A VALIDATED STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF RAMIPRIL AND TELMISARTAN

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ABSTRACT

Telmisartan, an angiotensin II antagonist and Ramipril, a long acting ACE inhibitor are found in combination in tablet dosage form used for the treatment of high blood pressure. The present study deals with development of validated stability indicating method for simultaneous estimation of Telmisartan and Ramipril using TLC plate precoated with Silica gel 60 F_{254} and the mobile phase consisting of Methanol: Chloroform in the ratio of 1:6v/v. Telmisartan and Ramipril were well resolved with R_f 0.68 ± 0.03 and 0.38±0.03 respectively Wavelength selected for quantization was 210nm. At this wavelength, Telmisartan and Ramipril show high absorbance. Inherent stability of these drugs was studied by exposing both drugs to various stress conditions as per ICH guidelines viz. Dry heat, oxidative, photolysis (UV and cool white fluorescent light), and hydrolytic conditions under different pH values. Both drugs were not degraded under acidic condition. Both drugs show degradation under alkaline, dry heat, oxidative condition and photolytic condition. The developed method is found to be simple, specific, precise and stability indicating. The specificity of the method was confirmed by peak purity profile of the resolved peaks.

Keywords: Ramipril, Telmisartan, Stress degradation, HPTLC, Validation.

INTRODUCTION

The drug stability test guidelines Q1A (R2) issued by International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples should be fully validated and the assays should be stability indicating. The aim of the present study accordingly was to establish inherent stability of the following two drugs in combination viz. Telmisartan and Ramipril through stress studies under a variety of ICH recommended test conditions and to develop and validate stability indicating assay method.

There is no report yet of stability indicating HPTLC method for these drugs in combination. Telmisartan (Angiotensin II antagonist) is chemically, 4′-[(1,4-dimethyl-2'-propyl [2,6'-1*H*-benimidazole] -1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid Ramipril (ACE inhibiter) is chemically [2S, 3As, 6aS]-1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl] amino]-1-oxopropyl] octohydrocyclopental (b) pyrrole-2-carboxilic acid (1).

MATERIALS AND METHODS

Ramipril was provided as a gift sample by Mepro pharmaceutical Ltd. Wardha and Telmisartan was provided as a gift sample by Glenmark pharmaceutical Ltd. Nashik. Drugs were used without any further purification.

All other reagent required for experimentation were of analytical reagent (AR) grade. Chemicals used for this experiment were Acetonitrile (AR grade), Methanol (AR grade), Chloroform (AR grade), NaOH (AR grade), HCl (AR grade), H_2O_2 (AR grade). These chemicals were purchased from MERCK Chemicals

INSTRUMENTATION

Chromatographic separation of drugs were performed on Merck TLC plates pre-coated with silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) from E. Merck, Germany. The samples were applied onto the plates as a band with 4 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland).

Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm). Densitometric scanning was performed using Camag TLC scanner 3 and operated by winCATS Software (V 1.4.2, Camag). Electronic balance (Make *SHIMDZU* Model *AY-120*) was used for weighing purpose.

Selection of Detection Wavelength

Wavelength selected was 210nm. At 210nm, Telmisartan and Ramipril show high absorbance. In the marketed formulation, Ramipril is found in the relatively low concentration as compared to Telmisartan, hence this wavelength suitable for Ramipril detection, was used.

Preparation of solution

Standard stock solution of Ramipril and Telmisartan were prepared by separately dissolving 25mg of each drug in 25ml of Methanol (1000 mcg/ml) and further diluted to get final concentration of 100 mcg/ml.

Sample was applied on pre-coated silica gel G plate as a band of length 4mm at a distance of 10mm from both x-axis and y-axis. This plate was developed in development chamber using selected mobile phase.

Stress Degradation studies

Degradation under acid catalysed hydrolytic condition

10 ml of working standard solutions of Ramipril and Telmisartan of conc. 1000 mcg/ml were separately mixed with 1 ml of 1 N HCl. The solutions were diluted to 25 ml with methanol. Appropriate volume of resultant solution was applied on TLC plate (Ramipril 2000 ng and Telmisartan 2400 ng per band) and densitograms were developed.

Degradation under alkali catalysed hydrolytic condition

10 ml of working standard solutions of Ramipril and Telmisartan of conc. 1000 mcg/ml were separately mixed with 1ml of 1N NaOH. The solutions were diluted to 25 ml with methanol and kept aside for 4 hours. Appropriate volume of resultant solution was applied on TLC plate (for Ramipril 2000 ng and 2400 ng for Telmisartan per band) and densitograms were developed.

Degradation under neutral hydrolytic condition

20 ml of working standard solutions of Ramipril and Telmisartan each of conc. 1000 mcg/ml were mixed separately with 5 ml of water. The solutions were diluted to 50 ml with methanol and refluxed at 60°C for 1 hour. Appropriate volume of resultant solution was applied on TLC plate (for Ramipril 2000 ng and 2400 for Telmisartan per band) and densitograms were developed.

Degradation under oxidative condition

20 ml of working standard solutions of Ramipril and Telmisartan of each conc. 1000 mcg/ml were separately mixed with 10 ml of 30% H_2O_2. The solutions were diluted to 50 ml with methanol and refluxed at 60 °C for 1 hour. Appropriate volume of resultant solution was applied on TLC plate (for Ramipril 2000 ng and 2400ng for Telmisartan per band) and densitograms were developed.

Degradation under dry heat

Dry heat studies were performed by keeping drug samples in oven (80^0 C) for 6 hrs. Samples were withdrawn, dissolved in methanol, diluted suitably and appropriate volumes of resultant solution (Ramipril 2000 ng/band and Telmisartan 2400 ng/band) were applied on TLC plate and densitograms were developed.

Photo-degradation studies

Photolytic studies were also carried out by exposure of drugs to UV light up to 200 watt hrs/sq. m. and subsequently cool fluorescent light to achieve an illumination of 1.2 million Lux.Hr.

Method validation

Linearity

2 ml Telmisartan and 0.8 ml Ramipril were taken from stock solution (1000 mcg/ml), transferred to 10 ml volumetric flask and the volume was made up using methanol. 5, 10, 15, 20 and 25 µL of spotting volume were applied on TLC plate and densitograms were developed. The data of peak area v/s drug amount were treated by linear least-square regression analysis.

Precision

Precision of the system and method was evaluated by analyzing six independent standard and sample preparations obtained from homogenous sample and % RSD value was calculated to determine any intra-day variation. These studies were also repeated on different days to determine inter-day variation.

Accuracy

To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Mean percentage recovery was determined.

Limit of detection and limit of quantification

The limit of detection (LOD) is smallest concentration of the analyte that gives the measurable response. The LOQ is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOD and LOQ were calculated using the following formulae:

$$LOD = \frac{3.3\ \sigma}{S} \qquad LOQ = \frac{10\ \sigma}{S}$$

Where σ is Standard deviation of the response and S is slope of the calibration curve.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peaks was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on winCATS software using statistical equation.

RESULTS AND DISCUSSION

Development of the optimum mobile phase

TLC procedure was optimized with a view to develop a stability-indicating assay method. The drug reference standards were spotted on the TLC plates and developed in different solvent systems. Different mobile phases were tried to resolve Ramipril and Telmisartan. Best suited mobile phase was found to be Methanol: Chloroform in the ratio of 1:6 v/v. Developed mobile phase resulted in resolution for two drugs with R_f 0.68 ± 0.03 and 0.38 ± 0.03 for Telmisartan and Ramipril respectively. Well-defined spots were obtained when the chamber was saturated with the mobile phase at room temperature. The representative densitogram is given in Figure1.

Validation of the developed stability-indicating method

The response for the drugs was found to be linear in the concentration range 400-2000 ng/band for Ramipril and 1000-5000 ng/band for Telmisartan. The RSD value for precision study was found to be not more than 1.804 % and 1.8417% for Ramipril and Telmisartan respectively, thus confirming precision of the method. The accuracy of the method was determined at 80, 100 and 120% level. The mean recovery ranged from 98 - 102% for both Ramipril and Telmisartan. The Limit of Detection was found to be 91 ng/band and 121 ng/band and Limit of Quantitation was found to be 300 ng/band and 400 ng/band for Ramipril and Telmisartan respectively.

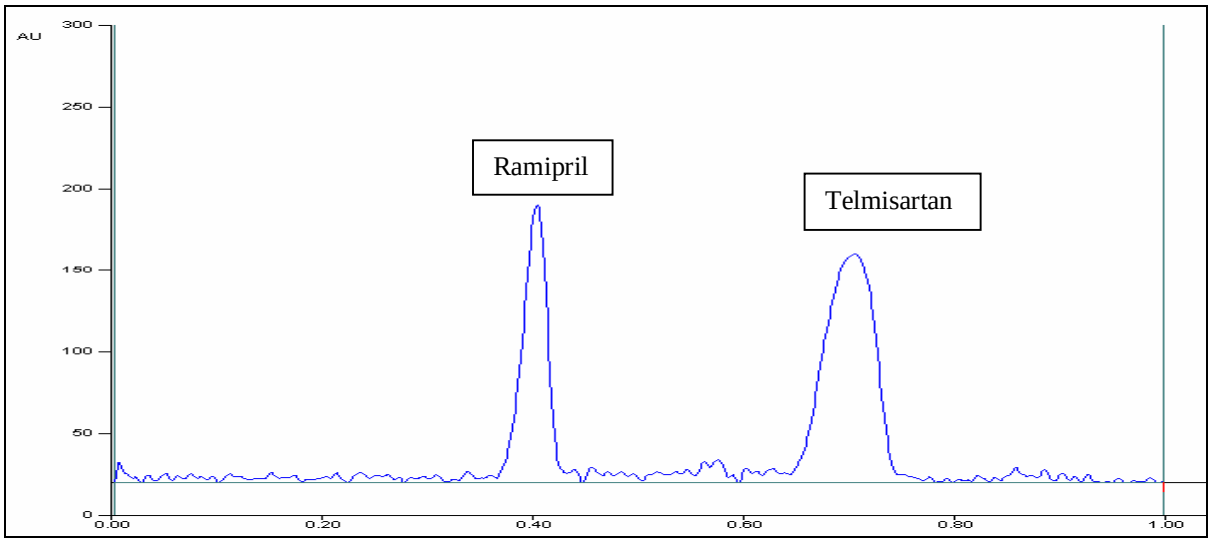

Fig.1: Representative Densitogram of Telmisartan and Ramipril of (conc.2000ng/band and 800ng/band resp.) at 210nm.

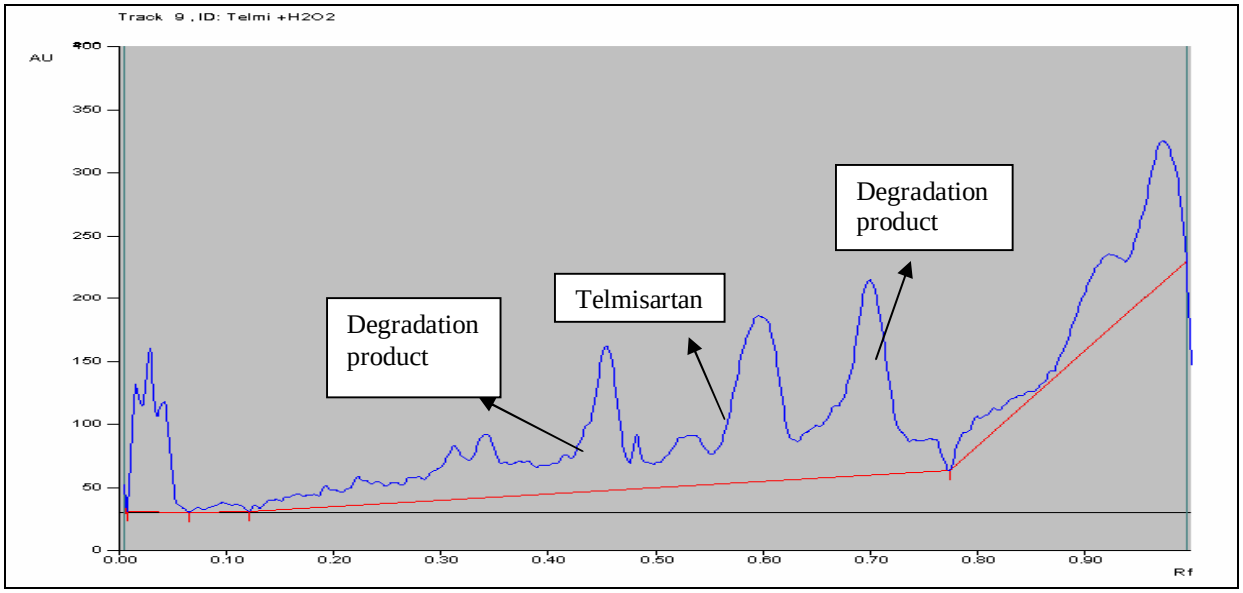

Fig.2: Representative Densitogram showing degradation of Telmisartan under oxidative condition.

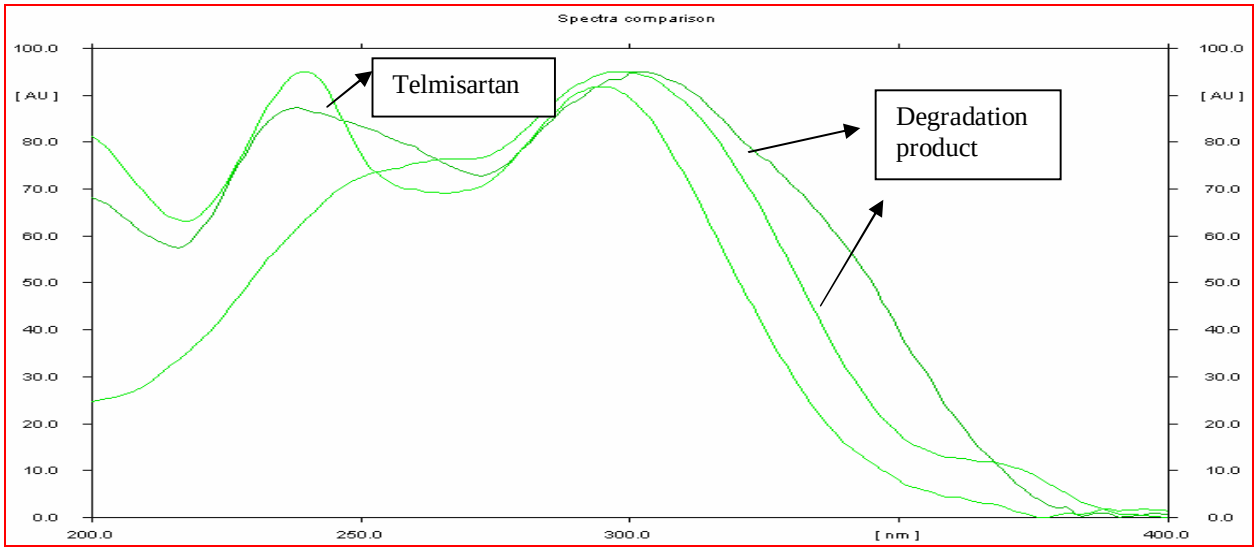

Fig.3: Representative Spectra of Telmisartan and its degradation products under oxidation condition.

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be r(s, m)= 0.9945 and r(m, e) = 0.9987, indicating the non interference of any other peak of degradation product, impurity or matrix (Table 1).

Table 1: Validation Summary

Validation Parameters	Telmisartan	Ramipril
Linearity (r^2) Linearity Equation	0.997 Y = 1.104x + 3398	0.998 Y = 3.022x+733.5
Precision (% RSD)	NMT 2 %	NMT 2 %
Accuracy (mean recovery)	98-102%	98-102%
LOD	121 ng/band	91 ng/band
LOQ	400 ng/band	300 ng/band
Specificity	Specific	Specific

Degradation observed

Major degradation of both drugs was observed under alkaline hydrolysis condition while Telmisartan showed degradation under oxidative condition and Ramipril showed degradation under dry heat also. Lesser degradation was observed under acidic hydrolysis, neutral hydrolysis, and photolytic condition.

47.50% degradation was observed for Ramipril and 8.89% for Telmisartan in alkaline hydrolysis (1ml of 1N NaOH) after keeping for 4Hrs.

Ramipril and Telmisartan both showed oxidative degradation under reflux condition (1 hr at 60^0c) of about 56% and 85%. Telmisartan showed degraded product peaks at R_f 0.46 and 0.69.

Under dry heat (Oven, 80 ^{0}C, 6 hr), Ramipril was found to be degraded up to 52.25% with decrease in area only, whereas Telmisartan showed negligible degradation.

Under Photolytic studies, no additional peaks were observed and drug peak area remained constant. This indicates stability of drugs in UV and Fluorescence light for specified period (Table 2).

Table 2: Forced Degradation Study Results for Telmisartan and Ramipril

Conditions	Degradation	
	Telmisartan	Ramipril
Dry Heat, 80^o C, 6 hrs	< 2%	54.2%
UV light, 200 lux hrs	< 2%	< 2%
Fluorescent light, 1200 lux hrs	< 2%	< 2%
1M NaOH (for 4 Hrs)	8.85%	47.77%
1N HCL	< 2%	< 2%
H_2O, (2 hrs, reflux)	< 2%	< 2%
30 % H_2O_2 (2 hrs, reflux)	85%	56%

CONCLUSION

From the above study we can conclude that the Ramipril and Telmisartan undergo degradation to different extent under different, above mentioned, stress conditions. In this study, the products formed after forced decomposition studies were resolved from the bulk drug response. From the peak purity profile studies, it was confirmed that the peak of the degradation product was not interfering with the peak of drugs. It confirms that peak for degradation product of drug can be resolved from the drug peak by this method. The developed method is simple, accurate, precise, and specific. It is proposed for routine analysis of these drugs in presence of degradation products in stability study.

Acknowledgment

The authors wish to express their gratitude to the management of AISSMS College of Pharmacy for providing the research facilities, University of Pune for providing research grant, M/s Glenmark pharmaceutical, Ltd, Nasik & M/s. Mepro pharmaceuticals, Wadhwan for providing the Telmisartan and Ramipril drug samples.

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