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Development and validation of stability indicating RP-HPLC method for simultaneous determination of Telmisartan and Hydrochlorothiazide from their combination drug product

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ABSTRACT

A stability-indicating RP-HPLC method has been established for analysis of telmisartan (TEL) and hydrochlorothiazide (HTZ) in the presence of degradation products formed under different stress conditions. The drug substances were subjected to stress by hydrolysis (1N NaOH, 1N HCl and neutral), oxidation (3% v/v H_2O_2), photochemical, thermal (100°C), and UV degradation (254 nm). Both the drugs were degraded under selected experimental conditions. Successful separation of the drugs from the degradation products was achieved on a hypersil gold C18 column with 40:60 (v/v) acetonitrile-aqueous 0.01M potassium dihydrogen o-phosphate buffer (pH 3 adjusted with 2% v/v o-phosphoric acid) as mobile phase. The method was linear over the concentration range of 6-36 µg mL^{-1} ($r > 0.991$), with limits of detection and quantitation (LOD and LOQ) of 0.495 and 1.50 µg mL^{-1}, respectively, for TEL and 2-12 µg mL^{-1} ($r > 0.991$) with LOD and LOQ of 0.873 and 2.64 µg mL^{-1}, respectively, for HTZ. The method was validated for specificity, selectivity, accuracy, and precision. This method was reproducible and selective. As the developed method could effectively separate the drugs from their degradation products, it can be used as stability-indicating.

Keywords: Telmisartan, Hydrochlorothiazide, Stability Indicating Studies, RP-HPLC, Degradation products.

INTRODUCTION

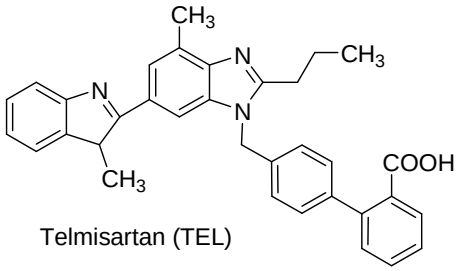
Telmisartan (TEL), 2-[4-[[4-Methyl-6-(1-methylbenzimidazol-2-yl)propylbenzimidazol-1-yl]-methyl]phenyl] benzoic acid, is an angiotensin II receptor antagonist highly selective for Type 1 angiotensin II receptors [1-5]. HPLC-fluorimetric method for quantitation of TEL in combination with HTZ in tablet dosage form is reported [6,7]. Two stability indicating assay methods for quantitation of TEL alone in bulk drug and in pharmaceuticals and in combination with ramipril are reported [8,9]. HPTLC procedures for quantification of TEL in combination with HTZ [10] and TEL alone [11] are reported.

HTZ is official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia. Few spectrophotometric methods for the quantitative determination of HTZ in combination with other drugs *viz.* by multivariate calibration method [12] and fourth order derivative spectrophotometric method for HTZ and Irbesartan [13] have been reported. There are several reports on liquid chromatographic determination of HTZ in combination with other drugs *i.e.* metoclopamide, imipramine, diclofenac [14], losartan [15], fosinopril [16], ramipril [17],

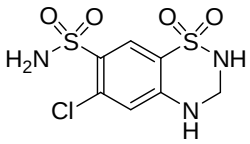
benazepril hydrochloride [18], valsartan [19], telmisartan [6,7], moexipril hydrochloride [20] and enalapril maleate [21]. Apart from this, a stability indicating assay method for quantitation of HTZ in oral suspension [22], simultaneous determination of losartan, HTZ and their degradation products [23] and a reverse phase liquid chromatography method for HTZ and captopril in human plasma [24] is reported. Few HPTLC methods for estimation of HTZ along with other drugs like telmisartan, valsartan, irbesartan, olmesartan and quinalapril are reported [25-29].

It was thought that a HPLC method of analysis that separates the drugs from the degradation products formed under ICH-suggested conditions (hydrolysis, oxidation, photolysis and thermal stress) would be of general interest. These studies provide valuable information about the inherent ability of a drug and help in the validation of analytical methods to be used in stability studies. Because of the requirement for separation of several components during stability analysis, chromatographic methods have taken precedence over conventional methods of analysis. Other than separation of multiple components, the advantage of chromatographic methods is that they are more accurate and sensitive, even for small quantities of degradation products.

After through literature survey, we concluded that two stability indicating assay procedures for estimation of TEL and a stability indicating assay method for quantitation of HTZ in oral suspension were reported. However, no stability indicating RP-HPLC method for simultaneous determination of telmisartan and hydrochlorothiazide has been reported till date. Therefore, the objective of this study was to develop a simple, rapid, precise, accurate, and specific stability-indicating RP-HPLC method for simultaneous determination of TEL and HTZ [30-32].



Telmisartan (TEL)



Hydrochlorthaizide (HTZ)

MATERIALS AND METHODS

Chemicals and Reagents

Emcure Pharmaceuticals (Pune, India) provided Pharmaceutical grade of HTZ. TEL was a gift from Glenmark Pharmaceuticals Pvt. Ltd. (Mumbai, India). We used them without further purification. Water, methanol and acetonitrile (ACN) were of HPLC grade and purchased from Merck Chemicals (Mumbai, India). We procured analytical reagent (AR) grade *o*-phosphoric acid and potassium *o*-dihydrogen phosphate from LOBA Chemie Pvt. Ltd. (Mumbai, India). Pharmaceutical finished dosage form used in the present work was Telma-H® (Glenmark Pharmaceuticals India. Ltd., batch no. 05900931, and Telpres-H® (Nicholas Piramal Healthcare Pvt. India, batch no. TH90808) tablets containing 12.5 mg of HTZ and 40 mg of TEL.

HPLC Instrumentation and Conditions

The HPLC system (Perkin Elmer, Model: Binary Series 200, USA) consisted of a pump and a manual injector with 20 µL loop. We operated the UV-Vis detector at a wavelength 225 nm. The software used was Total Chrom Navigator version 6.3. The column used was Thermo Hypersil GOLD (250 mm x 4.6 mm (i.d.), 5 µm particle size C18 column. The mobile phase was a 40:60 (v/v) mixture of ACN and aqueous 0.01 M potassium dihydrogen *o*-phosphate buffer (pH 3 adjusted with 2 % v/v *o*-phosphoric acid). Flow rate was 0.8 mL min^{-1}. Before use, the mobile phase was filtered through a 0.45 µm membrane filter (Millipore). We performed all the analysis at ambient temperature. 20 µL volume injected. The detector was operated at 225 nm.

Preparation of Stock and Standard Solutions

Stock standard solutions of TEL (1 mg mL^{-1}) and HTZ (1 mg mL^{-1}) were prepared by dissolving 10 mg each in 10 mL methanol. Appropriate volumes of the stock solutions were transferred to 10 mL volumetric flasks and were diluted to volume with mobile phase to furnish final concentrations in the ranges 6-36 µg mL^{-1} and 2-12 µg mL^{-1} for TEL and HTZ respectively.

Preparation of Pharmaceutical Formulations for Assay

We weighed ten tablets each of Telma-H ® and Telpers-H® and finely powdered. Accurately weighed powder equivalent to 10 mg HTZ (maintaining 32 mg of TEL) was transferred into separate 50 mL volumetric flasks, and to

this 40 mL of methanol was added. This mixture was shaked for complete extraction of drugs and the solution was made with same solvent to obtain concentrations of HTZ and TEL as 200 and 640 µg mL^{-1} respectively. The solutions were then filtered through Whatman filter paper (No. 41). Further dilutions of these solutions were carried out with mobile phase to reach the final concentrations of HTZ and TEL as 10 and 32 µg mL^{-1}, respectively. 20 µL of these solutions were injected in HPLC under described conditions.

Forced Degradation of TEL and HTZ

To determine whether the analytical method and assay were stability-indicating TEL and HTZ bulk powder were stressed under different forced degradation conditions in forced degradation studies. TEL and HTZ were weighed (10 mg) and dissolved with 10 mL of methanol to obtain solutions of 1000 µg mL^{-1} concentration. These stock solutions were used for forced degradation studies.

Acidic and Alkaline Degradation

HCl (1N, 10 mL) and NaOH (1N, 10 mL) were separately added to 10 mL methanolic stock solutions of TEL and HTZ. These mixtures were separately heated under reflux for 4 h at 70°C in the dark (to exclude the possible degradative effect of light). The solutions (10 mL) were transferred separately to volumetric flasks, neutralized by addition of 10 mL 1N NaOH and 1N HCl, and suitable volume was diluted with mobile phase.

Oxidative Degradation

Hydrogen peroxide (H_2O_2; 3% v/v, 10 mL) was added to 10 mL methanolic stock solutions of TEL and HTZ. These solutions were separately heated under reflux for 3 h at 70°C in dark. The solutions were then transferred separately to volumetric flasks, neutralized by addition of 1N NaOH and diluted to suitable volume with mobile phase.

Neutral Degradation

HPLC grade water (10 mL) was added to 10 mL methanolic stock solutions of TEL and HTZ. These solutions were separately heated under reflux for 4 h at 70°C. The experiment was performed in dark to exclude the possible degradative effect of light. The solutions were then transferred separately to volumetric flasks and suitably diluted with mobile phase.

Dry heat Degradation

For dry heat degradation, solid drugs were kept in petriplates in an oven at 100°C for 5 h. Thereafter, 10 mg of each TEL and HTZ was weighed and transferred into 10 mL volumetric flask and diluted up to the mark with methanol to obtain a solution of 1000 µg mL^{-1}. The solutions were then transferred separately to volumetric flasks and suitably diluted with mobile phase.

Photochemical and UV Degradation

10 mL methanolic stock solutions of TEL and HTZ were separately subjected to natural sunlight for 5 h to study the effect of photo degradation. For UV degradation studies, the same stock solutions of drugs (1000 µg mL^{-1}) were exposed to UV radiation of a wavelength of 256 nm and of 1.4 flux intensity for 5h in UV chamber. Suitable volumes from above stock solutions were diluted with mobile phase.

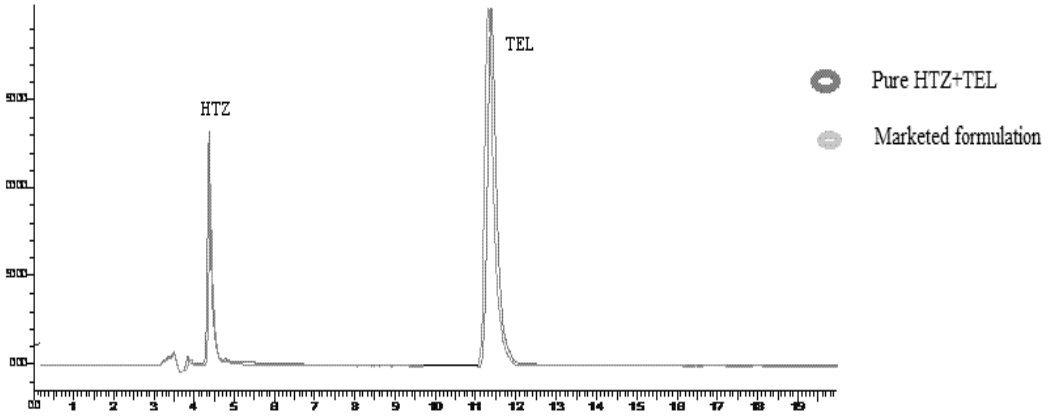
RESULTS AND DISCUSSION

HPLC Method Development

The HPLC procedure was optimized in a view to develop a method for stability-indicating assay. To optimize the method, different analytical columns, mobile phase composition, pH, flow rate, wavelength of detection, and ionic strength of the buffer were studied. During the optimization of HPLC method, three columns (Perkin Elmer C18 5 µm, 250 × 4.6 mm; Thermo Hypersil Gold C18 5 µm, 250 × 4.6 mm; Chromatopak peerless basic C18 5 µm, 250 × 4.6 mm), two organic solvents (methanol and acetonitrile) and four different pH values (3.0, 5.0, 6.0, 7.0) were tested. HTZ and TEL exhibited quite a similar behavior as both of them contain polar functional groups. Amongst the stationary phases tried, Thermo Hypersil Gold C18 gave the best results in terms of resolution, peak shape and analysis time. After several trials, a satisfactory separation was obtained with a mobile phase consisting of acetonitrile 0.01 M phosphate buffer (pH adjusted to 3 using 2 % v/v *o*-phosphoric acid) in a ratio of 40:60 v/v. The effect of organic modifiers on retention and peak shape of the drugs and their degradation products was studied and optimized. Increasing acetonitrile concentration to greater than 40 % led inadequate separation between HTZ and TEL. Lower acetonitrile concentration resulted in excessive tailing for two drugs with increased retention time for telmisartan peak. Use of water instead of buffer resulted in splitting of both the peaks. Mobile phase pH substantially affects the chromatographic behavior of both drugs and their degradation products. Variation of pH of the phosphate buffer between 4.0 to 7.0 resulted in poor separation and tailing for the two peaks and splitting for HTZ peak. At apparent pH 3-4 improved resolution for the three drugs was observed, but at actual pH 3 of the buffer

optimum resolution with reasonable retention time values (*i.e.* 4.3 ± 0.01 and 11.5 ± 0.01 min for HTZ and TEL respectively) were achieved (Fig. 1). Flow rate of 0.8 mL min^{-1} was the best for separation of the drugs and their degradation products. At 1 mL min^{-1} although sharp peaks were obtained, resolution between the peaks of drugs and degraded products was poor.

Fig. 1 Overlay chromatograms for HTZ and TEL



Using the optimized mobile phase, best results were obtained in terms of peak symmetry, selectivity and analysis time for the selected tablet formulation. This method has many advantages like isocratic conditions rather than gradient RP-HPLC which requires more sophisticated instrumentation and analysis time was also shorter. However, stability indicating methods are reported for quantitation of TEL and HTZ individually, there is no published method for study of the stability of the drugs simultaneously under conditions of forced degradation and quantitation hence there is a need for a new analytical method of this type.

Application to Pharmaceutical Formulations

The proposed method was successfully used to determine TEL and HTZ in their dosage forms Telma-H and Telpres-H tablets, respectively. Six replicate determinations were performed. Satisfactory results were obtained for each compound, in good agreement with label claims. The results are showed in Table 1.

Table 1. Results from analysis of marketed tablet formulations

	Tablet Component	Label Claim (mg/Tab)	Amount Found (%)	Recovery (%)	S.D*	% R. S. D.*
Telma-H	HTZ	12.5	12.5	100.6	0.64	0.63
	TEL	40	41.08	102.7	1.39	1.35
Telpres-H	HTZ	12.5	12.5	100.06	1.373	1.37
	TEL	40	41.05	102.63	1.466	1.42

** Results are mean of three determinations*

Validation of the Method

The method developed was validated in terms of linearity, accuracy, precision, limit of detection (LOD) and quantitation (LOQ) and system suitability as described in ICH guidelines Q2 (R1) [30-32].

Linearity and system suitability test (SST) parameters

The calibration curve was found to be linear over range of 6-36 µg mL^{-1} and 2-12 µg $mL^{-1}$for TEL and HTZ respectively. Six injections were done at each concentrations. The linearity of calibration curve was determined using the plot of peak areas vs. concentrations of analyte. Typical regression equations for the calibration plots were y = 103468x + 20468 and y = 97227x + 43691 for TEL and HTZ, respectively.

System suitability tests are an integral part of chromatographic method, which are used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drugs solution at the concentration level of 10 µg mL^{-1} for HTZ and 32 µg mL^{-1} for TEL to check the reproducibility of the system. All were satisfactory and indicated good specificity of the method for assessment of the stability of TEL and HTZ. The results for linear regression analysis of calibration curves and system suitability test parameters are shown in Table 2.

Table 2. Linearity, regression and SST parameters for determination of TEL and HTZ

Parameters (Units)	Linearity range (µg mL^{-1})	N	Slope	Intercept	r^2 ± SD	Retention time t_R (min)	Tailing factor (T)	Number of theoretical plates (N)
TEL	6-36	6	466451	86261	0.9927 ± 0.003	11.5 ± 0.01	1.3	11694.83
HTZ	2-12	6	91531	74290	0.9891 ± 0.006	4.37 ± 0.01	1.4	91694.83

Recovery studies

Accuracy was determined by adding known amounts of the drugs to commercial pharmaceutical tablet (Telpres-H) of known concentration (standard addition method). The resulting mixtures were analyzed and the results obtained were compared with the results expected. Satisfactory recoveries with small percentage relative standard deviations (RSD) were obtained, which indicated the high accuracy of the method (Table 3).

Table 3. Results of recovery experiment using proposed method

Spike (%)	TEL				HTZ			
	Amount taken	Amount Recovered	% Recovery	% RSD*	Amount taken	Amount Recovered	% Recovered	% RSD*
80	57.6	57.42	99.7	0.682	18	18	100	0.737
100	64	65.02	101.6	1.00	20	20.4	102	0.830
120	70.4	73.21	104	1.16	22	21.95	99.8	1.793

** Results are mean of three determinations*

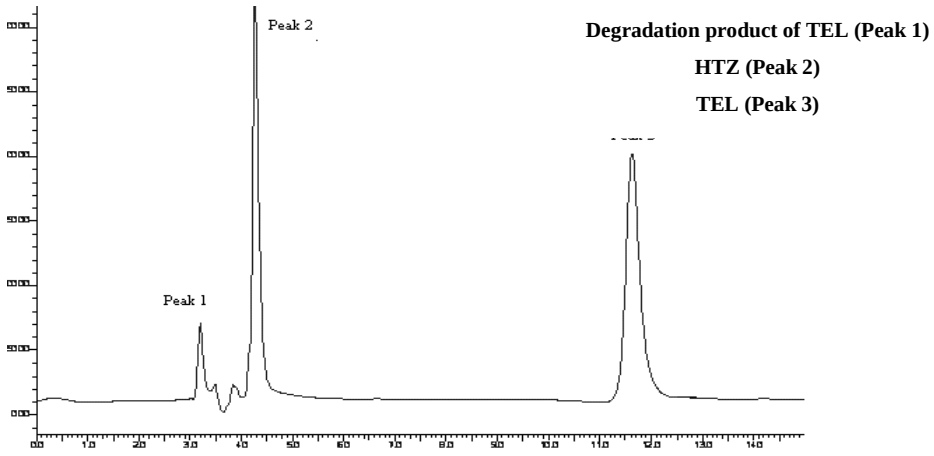
Precision

Precision was assessed at two levels, *i.e.* repeatability (intra-day precision) and intermediate precision, in accordance with ICH recommendations. Six injections, of three different concentrations, were given on the same day and the percent relative standard deviations (% RSD) were calculated to determine repeatability. These studies were also repeated on six consecutive days to determine inter-day precision. The % RSD values for the intra-day precision study for TEL and HTZ were 1.62 and 1.52 and for the inter-day study 1.92 and 1.25 respectively. Since the values were less than 2 %, it proved that the method was sufficiently precise.

Detection (LOD) and Quantification (LOQ) limits

In the present study, the LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s formulae, respectively; where σ is the standard deviation of the peak areas and is the slope of the corresponding calibration curve. The LOD was 0.495 and 0.873 µg mL^{-1} for TEL and HTZ respectively, and the respective LOQ were 1.50 and 2.64 µg mL^{-1}.

Fig. 2 Chromatogram of TEL and HTZ degraded under acidic hydrolysis



Robustness

Robustness is the measure of capacity of analytical methods to remain unaffected by small but deliberate variation of the operating conditions. Variation of the pH of the 0.01 M potassium dihydrogen *o*-phosphate buffer in the mobile phase by ± 0.2, of the amount of organic solvent in the mobile phase by ± 2%, and the detector wavelength by ± 2 nm had no significant effect on the chromatographic resolution of the method.

Degradation Studies

The degradation studies indicated that both TEL and HTZ degraded in acidic, basic and neutral hydrolysis, oxidation, photolysis, dry heat and UV degradation under experimental conditions with maximum degradation under neutral hydrolytic condition. TEL, an angiotensin II receptor antagonist, was relatively stable at basic, oxidative hydrolysis and dry heat stress conditions, resulting 8.4, 8 and 7.9 % degradation. However, it was moderately stable towards acid hydrolysis and photo degradation resulting in 18 and 27.6 % degradation. The chromatogram of acid degraded sample of TEL showed one additional degradation peak at t_R 3.15 min (Fig. 2).

The chromatograms of photochemical and oxidative degradation samples of TEL showed one additional peak at t_R 12.2 min and two additional peaks at t_R 5.0 and 5.2 mins respectively (Fig. 3 and 4).

Fig. 3 Chromatogram of TEL and HTZ degraded under sunlight

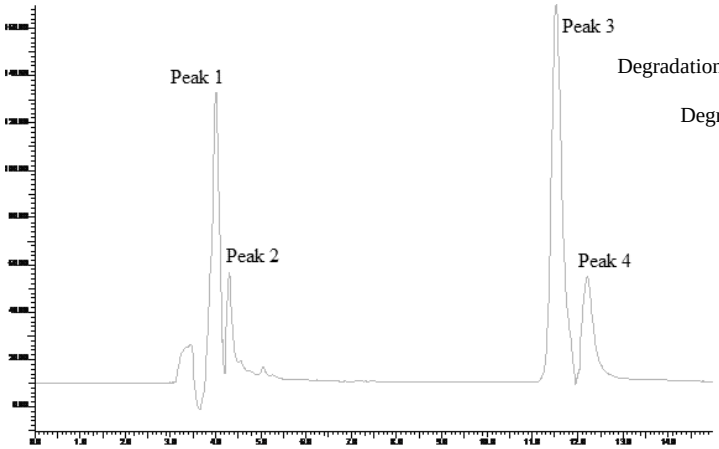
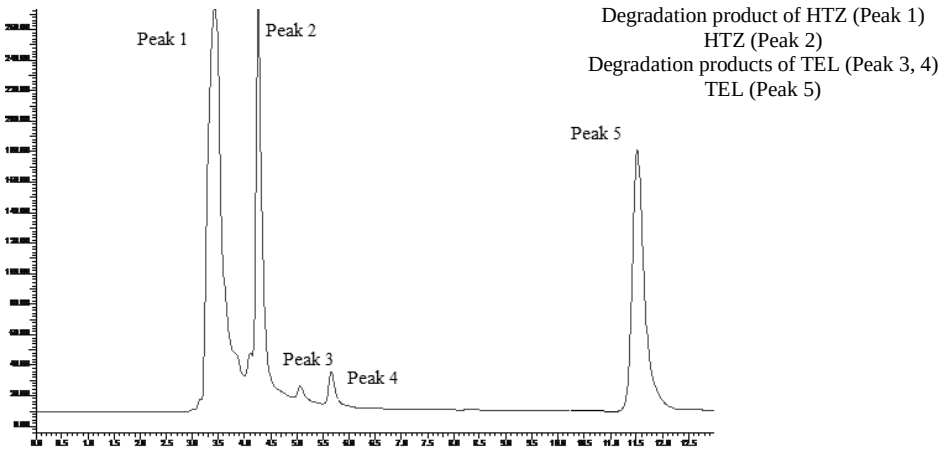


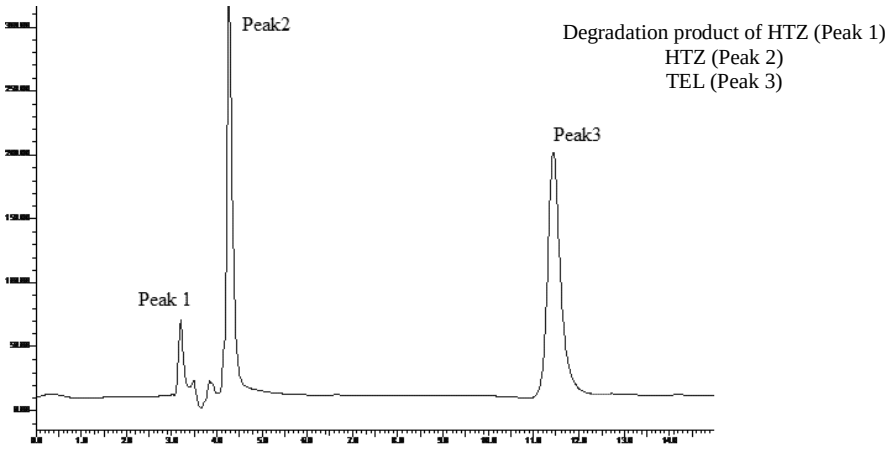
Fig. 4 Chromatogram of TEL and HTZ degraded under oxidative degradation



In basic hydrolysis, TEL degrades as observed by the decreased area of the same concentration of the nondegraded drug, without giving additional degradation peaks (Fig. 5). In UV degradation, TEL showed no additional degradation peak, however an increase in peak area in degraded sample was observed. This can be due to appearance of the degraded peak at the same retention time as that of TEL.

In contrast, HTZ was found to be moderately stable towards acidic, basic and oxidative hydrolysis, dry heat and UV degradation resulting 17.8, 17.5, 12.3, 15 and 18% degradation. The chromatogram of base degraded sample of HTZ showed one additional degradation peak at t_R 3.2 min (Fig. 5).

Fig. 5 Chromatogram of TEL and HTZ degraded under basic condition



The chromatograms of photochemical and oxidative degradation samples of HTZ showed one additional peak at t_R 4 min and at t_R 3.5 min respectively (Fig. 3 and 4). In acidic hydrolysis, HTZ degrades as observed by the decreased area of the same concentration of the nondegraded drug, without giving any additional degradation peaks (Fig. 2). In neutral hydrolysis, and dry heat degradation, the drug degrades as shown by the decreased areas in the peaks when compared with peak areas of the same concentration of the nondegraded drug, without giving any additional degradation peaks. Both TEL and HTZ were highly susceptible towards neutral hydrolysis, resulting in more than 50% degradation, however no additional degradation peak was observed. Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of the drugs under non degradation condition. Summary of degradation studies of the drugs is given in Table 4.

Table 4. Results from forced degradation of TEL and HTZ

Stress conditions	t_R of additional peaks for		Amount of degraded		Amount of recovered	
	TEL (min)	HTZ (min)	TEL (%)	HTZ (%)	TEL (%)	HTZ (%)
1N HCl (4 h)	3.15	_	18.61	17.86	82.39	82.14
1N NaOH (4 h)	_	3.2	8.42	17.53	91.57	82.47
3% H_2O_2 (3 h)	5.0, 5.2	3.5	7.91	12.3	92.09	87.7
Neutral (4 h)	-	-	59.09	53.56	40.91	46.44
Sunlight (5 h)	12.2	4.00,	27.65	0.0	27.65	100
UV light (4 h)	-	_	-	18	-	82
Dry Heat (100°C, 5 h)	-	_	8.45	15	91.55	84.18

CONCLUSION

In this work, the behavior of TEL and HTZ under different stress conditions was studied and a simple, accurate, and well-defined stability-indicating HPLC method was established for determination of TEL and HTZ in the presence of their degradation products. All the degraded products were well separated from both the drugs. The developed method was found to be simple for analysis of TEL and HTZ in combination without any interference from the excipients. The results of the degradation studies indicated the suitability of the method to study stability of TEL and HTZ under various forced degradation conditions *viz.* acid, base, dry heat, neutral, photolytic and UV degradation. The developed method can be applied to the analysis of stability samples of combination dosage form of TEL and HTZ.

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