



STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF TELMISARTAN AND RAMIPRIL IN TABLETS

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ABSTRACT

A simple, selective, rapid, precise and stability-indicating HPTLC method has been developed for the quantitative simultaneous estimation of Telmisartan and Ramipril in combined pharmaceutical dosage form and validation was done. The proposed HPTLC method involves the use of HPTLC plates (Merck) precoated with silica gel 60F₂₅₄ on aluminium sheets and a mobile phase comprising of toluene: acetonitrile: formic acid: water (5:5:0.3:1). Densitometric analysis of both the drugs was carried out in the absorbance mode at 212 nm. This method has been successfully applied for estimation of Telmisartan and Ramipril in combined tablets formulation. Both the drugs were subjected to acid-alkali hydrolysis, oxidation and photolytic degradation and both of them were found to be susceptible to acid-alkali hydrolysis, oxidation and photolytic degradation. Linearity of Telmisartan was found to be within the range of 500-2500 ng/spot and for Ramipril the range was found to be 250-1250 ng/spot, with significant high values of correlation coefficient for both the drugs. The method was validated for the precision, robustness and recovery. As method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one.

Keywords: HPTLC, Telmisartan, Ramipril, Simultaneous estimation

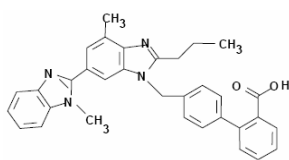
INTRODUCTION

Telmisartan, a nonpeptide molecule, is chemically 2-[4-[[4-methyl-6-(1-ethylbenzimidazol-2-yl)-2-propyl-benzimidazol-1-yl] methyl] phenyl] benzoic acid. Telmisartan is an angiotensin II receptor antagonist that is highly selective for type I angiotensin II receptor. Angiotensin II is the principle pressor agent of the rennin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium. It is used for treatment of hypertension in patients. Telmisartan is not official in any pharmacopoeia but listed in Merck Index¹.

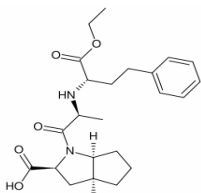
Ramipril is chemically (1S, 5S, 7S) -8- [(2S) -2- [(1S) -1-ethoxycarbonyl-3- phenyl- propyl] amino] propanoyl]-8-azabicyclo [3.3.0] octane-7-carboxylic acid. Ramipril is a highly lipophilic, long acting ACE inhibitor. The drug is used for treating blood pressure and congestive heart failure. It effectively reduces both supine and standing blood pressure without significant alteration in the pulse rate. Ramipril is official in BP² 2007 and USP³ 2007.

Combinations of Telmisartan and Ramipril are available commercially as tablets in medicinally recommended ratios of 40:5 and 40:2.5 respectively. Telmisartan and Ramipril combinations are used to lower blood pressure, and as anti-hypertensive agent.

The literature survey reveals that several methods were reported, including spectroscopic⁴⁻⁶ and chromatographic methods⁹⁻¹² for the individual estimation of Telmisartan and Ramipril or combination with other drugs. A stability-indicating LC method¹³ has been reported for simultaneous determination of Telmisartan and Ramipril in combined pharmaceutical dosage form in the presence of their degradants. Hence we attempted to develop a simple, accurate, precise and economic analytical method. This project work describes a simple HPTLC method for simultaneous estimation of Telmisartan and Ramipril in combination tablets.



TELMISARTAN



RAMIPRIL

MATERIALS AND METHODS

Instrument: A Camag, Linomat V Sample Applicator with Camag 100 µl Syringe; Camag Twin trough Glass Chamber (20 x 10); Camag TLC Scanner III with Wincat software.

Reagents and chemicals: Pure drug samples of Telmisartan and Ramipril were provided as a gift sample by Aurobindo Pharma Ltd, Hyderabad, India. Commercial pharmaceutical preparation TelmisartanTM – R 2.5 (Mkd. By Dr. REDDY'S LABORATORIES LTD) was procured from local pharmacy.

Preparation of standard stock solutions: For Telmisartan, standard stock solution was prepared by dissolving 250 mg of drug in 25 ml of 0.1 N NaOH, to give a concentration of 10 mg/ml. For Ramipril, standard stock solution was prepared by dissolving 125mg of drug in 25 ml of 0.1 N NaOH, to give a concentration of 5 mg/ml.

Chromatographic conditions: The experiment was performed on silica gel 60F₂₅₄ HPTLC plates using mobile phase comprising of toluene: acetonitrile: formic acid: methanol in the ratio of 5:5:0.3:1(v/v/v/v). The plates were prewashed with methanol and activated in an oven at 60°C for 1 hour before use. The sample solutions were applied as 1µl per spot and sharp bands of 6 mm width with the help of Camag Linomat V sample applicator in speed of 10 second/µl. After application of samples the plates were dried in oven and ascending development to distance of 90 mm was performed in a saturated 20cm x 10cm Camag twin trough chamber for 15 minutes at room temperature. Subsequent to the development, the HPTLC plates were air dried and densitometric scanning was performed at 212 nm using Camag TLC scanner III with help of Wincats software.

Linearity and range

Adequate dilutions were made from stock solutions to get five concentrations of mixed standards contains 500 – 2500 ng/spot for Telmisartan and 250 – 1250 ng/spot for Ramipril respectively using double distilled water. All these five dilutions were applied as spots of 1 µl each on a TLC plate. After development of the plate on the selected mobile phase it was dried in hot air oven. The plate was then scanned and quantified at 212 nm. The densitograms were recorded and the measured peak areas were plotted against concentrations.

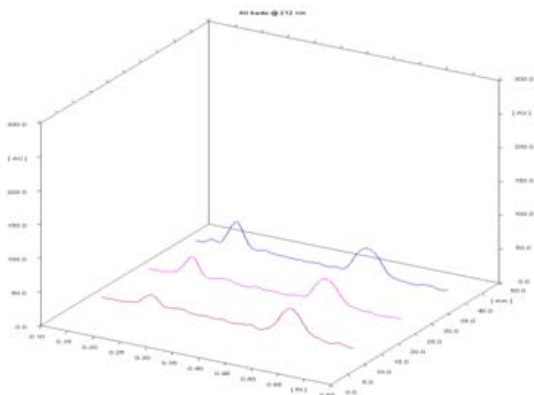


Fig. 1: Densitogram of Telmisartan and Ramipril showing R_f at 0.43 and 0.22 respectively

Preparation of tablet sample solutions

Accurately weighed and powdered 20 tablets of Telmisartan and Ramipril combination, each containing 40 mg of Telmisartan and 2.5 mg of Ramipril as per label claim. A weight of 1.7281 gm equivalent to 250 mg of Telmisartan and 15.625 mg of Ramipril was taken and dissolved in 0.1 N NaOH in a 25 ml standard flask. Solution was shaken for 30 min and filtered through Whatmann filter paper and finally made upto the mark using 0.1 N NaOH. The solution (stock) prepared above contains 10000 $\mu\text{g/ml}$ of Telmisartan and 625 $\mu\text{g/ml}$ of Ramipril.

For estimation of drug from commercial formulation, the tablet sample solution prepared were diluted to three different concentrations ranging 8000:500, 4000:250 and 2000:125 ng/spot of Telmisartan and Ramipril respectively using double distilled water. They were applied as spots of 1 μl each on same TLC plate. After development of plate on the selected mobile phase it was dried in hot air oven. Then peak areas of Telmisartan and Ramipril in those three dilutions were measured at 212 nm (Table 1).

Recovery experiments were carried out to check for the presence of any interferences from the excipients present in the formulations and also to find out the accuracy of the method. Recovery study was performed by the standard addition method. A series of three different dilutions were done in which fixed amount is 4000:250 ng/spot of Telmisartan and Ramipril, and the different amounts were added as 500:125, 1000:250 and 1500:375 $\mu\text{g/ml}$ of Telmisartan and Ramipril respectively. The peak areas of these three dilutions were measured by densitometric scanning at 212 nm from which the percentage recovery of both the drugs was estimated as shown in Table 2.

Degradation study using HPTLC technique

Forced degradation studies¹⁴ are an important part of the drug development process. Although the concept of stress testing is not new to the pharmaceutical industry, they are regularly been performed to determine stability and half-life of the drug products. The purpose of stability testing is to provide evidence on how quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and various storage conditions.

Table 1: Assay results of Telmisartan and Ramipril

Drug	Label claim (mg)	Amount present (ng/spot)	Amount estimated (ng/spot)	Percentage purity
Telmisartan	40	2000	2056.57	102.32
		4000	3986.87	99.67
		8000	7689.39	97.12
Ramipril	2.5	125	127.65	102.12
		250	246.44	98.57
		500	502.26	100.45

Table 2: Results of recovery studies

Drug	Amount of drug present (ng/spot)	Amount of drug added (ng/spot)	Amount recovered (ng/ μl)	Percentage recovery
Telmisartan	4000	500	4051.20	101.28
	4000	1000	3906.15	97.65
	4000	1500	3958.84	98.97
Ramipril	250	125	242.30	97.02
	250	250	257.60	102.04
	250	375	247.11	98.84

Acidic degradation: To 5 ml of stock solutions of both drugs, 10 ml of 1N HCl was added and refluxed at 60°C for 3 hrs.

Basic degradation: To 5 ml of stock solutions of both drugs, 10 ml of 1N NaOH was added and refluxed at 60°C for 3 hrs.

Oxidative degradation: To 5 ml of stock from each, added separately 10 ml of 30% H_2O_2 and the mixture was refluxed at 60°C for 3 hrs.

Photolytic degradation: About 250 mg of each of Telmisartan RS and Ramipril RS and 350 mg of powdered tablet formulation were taken in separate petridishes, and kept under direct sunlight for 24 hrs.

Estimation of degradation: The degraded samples were applied as spots of 1 μl each after suitable dilutions on separate TLC plates. The peak areas of Telmisartan, Ramipril and of degraded products were measured by densitometric scanning at 212 nm. Table 3 shows the percentage of degradation under each stress conditions.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple and rapid HPTLC method for the estimation of Telmisartan and Ramipril using precoated TLC plates. The R_f of telmisartan and ramipril was found to be at 0.43 and 0.22 respectively (Fig 1). The peaks were well resolved and symmetrical in shape.

Linearity experiment was performed and the response was found to be in the concentration range of 500 – 2500 ng/spot and 250 – 1250 ng/spot with the correlation coefficient 'r' values of 0.997 and 0.996 for telmisartan and ramipril respectively. Accuracy of method was determined by recovery studies (n=3). There was good repeatability of the proposed method as the precision of the method was less than 2% for both the drugs. The mean percentage recovery was found to be 97.65 – 101.28% for telmisartan and 97.02 – 102.04% for ramipril. This method is rapid, precise and accurate and hence can be employed for the routine quality control analysis.

Table 3: Results of degradation studies

Stress condition	% degraded (Ramipril)		% degraded (Telmisartan)	
	Standard	Formulation	Standard	Formulation
Acid	34.03	31.97	17.63	18.94
Alkali	12.07	19.54	3.25	4.93
Oxidative	16.26	22.26	27.69	35.61
Photolytic	2.06	15.42	2.76	6.93

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