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Forced Degradation Studies for Telmisartan and Hydrochlorothiazide

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ABSTRACT

Forced degradation studies were carried out for Telmisartan and Hydrochlorothiazide under different stress conditions like acidic, alkaline, oxidation, reduction, thermal, and photostability condition and found that degraded peaks did not interfere with the peaks of the drug under the study. Hence the developed and now validated stability-indicating HPLC method can be used routinely for the simultaneous determination of Telmisartan and Hydrochlorothiazide in both bulk drugs and marketed formulations (tablets).

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INTRODUCTION:

The forced degradation studies for Telmisartan and Hydrochlorothiazide were carried out to analyze the degree of degradation, to study whether the degraded products interfere with the actual drugs, and can be determined or analyzed by the same HPLC method developed.

The degradation of standard Telmisartan and Hydrochlorothiazide was carried out under various stress conditions such as acidic medium, basic medium, oxidation and thermal condition (60^{o}C), and Photostability condition.

Instrument Specification:

Chromatographic Conditions for Sample Analysis

Instrument	SHIMADZU UFLC-2000 Prominence LC-20AD Binary Gradient System SPDM 20 A Detector
Injector	Rheodyne
Column	Enable c-18 G column 250×4.6mm , 5µm
Detector	PDA Detector
Flow rate	1.0 ml/min
Injection volume	20µL
Mobile Phase	Acetonitrile: Potassium di hydrogen phosphate(60:40); pH3.0
Detection Wavelength	282 nm

Reagents and Chemical:

1. Acetonitrile, HPLC grade (Sd Fine-Chem Ltd)
2. Potassium Dihydrogen phosphate (Thermo Fisher Scientific India Pvt. Ltd, Mumbai)
3. Millipore water
4. Telmisartan (Hetero Pharma)
5. Hydrochlorothiazide (Aurobindo Pharma)

Degradation studies of Telmisartan and Hydrochlorothiazide in Acidic Condition:

Telmisartan and Hydrochlorothiazide were subjected to forced degradation in the acidic medium in presence of 0.1N HCl and heated at 60^0C for a period of 4 hrs.

Preparation of 0.1N Hydrochloric acid: 8.5 ml of concentrated hydrochloric acid was taken in a 1000ml volumetric flask and diluted to mark with water.

Procedure:

25mg each of Telmisartan and Hydrochlorothiazide was weighed and transferred into two separate 25 ml volumetric flasks and subjected to stress conditions in 2 ml of 0.1N HCl. The subjected solution was injected and the degradation was not found for 4 hrs. Further, the above solution was subjected to heat at 60^0C for a period of 4 hrs. The volume was made up to 25 ml with mobile phase (1000µg/ml).5ml of the above solution was diluted to 50ml in a volumetric flask with the mobile phase to get the concentration of 100µg/ml. At different time intervals, the sample aliquots were withdrawn at 2 and 4 hr and then neutralized with 2 ml of 0.1N NaOH. Take 2ml of each resulting solution (100µg/ml) and mixed in a separate flask.

20µL of these degraded solutions were injected into a chromatograph along with the control. The peak areas and the chromatograms obtained for Telmisartan, Hydrochlorothiazide, and degraded products were recorded. The % of degradation was calculated. The results obtained are presented in Table No. 21 & 22 (Page No.100) and Fig. 25 a, b & c (Page No. 101 & 102).

Degradation studies of Telmisartan and Hydrochlorothiazide in Alkaline Condition:

Telmisartan and Hydrochlorothiazide were subjected to forced degradation in the alkaline medium in presence of 0.1N NaOH and heated at 60^0C for a period of 4 hrs.

Preparation of 0.1N sodium hydroxide: 4 g of sodium hydroxide pellets were taken in 1000 ml volumetric flask and dissolved with water and volume was made to 1000 ml with water.

Procedure:

25 mg each of Telmisartan and Hydrochlorothiazide was weighed and transferred into two separate 25 ml volumetric flasks and subjected to stress conditions in 2ml of 0.1N NaOH.

The subjected solution was injected without heat at 0, 2, and 4 hr, and didn't find out the degradation. Further went for heated at 60^0C for a period of 4 hrs. The volume was made up to 25ml with mobile phase (1000µg/ml).5ml of the above solution was diluted to 50ml in a volumetric flask with the mobile phase to get the concentration of 100µg/ml. At different time intervals, the sample aliquots were withdrawn at 2 and 4hr and then neutralized with 2ml of 0.1N HCl. Take 2ml of each resulting solution (100µg/ml) and mixed in a separate flask.

20µL of these degraded solutions were injected into a chromatograph along with the control. The peak areas and the chromatograms obtained for Telmisartan, Hydrochlorothiazide, and degraded products were recorded. The % of degradation was calculated. The results obtained are presented in Table No. 23 & 24 (Page No.103) and Fig. 26 a, b, & c (Page No.104 & 105).

Degradation studies of Telmisartan and Hydrochlorothiazide in Oxidation condition:

Telmisartan and Hydrochlorothiazide were subjected to force degradation in a 3% v/v solution of hydrogen peroxide (oxidizing medium).

Preparation of 3% Hydrogen peroxide: 10ml of 30%Hydrogen peroxide was taken in 100 ml volumetric flask and dissolved with water and volume was made up to 100 ml with water.

Procedure:

25mg each of Telmisartan and Hydrochlorothiazide was weighed and transferred into two separate 25ml volumetric flasks and subjected to stress conditions in 2ml of 3% v/v hydrogen peroxide. The subjected solution was injected without heat at 0, 2, and 4 hr, and didn't find out the degradation. Further went for heated at 60^0C for a period of 4 hrs. The volume was made up to 25 ml with mobile phase (1000µg/ml).5ml of the above solution was diluted to 50 ml in a volumetric flask with the mobile phase to get the concentration of 100µg/ml. At different time intervals, the sample aliquots were withdrawn at 2 and 4 hr. Take 2ml of each resulting solution (100µg/ml) and mixed in a separate flask.

20µL of these degraded solutions were injected into a chromatograph along with the control. The peak areas and the chromatograms obtained for Telmisartan, Hydrochlorothiazide, and degraded products were recorded. The % of degradation was calculated.

Degradation studies of Telmisartan and Hydrochlorothiazide in Thermal conditions:

Thermal degradation studies for standard drugs Telmisartan and Hydrochlorothiazide were carried out in a dry hot air oven at 60^0C for 2 days by exposing the standard drug of 1 mm thickness in a Petri dish.

Procedure:

Standard Drug Telmisartan and Hydrochlorothiazide were subjected to dry heat condition, at 60^0C in a Petri dish. At different time intervals of 24hrs and 48hrs, accurately 25 mg each of Telmisartan and Hydrochlorothiazide was weighed into two separate 25 ml volumetric flasks, dissolved in a few drops of mobile phase, and finally, the volume was made up to 25ml with mobile phase (1000µg/ml). 5ml of the above solution was diluted to 50ml in a volumetric flask with the mobile phase to get a concentration of 100µg/ml.

20µL of these degraded solutions were injected into a chromatograph along with the control. The peak areas and the chromatograms obtained for Telmisartan, Hydrochlorothiazide, and degraded products were recorded. The % of degradation was calculated.

Degradation Studies of Telmisartan and Hydrochlorothiazide in Photostability Condition (UV light):

Photostability degradation studies for standard drugs Telmisartan and Hydrochlorothiazide were carried out in a photostability chamber by exposure to UV light in a Petri dish (1mm thickness).

Procedure:

Standard Drug Telmisartan and Hydrochlorothiazide were exposed to UV light in a Petri dish. At different time intervals of 24 hrs and 48 hrs, accurately 25mg each of Telmisartan and Hydrochlorothiazide, was weighed into two separate 25ml volumetric flasks, dissolved in a few drops of mobile phase, and finally, the volume was made up to 25ml with mobile phase (1000µg/ml). 5ml of the above solution was diluted to50ml in a volumetric flask with the mobile phase to get the concentration of 100µg/ml.

20µL of these degraded solutions were injected into a chromatograph along with the control. The peak areas and the chromatograms obtained for Telmisartan, Hydrochlorothiazide, and degraded products were recorded. The % of degradation was calculated.

Forced Degradation Studies for Telmisartan and Hydrochlorothiazide:

Forced degradation studies for Telmisartan and Hydrochlorothiazide were carried out in acidic, alkaline, oxidation, thermal, and UV condition.

5.4.1. Degradation Studies of Telmisartan and Hydrochlorothiazide in Acidic Condition:

Forced degradation studies for Telmisartan (100 µg/ml) and Hydrochlorothiazide (100 µg/ml) was carried out in 0.1N HCl and heated at 60^0C for a period of 4 hrs.

Degradation Data for Telmisartan in Acidic Condition

Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	2104288	101.27	101.27
2	2	2015039	97.03	97.03
3	4	1991829	95.93	95.93

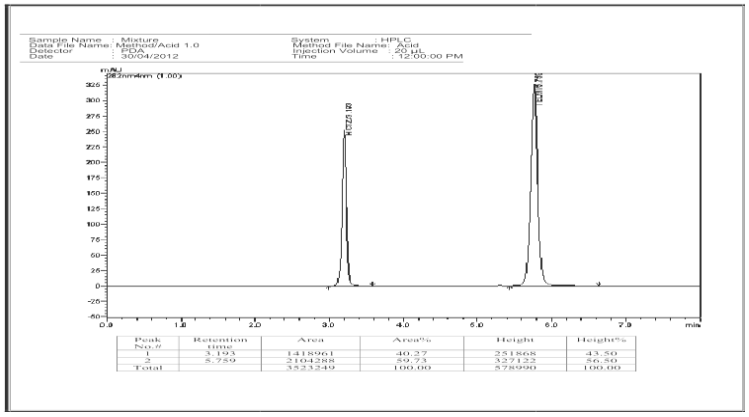
***Average of three readings**

Degradation Data for Hydrochlorothiazide in Acidic Condition

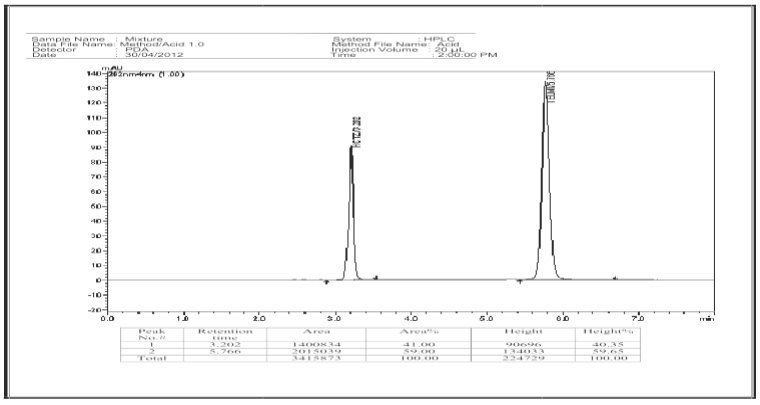
Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	1418961	102.51	102.51
2	2	1400834	101.16	101.16
3	4	1298366	93.53	93.53

***Average of three readings**

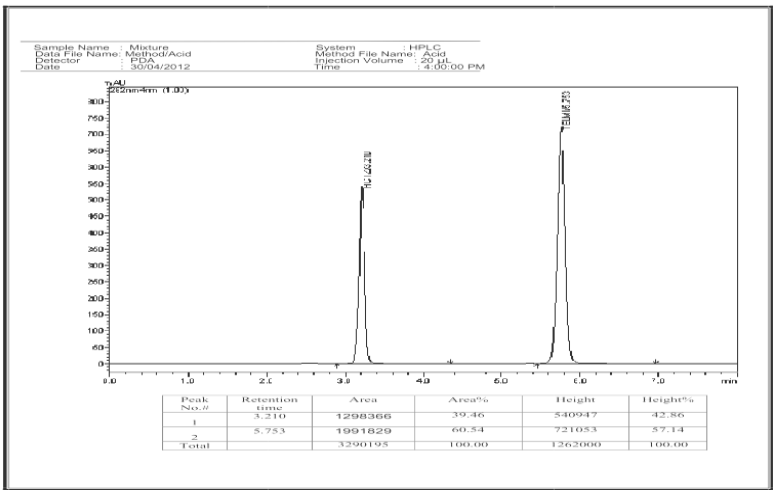
Chromatograms of Telmisartan and Hydrochlorothiazide in Acidic Condition:



(a) Chromatogram for TEL and HCTZ in Acidic Condt at 0 hr



(b) Chromatogram for TEL and HCTZ in Acidic Condt at 60^0C 2 hr



(c) Chromatogram for TEL and HCTZ in Acidic Condt at 60^0C 4 hr

Report:

The acidic degradation of standard Telmisartan drug in 0.1 N HCl was found to be Non-degraded for 4hrs and in heating conditions degraded was found to be 6.24 % at the 4th hour.

The acidic degradation of standard Hydrochlorothiazide drug in 0.1 N HCl was found to be Non-degraded for 4hrs and in heating conditions degraded 8.98 % at the 4th hour

Degradation Studies of Telmisartan and Hydrochlorothiazide in Alkaline Condition:

Forced degradation for Telmisartan and Hydrochlorothiazide were carried out in 0.1N NaOH and heated at 60^{0}C for a period of 4 hrs.

Degradation Data for Telmisartan in Alkaline Condition

Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	2142977	103.10	103.10
2	2	2003315	96.47	96.47
3	4	1896615	91.41	91.41

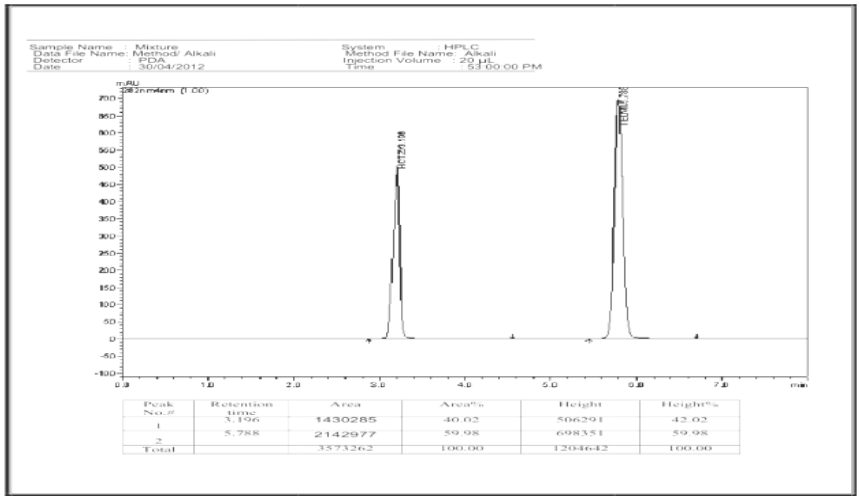
***Average of three readings**

Degradation Data for Hydrochlorothiazide in Alkaline Condition

Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	1430285	103.36	103.36
2	2	1403345	101.35	101.35
3	4	1371331	98.97	98.97

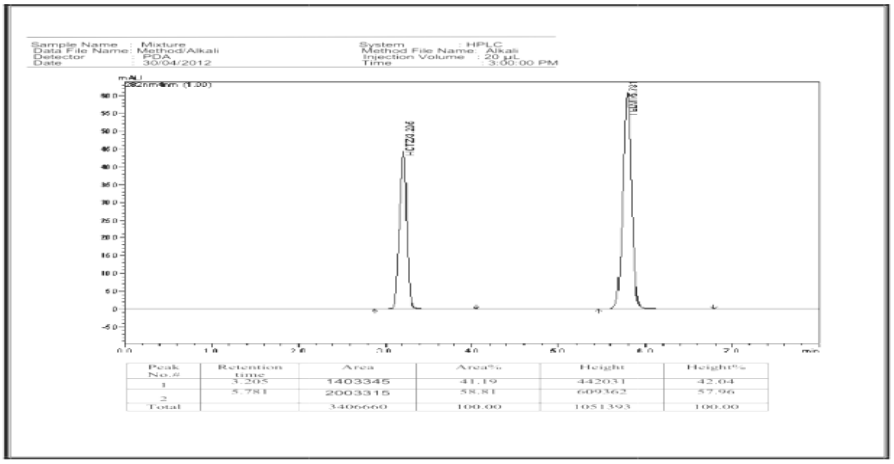
***Average of three readings**

Chromatograms of Telmisartan and Hydrochlorothiazide in Alkaline Condition:



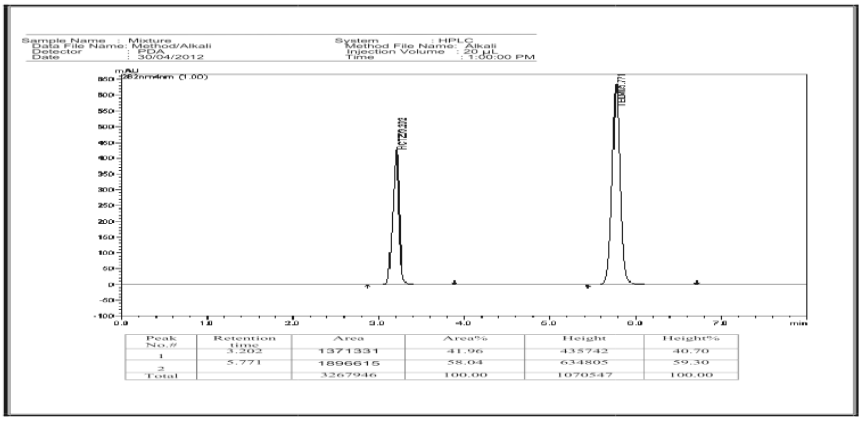
Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.196	1430285	40.02	506291	42.02
2	5.788	2142977	59.98	698351	59.98
Total		3573262	100.00	1204642	100.00

(a) Chromatogram for TEL and HCTZ in Alkaline Condt at 0 hr



Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.205	1403345	41.19	442031	42.04
2	5.781	2003315	58.81	609362	57.96
Total		3406660	100.00	1051393	100.00

(b) Chromatogram for TEL and HCTZ in Alkaline Condt at 60^0C 2hr



Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.203	1371331	41.96	435742	40.70
2	5.771	1896615	58.04	634805	59.30
Total		3267946	100.00	1070547	100.00

(c) Chromatogram for TEL and HCTZ in Alkaline Condt at 60^0C 4 hr

Report:

The alkaline degradation of standard Telmisartan was found to be Non-degraded for 4hrs and in heating conditions degradation was found to be 11.69% degraded in 0.1N NaOH at the 4th hour.

Whereas the degradation of standard drug Hydrochlorothiazide in alkaline medium was found to be Non-degraded for 4hrs and in heating condition degradation was found to be 4.39 % degraded in 0.1N NaOH at 4th hour.

Degradation Studies of Telmisartan and Hydrochlorothiazide in Oxidation Condition:

Forced degradation for Telmisartan and Hydrochlorothiazide was carried out in 3% Hydrogen peroxide.

Degradation Data for Telmisartan in Oxidation Condition

Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	2098671	101.0	101.0
2	2	2021429	97.33	97.33
3	4	1792875	86.49	86.49

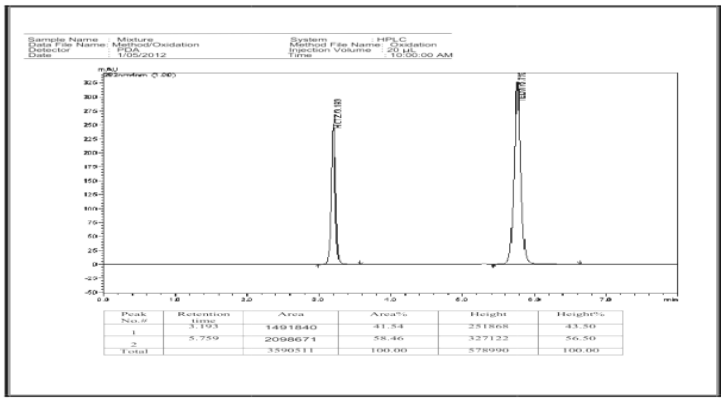
***Average of three readings**

Degradation Data for Hydrochlorothiazide in Oxidation Condition

Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	1491840	107.94	107.94
2	2	1217964	87.54	87.54
3	4	1006735	71.81	71.81

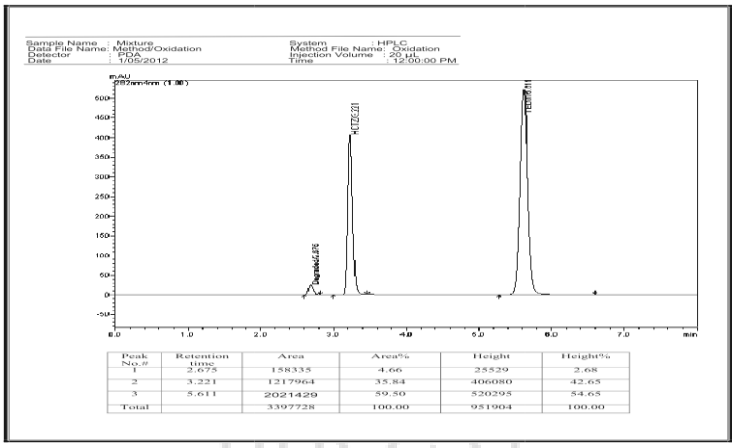
***Average of three readings**

Chromatograms of Telmisartan and Hydrochlorothiazide in Oxidation Condition:



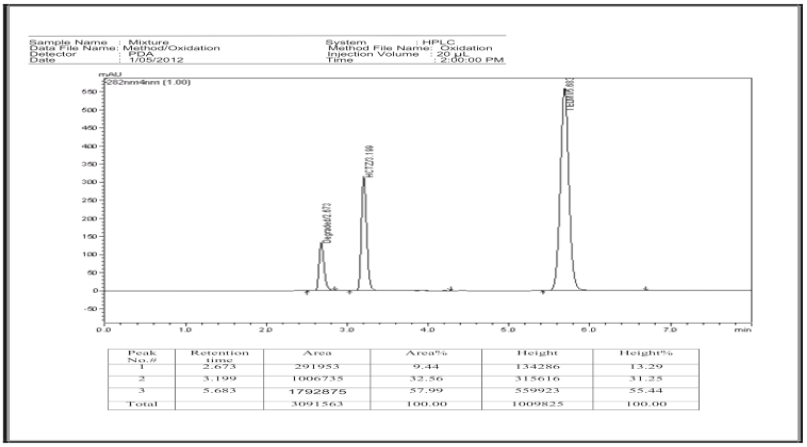
Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.193	1491840	41.54	251868	43.50
2	5.759	2098671	58.46	327122	56.50
Total		3590511	100.00	578990	100.00

(a) Chromatogram for TEL and HCTZ in Oxidation Condt at 0 hr



Peak No.#	Retention time	Area	Area%	Height	Height%
1	2.675	158335	4.66	25529	2.68
2	3.221	1217964	35.84	406080	42.65
3	5.611	20214429	59.50	520295	54.65
Total		3397728	100.00	951904	100.00

(b) Chromatogram for TEL and HCTZ in Oxidation Condt at 60^0C 2hr



Peak No.#	Retention time	Area	Area%	Height	Height%
1	2.673	291953	9.44	134286	13.29
2	3.199	1006735	32.56	315616	31.25
3	5.683	1792875	57.99	559923	55.44
Total		3091563	100.00	1009825	100.00

(c) Chromatogram for TEL an HCTZ in Oxidation Condt at 60^0C 4hr

Report:

The oxidation degradation of standard Telmisartan was found to be non-degraded for 4hrs and in heating condition degradation was found to be 14.51% degraded in oxidation condition at 4th hour.

Whereas the degradation of standard drug Hydrochlorothiazide in oxidation condition was found to be non-degraded for 4hrs and in heating condition degradation was found to be 36.13 % degraded in oxidation condition at 4th hr giving rise to a degraded peak at the retention time of 2.675 min.

Degradation Studies of Telmisartan and Hydrochlorothiazide in Thermal Condition at 60°C:

Thermal degradation studies for Telmisartan and Hydrochlorothiazide were carried out in a hot air oven at 60°C for two days.

Degradation Data for Telmisartan in Thermal Condition

Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	2118092	101.92	101.92
2	24	2035040	97.98	97.98
3	48	1804188	87.02	87.02

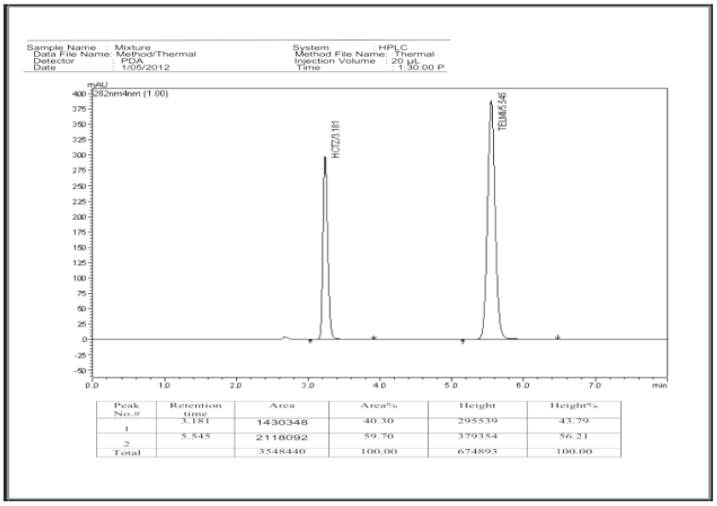
***Average of three readings**

Degradation Data for Hydrochlorothiazide in Thermal Condition

Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	1430348	103.36	103.36
2	24	1342115	96.79	96.79
3	48	1239373	89.142	89.142

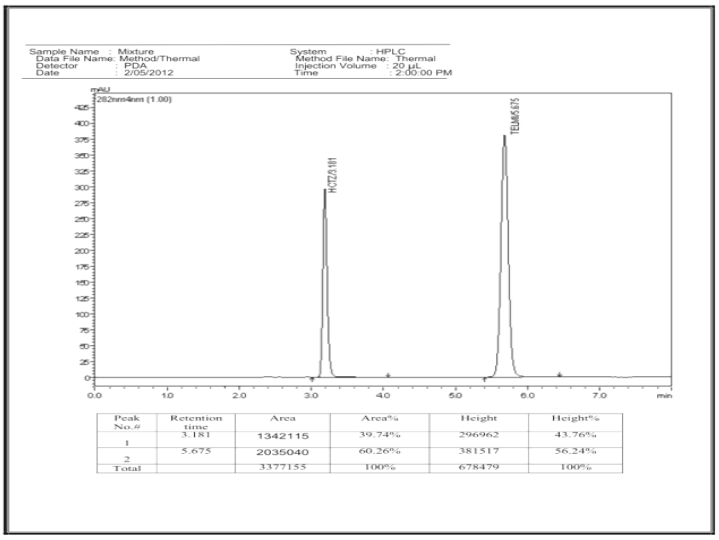
***Average of three readings**

Chromatograms of Telmisartan and Hydrochlorothiazide in Thermal Condition:



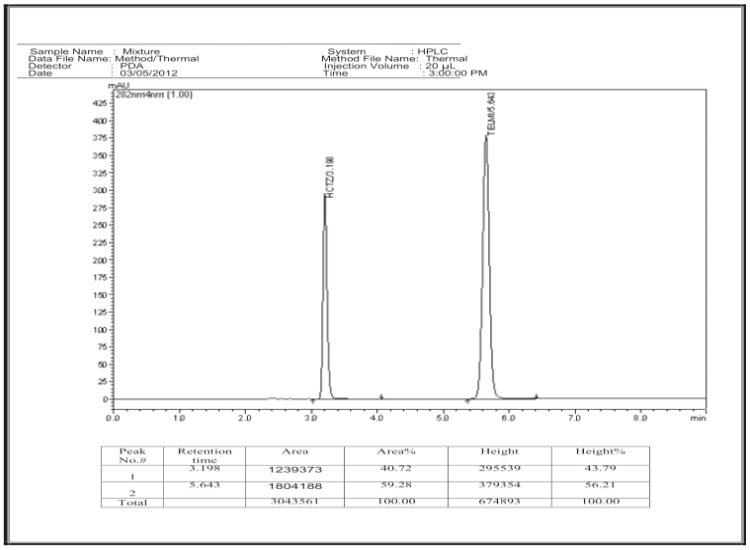
Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.181	1430348	40.30	295539	43.79
2	5.545	2118092	59.70	379354	56.21
Total		3548440	100.00	674893	100.00

(a) Chromatogram for TEL and HCTZ in Thermal Condt at 0 hrs



Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.181	1342115	39.74%	296962	43.76%
2	5.675	2035040	60.26%	381517	56.24%
Total		3377155	100%	678479	100%

(b) Chromatogram for TEL and HCTZ in Thermal Condt after 60°C at 24hrs



Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.198	1239373	40.72	295539	43.79
2	5.643	1804188	59.28	379354	56.21
Total		3043561	100.00	674893	100.00

(c) Chromatogram for TEL and HCTZ in Thermal Condition after 48 60°C hrs

Report:

The Thermal degradation of standard Telmisartan was found to be 14.9% degraded in Thermal conditions at 48 hours, at 60^0C.

The Thermal degradation of standard Hydrochlorothiazide was found to be 14.21% degraded in Thermal conditions at 48 hours, at 60^0C.

Degradation Studies of Telmisartan and Hydrochlorothiazide in Photostability Condition:

Photostability degradation studies for Telmisartan and Hydrochlorothiazide were carried out in the Photostability chamber by exposure to UV light for 48 hrs. The data obtained are presented below:

Degradation data for Telmisartan in Photostability Condition

Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	2113087	101.68	101.68
2	24	2039582	98.19	98.19
3	48	1801735	86.91	86.91

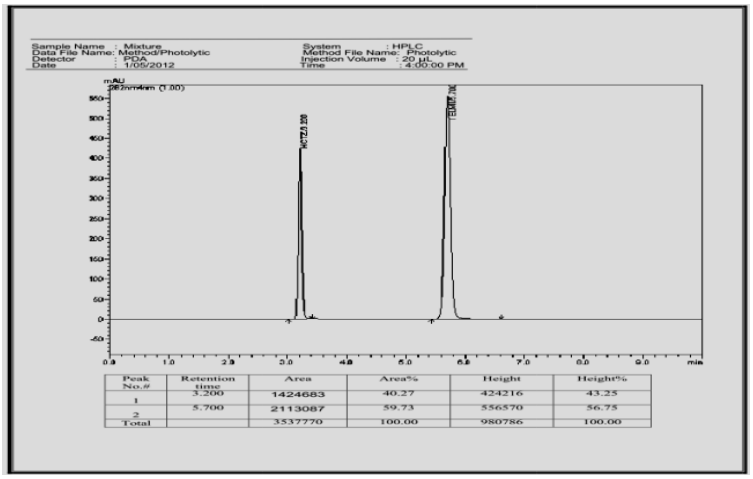
***Average of three readings**

Degradation Data for Hydrochlorothiazide in Photostability Condition

Sr No	Time interval (hrs)	Room Temperature		% Assay
		Peak Area*	Concentration (µg/ml)	
1	Control	1424683	102.94	102.94
2	24	1378421	99.49	99.49
3	48	1243726	89.46	89.46

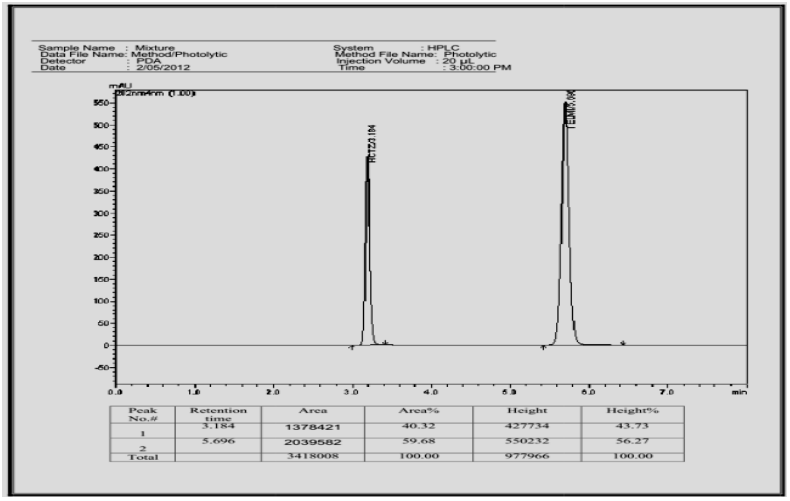
***Average of three readings**

Chromatograms of Telmisartan and Hydrochlorothiazide in Photo stability Condition:



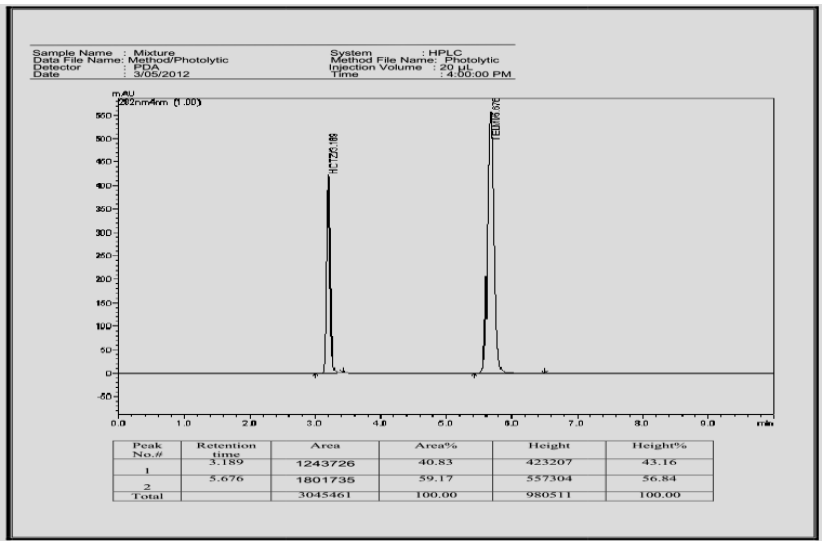
Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.200	1424683	40.27	424216	43.25
2	5.700	2113087	59.73	556570	56.75
Total		3537770	100.00	980786	100.00

(a) Chromatogram for TEL and HCTZ in Photostability Condition at 0hr



Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.184	1378421	40.32	427734	43.73
2	5.696	2039582	59.68	550232	56.27
Total		3418008	100.00	977966	100.00

(b) Chromatogram for TEL and HCTZ in Photostability Condition after 24 hrs



Peak No.#	Retention time	Area	Area%	Height	Height%
1	3.189	1243726	40.83	423207	43.16
2	5.676	1801735	59.17	557304	56.84
Total		3045461	100.00	980511	100.00

(c) Chromatogram for TEL and HCTZ in Photostability Condition after 48 hrs

Report:

The Photostability degradation of standard Telmisartan was found to be 14.77% degraded in Photostability conditions at 48 hours.

The Photostability degradation of standard Hydrochlorothiazide was found to be 13.48% degraded in the Photostability condition at 48 hours.

DISCUSSION AND SUMMARY

The market is flooded with various combinations of drugs for the treatment of disease and simultaneous estimation of such combinations in a dosage form plays a very important role in the field of pharmacy. One such combination is Telmisartan and Hydrochlorothiazide which is widely used in the treatment of Antihypertensive and Diuretic. Telmisartan and Hydrochlorothiazide combination dosage forms are marketed as Telma-H, CRESAR-H, INDITEL H, SARTEL-H, TALY-H, TARGIT-H.

From the literature survey, it was found that many methods had been reported for the determination of Telmisartan and Hydrochlorothiazide individually and in combination with other drugs. In this present research work, it was proposed to develop and validate a stability-indicating HPLC and UV method for simultaneous estimation of Telmisartan and Hydrochlorothiazide in bulk drugs and marketed formulations.

A standard drug of Telmisartan (Hetero Pharma) and Hydrochlorothiazide (Aurobindo Pharma) was obtained from Hyderabad. Telma-H Tablet formulation was purchased from the local market, Containing 40 mg Telmisartan and 12.5 mg Hydrochlorothiazide.

The objective of the proposed project was to develop and validate a stability-indicating HPLC and UV method for simultaneous estimation of Telmisartan and Hydrochlorothiazide in bulk drugs marketed formulation and to carry out the forced degradation of the drugs and study the effect of degraded products on the development method.

Various combinations of the solvent system in different ratios of mobile phase were tried out the forced degradation of drugs and study the effect of degraded products on the developed method. And the mobile phase of Acetonitrile: Potassium dihydrogen phosphate (pH: 3.0) in the ratio of 60:40 v/v was selected and standardized as it evoked satisfactory resolution and sharp peaks.

The overlaid spectrum of standard drugs of Telmisartan and Hydrochlorothiazide showed an isobestic point at 282 nm, which was selected, standardized, and used as the optimum wavelength. Various trials were also carried out by altering the flow rates from 0.8 to 1.2 ml/min with the objective to get a good resolution for Telmisartan and Hydrochlorothiazide. A good resolution and sharp peaks were observed for Telmisartan and Hydrochlorothiazide at the optimum flow rate of 1.0 ml/min which was selected, standardized, and used for our further study.

Stability indicating HPLC method was developed for the simultaneous estimation of Telmisartan and Hydrochlorothiazide using a C-18 column (Enable C-18 G, 250 mm x 4.6 mm, 5 µm), mobile phase consisting of Acetonitrile: Potassium dihydrogen phosphate (pH: 3.0) in the ratio of 60:40 Flow rate of 1.0 ml/ min, PDA detection at a wavelength of 282 nm. The retention time of Telmisartan and Hydrochlorothiazide was observed at 5.7 and 3.1 min respectively. The developed method was then validated by using various parameters like accuracy, precision, linearity, specificity, ruggedness, robustness, etc as per ICH guidelines.

System suitability:

System Suitability Parameters data

System Suitability Factor	Telmisartan	Hydrochlorothiazide	Acceptance Criteria
Tailing factor	1.146	1.350	2
HETP (mm)	13.32	17.50	-
Resolution	14.608		-
Theoretical plates	11256.398	8570.393	>6000
Asymmetry	0.26	0.57	1

The system suitability parameters were calculated to ascertain the suitability of the proposed method in the mobile phase of Acetonitrile and Potassium dihydrogen Phosphate (pH: 3.0) in the ratio of 60:40 Flow rate of 1.0 ml/ min, PDA detection at a wavelength of 282 nm were injected into the chromatograph.

The number of plates was found to be **11256.39** and **8570.39** for Telmisartan and Hydrochlorothiazide respectively. The HETP was found to be **13.326** and **17.502** for Telmisartan and Hydrochlorothiazide respectively, indicating the system suitability of the

method. The asymmetry factor was calculated statistically and found to be **0.26** and **0.57** for Telmisartan and Hydrochlorothiazide. The resolution of the method was good as found from the value of **14.608** indicating good and complete separation of the two components from each other with the well-defined baseline.

The developed HPLC method was applied for the simultaneous estimation of Telmisartan and Hydrochlorothiazide in marketed formulation (Telma – H tablets). The % recovery of Telmisartan and Hydrochlorothiazide was found **97.87** to **100.75%** and **95.50** to **98.33 %** respectively. As recovery ranges within the acceptable limits, indicating that the method can be applied for simultaneous determination of Telmisartan and Hydrochlorothiazide in marketed formulations (tablets).

Validation of HPLC Method:

Accuracy:

The accuracy was determined through a recovery study of the drugs by spiking the standard drug of Telmisartan and Hydrochlorothiazide at three different levels 80%, 100%, and 120% with the previously assayed samples of known fixed concentration.

The percentage recovery was found to be **98.81% to 102.75%** for Telmisartan and **99.83% to 103.85%** for Hydrochlorothiazide indicating no interference of excipients in the developed HPLC method for the determination of Telmisartan and Hydrochlorothiazide, the percentage recovery was in total agreement with acceptance criteria of **95- 105%.**

Validation Parameters of the HPLC Method

Parameters		**Telmisartan**	**Hydrochlorothiazide**	**Acceptance criteria**
Specificity		No peak was detected		No peak was detected
LOD (ng/ml)		0.99ng/ml	1.55ng/ml	-
LOQ (ng/ml)		3ng/ml	4.7ng/ml	-
Linearity & range		4 – 20 µg/ml	4–20 µg/ml	-
Precision	**System**	0.340 %	0.461 %	NMT 2%
	Method	0.388 %	0.607 %	
	Inter day	1.17 %	0.527%	
	Intra day	1.17 %	0.306 %	
R0bustness	**0.8 ml/min**	100.19 %	101.45 %	90-110 %
	1.2 ml/min	101.54 %	101.98 %	
	280 nm	101.06 %	100.56 %	90-110 %
	284 nm	100.07 %	100.55 %	
Accuracy (% Recovery)		98.81–102.75 %	99.83–103.85 %	90-110 %

Precision:

The precision of the method and system was determined to study the concordance of data between the series of measurements.

In system precision, the % RSD value of peak area was found to be **0.340%** for Telmisartan and **0.461%** for Hydrochlorothiazide.

In method precision, the % RSD value of peak area was found to be **0.388 %** for Telmisartan and **0.607 %** for Hydrochlorothiazide.

The intermediate precision of the method was determined by performing the assay on two different days (inter-day and intraday) to check the reproducibility. On intraday % the RSD value of peak area was found to be **1.17 %** for Telmisartan and **0.306%** for Hydrochlorothiazide. On inter-day % RSD value of peak area was found to be **0.17 %** for Telmisartan and **0.527 %** for Hydrochlorothiazide.

All the values of % RSD for the precision study obtained were well within the acceptance criteria of NMT 2%. Thus the proposed method was found to be providing a high degree of precision and reproducibility.

Specificity:

The specificity of the proposed method was determined by studying the effect of excipients, impurities, etc at the retention time of Telmisartan and Hydrochlorothiazide. Hence there was no interference from diluents, excipients, and impurities with the peaks of Telmisartan and Hydrochlorothiazide, indicating a high degree of specificity for the proposed method.

LOD and LOQ:

The LOD and LOQ were determined by the visualization method. The LOD was determined to find out the lowest amount of Telmisartan and Hydrochlorothiazide that can be detected and it was found to be **0.99ng/ml** and **1.55ng/ml** respectively. The LOQ was determined to find the lowest amount of Telmisartan and Hydrochlorothiazide that can be quantified and it was found to be **3ng/ml and 4.7ng /ml** for Telmisartan and Hydrochlorothiazide respectively indicating that the small concentration in micrograms level can be determined with acceptable accuracy and precision.

Linearity and Range:

The linearity for the drugs by the proposed method was determined to study its ability to elicit test results that are directly proportional to the concentration of the analyte in the sample.

Standard solutions in the concentration range of **4 - 20 µg/ml** of Telmisartan and Hydrochlorothiazide in the mobile phase of Acetonitrile: Potassium dihydrogen phosphate (pH: 3.0) in the ratio of 60:40 Flow rate of 1.0 ml/ min, PDA detection at a wavelength of 282 nm were injected into the chromatograph. From the peak areas obtained the standard calibration curve was prepared.

The proposed method is found to be linear at the concentration range of **4–20 µg/ml** for Telmisartan and Hydrochlorothiazide respectively. The percentage curve fittings were found to be **99.9%** for Telmisartan and **99.8%** for Hydrochlorothiazide.

Robustness:

The robustness of the method was determined by carrying out the assay after performing deliberate changes in, flow rate and change in wavelength.

The flow rate was slightly altered from 1.0ml/min to **0.8ml/min** and **by 1.2ml/min** the % assay for Telmisartan and Hydrochlorothiazide was found to be **100.19%, 101.45%,** and **101.54%, and 101.98%** respectively.

The wavelength was deliberately changed from 282 nm to **280 nm, and 284 nm** the % assay for Telmisartan and Hydrochlorothiazide was found to be in the range of **101.06 %, 100.56%,** and **100.07%, 100.55%** respectively.

All the robustness results indicated that the new method developed was robust and did not show significant variations on deliberate changes in the mobile phase flow rate and wavelength indicating a lack of influence on the test results by operational variables for the proposed method.

Forced degradation studies:

Forced degradation studies were carried out under different stress conditions like acidic, alkali, oxidation, thermal, and photolytic condition for Telmisartan and Hydrochlorothiazide, and to study whether the degraded products interfere with the method.

Forced Degradation Studies for Telmisartan and Hydrochlorothiazide.

Degradation condition	Drug Peak Area at control		Drug Peak Area at Stress Cond.		RT of degraded Products		% Degradation	
	TEL	HCTZ	TEL	HCTZ	TEL	HCTZ	TEL	HCTZ
Acidic 0.1N HCl	2104288	1418961	1991829	1298366	……	……	6.24%	8.98%
Alkaline 0.1 N NaOH	2142977	1430285	1896615	1371331	……	……	11.69%	4.39%
Oxidation 3% v/v H_2O_2	2098671	1491840	1792875	1217964	……	2.67	14.51%	36.13%
Thermal Condition	2118092	1430348	1804188	1239373	……	……	14.9%	14.21%
Photo Stability	2113087	1424683	1801735	1243726	……	……	14.77%	13.48%

In **Acidic conditions** standard drugs Telmisartan and Hydrochlorothiazide were found to be 6.24% and 8.98% degraded 4th hour at 60^{0}C heat.

In **Alkaline conditions** standard drug of Telmisartan and Hydrochlorothiazide was found to be 11.69% and 4.39% degraded 4th hour at 60^{0}C heat.

In **Oxidative conditions** standard drugs of Telmisartan and Hydrochlorothiazide were found to be 14.51% and 36.13% degraded 4th hour at 60^{0}C heat.

In **Thermal Studies** standard drugs of Telmisartan and Hydrochlorothiazide were found to be 14.9% and 14.21% degraded at 60^{0}C for 48 hr.

In **Photostability Studies** standard drugs Telmisartan and Hydrochlorothiazide were found to be 14.77% and 13.48% degraded for 48 hr.

From the degradation studies data, it was found that Telmisartan and Hydrochlorothiazide were found to be degraded in all stress conditions.

Telmisartan and Hydrochlorothiazide were found to be Non-degraded for 4hrs at Non-heating and in heating, the condition was found to be degraded for 4hrs period.

Hence stress testing should be given importance for such a combination of drugs and quantification of degraded products of such drugs helps us to maintain the quality, safety, and efficacy of drugs in formulations.

CONCLUSION

An HPLC method was developed for the simultaneous estimation of Telmisartan and Hydrochlorothiazide in bulk drugs and marketed formulations. The HPLC system used was SHIMADZU UFLC-2000 Prominence LC-20AD Binary Gradient System. SPDM 20A detector with Rheodyne injector and Enable C18 G column 250x 4.6mm,5µm.Injection volume of20µL was injected and eluted with the mobile phase of Acetonitrile and Potassium dihydrogen phosphate (pH3.0)in the ratio of 60:40 v/v at the flow rate of 1.0ml/min and UV detection at 282nm. The peaks of Telmisartan and Hydrochlorothiazide were found well separated with retention times of 5.7 min and 3.1min respectively.

The developed methods were validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, ruggedness, and robustness. The results obtained were well within the acceptance criteria for all the parameters. The proposed methods were applied for the determination of Telmisartan and Hydrochlorothiazide in bulk drug and marketed formulations (Tablets). The assay results conformed to the label claim of the formulation. Hence the proposed method can be used for the routine analysis of Telmisartan and Hydrochlorothiazide in their marketed tablet dosage formulations.

Forced degradation studies were carried out for Telmisartan and Hydrochlorothiazide under different stress conditions like acidic medium, alkaline medium, oxidation condition, thermal condition, and photo stability condition. And it was found that the peaks of degraded products did not interfere with the peaks of the drug under the study. Hence the stability-indicating HPLC method developed and validated can be used routinely for the simultaneous determination of Telmisartan and Hydrochlorothiazide in bulk drugs and marketed formulations (tablets).

The developed methods were validated for various parameters as per ICH guidelines like accuracy, specificity, LOD, LOQ, linearity, range, and sensitivity. The results obtained were within the acceptance criteria for the parameter. The proposed methods were applied for the

estimation of Telmisartan and Hydrochlorothiazide in marketed formulations. The assay results confirmed the label claim of the formulation. Hence the proposed method was found to be satisfactory and could be used for the routine analysis of Telmisartan and Hydrochlorothiazide in their marketed tablet dosage formulations.

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