STABILITY-INDICATING HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LOW LEVEL IMPURITIES OF TELMISARTAN AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORMS

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ABSTRACT

The present work describes development and validation of a stability-indicating reverse-phase high performance liquid chromatographic (RP-HPLC) method for simultaneous determination of trace level impurities of telmisartan (TLM) and hydrochlorothiazide (HCTZ) in their tablets. The stressed degradation study including acid, base, H_2O_2, humidity, thermal and photolytic conditions were performed on TLM and HCTZ tablets as per ICH guidelines to prove the stability-indicating capability of the developed method. Slight degradation was observed in acid and humidity degradations while no degradation in other conditions. Chromatographic separation was achieved on Inertsil ODS 3V (250 x 4.6 mm, 5 µm) column using 1 % (v/v) triethylamine in potassium hydrogen orthophosphate (pH 2.5) and acetonitrile buffer using linear gradient programme. Flow rate was 1.3 mL min^{-1} and detection wavelength was carried out at 271 nm. All the known and degradation impurities were separated within 65 minutes. The LOD and LOQ values of impurities were found between 0.005 µg mL^{-1} to 0.029 µg mL^{-1} and the percentage recovery values were in the range of 97.3 to 101.8 at different concentration levels.

Keywords: TLM and HCTZ tablets, RP-HPLC stability method, Development and validation.

INTRODUCTION

Telmisartan (TLM) is an angiotensin receptor blocker that shows high affinity for the angiotensin II type 1 (AT$_1$) receptors, has a long duration of action and has the longest half-life of any ARB[1]. It is chemically named as 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid. It acts as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. It is believed that telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease[1].

Hydrochlorothiazide shortly named as HCTZ, is a first line diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water. It is chemically named as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. It belongs to the thiazide class of diuretics, acting on the kidneys to reduce sodium (Na) re-absorption in the distal convoluted tubule. This increases the osmolarity in the lumen, causing less water to be reabsorbed from the collecting ducts. This leads to increased urinary output. Thiazides decrease mineral bone loss by promoting calcium retention in the kidney and by

directly stimulating osteoblast differentiation and bone mineral formation[2].

The literature survey reveals that no methods were available for the quantification of impurities of TLM and HCTZ in solid dosage forms. Bhat *et al.*[3] reported a simultaneous method for the quantification TLM and HCTZ in their dosage forms. Babewy *et al.* determined the TLM and HCTZ by the application of first-derivative, ratio derivative spectrophotometry, TLC-densitometry and spectrofluorimetry in pharmaceutical dosage forms and plasma[4]. Various HPLC and spectroscopic methods have been described[5-11] for the quantification of either TLM or HCTZ in their dosage forms. All these methods were useful for the quantification of either TLM or HCTZ but none of those are useful for the quantification of related impurities or the degradation impurities of TLM or HCTZ in their dosage forms.

The present work describes a sensitive, specific and stability-indicating HPLC method for the determination of impurities of TLM as well as HCTZ in a single method. Developed LC method was validated with respect to LOD, LOQ, linearity, precision, accuracy and robustness. Forced degradation studies were carried out to verify the stability-indicating nature of the LC method. The compatibility studies on stability samples and excipients were also performed using the developed method.

TLM

TLM impurity A

TLM impurity B

TLM impurity C

TLM impurity D

TLM impurity E
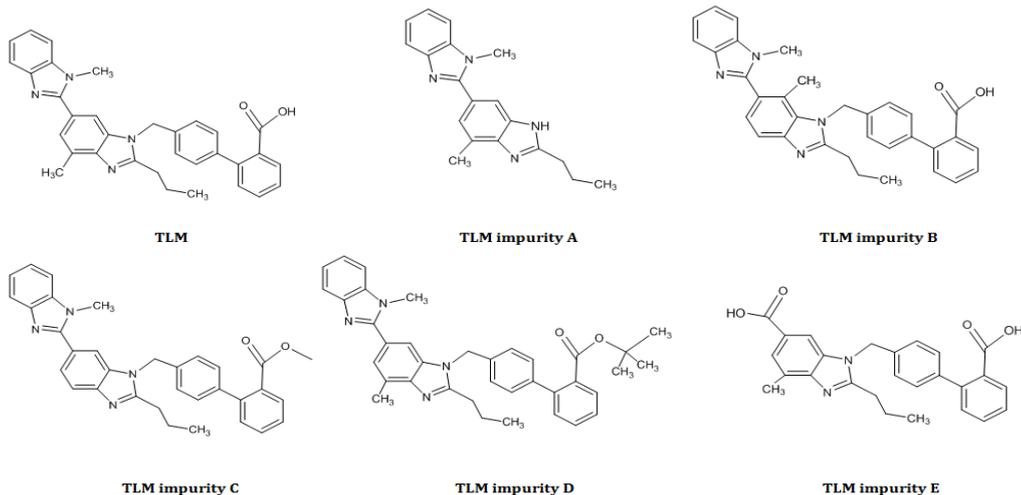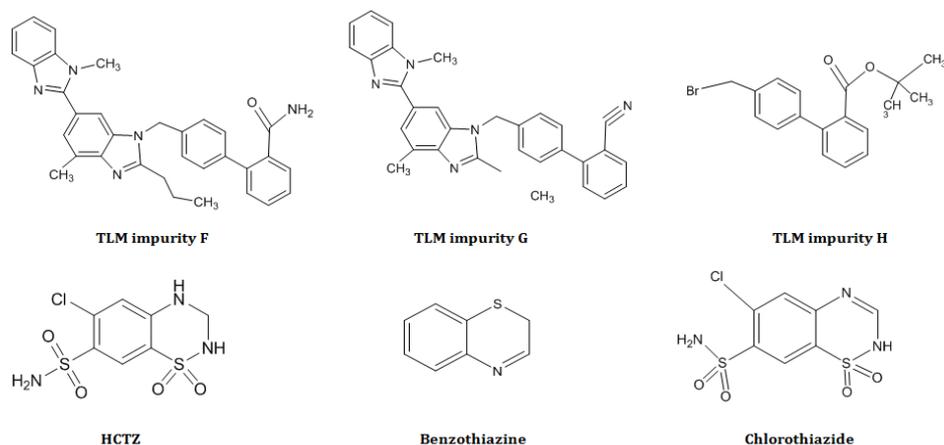
Fig. 1: Structures of TLM, HCTZ and their related impurities

MATERIALS AND METHODS

Chemicals and reagents

Standards of TLM, HCTZ and their related impurities, and tablet samples of TLM and HCTZ were received from Sun Pharmaceutical Limited (Gujarat, India). HPLC grade acetonitrile (ACN) and methanol (MeOH) were obtained from Rankem (Mumbai, India). Orthophosphoric acid (OPA) was obtained from Qualigens Fine chemicals (Mumbai, India). Potassium dihydrogen orthophosphate (KH_2PO_4) and tetrabutyl ammonium hydroxide (TBAH) were purchased from Merck specialties Pvt. Ltd (Worli, Mumbai). Triethylamine (TEA) and tetrahydrofuran (THF) were purchased from Spectrochem Pvt. Ltd (Mumbai, India). Ammonium acetate and formic acid were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Instrumentation

The Waters LC system (Milford, MA, USA) equipped with a diode array detector was used for method development and forced degradation studies. The output signal was monitored and processed using Empower software. Waters LC consists of 2695 separation module and 2996 PDA detector used for validation study. Intermediate precision was carried out using waters 2695 separation module with 2487 dual wavelength detector. Photolytic chamber was used for photolytic degradation and thermal degradation samples were kept at 105°C in thermal oven.

Chromatographic conditions

The chromatographic separation was achieved on Inertsil ODS 3V 250 x 4.6 mm, 5 µm column using mobile phase-A composed of 10 mM KH_2PO_4 having 1% (v/v) of TEA (pH adjusted to 2.5 with OPA) and mobile phase-B was ACN. Gradient programme used for chromatographic separation was presented in Table 1. Flow rate was set to 1.3 mL min^{-1} with a column temperature of 35°C and detection wavelength was carried out at 271 nm. The injection volume was 10 µL. MeOH and water in the ratio of 80:20 was used as diluent for the preparation of standards and samples.

Table 1: Gradient program

Time	Buffer	ACN
0	93	7
15	93	7
45	35	65
50	35	65
53	93	7
60	93	7

Preparation of solutions

Standard stock solution of TLM

Accurately weighed and transferred 80 mg of TLM working standard into a 100 mL volumetric flask. Added about 70 mL of MeOH and sonicated for 5 min. To this further added 20 mL water and sonicated for 2 min and diluted to the mark with MeOH.

Standard stock solution of HCTZ

Accurately weighed and transferred 50 mg of HCTZ working standard into a 200 mL volumetric flask. Added about 70 mL of MeOH and sonicated for 5 min. To this added 20 mL water, sonicated for 2 min and made up to the mark with MeOH.

Preparation of standard solution

Transferred 5 mL of each of TLM and HCTZ standard stock solutions into a 50 mL volumetric flask and diluted up to the mark with the diluent. Further diluted 5 mL of this solution into 50 mL with the diluent.

Preparation of sample solution

Determined the average weight of 20 tablets and crushed to fine powder. Accurately weighed and transferred a sample powder equivalent to 25 mg of HCTZ into a 100 mL volumetric flask. Added about 70 mL of MeOH and sonicate for 30 min with intermittent shaking. Again added 20 mL water and sonicated for 15 min. Made up the volume of 100 mL volumetric flask with MeOH and then filtered the solution through 0.45 µm PTFE membrane filter.

Preparation of placebo solution

Common placebo (Placebo without any API)

Weighed the placebo powder equivalent to 25 mg of HCTZ and transferred into a 100 mL volumetric flask. Added about 70 mL of MeOH and sonicated for 30 min with intermittent shaking. Further added 20 mL water and sonicated for 15 min. Made up the volume of 100 mL volumetric flask with MeOH and then filtered the solution through 0.45 µm PTFE membrane filter.

Placebo for HCTZ (Placebo with TLM)

Weighed the placebo powder equivalent to 25 mg of the HCTZ and transferred into a 100 mL volumetric flask. Added about 70 mL of MeOH and sonicated for 30 min with intermittent shaking. Again added 20 mL water and further sonicated for 15 min. Made up the volume of 100 mL volumetric flask with MeOH and then filtered the solution through 0.45 µm PTFE membrane filter.

Placebo for TLM (Placebo with HCTZ API)

Weighed the placebo powder equivalent to 25 mg of the HCTZ and transferred it to a 100 mL volumetric flask. Added about 70 mL of MeOH and sonicated for 30 min with intermittent shaking. Again added 20 mL water and further sonicate for 15 min. Made up the volume of 100 mL volumetric flask with MeOH and then filtered the solution through 0.45 µm PTFE membrane filter.

Preparation of spiked sample solution

Accurately weighed 80 mg of TLM and 25 mg of HCTZ were transferred into a 100 mL volumetric flask, to this added 0.5 mL of HCTZ impurities and 2 mL of individual impurities of TLM. To this added about 70 mL of MeOH and sonicated for 5 min. Again added 20 mL water, sonicated for 2 min and finally made up to the mark with MeOH.

Method validation

After method development, it was necessary to perform the method validation to ensure that the developed method was capable of giving reproducible and reliable results when used by different operators employing in the same equipment of same lab or different laboratories. As per ICH guidelines[12], stress testing needs to be performed to elucidate the inherent stability characteristics of the active substance and also to prove the stability indicating capability of the method.

The developed HPLC method was validated to quantify the related substances of TLM and HCTZ in their tablet dosage forms by determining the parameters including specificity, LOD, LOQ, linearity, accuracy, precision and robustness according to the ICH guidelines[12].

Specificity

The specificity of the method was established to prove the absence of interference from excipients which take part in the pharmaceutical preparation. Degradation study was also performed as per ICH guidelines by subjecting the tablet powder to accelerated degradations such as acid, alkaline, oxidative, thermal, humidity and photolytic conditions to evaluate the interference of degradation impurities. Thermal degradation was performed by keeping the placebo and tablet powders in different Petri dishes and then placed them in a thermal oven at 105°C for 3 days. Humidity degradation was done by placing the tablet and placebo powders in two Petri dishes and kept in a humidity chamber at 90% RH, 25°C for 7 days. Photolytic study was carried out by placing the placebo and tablet powder in a photolytic chamber at 1.2 million lux hours, 200 wh/m^2 for 7 days. The acid, base and oxidative degradations were performed by adding 5 mL of 5 N HCl, 5 mL of 5 N NaOH and 5 mL of 30% H_2O_2, respectively, to the placebo and tablet powders and refluxed at 70°C for 3 h.

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) were important for the impurity tests and the assays of dosages containing low drug levels and placebos. The LOD is generally quoted as the concentration yielding a signal-to-noise ratio of 2:1 or 3:1 and LOQ is quoted as the concentration yielding a signal-to-noise ratio of 10:1. The signal-to-noise ratio is determined by the following equation:

$$s = H/h$$

Where, H = height of the peak corresponding to the component

h = absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.

Linearity

Linearity is the method's ability to obtain results which are either directly, or after mathematical transformation proportional to the concentration of the analyte within a given range. The linearity of response for TLM, HCTZ and their related impurities were determined in the range from LOQ to 150%. A graph was plotted between the peak areas versus concentration to obtain the calibration curve. The five concentrations of each component were subjected to regression analysis by least-squares method to calculate correlation co-efficient and calibration equation. The relative response factor for all the impurities was determined against their respective standard. The method of linear regression was used for the data evaluation. Peak area of standard compounds was plotted against respective concentrations.

Precision

Precision is a measure of the reproducibility of the whole analytical method under normal operating circumstances. The precision was expressed as the relative standard deviation (RSD).

% RSD = (Standard deviation/ average) x 100

Precision of the developed method was determined by injecting the impurities spiked solution at six times and calculated the % RSD for each impurity.

Accuracy

Accuracy was determined by applying the method to samples in which known amounts of analyte have been added. These should be analyzed against standard and blank solutions to ensure that no interference exists. The accuracy was calculated from the test results as a percentage of the analyte recovered by the assay.

Accuracy of the present method was carried out by injecting the impurities spiked solution at three different concentration levels of 50%, 100% and 150% to their specification limit, in triplicate. The % recovery was calculated for each impurity. The mean of percentage recovery was designed.

Robustness

Robustness of the method indicates the reliability of an analysis with respect to deliberate variations in method parameters. It was performed by injecting the impurities spiked solution and the stressed degradation sample solutions by changing several parameters including pH of the buffer solution, different batch of the same column, flow rate, temperature and mobile phase ratio.

Solution stability

Prepared the impurities spiked sample solution, standard solution, and placebo solution containing TLM and placebo solution containing HCTZ in duplicate. All these solutions were divided into two portions; one portion was kept at room temperature and the other under refrigerator (2-8°C). The freshly prepared solutions, and the solutions which were stored at room temperature and freeze up to 24 h were injected at different time intervals. Compared the % impurity obtained at initial against the % impurity obtained at different time intervals.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main purpose of the current chromatographic method was to develop a LC method for the separation and quantification of known and unknown impurities of TLM and HCTZ at trace level. From the structures of TLM and HCTZ, it was observed that TLM has an acid functional group of pK_a values of 3.5, 4.1 and 6.0 while HCTZ has a basic functional group of 7.9 and 9.2 pK_a values. In spite of the fact that in the reversed-phase separations the pH of selected buffer should have the pH ± 1.5 units from the pK_a values of the analytes[15]. The selection of buffer with proper pH leads to ionization of analytes which consequences the sharp and symmetric peak shapes and reproducible retention times (RT). The KH_2PO_4 has a wide range of pK_a values, so, initially selected a buffer of 0.01 M KH_2PO_4 composed of 0.1% TEA and set the pH of this solution to 2.5 with orthophosphoric acid. The method development trials were carried on more than eight different HPLC columns and corresponding chromatograms were shown in Fig. 2 and data were presented in Table 2.

The screening studies were performed on variety of columns to cover a wide range of stationary phase properties including carbon chain length, carbon loading, surface area, temperature and pH range. Column information and highlight of the scientific rationale for selection of the most appropriate column for the intended purpose of this method were provided in Table 2. Each of the selected columns was screened with different mobile phase ratios, different column temperatures, different type of organic solvents including MeOH, ACN and THF, and different types of mobile phase additives like TEA, TBAH at different concentration levels. In most of the trials major impurities of TLM are separated but not the impurities related to HCTZ.
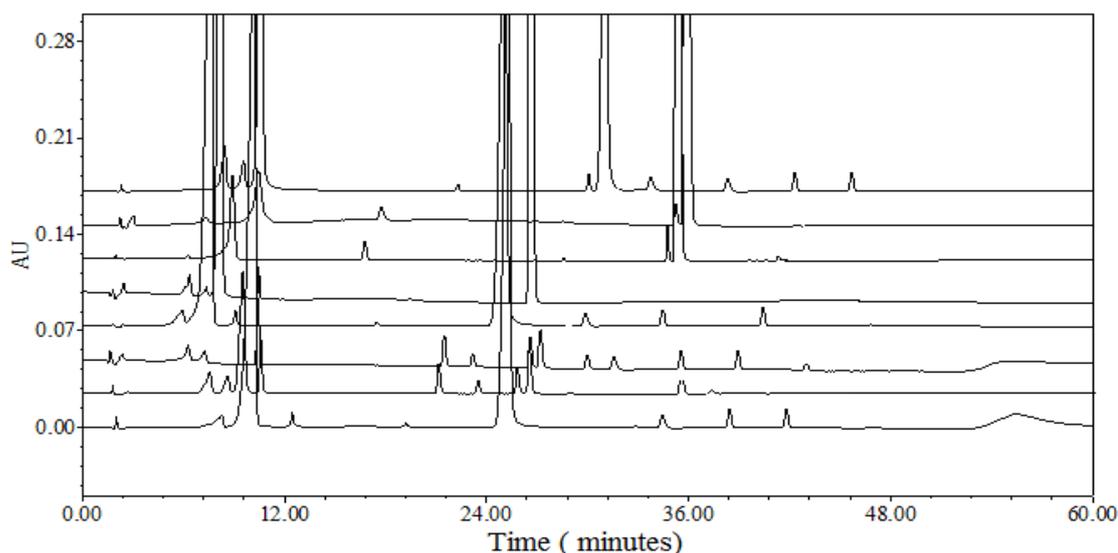
Fig. 2: Chromatograms obtained for impurities spiked solution on different HPLC columns during development

During the method development trials on most of these columns, TLM and its related impurities were separated except its positional isomer (Imp-B) while HCTZ and its impurities does not separated well enough and also peak shape of HCTZ found to be broad. Certain columns such as the Inertsil ODS 3V 150 x 4.6 mm, 5 µm column provided the separation of TLM from its positional isomer but the resolution between HCTZ and its impurities was found to be very less. To enhance the resolution between HCTZ and its impurities increased the column length from 150 to 250 cm and performed the analysis with a gradient program shown in Table 3.

Table 2: LC Columns screened for alternate column selection

Trial	Column used	Column specifications	Observation
1	Zorbax SB C-18 150 x 4.6 mm, 3.5 µm	Pore size : 80 A° Surface area : 180 m²/g Temp limit : 90 °C pH range : 1.0-8.0 Carbon loading : 10%	1. Chlorothiazide impurity was merging with HCTZ. 2. TLM Imp-B co-eluting with TLM.
2	Phenomenex Luna C-18 250 x 4.6 mm, 5 µm	Pore size : 80 A° Surface area : 440 m²/g Temp limit : 60 °C pH range : 1.5-10.0 Carbon loading : 19%	TLM Imp-C and Imp-D in peak identification solution were eluting at the same retention time.
3	Kromasil C-18 150 x 4.6 mm, 5 µm	Pore size : 100 A° Surface area : 330 m²/g Temp limit : 60 °C pH range : 1.5-10.0 Carbon loading : 19%	Chlorothiazide impurity does not well separated from HCTZ and broad peak shape was observed for HCTZ and its impurities.
4	Inertsil ODS-3 150 x 4.6 mm, 5 µm	Pore size : 100 A° Surface area : 450 m²/g Temp limit : 60 °C pH range : 2.0-7.5 Carbon loading : 15%	TLM Imp-C was merging with Imp-G chlorothiazide is merging with HCTZ.
5	ACE-5 C-18 150 x 4.6 mm, 3.5 µm	Pore size : 300 A° Surface area : 100 m²/g pH range : 1.5-11.0 Carbon loading : 9%	Chlorothiazide peak co-eluting with HCTZ and broad peak shape was observed for HCTZ.
6	Hypersil BDS C-18 250 x 4.6 mm, 5 µm	Pore size : 130 A° Surface area : 170 m²/g pH range : 1.5-11.0 Carbon loading : 11%	TLM imp-B was co-eluting with TLM and Imp-H was not eluted.
7	Symmetry C-18 250 x 4.6 mm, 5 µm	Pore size : 100 A° Surface area : 335 m²/g Carbon loading : 19%	Broad peak shape was observed for HCTZ and chlorothoazide impurity was co-eluting with HCTZ.
8	Inertsil ODS-3V 250 x 4.6 mm, 5 µm	Pore size : 100 A° Surface area : 450 m²/g Temp limit : 60 °C pH range : 2.0-7.5 Carbon loading : 15%	All impurities of HCTZ and TLM were well separated and the resolution between the impurities was not less than 2.

Using Inertsil ODS-3V 250 x 4.6 mm, 5 µm column, all the known impurities of TLM and HCTZ are well separated and so, injected the stress degradation samples to verify any unknown impurities co-eluting with the known impurities.

Table 3: Gradient program

Time	Buffer	ACN
0	100	0
45	25	75
50	25	75
53	100	0
65	100	0

Optimization of column temperature

To study the temperature effect on resolution between the impurity peaks of TLM and HCTZ, injected the impurities' spiked solution at different column temperatures. It was observed that at a column temperature of 45°C all the impurities have well separated than the other column temperatures. The resolution between closely eluting positional isomer of TLM imp-B and TLM was found to be not less than 2.

Specificity

Specificity of the developed method was performed by injecting the stressed degradation samples and the impurities spiked solutions. The degradation study was carried out using the samples which include a. Tablet powder containing TLM and HCTZ, b. Placebo powder containing HCTZ, c. Placebo powder containing TLM and d. Placebo without TLM and HCTZ. The corresponding chromatograms were presented in shown Fig. 3.

TLM was found to be stable in all the degradation conditions except in acid degradation while HCTZ undergoes degradation in all conditions and forms benzothiadiazine as a major degradant (Table 4). Spectral homogeneity of TLM and HCTZ, and their known and unknown impurities were checked. Purity angle value was greater than the purity threshold for all peaks indicates all peaks are spectrally homogeneous. Also spectral homogeneity of known impurities in degradation samples found to be similar with those obtained for the individual impurities, indicates that no peak was co-eluting at the retention time of respective known impurities.

Impurities Spiked Solution

Acid degradation sample

Base degradation chromatogram

Peroxide degradation chromatogram
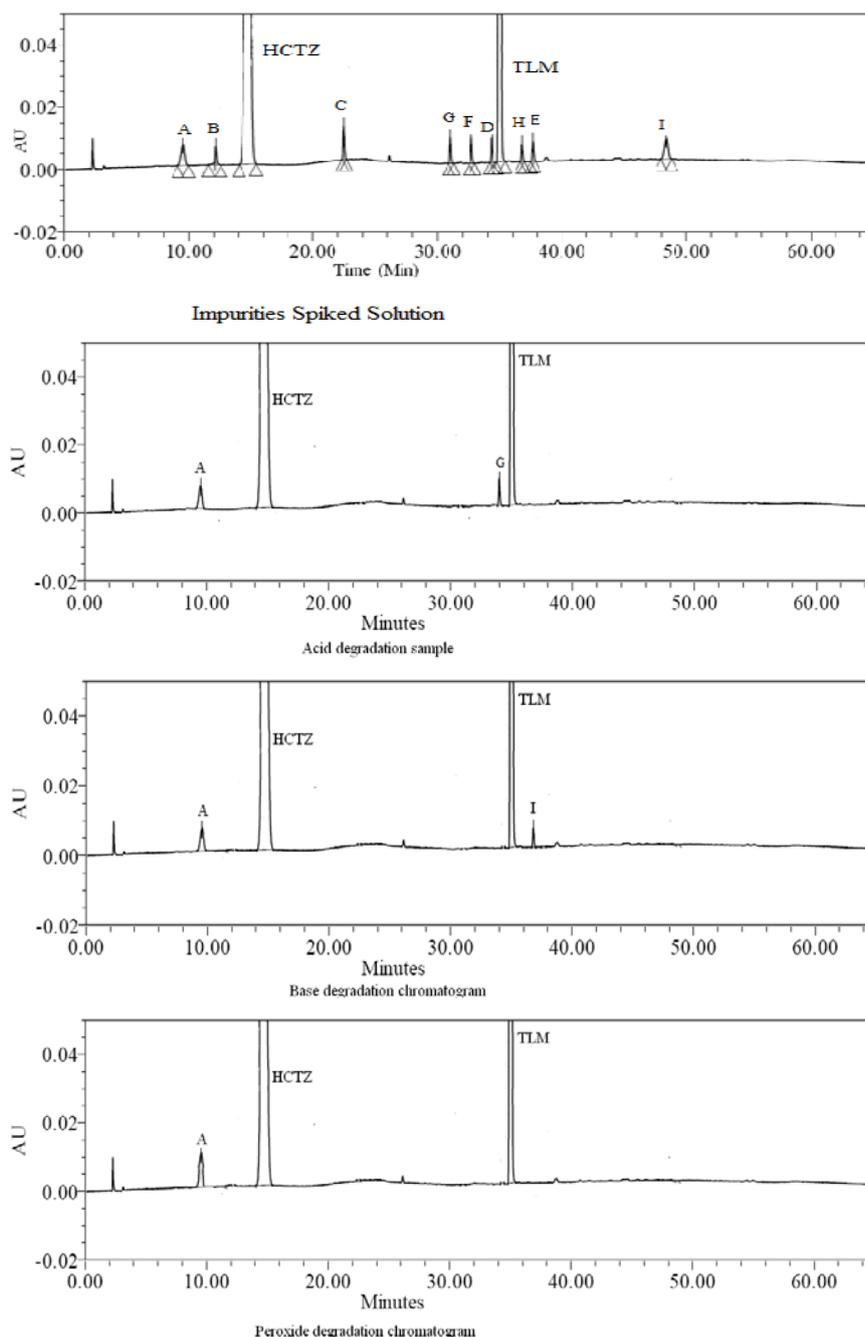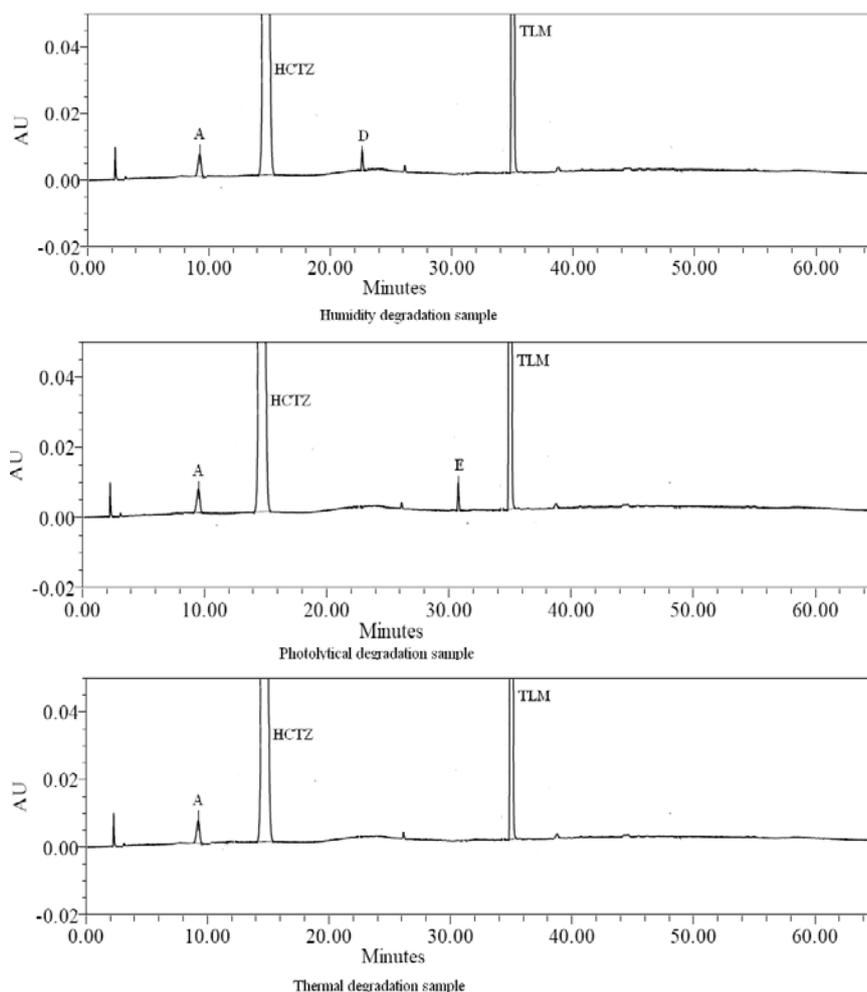
Fig. 3: Degradation chromatograms of impurities spiked solution

The degradation results of TLM and HCTZ in various stress conditions were mentioned in the Table 4. The results indicate TLM undergoes degradation only in presence of acid to forms Imp C.

HCTZ undergoes degradation in all the stress conditions including acid, base, thermal, humidity, photolytic and peroxide degradations and the major degradant formed is benzothiadiazine.

Table 4: Degradation data of TLM and HCTZ tablet

Degradation conditions	% Benzothiadiazine	% TLM Imp-C	% Other known impurities	% Major unknown degradation product	Mass balance
Acid treatment (5N HCl, 70°C, 3 hrs)	7.12	0.21	0.12	0.08	98.6
Base treatment (5N NaOH, 70 ºC, 3 hrs)	6.84	0.08	0.14	0.07	98.9
H_2O_2 treatment (5% H_2O_2, 70 °C, 3 hrs)	3.14	0.06	0.11	0.11	99.1
Thermal-105 °C, 3 days	1.12	0.05	0.08	0.10	98.3
Humidity-90% RH, 25 °C, 7 days	0.89	0.08	0.09	0.11	99.0
Photolytic-1.2 m lux hrs, 200 Whrs/m², 7 days	0.54	0.05	0.08	0.10	99.3

LOD and LOQ

The LOD and LOQ have determined for TLM, HCTZ and their impurities by injecting a series of solutions with known concentration. Calculated the S/N ratio for these solutions and selected the concentration at which level S/N was about 3 as LOD and the S/N ratio was about 10 as LOQ. The obtained LOD and LOQ values for TLM, HCTZ and their impurities were mentioned in Table 5.

Linearity

The response obtained for all compounds was found to be linear from LOQ to 150% of standard concentration. Correlation co-efficient for all compounds was not less than 0.99. Correlation coefficient values of all compounds were presented in Table 5. The

results demonstrate that an excellent correlation between the peak area and concentration of all impurities.

Precision

System precision was determined by injecting the impurities' spiked solution of six injections and the observed value reported in Table 5. The % RSD for the area of all compounds in impurities' spiked solution for six injections was not more than 1.6. The intermediate precision of the method was studied by injecting the impurities spiked solution of six replicate injections and the values were reported in Table 5. Less difference between the two analysts shows that this method has good intermediate precision. Difference in results between two analysts found to be less indicates that method is precise.

Table 5: LOD, LOQ, linearity and precision data

Parameter	HCTZ	TLM	%Benzo	%Chloro	% Imp-A	% Imp-B	% Imp-C	%Imp-D	% Imp-E	% Imp-F	% Imp-G	% Imp-H
LOD (µg mL^{-1})	0.005	0.007	0.008	0.006	0.009	0.008	0.007	0.008	0.009	0.007	0.007	0.009
S/N ratio	2.7	3.2	3.3	2.9	3.0	3.1	3.3	2.9	3.2	3.3	3.0	3.2
LOQ (µg mL^{-1})	0.011	0.022	0.025	0.023	0.026	0.025	0.024	0.024	0.029	0.022	0.021	0.026
S/N ratio	9.9	10.1	10.5	10.1	10.2	10.6	10.4	9.9	10.2	10.3	10.6	10.4
Regression statistics												
Slope	465965	388733	456741	471970	394615	406269	344374	386706	364374	396706	344345	386433
Intercept	-847.5	1472.34	-815.22	-2585.31	1813.05	240.90	5152.79	6164.21	5652.79	1764.21	5132.77	1453.31
Correlation coefficient	0.9996	0.9995	0.9998	0.9996	0.9955	0.9968	0.9953	0.9924	0.9993	0.9944	0.9973	0.9955
Method precision (% RSD)[a]	1.12	1.35	1.54	1.52	1.25	1.21	1.11	1.32	1.25	1.51	1.38	1.34
Intermediate precision (% RSD)[b]	0.96	0.78	0.93	0.94	0.68	0.91	0.94	0.98	0.79	0.93	0.88	0.85

[a]Method precision calculated from six replicate injections of impurities spiked solution

[b]Sytem precision calculated from six injections of impurities spiked solution

Accuracy

The percentage recovery results for impurities of TLM and HCTZ were varied from 97.3-101.8% at three different concentration levels and shown in Table 6. Based on the % recovery data, concluded that the developed method is capable for the estimation of its related substances and is adequate for routine analysis in a quality control laboratory.

Robustness

In all the robust conditions (flow rate, temperature, pH of the mobile phase and organic solvent composition) resolution between two critical pairs (resolution between chlorothiazide and HCTZ, resolution between TLM and TLM Imp-B) was not less than 2.0. Also the resolution between the remaining impurities from analytes was not significantly affected and elution pattern of the impurities remained unchanged. The peak shape for all the impurities was found to be good. Peak purity for all impurities also tested to observe any placebo peaks interference in all the robust conditions. The average peak area percentages of all compounds under various robust conditions were found to be within ±0.1% of the average result obtained using the normal conditions.

Solution stability

The impurity percent difference was determined for solutions stored at room temperature and at refrigerator samples in different time intervals up to 24 hrs. Except benzothiadiazine all impurities found to be stable up to 24 hrs at room temperature and also at refrigerator where as benzothiadiazine found to be stable up to 12 hrs in both the conditions.

Table 6: Accuracy results

Amount added	% Recovery range for triplicate injections									
	%Benzo	%Chloro	% Imp-A	% Imp-B	% Imp-C	%Imp-D	% Imp-E	% Imp-F	% Imp-G	% Imp-H
LOQ	99.1–100.3	98.3–100.2	97.8–99.4	98.1–100.1	97.3–100.2	97.8–99.9	99.1–101.3	97.3–100.8	99.3–100.2	99.3–100.8
50%	99.3–99.8	97.3–99.7	98.2–99.5	97.3–99.9	97.8–99.7	98.2–100.1	99.3–100.8	99.3–100.2	98.2–99.5	99.7–101.8
100%	99.7–100.8	98.6–99.4	98.7–99.4	98.7–100.1	99.6–100.1	98.7–100.5	99.7–101.8	98.6–99.4	97.8–99.7	98.3–100.2
150%	99.6–100.5	99.1–99.6	99.2–99.8	97.6–100.2	99.5–100.1	99.2–101.2	99.6–100.5	98.1–100.9	99.1–99.6	98.7–99.4

CONCLUSIONS

A novel RP-HPLC method was developed for the separation and quantification of related substances of TLM and HCTZ in their pharmaceutical dosage forms. Degradation behaviour of HCTZ and TLM was studied under various degradation conditions. Benzothiadiazine is the major degradant formed from HCTZ and TLM showing a minor degradation in acid condition and stable in rest of the degradation conditions. All the process impurities and the degradation impurities were well separated from TLM and HCTZ revealed the stability-indicating capability of the method. The developed method can be used for the quantification of related substances of TLM and HCTZ in routine analysis.

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