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A NEW METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN AND RAMIPRIL BY RP-HPLC

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the determination of Telmisartan and Ramipril in combined tablet dosage form. A reverse phase C_{18} column was used as stationary phase of 250x4.6 mm i.d and 5mm partial size, with mobile phase consisting of phosphate buffer of P^H 3.0 and acetonitrile in the proportion of 60:40 v/v respectively was used. The flow rate was 1.0 ml/min and the effluents were monitored at 245nm. The retention time was 5.35 and 10.3 minutes. The detector response was linear in the concentration of 16 to 24µg/ml for Telmisartan with 0.9998 as the value of correlation coefficient and for Ramipril the linearity in the range of 2 to 3µg/ml with 0.9998 as the value of correlation coefficient**.** The Limit of measurements such as limit of detection and limit of quantification were found to be 9.47µg and 31.57µg for Telmisartan and For Ramipril 1.16µg and 3.88µg, respectively. The percentage relative standard deviations for the assay values were found to be 1.11 and 0.518 for Telmisartan and Ramipril. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate. Hence, this method can be easily and conveniently adopted for routine analysis of Telmisartan and Ramipril in combined tablet dosage form.

Keywords: RP-HPLC, LOD, LOQ, Telmisartan, Ramipril.

INTRODUCTION

The present work deals with the studies carried out on the development, optimization and validation of RP-HPLC and HPTLC methods for the simultaneous estimation of Telmisartan and Ramipril in combined dosage form. [1-2]Market is folded with combination of drugs in various dosage forms. The multi-components formulations have gained a lot of importance now a days due to greater patient acceptability, increased potency, multiple action, fewer side effects and quicker relief.

Telmisartan[3-7] is an Angiotensin – II antagonist (ARB). The effect of Angiotensin –II include vasoconstriction that leads to increase in sympathetic activity and stimulates the secretion of aldosterone that leads to increase in sodium and water retention and left ventricle hypotrophy. Telmisartan blocks the binding of Angiotensin – II to the Angiotensin – I (AT_1) receptors in many tissues, such as vascular smooth muscle and the adrenal glands. Blockade of Angiotensin – I receptor inhibits the negative feedback of Angiotensin - II on rennin secretion, but the resulting increased plasma rennin activity and Angiotensin II circulation levels do not overcome the effect of Telmisartan on blood pressure. Telmisartan indicated for the treatment of hypertension. It may use alone or in combination with other antihypertensive agents. The drug is White to yellowish solid having the Chemical name 4^1-[(1, 4^1-dimethyl-2^1-propyl [2, 6^1-bi-1H Benzimidazol]-1^1-yl methyl]-[1, 1-biphnyl] 2-carboxylic aid. Empirical formula of $C_{33}H_{30}N_4O_2$ with Molecular weight -514.63gms

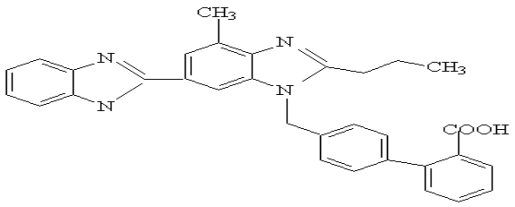

Fig No: 01 Showing the Structure of Telmisartan

Ramipril is a non sulfhydryl Angiotensin converting enzyme (ACEI). The mechanism action of Ramipril is Blockade of conversion of Angiotensin –I to Angiotensin –II and helps in reducing blood pressure in hypertensive patients. Ramiprilat is the diacid metabolite of Ramipril. Ramiprilat produced from Ramipril by hepatic cleavage of ester group in liver. Ramipril indicated for the treatment of hypertension. It may used alone or in combination with other antihypertensive agents. It is a White crystalline solid with Empirical formula $C_{23}H_{32}N_2O_5$ having the Chemical name 4-(2-(1-ethoxy carbonyl aminopropanol)-4-

Azabicyclo (3-3.0) octane-3- carboxylic acid with Molecular weight of 416.5gms, Slightly insoluble in water and Freely soluble methanol

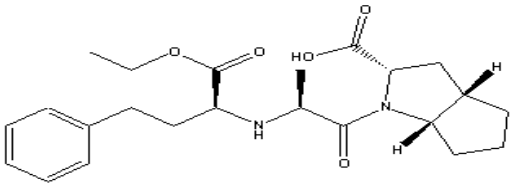

Fig N0: 02- Showing the structure of Ramipril

Telmisartan and Ramipril is the combination of Angiotensin receptor blocker (ARB) and Angiotensin converting enzyme inhibitor (ACEI). This combination completely blocks the Rennin Angiotensin Aldosterone system (RAAS) in hypertensive. This combination particularly useful to treat high blood pressure, that can damage the blood vessels of the Brain, Heart and Kidneys resulting in a stroke, Heart failure and kidney failure. By lowering blood pressure, Telmisartan and Ramipril can reduce the risk of having damage to Kidneys, Heart and other organs.

MATERIALS AND METHODS[8-15]

Instrumentation: Shimadzu HPLC-LC 2010 CHT with class VP version 6.12 with chemstation software.

Reagents and Chemicals: Acetonitrile HPLC grade**,** Orthophosphoric acid AR grade**,** Potassium di-hydrogen phosphate AR grade, Methanol HPLC grade**,** Water –Milli Q grade Reference Standards

Optimized chromatographic conditions

Column : Hypersil ODS C_{18}; 4.6 x 150 mm, 5microns
Mobile Phase : 10 mM pot. Di hydrogen phosphate: acetonitrile (60:40).
PH : 3.0 ±0.01
Flow rate : 1 ml/min
Detector : UV
Injection volume : 20µl
Column temperature : Ambient
Wavelength : 245 nm
Run time : 15 minutes.

Label claim: TELM - 40 mg and RAMI - 5 mg

Preparation of buffer

10mM potassium di-hydrogen phosphate solution is prepared in water. I.e 1360.1 mg dissolved in 1000 ml of distilled water PH is adjusted to 3.0 ±0.01with orthophosphoric acid and filtered through 0.45 µm membrane filter.

Preparation of Mobile Phase

Mobile phase is prepared by mixing 600 ml of buffer and 400 ml of acetonitrile (60:40).

Preparation of Standard Stock Solution

An accurately weighed quantity of 40 mg of Telmisartan and 5 mg of Ramipril is transferred into a 100 ml volumetric flask. Dissolved with 25 ml of methanol and diluted to required volume with mobile phase, having the concentration of 0.4 mg/ml of Telmisartan and 0.05 mg/ml of Ramipril.

Preparation of Standard Solution

From the standard stock solution 5 ml is pipette out into 100 ml volumetric flask and made up the volume with mobile phase, having the concentration of 0.02 mg/ml of Telmisartan and 0.0025 mg/ml of Ramipril.

Preparation of Sample Solution

Twenty tablets were weighed and ground to a fine powder. An amount of power equivalent to 40 mg of Telmisartan and 5 mg of Ramipril were weighed accurately and transferred into a 100 ml volumetric flask containing 25 ml of methanol and sonicated for 30 min. and diluted to 100 ml with mobile phase, then the solution was filtered through 0.45 µm membrane filter and 5 ml of filtrate taken into 100 ml volumetric flask and made up to the volume with mobile phase.

RESULTS AND DISCUSSION

After several trails with various solvents, mobile phase system composed of phosphate buffer of P^H 3.0 and Acetonitrile in the proportion of 60:40 respectively was chosen for the simultaneous estimation of Telmisartan and Ramipril in combined dosage form by RP-HPLC. This mobile phase composition offered maximum resolution for the drug at the detection wavelength of 245nm.

Mobile phase with the flow rate of 1 ml/min gave optimum separation with good resolution between the peaks. A reverse phase C_{18} column was used as stationary phase. The retention time of Telmisartan and Ramipril were found to be 5.35 and 10.3 minutes, respectively. **(Fig 3-6)** The total time of analysis was less than 12 minutes. The percentage label claim for Telmisartan and Ramipril were found to be 100.49 and 100.19, respectively **(Table 1) & (Fig 6).**

From the calibration curve constructed by plotting concentration vs. peak area, it was found that there exists a linear relationship in the concentration range of 16 to 24µg/ml for Telmisartan **(Table 2)**, with 0.9998 as the value of correlation coefficient **(Table 2a)** and for Ramipril the linearity in the range of 2 to 3µg/ml **(Table 3),** with 0.9998 as the value of correlation coefficient **(Table 3a) & (Fig 7 & 8).**

System suitability studies were carried out in which the Resolution between the peaks was found to be 4.38. The asymmetric factors for Telmisartan and Ramipril were 1.48 and 1.56, respectively. Telmisartan was found to have a value of 2945 as its number of Theoretical plates and for Ramipril it was 4738 **(Table 4).**

For system precision studies, the standard solution was prepared at working concentration and analysis was carried at replicate. The percentage relative standard deviation was calculated for the peak areas of each drug and it was found to be 1.24 for Telmisartan and 0.5 for Ramipril **(Table 6).**

For method precision, the sample solution at working concentration was analyzed in replicate as per the assay method. The percentage relative standard deviations for the assay values were found to be 1.11 and 0.518 for Telmisartan and Ramipril, respectively **(Table 7).**

The accuracy of the method was studied by performing recovery studies at 5%, 10% and 15% level. The standard drug at the concentration level of 5%, 10% and 15% were added to the sample and the analysis was carried out as per the assay method. The results were expressed in terms of percentage recovery. The values were found to be 99.16 and 99.06 at 5% level, 98.90 and 99.50 at 10% level and 99.31 and 99.11 for Telmisartan and Ramipril, respectively **(Table 5) & (Fig 9,10 & 11).**

Limit of measurements such as limit of detection and limit of quantification were found to be 9.47µg and 31.57µg for Telmisartan and For Ramipril 1.16µg and 3.88µg, respectively **(Table 7)**

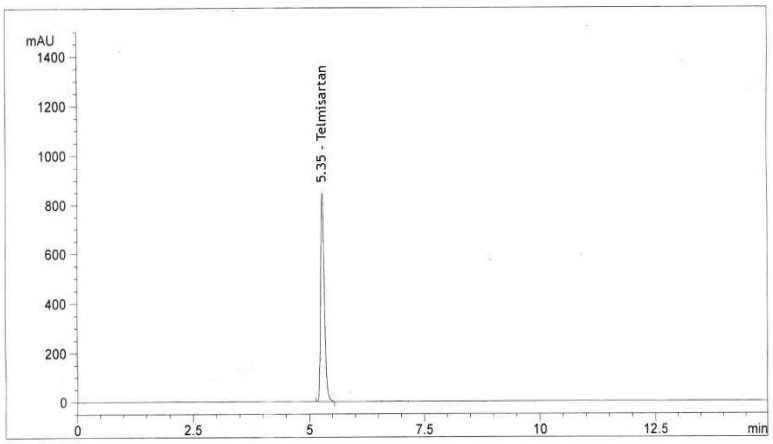

Fig No. 3: Chromatogram of Standard Telmisartan

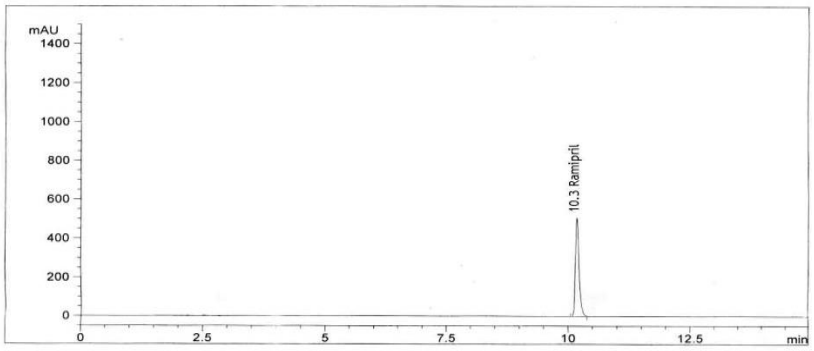

Fig No. 4: Chromatogram of Standard Ramipril

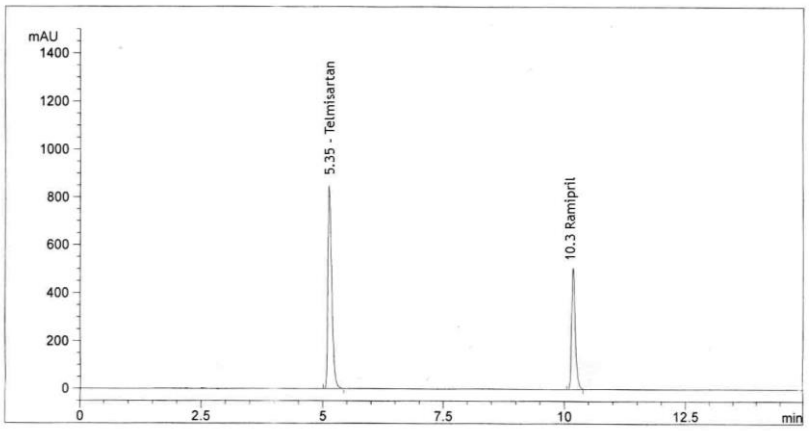

Fig No. 5: Chromatogram of Standard Telmisartan & Ramipril

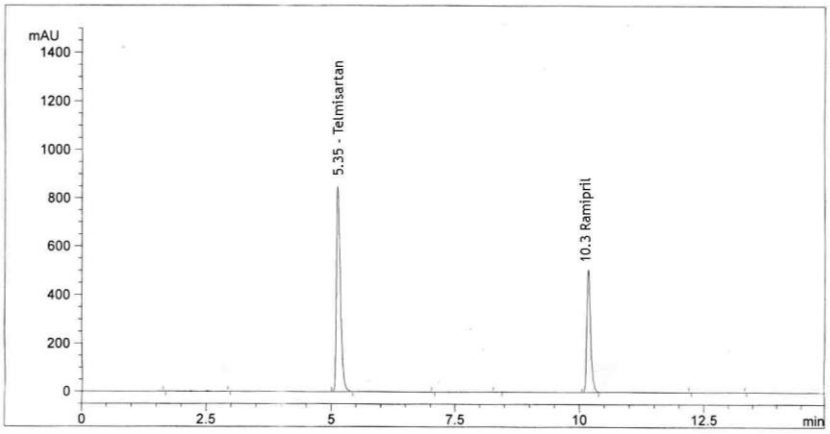

Fig No 6: Chromatogram of Telmisartan & Ramipril Formulation

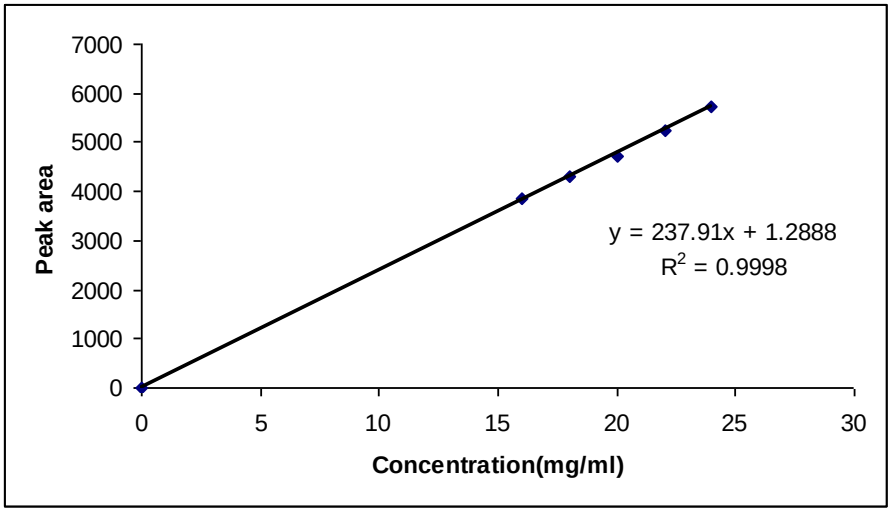

Fig No. 7: Linearity of Telmisartan

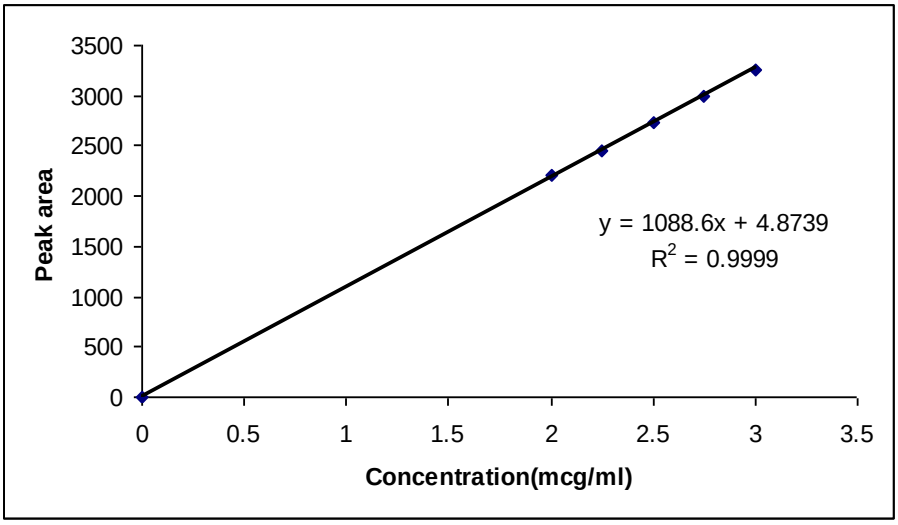

Fig No. 8: Linearity of Ramipril

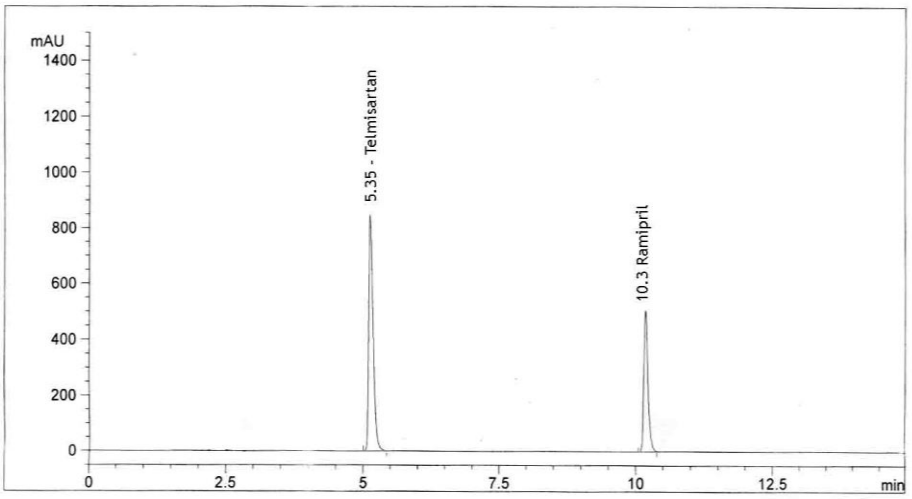

Fig No. 9: Chromatogram of Recovery Studies at 5% Level

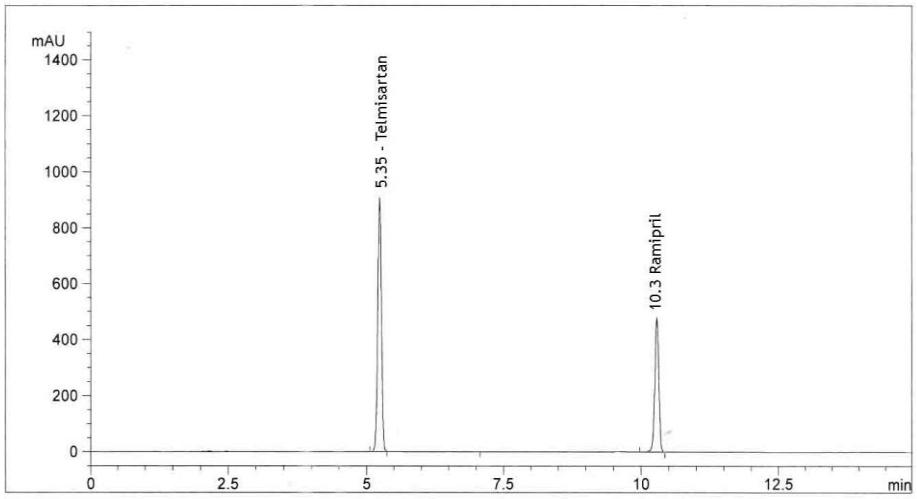

Fig No. 10: Chromatogram of Recovery Studies at 10% Level

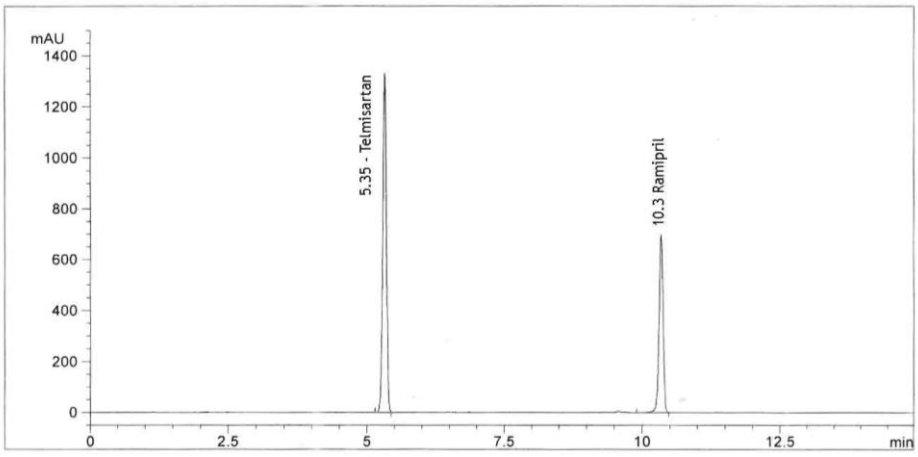

Fig No. 11: Chromatogram of Recovery Studies at 15% Level

TABLE 1: QUANTITATIVE ESTIMATION

Tablet Sample	Label Claim (mg)	Amount present (mg/tablet)	% Label Claim	%Deviation
TELM	40	40.20	100.49	+ 0.49
RAMI	5	5.0099	100.19	+0.19

Each value is mean of three readings

The values obtained for the assay are statistically validated and tabulated in Table 2

TABLE 2: LINEARITY DATA FOR TELMISARTAN

CONCENTRATION (µg/ml)	PEAK AREA
16	3838.48
18	4286.70
20	4700.65
22	5237.62
24	5735.72

From the data obtained correlation coefficient, y-intercept and slope were calculated to provide mathematical estimates of linearity for Telmisartan and tabulated in Table 2a

TABLE 2a: PARA METERS OF TELMISARTAN

PARAMETERS	TELMISARTAN
Linear Dynamic range	16-24µg/ml
Correlation coefficient	0.9998
Slope(m)	237.91
Intercept(c)	1.288

TABLE 3: LINEARITY DATA FOR RAMIPRIL

CONCENTRATION (µg/ml)	PEAK AREA
2.00	2200.66
2.25	2450.88
2.50	2725.44
2.75	2995.46
3.00	3264.802

From the data obtained correlation coefficient, y-intercept and slope were calculated to provide mathematical estimates of linearity for Ramipril and tabulated in Table 3a

TABLE -3a ANALYTICAL PERFORMANCE PARA METERS OF RAMIPRIL

PARAMETER	RAMIPRAMIL
Linear Dynamic range	2 – 3 µg/ml
Correlation coefficient (r)	0.999
Slope (m)	1088.6
Intercept (c)	4.8739

SYSTEM SUITABILITY

System suitability parameters like resolution and asymmetry factor or tailing factor are studied and tabulated in **Table 5**

TABLE 5: SYSTEM SUITABILITY DATA

Parameter	TELM	RAMI
Resolution	4.38	
Asymmetry factor	1.48	1.56
No. of Theoretical plates	2945	4738
Tailing factor	1.2	1.32

RECOVERY STUDIES

TABLE 6: RECOVERY STUDIES -The results are tabulated in Table 6

Sample	Amt. of standard added (mg)	Amt. of drug recovered (mg)	% Recovery	% Mean Recovery	% RSD
TELMI	2 2 2	1.986 1.982 1.970	99.30 99.70 98.50	99.16	0.209
	4 4 4	3.938 3.968 3.963	98.45 99.20 99.07	98.90	
	6 6 6	5.98 5.94 5.96	99.6 99.00 99.33	99.31	
RAMI	0.25 0.25 0.25	0.246 0.249 0.248	98.40 99.60 99.20	99.06	0.242
	0.5 0.5 0.5	0.498 0.492 0.495	99.60 98.40 99.00	99.50	
	0.75 0.75 0.75	0.747 0.735 0.748	99.60 98.00 99.73	99.11	
RAMI	0.5 0.5 0.5	0.496 0.501 0.499	99.2 100.2 99.8	99.73	

TABLE 7: LIMITS OF MEASUREMENTS

Sample	Limit of Detection (µg)	Limit of Quantification (µg)
TELM	9.47	31.57
RAMI	1.16	3.88

CONCLUSION

The proposed HPLC method were found to be simple, specific, precise, accurate and rapid for determination of Telmisartan and Ramipril in combined tablet dosage form. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in

good agreement with their respective label claims and they suggested non –interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Telmisartan and Ramipril in combined tablet dosage form.

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