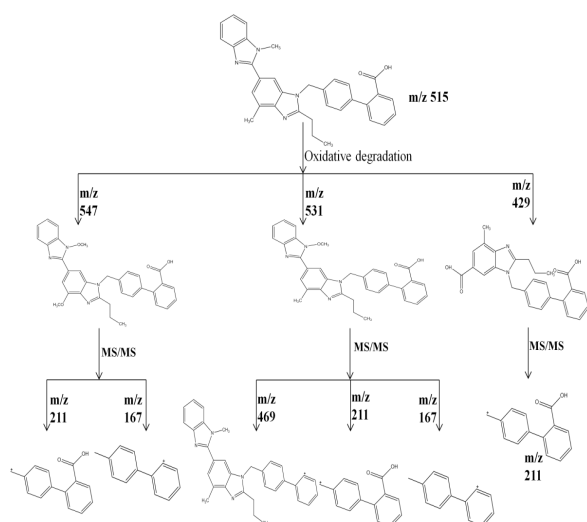

Figure - VII: Oxidative degradation products of Telmisartan and their fragments from MS/MS.
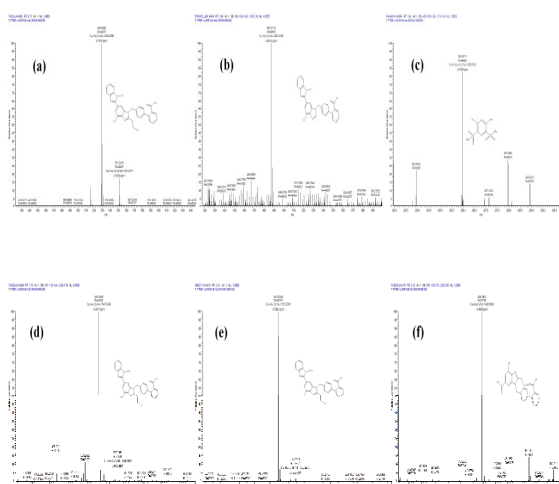

Figure - VIII: High resolution mass spectra of all identified degradants of Telmisartan and Hydrochlorothiazide.

3.3.4. Oxidative Degradation

On oxidative degradation for 6hr at 80°C with 15% H_2O_2, three degradation products of Telmisartan with *m/z* 531, 547, 429 were observed. On MS/MS analysis the degradant with *m/z* 531 gave fragments at *m/z* 469, 211, 167, for *m/z* 547 the fragments were *m/z* 211, 167 and for *m/z* 429 the fragment was observed at *m/z* 211. Figure VII represents structures of Telmisartan oxidative degradation products and their fragments. Hydrochlorothiazide has not given any oxidative degradant. This show Hydrochlorothiazide is stable under oxidative degradation. Figure VIII represents high resolution mass spectra of all identified degradants of Telmisartan and Hydrochlorothiazide.

4. CONCLUSION

Telmisartan and Hydrochlorothiazide in tablet dosage form was validated and found to be accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages including simple mobile phase, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The method can be used for routine analysis of marketed products of Telmisartan and Hydrochlorothiazide in combined tablet formulation, for stability studies and also in the pharmacokinetic study.

The developed and validated UHPLC-MS method was applied for the degradation studies of Telmisartan and Hydrochlorothiazide. The identified major degradation products of Telmisartan and Hydrochlorothiazide, under different stress conditions were subjected to fragment analysis using UHPLC-MS/MS method to get structural information.

Fragment ions with *m/z* 211 and 167 were common for most of the degradation products of Telmisartan. In acid, oxidative and neutral conditions Hydrochlorothiazide was stable. For Telmisartan together five degradation products (m/z 529, 487, 531, 547 and 428) were observed in acid and oxidative degradation conditions, among these m/z 531 and 547 are new and were not reported so for. Whereas in alkali and neutral conditions no degradation was observed, it means the drug is stable in both these conditions.

Acknowledgement

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