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Simultaneous estimation of telmisartan and ramipril in combined dosage form by using HPTLC

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ABSTRACT

In this paper describes developed and validated thin layer liquid chromatography (TLC) method for the simultaneous estimation of telmisartan and ramipril in a combined dosage form. Telmisartan and Ramipril were determined by High Performance Thin Layer chromatography method (HPTLC) in tablet dosage form. The method was carried out in TLC Precoated silica gel on aluminum plate 60 F 254, (10 cm × 10 cm, prewashed by methanol and activated at 60° C for 5 min prior to chromatography). The solvent system was Acetone: Benzene: Ethyl acetate: Glacial acetic acid in the proportion of 6:4:1:0.05, (v/v/v/v) with R_f Value for telmisartan and ramipril was 0.673 and 0.353 respectively. The linearity regression analysis for calibration showed 0.999 and 0.998 for telmisartan and ramipril with respect to peak area and height in the concentration range of 150- 1700 ng/spot and 300-1900 ng/spot respectively. The method developed can be used for routine analysis of drugs content in tablet dosage form.

Key words: Telmisartan and Ramipril; High Performance Thin Layer Chromatography; Quantitative Analysis.

Abbreviations: RAMI, ramipril; TELMI, telmisartan; HPTLC, high performance thin layer chromatography

INTRODUCTION

Telmisartan is a angiotensin II antagonist used as antihypertensive agent with a chemical name 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1Hbenzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid. Telmisartan blocks the vasoconstrictor and aldosterone secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Telmisartan is indicated for the treatment of hypertension.[1]

Ramipril is angiotensin converting enzyme inhibitors (ACE) used as antihypertensive agent with a chemical name (1S, 5S, 7S)-8-[(2S)-2-[[[(1S)-1-ethoxycarbonyl-3-phenylpropyl] amino] propanoyl]-8-azabicyclo [3.3.0] octane-7-carboxylic acid. Ramipril inhibit the angiotensin converting enzyme (ACE) results in decreased human plasma angiotensin II which leads to decreased vasopressor activity and to decrease aldosterone secretion. Ramipril is indicated for the treatment of Mild to moderate hypertension, Congestive heart failure, Following myocardial infarction in patients with clinical evidence of heart failure.[2]

Some analytical methods for the quantitative determination of telmisartan in pharmaceutical formulations are described in literature like stability indicating liquid chromatography (LC) method [3], LCMS method in human plasma[4], LC-Tendom MS in human plasma[5], LC method for tablet and plasma[6,7], in urine by HPLC fluorimetry[8], difference spectrophotometric method in tablet[9].

Some analytical methods for the quantitative determination of ramipril an in pharmaceutical formulations are described in literature like Chemometrically-Assisted Spectrophotometric Estimation in binary mixture with felodipine[10], voltametry and polarography in dosage forms and biological fluids[11], kinetic spectrophotometric method[12], Spectrophotometry & spectrofluorimetry in dosage forms[13], Spectrophotometry & atomic absorption spectrometry in combination with perindropil[14], Spectrophotometry and liquid chromatography in dosage forms[15,16]. Only one stability indicating HPLC method is reported in literature for ramipril and telmisartan for solid dosage form [17]. Some methods like potentiometry and HPLC are official for the estimation of ramipril in United State of Pharmacopeia, British Pharmacopeia.

The aim of the present investigation was to develop a simple, precise and accurate HPTLC method for determination of telmisartan and ramipril in tablet dosage forms.

MATERIALS AND METHODS

Chemicals

RAMI and TELMI were obtained as a gift samples by Torrent Research Center, Gandhinagar. Drug formulation Brand I (Telma R) and Brand II (Telista RM) were obtained from the market. Chemicals was obtained from Merck LTD.

Instrumentation

A Camag TLC system (Muttens, Switzerland) comprising of Camang Linomate V automatic sample applicator, Hamilton syringe (100 μ l), Camang TLC scanner 3, Camang WinCATS software, Camang twin trough chamber (10 x 10 and 20 x 10) and ultrasonicator was used during the study. TLC plates used were precoated silica gel aluminium plate 60 F 254, (10 cm x 10 cm with 250 μ m thicknesses (E.Merck, Mumbai, India).

Chromatographic condition

Stationary phase	: Precoated silica gel on aluminium plate 60 F 254, (10 cm x 10 cm, activated at 60° C for 5 min prior to chromatography)
Mobile Phase	: Acetone: Benzene: Ethyl acetate: Glacial acetic acid in the proportion of 6:4:1:0.05, (v/v/v/v), for the RAMI and TELMI.
Vol. of Mobile phase	: 10 mL
Saturation Time	: 15 min at room temperature (30 \pm 1° C) and RH 60 % \pm 5
Application rate	: 0.1 μ L/s
Scanner band width	: 6 mm
Slit dimension	: 5 mm x 0.45 mm
Scanning speed	: 10 mm/s
Detection	: Densitometrically using a UV detector at 210 & 296 nm for ramipril and telmisartan respectively in the reflectance-absorption mode.

Solutions

Preparation of Standard Stock Solutions

Standard Stock solutions of Ramipril and telmisartan were prepared by accurate weighing of 100 mg for both the drugs in the separate 100 ml volumetric flask and dissolving in a methanol and then made up to mark with methanol. From the above stock solutions transfer suitable aliquots and prepare standard mixture solution having concentration of 500 μ g/mL of ramipril and telmisartan For simultaneous quantitative studies of both drugs, a series of standard solutions containing both the drugs were prepared by appropriate dilution of mixture of working standard stock solutions.

Preparation of Standard Working Solutions for Assay

Standard Working concentration prepared from the standard stock solution for the determination of assay of both drugs was 600 ng/spot used for quantitative studies.

HPTLC Method development

For HPTLC analysis, initially various mobile phases and stationary phases were tried in attempts to obtain the best separation and resolution between ramipril and telmisartan. The mobile phase consisting Acetone: Benzene: Ethyl acetate: Glacial acetic acid in the proportion of (6:4:1:0.05, v/v/v/v) selected that gave satisfactory separation and gave two well resolved peaks for ramipril and telmisartan which is shown in Fig. I. As ramipril and telmisartan exhibit significant absorbance at wavelength 210 nm and 296 nm were selected as detection of wavelength for the simultaneous determination of ramipril and telmisartan, respectively. The R_f value for ramipril and telmisartan was 0.35 and 0.67 respectively. Various system suitability test parameters were calculated and are shown in Table I.

Method Validation

The developed method was method validated for the simultaneous assay determination of ramipril and telmisartan using following parameters.

Linearity (calibration curve)

Linearity was checked by preparing standard solutions of both ramipril and telmisartan at five different concentration levels in the same volumetric flasks using their respective stock solutions. The calibration curves for ramipril and telmisartan were drawn in the concentration range of 300 to 1800 ng/spot and 100 to 1800 ng/spot respectively. The calibration curves were constructed by plotting peak areas versus concentrations with the help of win-CATS software which are shown in graph I and graph II. Each reading was the average of three determinations. The regression coefficient (R^2) for calibration curve of ramipril and telmisartan was 0.998 and 0.996 respectively

Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of ramipril and telmisartan by the standard addition method. For that Known amounts of standard solutions of ramipril and telmisartan (1, 2, and 3 μ L), were added to prequantified sample solutions of capsule dosage forms and determining their assay The mean recovery data for each level (at 95% confidence limits) and its percentage recoveries are presented in Table III.

Precision

Intra-day precision of the method were evaluated for synthetic mixtures of ramipril and telmisartan by repeated injecting (n=6) at three different independent concentrations i.e. 2000, 3000, 4000 ng/spot without the changing the position of the plate. The R.S.D.values ranged from 0.72-1.16 % and 0.73 – 1.01% for ramipril and telmisartan, respectively as shown in Table III. Interday precisions of the proposed method were determined by estimating the corresponding responses 3 times on the 3 different days over a period of 1 week for 3 different concentrations of ramipril and telmisartan (2000, 3000 and 4000 ng/spot). The R.S.D.values ranged from 0.83 – 1.29 % and 0.74 – 1.05 % for ramipril and telmisartan, respectively as shown in Table III.

*Assay determination for ramipril and telmisartan from formulations**Sample Stock Solution Preparation*

Accurately weighed and transferred one intact capsule into 250 ml volumetric flask and dissolved by water. The suspension sonicated for 15minutes and the final volume was made up to mark with water. Filter the resulting solution through Nylon filter having a pore size 0.45 μ . Discard first few ml of filtrate and further dilute 2 ml of this solution to 10ml with methanol to give a solution containing 200 μ g/ml for ramipril and telmisartan.

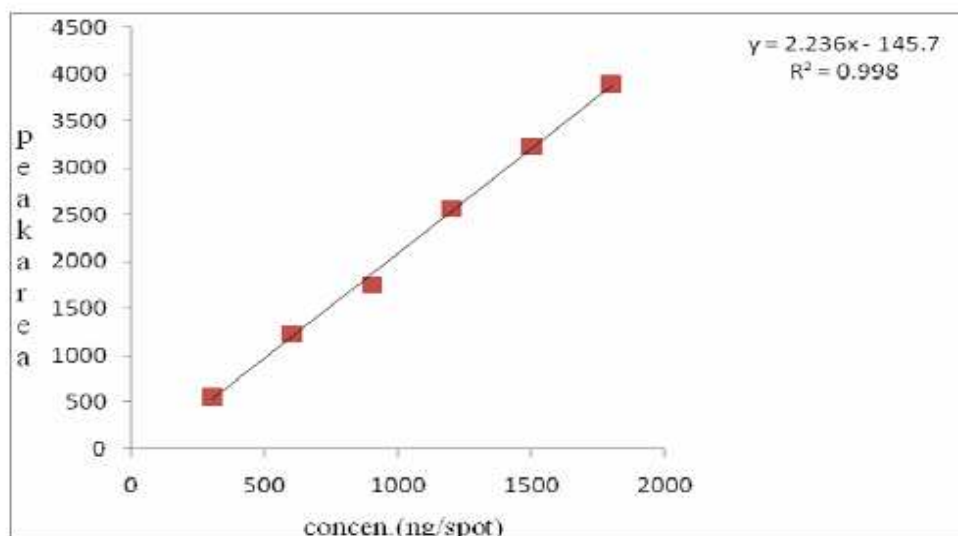
Simultaneous quantification of ramipril and telmisartan in combined Capsules dosage form

The proposed validated method was successfully applied to determine ampicillin and dicloxacillin in their combined capsule dosage form. From the above sample stock solution 15 μ l was applied on pre-washed TLC plate, developed in the above mobile phase, dried in air and photometrically analyzed as described above. From the peak area obtained in the chromatogram, the amounts of all the drugs were calculated and results of assay shown in Table IV.

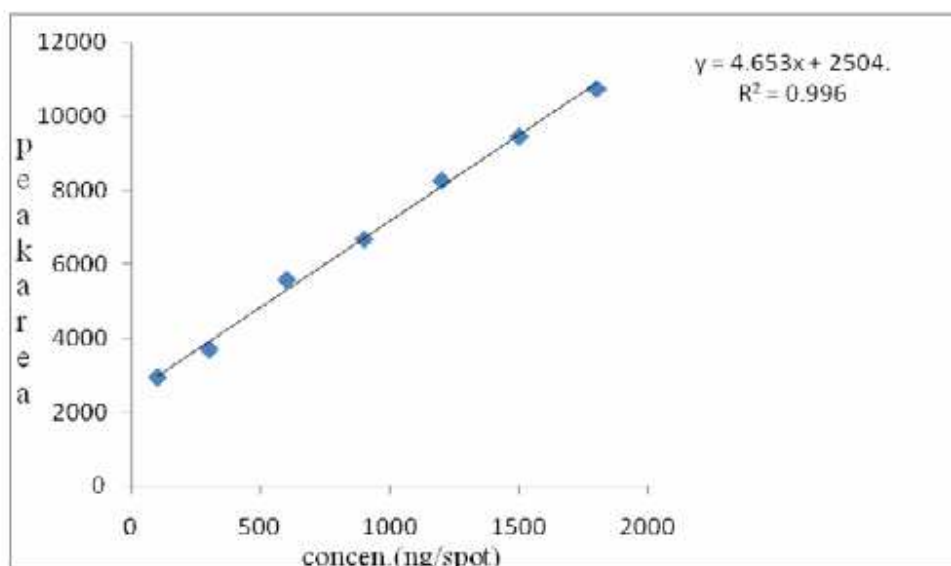
RESULTS AND DISCUSSION

Literature survey reveals that no HPTLC method has been reported for simultaneous determination of ramipril and telmisartan. So, the proposed HPTLC method was optimized with several solvent systems. The mobile phase Acetone: Benzene: Ethyl acetate (5:3:2, v/v/v) gave good resolution with R_f values of 0.35 and 0.68 for ramipril and telmisartan, respectively. In order to reduce the broadening of peak, 0.03 mL of glacial acetic acid was added. Resolution of the peaks for mixture of standard drugs with clear baseline separation was obtained and for individual

drug shown in Fig. II and Fig. III. The calibration curves for ramipril and telmisartan were constructed by plotting area and concentration which is shown in graph I and graph II. The validation parameters were studied for proposed method. The method was found to be accurate with % recovery 99.9% – 100.48% for ramipril and 99.07% – 99.62% for telmisartan. The method was found to be precise with CV 1.63-1.92 for intraday (n=3) and CV 1.16-1.83 for interday (n=3) for ramipril and CV 1.40-1.81 for intraday (n=3) and CV 1.13-1.32 for interday (n=3) for telmisartan. Summary of validation parameters is shown in Table III. This proposed validated method was applied for the estimation of marketed formulations and assay results shown in Table IV. The results of analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of ramipril and telmisartan. The methods can be used for the routine simultaneous analysis of the ramipril and telmisartan in pharmaceutical preparations.



Graph I. Calibration Curve for ramipril



Graph II. Calibration Curve for telmisartan

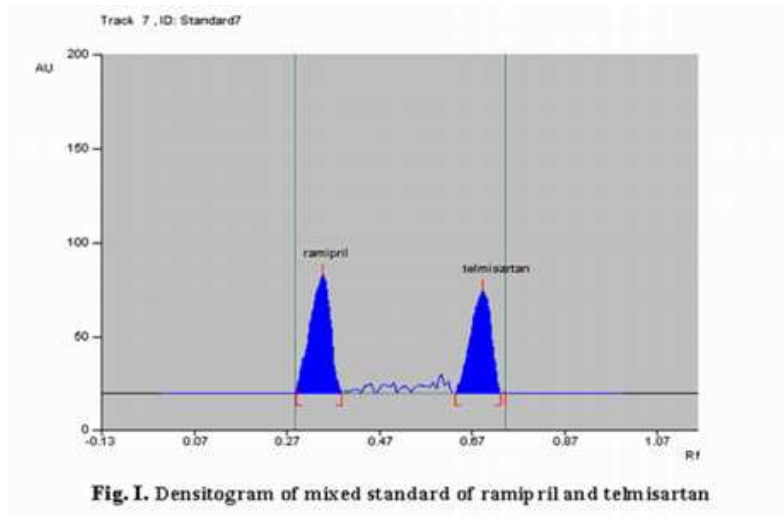


Fig. I. Densitogram of mixed standard of ramipril and telmisartan

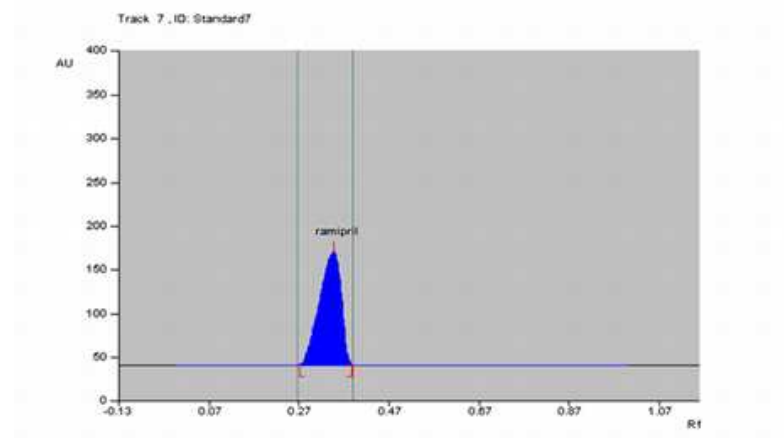


Fig. II. Densitogram of ramipril

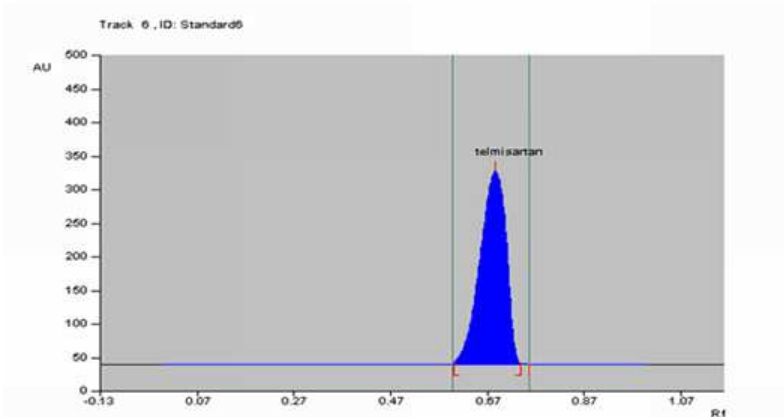


Fig. III. Densitogram of telmisartan

Table I System suitability parameters

System suitability parameters	Proposed method	
	Ramipril	Telmisartan
R _f	0.35	0.67

Number of samples analyzed is three

Table II Regression analysis of the calibration curves for ramipril and telmisartan for the proposed HPTLC method

Parameters	Ramipril	Telmisartan
Linear Range(ng/spot)	300-1800	100-1800
Slope	2.236	4.653
Intercept	-145.7	2504
Regression Co-efficient R ²	0.998	0.996

Table III Summary of validation parameters of HPTLC

Parameters	Ramipril	Telmisartan
%Recovery	99.9± 0.078 to 100.96 ± 0.672	99.07 ± 0.663 to 100.56 ± 0.393
Precision(CV)		
Intra-day (n=3)	1.63-1.92	1.40-1.81
Inter-day (n=3)	1.16-1.83	1.13-1.32
LOD (ng/spot)	130	43
LOQ (ng/spot)	300	100

^a LOD = Limit of detection.

^b LOQ = Limit of quantification.

^c RSD = Relative standard deviation

Table IV Assay results for the combined dosage form using the proposed HPTLC method

Formulation	Labeled Amount (mg)		Amount Found (mg)		% Ramipril	%Telmisartan
	Ramipril	Telmisartan	Ramipril	Telmisartan		
Brand-I	5	40	4.90	39.5	98.0 ±1.42	98.75 ±1.32
Brand-II	5	40	4.95	38.85	99.0 ±0.71	99.52 ±0.18

REFERENCES

- [1] Frampton JE, Peters DH. *Drugs*. **1995**; 49:440–66.
- [2] Benson SC. *Hypertension*. **2004**; 43: 993-1002.
- [3] Kumar VM, Muley PR. *Indian pharm*. **2005**; 36: 69-72.
- [4] Chen B, Liang Y, Wang Y, Deng F, Ping Z. *Anal. Chim. Acta*. **2005**; 540: 367-373.
- [5] Hempen C, Glasle L, Kunz U, Karst U. *Anal. Chim. Acta*. **2006**; 560: 41-49.
- [6] Robert ED, Peter S, Herbert N, Kenneth S, William IF. *J Pharm. Sci*. **2000**; 89: 1465-1479.
- [7] Shen J, Jiao Z, Liz D, Shix J, Zhong M. *Pharmazie* **2005**; 60: 418-420.
- [8] Palled MS, Chatter M, Bhat AR. *Indian J Pharma. Sci*. **2005**; 67: 108-110.
- [9] Torrealday N, Gonzalez L, Alonso RM, Jimenez RM, Ortiz LE. *J Pharm. Biomed. Anal*. **2003**; 32: 847-857.
- [10] Rontogianni MA, Markopoulou CK, Koundourellis JE. *J. Liq. Chromato. & Related Tech*. **2006**; 29: 2701-2719.
- [11] Majed AA, Belal F, Abadi A, AlObaid AM. *Farmaco*. **2000**; 55: 233-238
- [12] Rahman N, Ahmad Y. *AAPS Pharm. Sci. Tech*. **2005**; 6(3): E543-E551.
- [13] Abdellatef HE. *Spectrochim. Acta. Part A: Mol. and Biomol. Spectro*. **2007**; 66: 701-706.
- [14] Abdellatef HE, Ayad MM, Taha EA. *J Pharm. Biomed. Anal*. **1999**; 18(6): 1021- 1027.
- [15] AlMajed A, Belal F, AlWarthan AA. *Spectrosc. Letters* **2001**; 31: 211-220.
- [16] Bhushan R, Gupta D, Singh S. *Biomed. Chromato*. **2005**; 20: 217-224.
- [17] Patil KR, Rane VP, Jaiprakash N, Shinde DB. *J Chromat*. **2008**; 67: 575-582.