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Stability-Indicating RP-HPLC Method for Analysis of Telmisartan in the Bulk Drug and in Formulations

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Summary. A sensitive and reproducible HPLC method has been developed for quantitative analysis of telmisartan. The drug was separated from its degradation products on a C_{18} column at ambient temperature with methanol–water 80:20 (*v*/*v*), pH 4.0 (adjusted by addition of orthophosphoric acid), as mobile phase at a flow rate of 1.0 mL min^{-1}. Under these conditions the retention time of telmisartan was 4.85 ± 0.05 min. Quantification on the basis of peak area was achieved by UV detection at 225 nm; calibration plots were linear in the concentration range 10–60 μg mL^{-1}. When the method was applied to a pharmaceutical formulation there was no chromatographic interference from tablet excipients. The method was validated for precision, robustness, recovery, and limits of detection and quantification. The drug was subjected to acidic and alkaline hydrolysis, and oxidising, dry heat, wet heat, and photodegrading conditions. Because the method could effectively separate the drug from its degradation products, it can be regarded as stability indicating.

Key Words: telmisartan, RP- HPLC, validation, stress study

Introduction

Telmisartan (chemically 4′-[(1,4′-dimethyl-2′-propyl-[2,6′-bi-1*H*-benzimidazol]-1′-yl)methyl][1,1′biphenyl]-2-carboxylic acid; *Fig. 1*) is a highly selective, non-peptide angiotensin II type-1 receptor antagonist. It selectively blocks the angiotensin type 1 receptor, which is responsible for vasoconstriction and for salt and water retention [1].

Because telmisartan is a novel drug, few analytical methods have been reported for its quantitative analysis. Capillary zone electrophoretic [2], micellar electrokinetic chromatographic [3], and polarographic [4] methods have been used for analysis of telmisartan in human plasma and in formu-

lations. An RP-HPLC method for analysis in tablets and HPLC coupled with fluorescence detection have also been reported [5, 6]. LC–MS has also been used for quantitative analysis of telmisartan in human plasma [7–10].

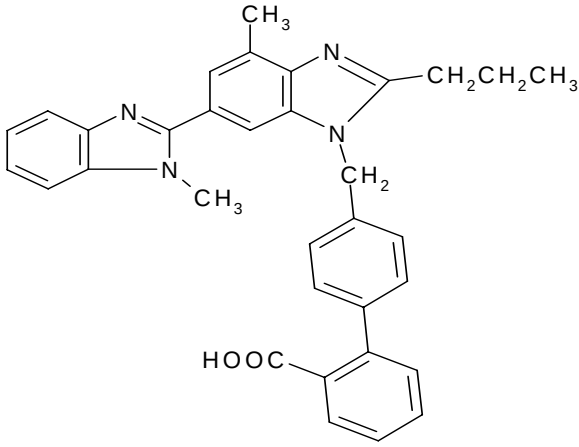

Fig. 1. The structure of telmisartan

International Conference on Harmonization (ICH) guidelines require stress testing be performed to elucidate the inherent stability characteristics of active substances [11]. Because a thorough literature survey revealed no stability-indicating chromatographic method for analysis of telmisartan in pharmaceutical dosage forms, the objective of this work was to develop an HPLC method for analysis of telmisartan in the presence of its degradation products and related impurities, for assessment of the purity of the bulk drug and stability of its bulk dosage form. The method is rapid, simple, accurate, specific, repeatable, and stability-indicating, and is suitable for routine analysis of telmisartan in tablet dosage forms. The method was validated to ensure compliance with ICH guidelines [12, 13] and its updated international convention [14].

Experimental

Chemicals, Reagents, and Solutions

Pharmaceutical grade telmisartan, kindly supplied as a gift by Glenmark Pharmaceuticals, Mumbai, India, was certified to contain 99.65% (*w*/*w*) on a dry-weight basis, and was used without further purification. All chemicals

and reagents used were HPLC-grade and were purchased from Merck Chemicals, India.

Stock solution (1000 μg mL^{-1}) was prepared by dissolving 100 mg telmisartan in 100 mL methanol. Six calibration solutions with concentrations in the range 10–60 μg mL^{-1} were prepared by dilution of the stock solution with methanol.

Sample Preparation

To determine the telmisartan content of conventional tablets (label claim 40 mg telmisartan per tablet), twenty tablets were weighed and the mean weight was determined. The tablets were finely powdered and powder equivalent to 100 mg telmisartan was placed in a 100-mL volumetric flask containing 50 mL methanol. The mixture was sonicated for 30 min then diluted to 100 mL with methanol and the resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and, after suitable dilution, filtered through a 0.45-μm pore size filter (Millipore, Milford, MA, USA).

Chromatography

HPLC was performed with a Jasco model PU 1580, intelligent HPLC pump, an AS-1555 sampler with auto injecting facility, programmed at 20 μL per injection, and a Jasco model UV 1575 UV–visible detector. The software used was Jasco Borwin version 1.5, LC-Net II/ADC system. Compounds were separated on a 250 mm × 4.6 mm, 5.0-μm particle, Hypersil C_{18} ODS column (Thermo Electron Corporation, Japan) and on a 250 mm × 4.6 mm, 5.0-μm particle, Finepak SIL-5, C_{18} column (Jasco, Japan). The mobile phase was methanol–water 80:20 (*v*/*v*), pH 4.0 (adjusted with orthophosphoric acid) at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 225 nm. The mobile phase and samples were filtered using a 0.45-μm membrane filter. Before use the mobile phase was degassed ultrasonically. All analyses were performed at ambient temperature.

Method Validation

System suitability was assessed by replicate analysis of six injections of the drug at a concentration of 50 μg mL^{-1}. For calibration, each of the standard solutions (10–60 μg mL^{-1}) prepared as described above was injected in triplicate and calibration plots were constructed by plotting peak area against

concentration. Method repeatability, as RSD, was assessed by assay of 20, 40, and 60 μg mL^{-1} standard solutions six times on the same day for intra-day precision and on two different days for intermediate (inter-day) precision. Robustness was evaluated by deliberately varying some of the method conditions (flow rate, percentage of methanol in the mobile phase, and mobile phase pH) and analysis of 20, 40, and 60 μg mL^{-1} standard solutions under the different conditions. The limits of detection (LOD) and quantification (LOQ) were determined as the amounts for which the signal-to-noise ratios (S/N) were 3 and 10, respectively. The values obtained were verified experimentally by diluting a solution of telmisartan of known concentrations until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate analyses. The specificity of the method was determined by complete separation of telmisartan in the presence of its degradation products and determination of retention times (t_R), capacity factors (k), and tailing or asymmetry factor (T) etc. Recovery as a measure of the accuracy of the method was studied by analysis of drug sample to which known amounts of telmisartan corresponding to 80, 100, and 120% of the label claim had been added (standard addition method). At each level the amount was determined six times and the results obtained were compared with the expected results.

Forced Degradation Study

A stock solution containing 1 mg mL^{-1} telmisartan was used for forced degradation to provide an indication of the stability indicating property and specificity of the method. In all degradation studies the average peak area of telmisartan was calculated after analysis of seven replicates (50 μg mL^{-1}). To assess acid and base-induced degradation 10 mL 1 M HCl or 5 M NaOH was added to 10 mL methanolic stock solution and the mixtures were heated under reflux for 3 h at 70°C. Forced degradation in acidic and basic media was performed in the dark to exclude the possible degradative effect of light. To assess oxidative degradation 10 mL 6.0% (w/v) or 50% (w/v) hydrogen peroxide was added to 10 mL methanolic stock solution and the solutions were heated under reflux for 3.0 h at 70°C and then heated in a boiling water bath for 10 min to remove excess hydrogen peroxide completely. To assess degradation by dry heat and wet heat the drug standard was placed in oven at 100°C for 24 h to study dry heat degradation and the stock solution was heated under reflux for 3.0 h on a boiling water bath for wet heat degradation. To study the photochemical stability of the drug the stock solution was exposed to direct sunlight for 15 days. Neutral hydrolysis was assessed by adding 10 mL double-distilled water to 10 mL of

methanolic stock solution and heating the mixture under reflux for 3 h at 70°C.

Results and Discussion

The HPLC procedure was optimized to develop a stability-indicating assay. Pure drug and its degradation products were injected and chromatographed using different mobile phases. During optimization of the HPLC method, two columns (250 mm × 4.6 mm, 5-µm particle, Finepak C_{18} and ODS Hypersil C_{18}), two organic solvents (acetonitrile and methanol), and two buffers (acetate and phosphate) at two different pH values (3 and 4) were tested. Initially methanol–water, acetonitrile–water, acetonitrile–acetate buffer, methanol–acetate buffer, acetonitrile–phosphate buffer, and methanol–phosphate buffer were tried in different ratios at pH 3 and 4. A methanol-to-water ratio of 80:20 (v/v) at pH 4.0, adjusted with orthophosphoric acid, at a flow rate of 1.0 mL min^{-1} and with detection at 225 nm, resulted in an acceptable retention time (t_R = 4.85 min) and symmetrical peak shape (*Fig. 2*), so this mobile phase was selected for validation purposes and stability studies.

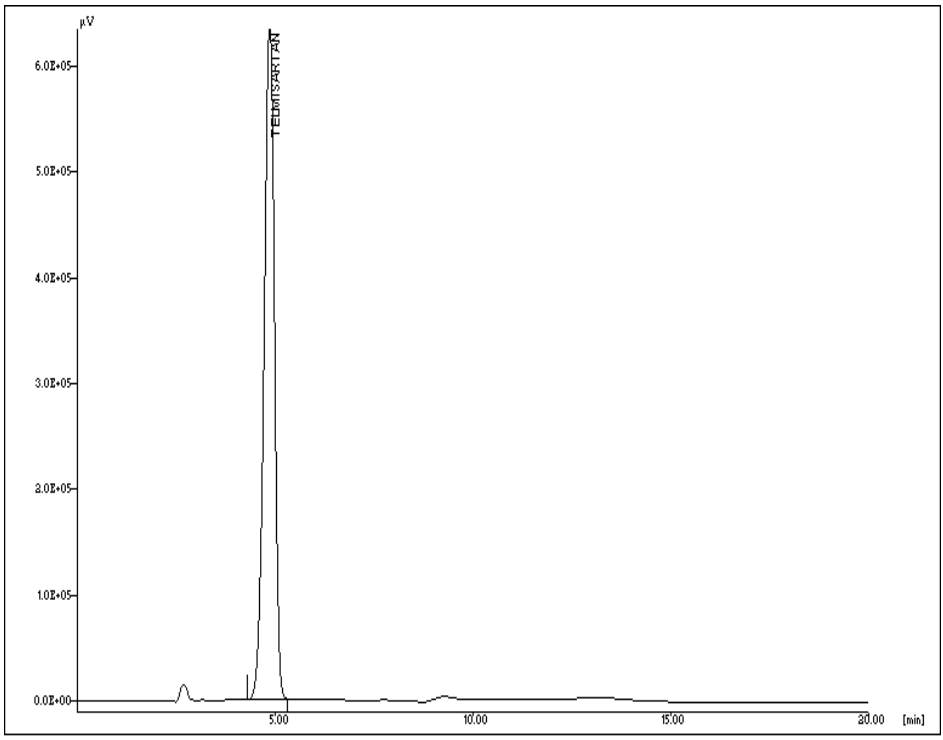

Fig. 2. Chromatogram obtained from telmisartan standard

CV for peak area and retention time was less than 2%. The number of theoretical plates was approximately 3000 ± 0.025 (mean ± %*CV*). Tailing factor for the telmisartan peak was less than 2% and resolution of the standard from the degradation products was satisfactory. Response was a linear function of concentration in the range 10–60 μg mL^{-1} with a good correlation coefficient (0.9991 ± 0.68). Typically, the regression equation was $y = 0.10x + 22$.

The within-run and between-run precision, as RSD, was 0.44 and 0.49% for intra-day and inter-day analysis, respectively. In assessment of robustness in accordance with ICH guidelines [14], flow rate, and mobile phase composition and pH (but not columns from different manufacturers or solvents from different lots) were changed at three levels (−1, 0, and 1), one at a time, and mixed standard solutions at three concentrations (20, 40, and 50 μg mL^{-1}) were injected six times. The results obtained, presented in *Table 1*, were not affected by small variation of the conditions.

Table I. Results from evaluation of the robustness of the method ($n = 6$)

Condition	Level	t_R (min)	k
Flow rate (mL min^{-1})			
0.90	−1	4.80	2.20
1.0	0	4.85	2.23
1.1	+1	5.00	2.3
Mean ± SD ($n = 6$)		4.88 ± 0.10	2.24 ± 0.06
Percentage of methanol in the mobile phase (v/v)			
78	−1	4.93	2.28
80	0	4.85	2.23
82	+1	4.70	2.08
Mean ± SD ($n = 6$)		4.83 ± 0.11	2.20 ± 0.10
Mobile phase pH			
3.9	−1	4.76	2.17
4.0	0	4.85	2.23
4.1	+1	4.95	2.30
Mean ± SD ($n = 6$)		4.85 ± 0.09	2.23 ± 0.06

t_R is retention time, k is capacity factor, and SD is standard deviation

LOD and LOQ were 0.20 and 0.50 μg mL^{-1}, respectively. The specificity of the method is illustrated in *Fig. 3*, which illustrates complete separation

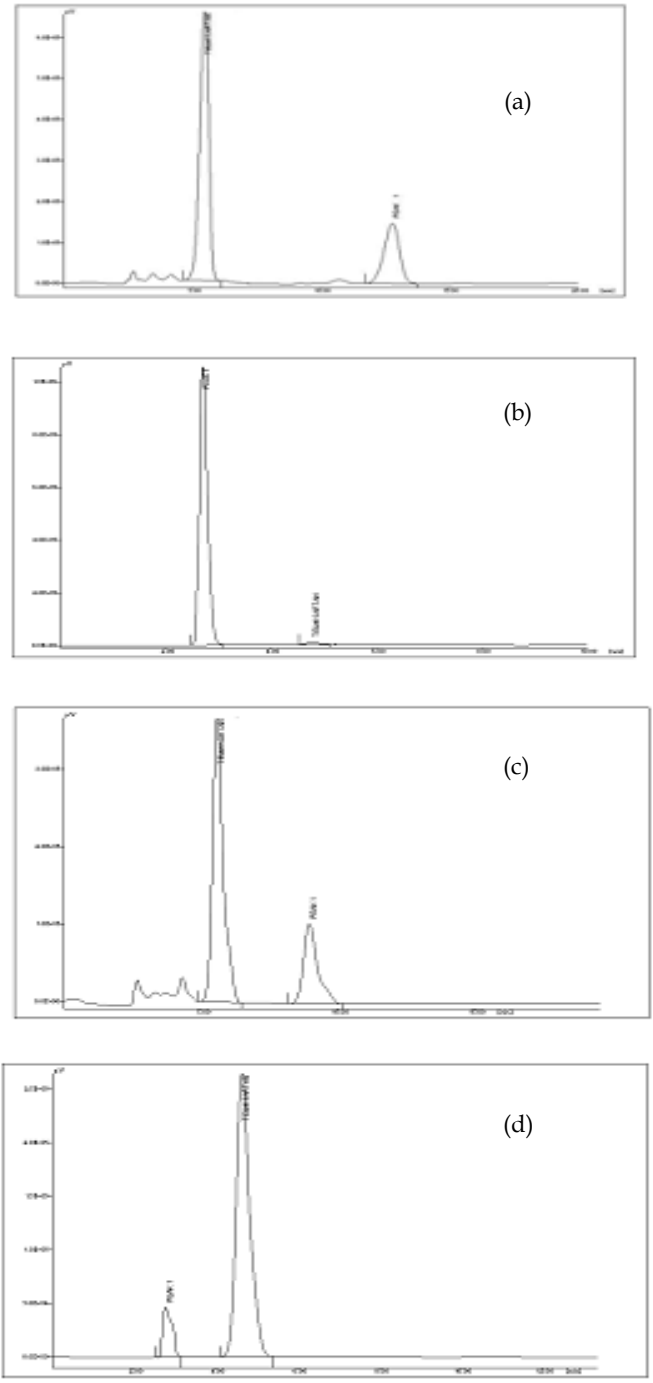

Fig. 3. Chromatograms obtained after forced degradation: (a) acid hydrolysis, (b) oxidation, (c) photodegradation, (d) neutral degradation

of telmisartan from its degradation products. Chromatograms obtained from samples treated under acidic, oxidising, photolytic, and neutral conditions each contained an additional peak at 12.75, 2.95, 9.25, and 2.80 min, respectively. No additional peak was observed in HPLC of samples treated under basic, dry heat, and wet heat conditions, although it was evident from the amount of drug remaining that substantial degradation of the drug was occurring. This indicates that the drug is susceptible to acidic and basic hydrolysis, oxidation, dry and wet heat degradation, and photodegradation. An attempt was made to isolate and characterize major degradation products of telmisartan by use of LC–ESI-MS, and to suggest possible degradation pathways. It was established that hydrolysis with methanolic acid and base results in esterification of the carboxyl group of telmisartan at C-1, thereby yielding a novel derivative of m/z 530. Photodegradation of telmisartan results in dehydrogenation of the propyl chain, i.e. formation of double bond at C-18, giving a product of m/z 513.8. Oxidative degradation of telmisartan results in fragmentation at the C-14 methyl group, leaving behind a two-benzimidazole-ring structure with m/z 302. Degradation of telmisartan occurs mainly because of hydrolysis during manufacturing, transport, or storage of the bulk drug under excessively humid conditions for prolonged period of time.

When the method was used for extraction and subsequent analysis of telmisartan from a pharmaceutical dosage form after spiking with additional drug, recovery was 99–101% *(Table II).*

Table II. Results from determination of the recovery of the method by use of standard addition technique (n = 6)

Excess drug added to the analyte (%)	Theoretical content (ng)	Recovery (%)	RSD (%)	SE
0	40	88.00	1.47	0.60
80	72	99.98	0.74	0.30
100	80	99.45	1.07	0.43
120	88	99.27	1.48	0.60

When solutions of telmisartan at three different concentrations (20, 40, and 60 μg mL^{-1}) were prepared from stock solution and analysed after storage at room temperature for three days, no additional peaks were observed in the chromatograms, indicating the stability of telmisartan in solution. The SD of peak areas and RSD were 1.29 and 0.34%, respectively.

Telmisartan peaks at t_R 4.87 min were observed in chromatograms obtained from tablet extracts. Amounts of telmisartan in tablets were in good

agreement with the label claims, suggesting there was no interference from any of the excipients normally present in the tablets. The drug content was found to be 98.95 ± 0.66% (RSD 0.67%) with SE 0.27, *t*-value 2.1, and *F*-value 1.0. The theoretical *t* and *F*-values were 2.57 and 5.0, respectively ($P = 0.05$).

Conclusion

This HPLC method enables simple, accurate, reproducible, and stability-indicating quantitative analysis of telmisartan in pharmaceutical tablets, without interference from excipients or from its acidic, alkaline, oxidative and photolytic degradation products. The method was validated in accordance with ICH guidelines. Six real samples of tablets were analysed and the results correlated. The method reduces the duration of analysis and seems to be equally suitable for routine analysis of telmisartan in pharmaceutical formulations in quality-control laboratories, where economy and speed are essential. This study is a typical example of the development of a stability-indicating assay in accordance with the recommendations of the ICH guidelines. It is one of the rare studies in which forced decomposition was achieved under all the different conditions suggested and the degradation products were resolved.

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