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Stress Degradation Studies of Telmisartan and Hydrochlorothiazide and Development of Validated Stability Indicating Method

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ABSTRACT

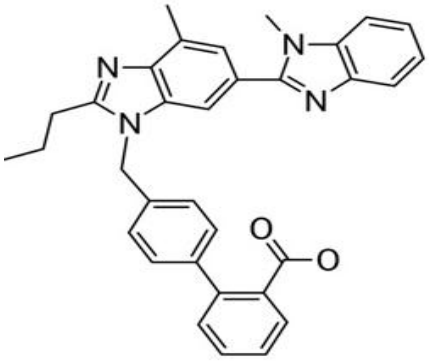
Telmisartan and Hydrochlorothiazide were subjected to different ICH prescribed stress conditions like acidic, alkaline, oxidation, reduction, thermal and photostability condition and found that degraded peaks did not interfere with the peaks of drug under the study. A stability indicating HPLC method was developed for analysis of the drug in the presence of degradation products involved a Enable C-18 G column 250x 4.6mm, 5 µm. Injection volume of 20 µL and a mobile phase composed of acetonitrile: potassium dihydrogen phosphate (pH 3.5) in the ratio of 60:40 v/v, which was pumped through the column in isocratic mode at the flow rate of 1.0 ml/min. The detection was carried out at 282 nm. The method was validated for linearity, range, precision, accuracy, specificity, selectivity and intermediate precision.

INTRODUCTION

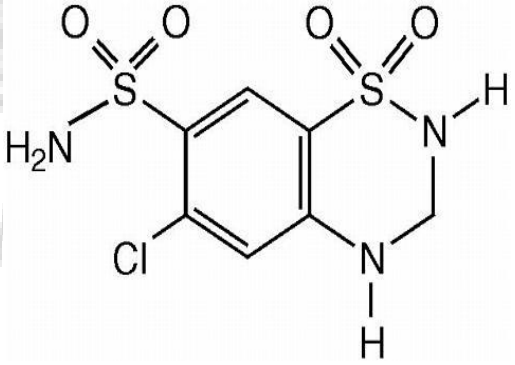
The parent drug stability test guideline Q1QA (R2) issued by International conference on Harmonization (ICH) [1] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence, supporting the suitability of the proposed analytical procedures. It also requires analytical test procedures for stability, samples should be stability indicating and they should be fully validated.

Accordingly, the aims of the present study were to establish inherent stability of Telmisartan and Hydrochlorothiazide through stress studies under a variety of ICH recommended test conditions[2] and to develop stability indicating assay.

DRUG PROFILES [3-6]



TELMISARTAN



HYDROCHLOROTHIAZIDE

PARAMETERS	TELMISARTAN	HYDROCHLOROTHIAZIDE
IUPAC NAME	*2-(4-{[4-Methyl-6-(1-methyl-1H-1, 3benzodiazole-2-yl)-2-propyl-1H-1, 3-benzodiazole1-yl] methyl} phenyl) benzoic acid.*	*6-chloro-1, 1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadi azine-7-Sulfonamide*.
MOLECULAR FORMULA	$C_{33}H_{30}N_4O_2$	$C_7H_8ClN_3O_4S_2$
MOLECULAR WEIGHT	514.617 g/mol	297.74 g/mol
SOLUBILITY	Freely soluble in DMSO> 5mg/ml at 60^0	Slightly soluble in water and soluble in alcohol.
pKa	3.5, 4.1, and 6.0	7.9 and 9.2
CATEGORY	Anti-hypertensive	Anti-hypertensive, Diuretic
CHEMICAL NATURE	Weak acid	Neutral
BIOAVAILABILITY	42-100%	Variably absorbed from GI tract
PROTEIN BINDING	≥99.5%	67.9%
METABOLISM	Minimal hepatic	Does not undergo significant metabolism (>95% excreted unchanged in urine)
HALF LIFE	24 hours	5.6-14.8 hours
EXCRETION	Faecal 97%	Primary excreted unchanged in urine

MATERIALS AND METHODS[7-9]

A stability indicating HPLC method for simultaneous estimation of Telmisartan and Hydrochlorothiazide was developed and validated. The chemicals were Acetonitrile, HPLC grade was procured from (Sd Fine-Chem Ltd), Methanol, HPLC grade (Sd Fine-Chem Ltd), Potassium Dihydrogen phosphate (Thermo fisher Scientific India Pvt. Ltd, Mumbai), Millipore water, Telmisartan (Hetero Pharma), Hydrochlorothiazide (Aurobindo Pharma) and Telma – H tablets was made by Glenmark Pharmaceuticals Ltd, Baddi, India, Purchased from local market.

Degradation Studies[9-12]

All stress decomposition studies were performed at an initial drug concentration of 1 mg/ml in acetonitrile: potassium dihydrogen phosphate (pH 3.5) in the ratio of 60:40 v/v.

Degradation Studies of Telmisartan and Hydrochlorothiazide in Acidic Condition

Telmisartan and Hydrochlorothiazide were subjected to forced degradation in acidic medium in presence of 0.1N HCl and heated at 60^0C for a period of 4 hrs. 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 2 mL of each resulting solutions (100 µg/mL) and mixed in a separate flask. 20 µL of this 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.

Degradation Studies of Telmisartan and Hydrochlorothiazide in Alkaline Condition

Telmisartan and Hydrochlorothiazide were subjected to forced degradation in alkaline medium in presence of 0.1N NaOH and heated at 60^0C for a period of 4 hrs. At different time intervals the sample aliquots were withdrawn at 2 and 4hr, and then neutralized with 2 mL of 0.1N HCl. 20 µL of this 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.

Degradation Studies of Telmisartan and Hydrochlorothiazide in Oxidation condition

Telmisartan and Hydrochlorothiazide were subjected to force degradation in 3 % v/v solution of hydrogen peroxide (oxidizing medium). Subjected solution was injected without heat at 0, 2 and

4 hr, didn't find out the degradation. Further went for heated at 60^0C for a period of 4 hrs. At different time intervals the sample aliquots were withdrawn at 2 and 4 hr. Take 2 mL of each resulting solutions (100 µg/mL) and mixed in a separate flask. 20 µL of this 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.

Degradation Studies of Telmisartan and Hydrochlorothiazide in Thermal condition

Thermal degradation studies for standard drug 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. 20µL of this 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.

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

Photostability degradation studies for standard drug Telmisartan and Hydrochlorothiazide were carried out in a photo stability chamber by exposing to UV light in a Petri dish (1mm thickness). At different time intervals of 24 hrs and 48 hrs, 20 µL of this 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.

Separation Studies

HPLC studies were carried out first on all reaction solutions individually and on a mixture of those solutions in which decomposition was observed. Separation were achieved by isocratic elution using the instrument System, SHIMADZU UFLC-2000 Prominance LC-20AD SPDM 20A Binary Gradient System with Enable C-18 G column 250×4.6 mm,5 µm, detector was PDA with detection wave length 282 nm, a mobile phase composed of acetonitrile: potassium dihydrogen phosphate (pH 3.5) in the ratio of 60:40 v/v at the flow rate of 1.0 ml/min.

Validation of the Method

Linearity and Range

Stock solutions of the drugs were prepared at strength of 1 mg/ml. It was diluted to obtain a solutions containing. The volume was made up with the mobile phase to get concentrations ranging from 4–20 µg/mL. The solutions were injected I triplicate into the HPLC column, keeping the injection volume constant (20 µl).

Accuracy

Accuracy was evaluated by fortifying a mixture of degraded solutions with three known concentrations of the drug. The recovery of the added drug was determined. The accuracy was determined through recovery study of the drug by spiking the standard drug at three different levels 80 %, 100 %, 120 % with the assayed samples of known concentration.

Precision

Six injections, of three different concentrations were injected on the same day and the values of relative standard deviation (RSD) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

System Precision

The system precision is checked by using standard substance to ensure that the analytical system is working properly. The peak area of six determinations is to be measured and % RSD.

Method Precision (Repeatability)

Method precision indicates whether a method is giving consistent results for a single batch, usually applied to standardization of methodology.

Intermediate Precision (Ruggedness)

Intermediate precision expresses variations like different days and different analysts. For proposed method intermediate precision different days like inter day (in between the days) and intraday (within the day) was determined.

Specificity

Specificity is the ability to assess unequivocally that the analyte in the presence of components which may be expected to be present; typically these might include impurities, degradation products and matrix components.

RESULTS AND DISCUSSION

HPLC studies on Telmisartan and Hydrochlorothiazide under different stress conditions suggested the following degradation behavior:

Validation of Developed Stability-Indicating Method [13-15]

Accuracy

The accuracy was determined through 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 %	
Robustness	**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 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 at two different days (inter day and intraday) to check the reproducibility. On intraday % 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 precision study obtained were well within the acceptance criteria of NMT 2 %. Thus the proposed method was found to be providing 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 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 0.99 ng/mL and 1.55 ng/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 3 ng/mL and 4.7 ng /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 which are directly proportional to 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 ratio of 60:40 Flow rate of 1.0 mL/ min, PDA detection at 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 1.0 mL/min to 0.8 mL/min and 1.2 mL/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 deliberate changed from 282 nm to 280 nm, 284 nm, the % assay for Telmisartan and Hydrochlorothiazide was found to be in the range from 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 of indicating lack of influence on the test results by operational variables for the proposed method.

In acidic condition, standard drug of Telmisartan and Hydrochlorothiazide were found to be 6.24 % and 8.98 % degraded 4^{th} hour at 60^0C heat.

In alkaline condition, standard drug of Telmisartan and Hydrochlorothiazide were found to be 11.69 % and 4.39 % degraded 4^{th} hour at 60^0C heat.

In oxidative condition, 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 4 hrs at non-heating and in heating condition was found to be degradation for 4 hrs period.

Hence stress testing should be given importance for such combination of drugs and quantification of degraded products of such drugs help us to maintain the quality, safety and efficacy of drugs in formulations. In acidic condition, standard drug of Telmisartan and Hydrochlorothiazide were found to be 6.24 % and 8.98 % degraded 4th hour at 60^{0}C heat.

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