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SIMULTANEOUS ESTIMATION OF RAMIPRIL AND ATORVASTATIN IN TABLET DOSAGE FORM USING HPTLC.

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ABSTRACT:
A simple, sensitive, precise and accurate high performance thin layer chromatographic (HPTLC) method has been developed for the simultaneous estimation of Ramipril and Atorvastatin in combined dosage form. The method was developed using precoated silica gel 60F254 as stationary phase. The mobile phase used was a mixture of n-Hexane Ethyl acetate(8 2 v/ v). Detection was carried out with ultra-violet detection at 240 nm. The Rf values were about 0.40 ±0.09 and 0.64 ±0.18 for Ramipril and Atorvastatin independently. The advanced method was validated for particularity, range, linearity, accuracy, precision, assay, Limit of Detection(LOD), Limit of Quantification(LOQ) and robustness. The proposed method can be used for the estimation of these medicines in combined dosage forms.

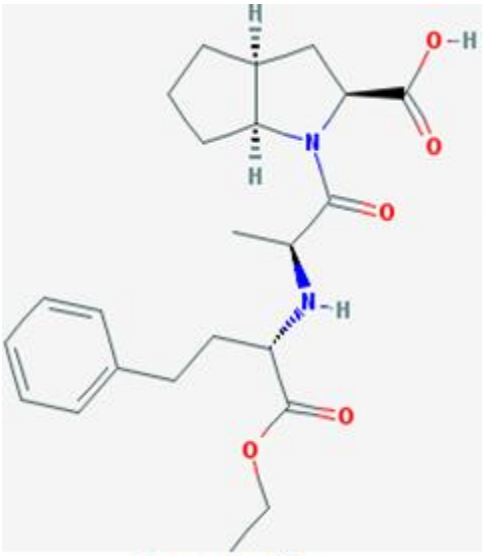
***INDEX TERMS*: Atorvastatin, Ramipril, Tablet and HPTLC.**

INTRODUCTION:

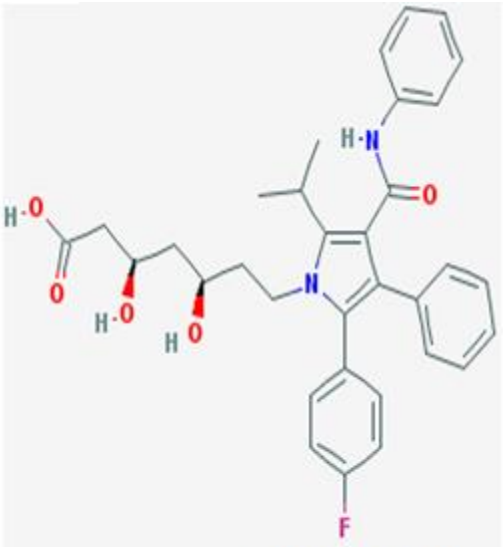
Ramipril(RA) is(1S, 5S, 7S)- 8((2S- 2-(((1S)-1-ethoxycarbonyl-3-phenyl propyl) amino) propanoyl)-8-azabicyclo(3.3.0) octane-7-carboxylic acid, an angiotensin converting enzyme asset, used to treat hypertension and congestive heart failure. Atorvastatin(AT) is(R-(R *, R *))- 2-(4- fluorophenyl)- β, δ- dihydroxy- 5-(1- methyl-ethyl)- 3- phenyl- 4-((phenyl amino) carbonyl)- 1H- pyrrole-1-heptanoic acid, a synthetic lipid- lowering agent which is about a 100 times as potent as the other medicines in its class and at lower costs than utmost of the others. Atorvastatin is an asset of 3- hydroxy- 3- methylglutaryl- coenzyme A reductase.(1)

Literature check revealed that several analytical methods similar as spectrophotometry, spectrofluorimetry, High performance Liquid Chromatography(HPLC), Raman spectroscopy, LCMS- MS and LC- ESI- MS have been reported for the determination of Ramipril and Atorvastatin in pharmaceutical dosage forms and natural samples. The single RP- LC system has been reported to determine Ramipril and Atorvastatin in combined tablet dosage

form. There's no single HPTLC method reported for the estimation of Ramipril and Atorvastatin. Hence, the end was to develop a selective, sensitive and accurate method which can estimate the two factors simultaneously by HPTLC. The proposed method describes a simple, accurate, sensitive and precise HPTLC method for the simultaneous estimation of Rampiril and Atorvastatin in retailed pharmaceutical dosage form.[2]



Ramipril



Atorvastatin

MATERIALS AND METHODS:

The instruments used were as follows:

A. **Camag HPTLC Method**

a. Linomat - 5 sample applicator.
b. Camag TLC Scanner 3
c. Win CATS software V- 1.4.2
d. Hamilton syringe (100 µl)
e. UV-Visible Double beam spectrophotometer (Jasco Model V-730) with single Monochromator.

B. **Marketed Formulation**

a. Brand Name: Cardace Protect 5

Manufactured by: Sanofi.

Label Claim: Each uncoated tablet containsRamipril 5 mg&Atorvastatin10 mg

Ramipril and Atorvastatin pure powder were procured as a gift sample from Arti Pharmaceutical Pvt.Ltd., Bhandup, Mumbai(Maharashtra, India),used as a sample.

1.METHOD DEVELOPMENT:

1.1 Selection of mobile phase and chromatographic conditions:

Chromatographic separation studies were carried out on the working standard solution of Ramipril (200 ng/band) and Atorvastatin (400 ng/band). Initially, trials were carried out using various solvents in different proportions on HPTLC plates, to obtain the desired method suitability parameters. After a few trials, n-Hexane: Ethyl acetate (8: 2 v/v) was chosen as the mobile phase which gave good resolution and acceptable peak parameters. Other chromatographic conditions like chamber saturation time, run length, the distance between tracks, and detection wavelength, were optimized to give reproducible R_f values and symmetrical peak shape for the drug peak.

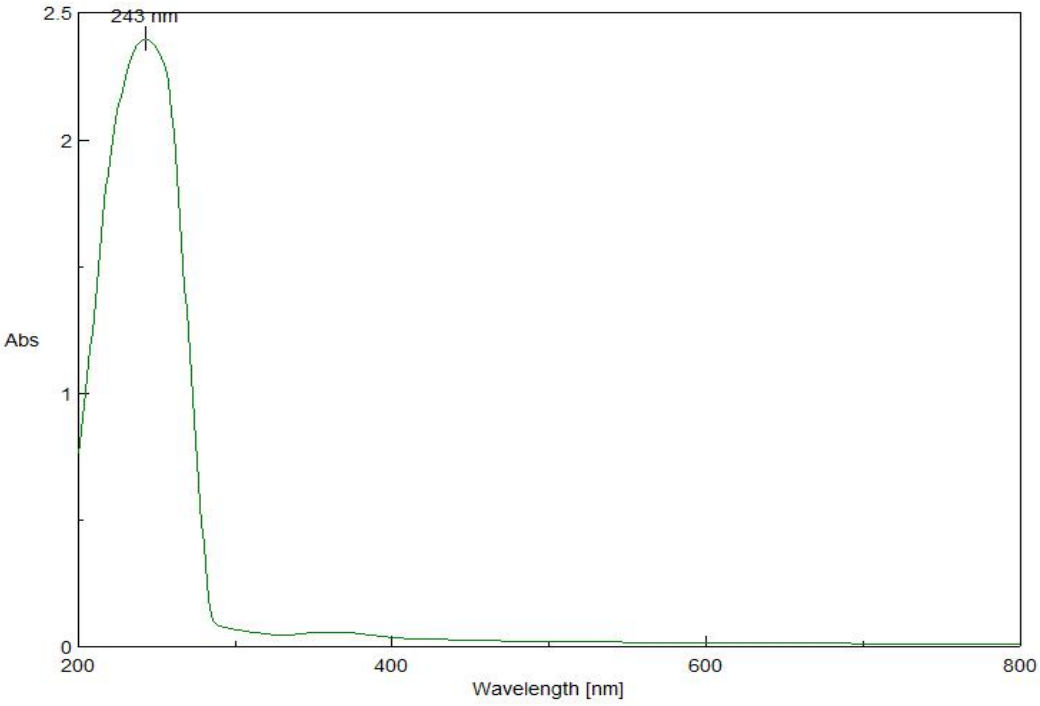
The samples were spotted in the form of bands of width of 6 mm with space between bands of 8 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminum plate 60 F_{254} (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5mm × 0.45mm and a scanning speed of 20mm/sec were employed. The linear ascending development was carried out in a 10cm × 10cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) used as the mobile phase. The optimized chamber saturation time for the mobile phase was 10 min. The length of the chromatogram run was 8cm and the development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometry scanning was performed on CAMAG thin-layer chromatography scanner at 240 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was a deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

1.2 Preparation of Standard stock solution:

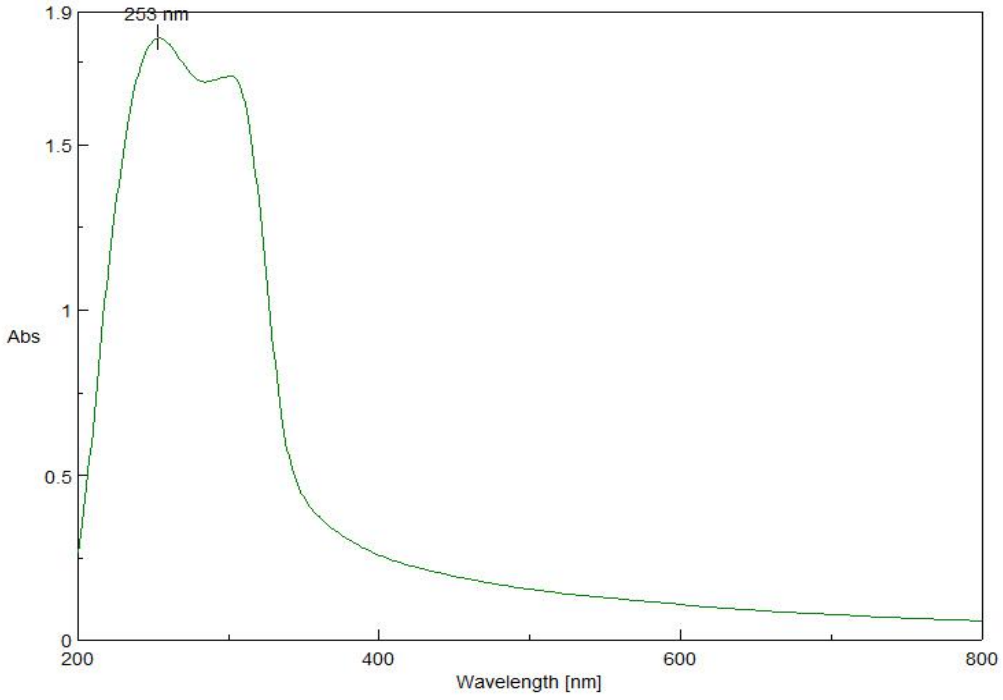
A standard stock solution of Ramipril and Atorvastatinwas prepared separately by dissolving 100mg of the drug in 10 ml of methanol to get a concentration of 1000 µg/ml. From the respective standard stock solution, working standard solution was prepared containing 100 µg/ml (100 ng/µl) of each Ramipril and of Atorvastatin, separately in methanol.

1.3 Selection of Detection Wavelength:

From the standard stock solution, further dilutions were made using methanol and scanned over the range of 200 - 800 nm, and the spectra were obtained. It was observed that both drugs showed considerable absorbance at 240 nm.



(A)



(B)

Fig. 1:UV-VIS Spectra of A) Ramipril and B) Atorvastatin

1.4 Preparation of sample solution(Tablet Formulation Analysis):

Twenty tablets each containing 5 mg of Ramipril and 10 mg of Atorvastatin were weighed and powdered. Powder equivalent to 10 mg of Ramipril (20 mg of Atorvastatin) was transferred to a 10 ml volumetric flask and was diluted with methanol and volume made to 10 ml (1000 µg/ml of Ramipril and 2000 µg/ml of Atorvastatin) with

methanol. The solution was filtered, and further dilutions were made with the mobile phase to get the final concentration of 100 µg/ml of Ramipril and 200 µg/ml of Atorvastatin. 2 µl volume was applied on a TLC plate and developed under optimized conditions.

1.5 Chromatogram and method suitability parameters of the drug:

Under the optimized chromatographic conditions 200 ng/band of Ramipril and 400 ng/band of Atorvastatinwere applied on the TLC plate and the retention factor of repeated applications was found.

Table 1: Chromatographic parameters

Sr. No.	Parameter	Conditions used for Analysis
1	Stationary phase	TLC aluminum plate precoated with silica gel 60 F_{254}
2.	Mobile phase	n-Hexane: Ethyl acetate (8: 2 v/v)
3.	Detection Wavelength	240 nm
4.	Saturation time	10 mins

2.METHOD VALIDATION:

The proposed analytical method was validated as per the International Conference on Harmonization (ICH) guidelines Q2 (R1).

2.1 Specificity:

The peak purity of Ramipril and Atorvastatin was assessed by comparing their separate analytes at the peak apex, peak launch and end positions of the peak. A good correlation was attained for both drugs. Good correlation values and satisfactory peak purity suggest that there's no hindrance in the quantification of the medicines in sample results. This verifies that the method is specific.

2.2 Linearity:

From the standard stock solution (1000 µg/ml) of Ramipril and Atorvastatin, the solution was prepared to contain 100 µg/ml of Ramipril and 200 µg/ml of Atorvastatin, separately. Different volumes were applied on the TLC plate to obtain a linear range. Six replicates per concentration were applied. The linearity (relationship between peak area and concentration) was determined over the concentration range of 100-600 ng/band for Ramipril and 200-1200 ng/band for Atorvastatin.

2.3 Range:

The specified range is normally derived from linearity studies. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical method has suitable levels of precision, accuracy, and linearity.[8]

2.4 Precision:

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter-day variation studies, 3 different concentrations were analyzed on 3 consecutive days, and percentage RSD was calculated.

2.5 Assay:

Tablet formulation analysis was carried out as mentioned under section Tablet Formulation Analysis. Procedure was repeated for six times. Sample solution was applied and area was recorded for each drug. Concentration and % purity was determined from linear equation.

2.6 Accuracy:

To check the accuracy of the method, recovery studies were carried out by adding the standard drugsto the sample at three different levels 50, 100 and 150 %. The basic concentrations of the sample chosen were 2 µl of 100 µg/ml of Ramipril and 2 µl of 1600 µg/ml of Atorvastatin. These solutions were applied on TLC plates in triplicate to obtain the densitogram. The drug concentrations of Ramipril and Atorvastatin were calculated by using linearity equations of Ramipril and Atorvastatin.

2.7 LOD and LOQ:

The limit of detection(LOD) is the smallest amount of analyte that can be detected in a sample, but not to be necessarily quantified, under the stated experimental conditions.The limit of quantification(LOQ) was identified as the smallest quantum of analyte that can be detected and quantified with acceptable accuracy, precision, and variability.The LOD and LOQ are calculated by the signal to noise method.[19]

LOD is calculated from the formula: -

$$LOD = \frac{3.3\,\sigma}{S}$$

Where,

σ = standard deviation of response for the lowest conc. in the range

S = slope of the calibration curve.

The Quantitation limit is expressed as:

$$LOQ = \frac{10\sigma}{S}$$

Where,

σ = standard deviation of response for the lowest conc. in the range

S = slope of the calibration curve.

2.9 Robustness:

The robustness study was done by making small changes in the optimized method parameters like ±0.1 change in pH 1± changes in mobile phase ratio and column temperature.The was no significant impact on the retention time and tailing factor.[12]

RESULT AND DISCUSSION:

3.2 RETENTION FACTOR:

Ramipril = 0.40 ± 0.09

Atorvastatin = 0.64 ± 0.18

Chromatogram of Methanol blank, Ramipril, Atorvastatin and Mixture are shown in Figure 2, 3, 4 and 5

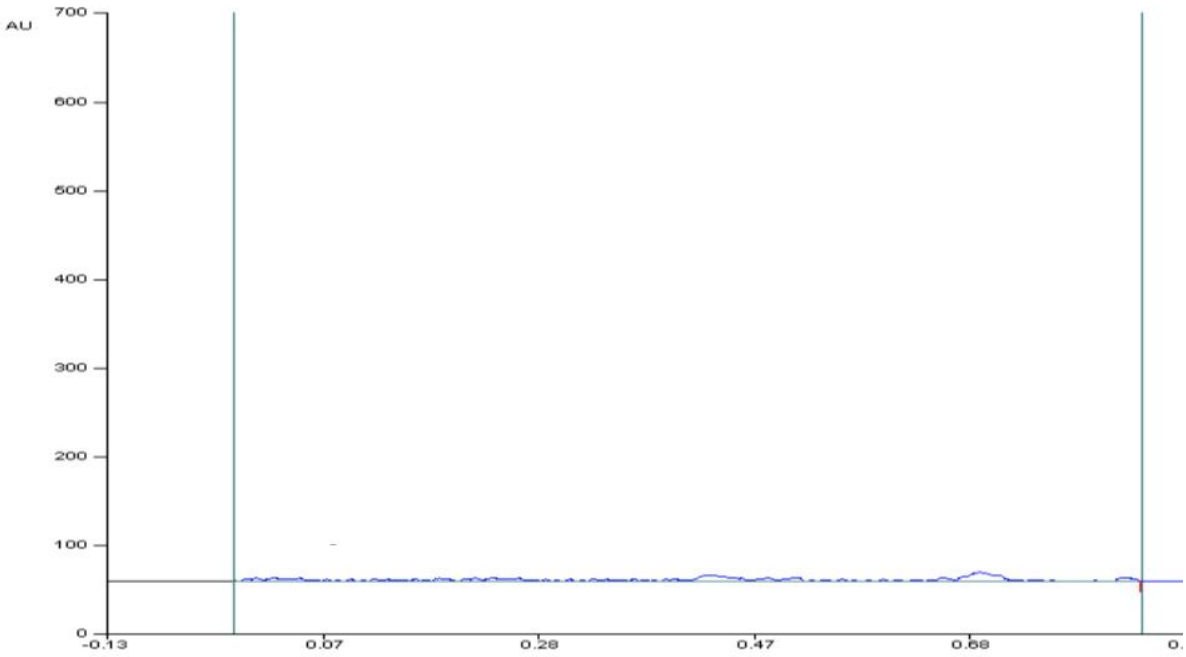


Fig 2.: Densitogram of Mobile Phase blank (Methanol)

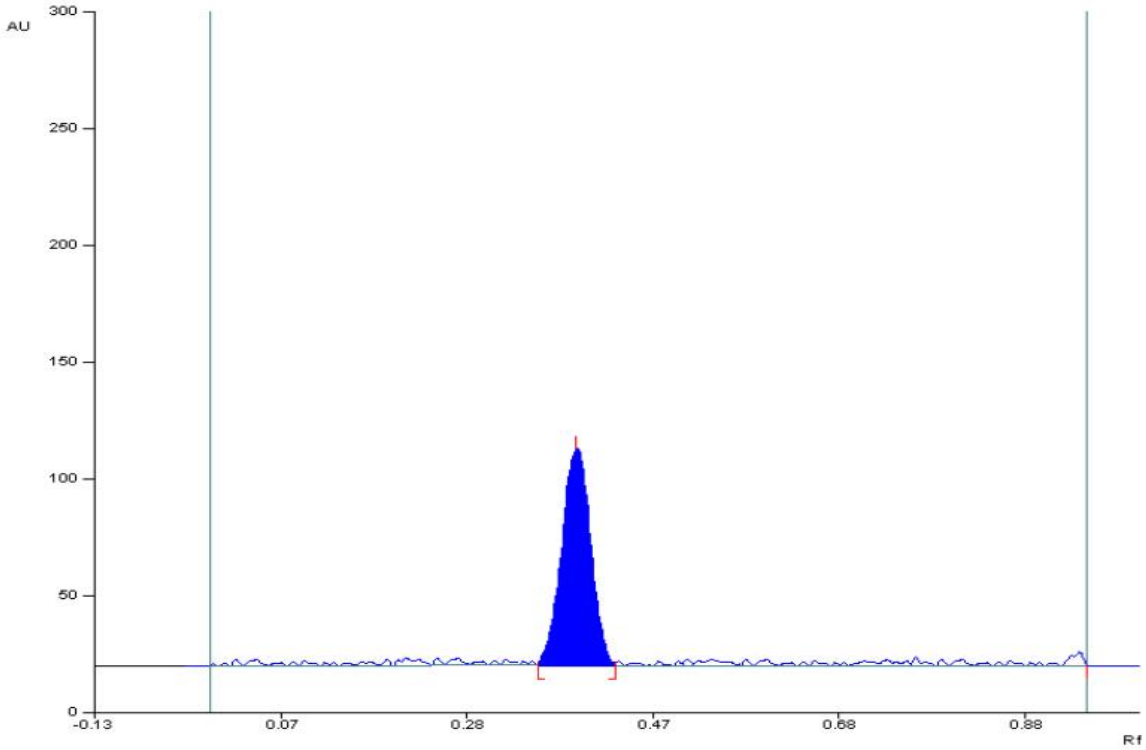


Fig 3:Densitogram of Ramipril (200 ng/band).

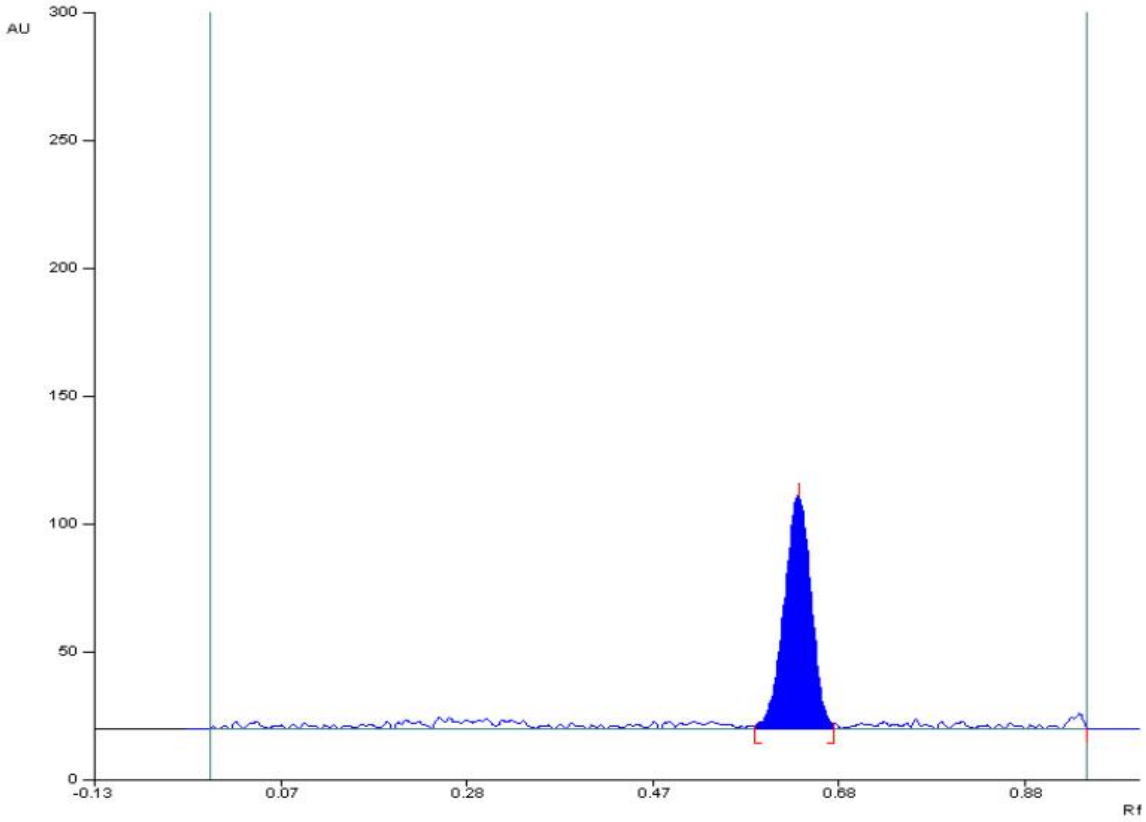


Fig 4:Densitogram of Atorvastatin (400 ng/band).

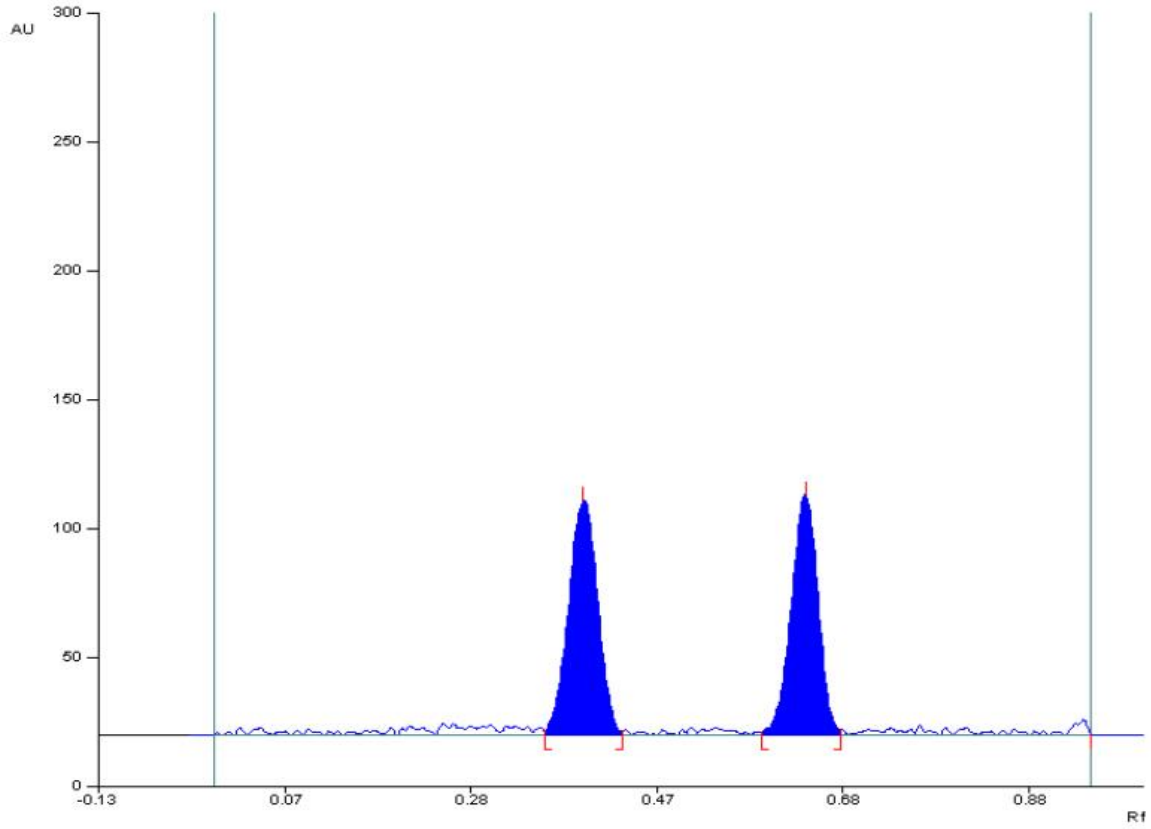


Fig 5:Densitogram of Standard mixture of Ramipril (200 ng/band) and Atorvastatin (400 ng/band)

Table 1: Method Suitability Parameters

Name	**Rf Mean ± % RSD**	**Concentration (µg/ml)**	**Area**	**Asymmetry**
RAMIPRIL	0.40 ± 0.09	200	3272.82	1.07
ATORVASTATIN	0.64 ± 0.18	400	4828.24	1.05

3.2 SPECIFICITY:

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.997, indicating the non-interference of any other peak of degradation product or impurity.

3.3 LINEARITY:

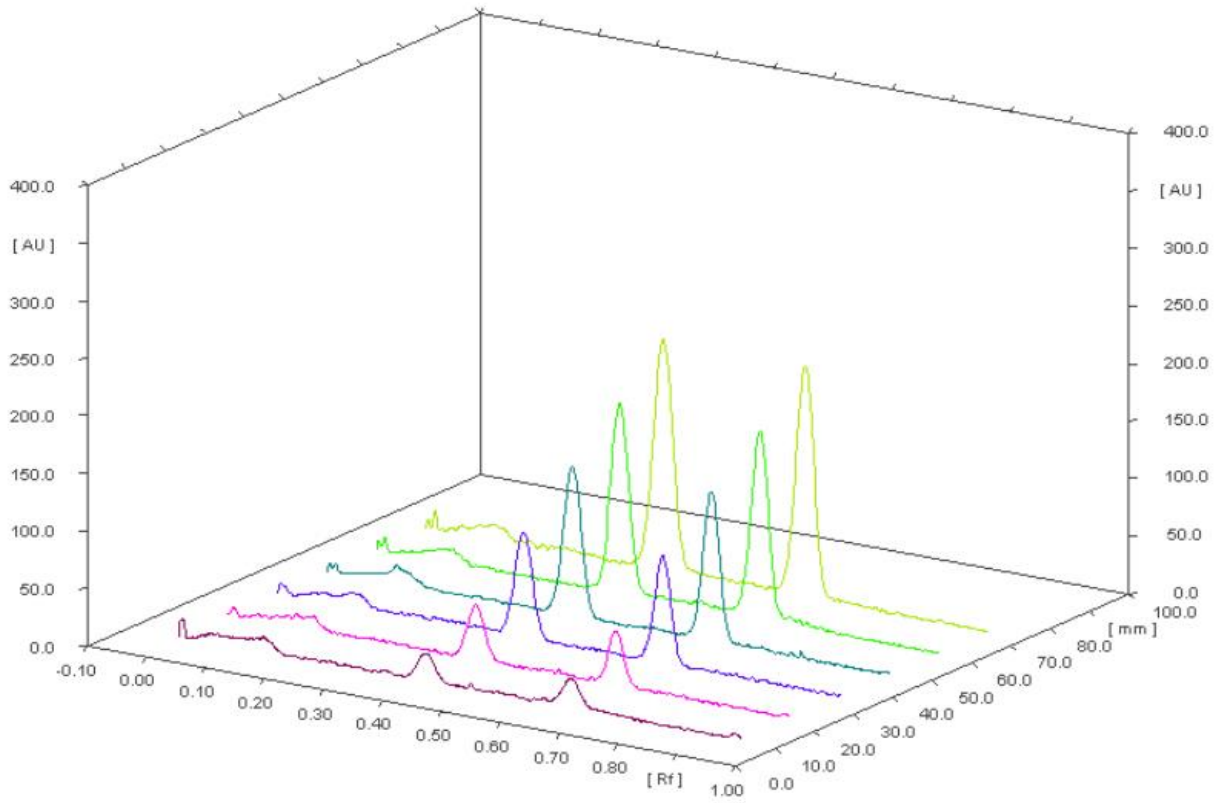


Fig 6:3D Densitogram of Linearity for Ramipril (Rf - 0.40)and Atorvastatin (Rf - 0.64)

Table 2: Linearity study for Ramipril

Replicates	**Concentrations of Ramipril (ng/ band)**					
	100	**200**	**300**	**400**	**500**	**600**
	Peak Area					
1	1722.09	3272.82	4729.05	6322.76	8217.3	9498.4
2	1720.92	3231.9	4803.8	6327.1	8193.3	9572.8
3	1690.22	3184.4	4780.2	6243.4	8128.2	9436.4
4	1711.92	3256.7	4755.4	6286.8	8187.1	9424
5	1688.13	3210.2	4684.2	6301.5	8212.2	9534.1
6	1705.72	3258.2	4842.2	6510.5	8190.2	9470.5
Mean	1706.498	3235.703	4765.792	6332.010	8188.050	9489.367
Std.dev.	14.719	33.531	55.896	92.515	31.793	57.407
%RSD	0.862	1.036	1.173	1.461	0.388	0.605

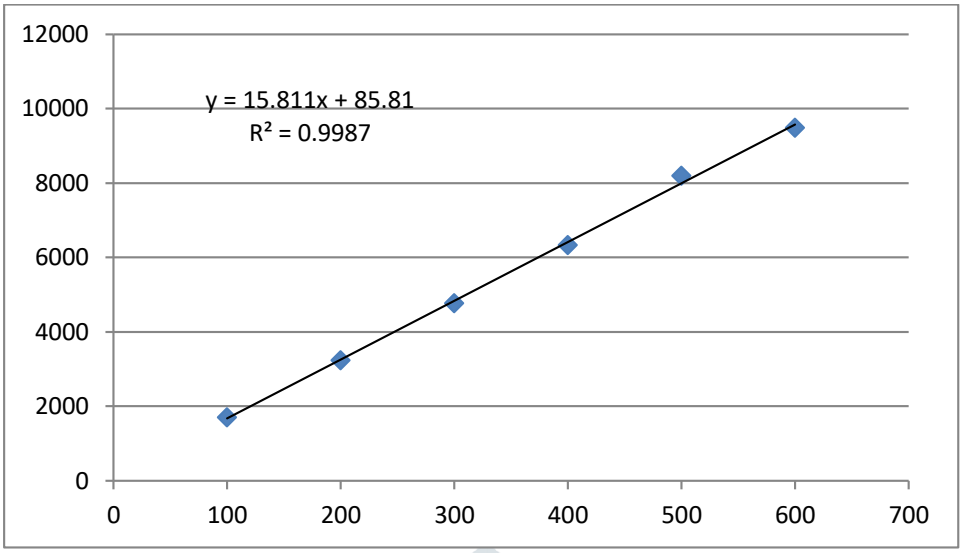


Fig7: Calibration curve for Ramipril

Table 3: Linearity study for Atorvastatin

Replicates	Concentrations of Atorvastatin (ng/band)					
	200	400	600	800	1000	1200
	Peak Area					
1	2904.72	4828.24	6837.6	8461.2	10241.84	11777.88
2	2892.24	4764.27	6732.56	8438.4	10218.89	11766.23
3	2882.4	4694.4	6718.64	8451.72	10162.8	11768.4
4	2942.4	4634.4	6750.24	8391.72	10194.24	11906.44
5	2967.45	4686.4	6732.24	8273.04	10315.32	11821.2
6	2897.39	4663.2	6977.04	8318.64	10197.96	11774.64
Mean	2914.433	4711.818	6791.387	8389.120	10221.842	11802.465
Std.dev.	33.155	71.571	100.545	77.449	52.849	54,798
%RSD	1.138	1.519	1.480	0.517	0.517	0.464

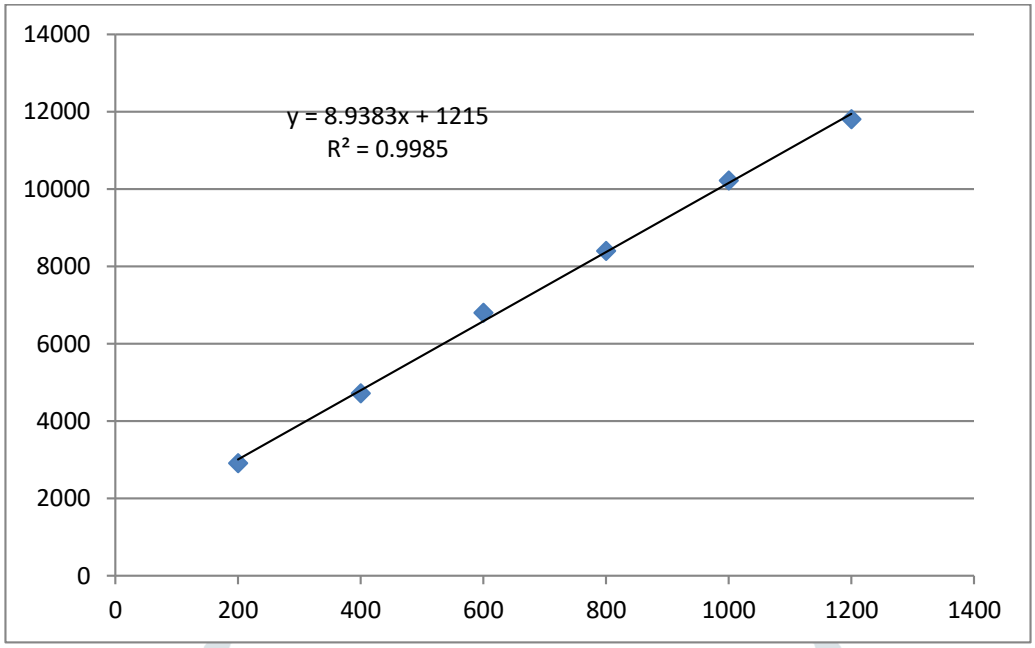


Fig 8: Calibration curve for Atorvastatin

3.4 RANGE:

Ramipril = 100 - 600 ng/band

Atorvastatin =200- 1200 ng/band

3.5 PRECISION:

The results were obtained for Intraday and Inter day variations:

Table 4: Intra-day precision study Ramipril

Concentration (ng/band)	Area	% Recovery	Avg % Recovery ± % RSD
200	3220.4	99.127	99.574 ± 0.416
	3236.9	99.649	
	3246.3	99.946	
400	6460.4	100.794	99.932 ± 0.811
	6398.8	99.820	
	6358.6	99.184	
600	9600.8	100.299	99.417 ± 0.922
	9523.2	99.481	
	9427.2	98.469	

Table 5:Inter-day precision of Ramipril

Concentration (ng/band)	Area	% Recovery	Avg % Recovery ± % RSD
100	3282.8	101.100	100.079 ± 1.139
	3211.6	98.849	

	3257.2	100.289	
200	6494.6	101.334	100.782 ± 0.574
	6462.8	100.831	
	6421.6	100.180	
300	9686.4	101.202	100.383 ± 0.870
	9618.4	100.485	
	9521.5	99.463	

Table 6: Intra-day precision study Atorvastatin

Concentration (ng/band)	**Area**	**% Recovery**	**Avg % Recovery ± % RSD**
200	4776.6	99.616	100.352 ± 0.642
	4819.4	100.813	
	4812.7	100.626	
400	8389.5	100.334	100.386 ± 0.392
	8423.1	100.804	
	8367.2	100.022	
600	11934.2	99.937	99.786 ± 0.379
	11871.8	99.355	
	11947.9	100.065	

Table 7: Inter-day precision study Atorvastatin

Concentration (ng/band)	Area	% Recovery	Avg % Recovery ± % RSD
200	4824.4	100.952	101.507 ± 0.508
	4847.4	101.596	
	4860.8	101.972	
400	8473.4	101.507	100.751 ± 0.234
	8507.3	101.982	
	8491.9	101.765	
600	11850.3	99.155	99.588 ± 0.452
	11946.7	100.054	
	11893.4	99.556	

3.6 ASSAY:

Concentration and % purity was determined from a linear equation. Representative chromatograph of sample analysis was plotted.

Table 8: Assay results of tablet formulation

Sr. no.	Ramipril			Atorvastatin		
	Peak area	Amount recovered (ng/band)	% Recovery	Peak area	Amount recovered (ng/band)	% Recovery
1	3213.3	197.805	98.902	4780.8	398.935	99.734
2	3256.6	200.543	100.272	4814.4	402.694	100.674
3	3230.4	198.886	99.443	4757.2	396.295	99.074
4	3236.8	199.291	99.645	4756.8	396.250	99.062
5	3229.4	198.823	99.411	4818.2	403.119	100.780
6	3244.7	199.791	99.895	4778.4	398.666	99.667
Mean	3235.20	199.190	99.595	4784.30	399.326	99.832
SD	14.74	0.933	0.466	26.81	2.999	0.750
% RSD	0.456	0.468	0.468	0.560	0.751	0.751

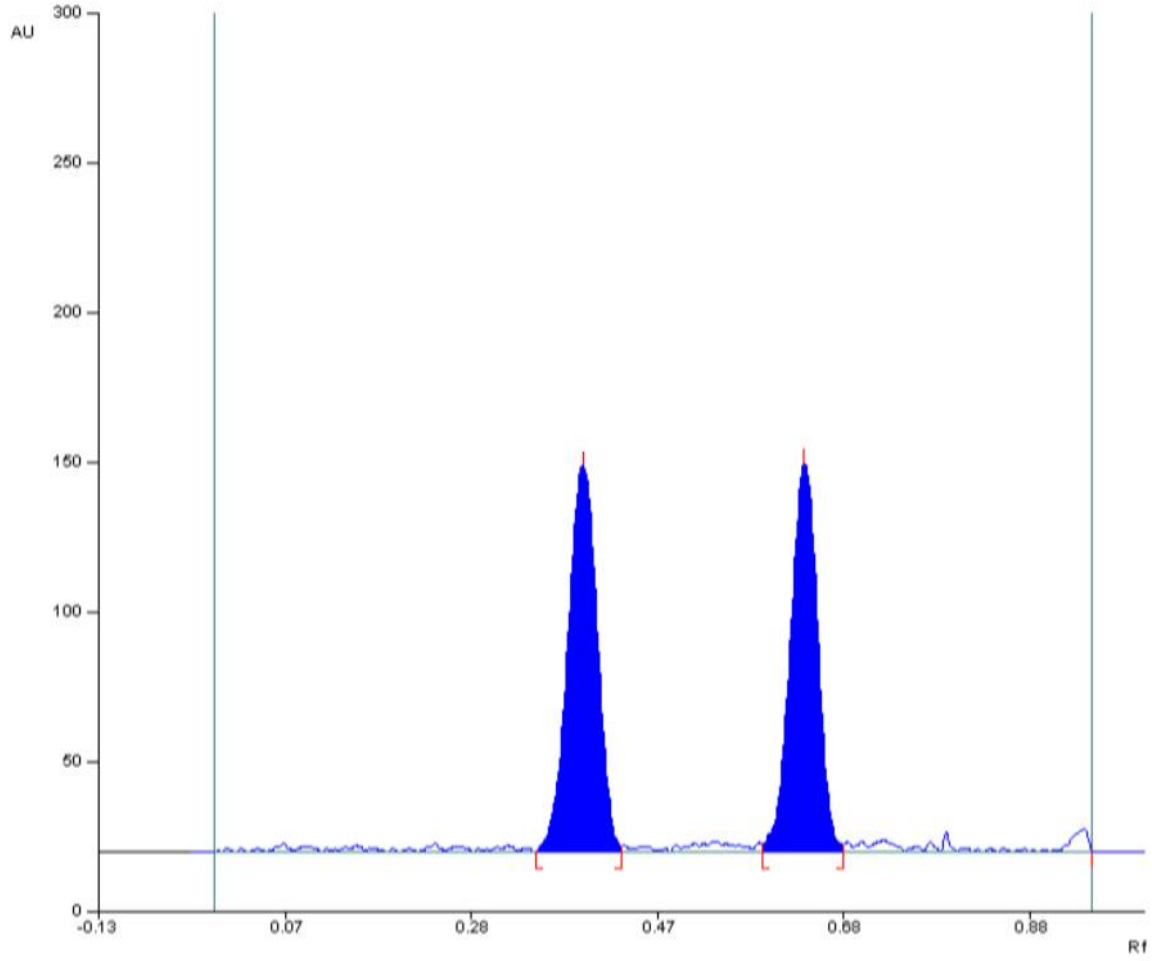


Fig 9:Densitogram of Test Solution Ramipril (200 ng/band) and Atorvastatin (400 ng/band)

3.7 ACCURACY:

Table 9: Recovery studies of Ramipril

Level	Conc. (ng/band)		Area	% Recovery	Mean % Recovery ± % RSD
	Sample	Std.			
50 %	**200**	**100**	**4890.6**	**101.296**	**100.464 ± 0.981**
			4799.5	**99.376**	
			4863.2	**100.719**	
100 %	**200**	**200**	**6448.5**	**100.605**	**100.023 ± 0.735**
			6427.1	**100.267**	
			6359.4	**99.197**	
150 %	**200**	**300**	**8014.1**	**100.288**	**100.326 ± 0.820**
			7953.6	**99.523**	
			8083.5	**101.166**	

Table 10: Recovery studies of Atorvastatin

Level	Conc. (ng/band)		Area	% Recovery	Mean % Recovery ± % RSD
	Sample	Std.			
50 %	400	200	6588.8	100.202	99.563 ± 0.641
			6520.4	98.926	
			6554.4	99.560	
100 %	400	400	8416.8	100.715	101.100 ± 0.416
			8476.4	101.549	
			8439.6	101.034	
150 %	400	600	10172.8	100.218	99.969 ± 0.598
			10189.2	100.402	
			10089.6	99.287	

3.8 LOD:

Limit of detection was found to be:

LOD of Ramipril = 8.308ng/band

LOD of Atorvastatin = 16.608ng/band

3.9 LOQ:

Limit of quantitation was found to be:

LOQ of Ramipril = 25.176ng/ band

LOQ of Atorvastatin = 50.328ng/band

3.10 ROBUSTNESS:

Table 11: Robustness study

Drug	% RSD found for Robustness study (Peak Area)								
	Wavelength			Chamber Saturation Time (min)			Time from application to development (min)		
	239	240	241	9	10	11	0	30	60
Ramipril	0.331	0.471	1.330	0.432	0.484	0.761	1.140	0.428	0.515
Atorvastatin	0.551	0.793	0.679	0.909	0.889	1.501	0.943	1.703	0.860

3.11 SUMMARY OF VALIDATION STUDY:

Table 12: Summary of Validation Study

Sr. No.	Validation Parameter	Results	
		RAMIPRIL	ATORVASTATIN
1.	Linearity	Y= 15.811x + 85.81 R^2 = 0.9987	Y = 8.9383x + 1215 R^2 = 0.9985
2.	Range	100-600 ng/band	200-1200 ng/band
3.	Assay (Mean ± % RSD)	99.595 ± 0.468	99.832 ± 0.751
4.	Precision	% RSD	% RSD
	A) Intraday precision	0.419 – 0.603 %	0.473 – 0.643 %
	B) Interday precision	0.691 – 0.859 %	0.413 – 0.520 %
5.	Accuracy	% Recovery	% Recovery
	50 %	100.464 ± 0.981	99.563 ± 0.641
	100 %	100.023 ± 0.735	101.100 ± 0.416
	150 %	100.326 ± 0.820	99.969 ± 0.598
6.	LOD	8.308 ng/band	16.608 ng/band
7.	LOQ	25.176 ng/band	50.328 ng/band
8.	Specificity	Specific	Specific
9.	Robustness	Robust	Robust

The present study illustrates a simple, accurate and precise HPTLC method for the simultaneous estimation of Ramipril and Atorvastatin. Additionally, the proposed method doesn't bear a complex treatment or sophisticated analytical units, which are generally associated with HPLC and LC – MS/ MS analyses. Reliability was found on by testing a range of validation parameters of the proposed method. Eventually, the proposed method was sensitive and specific; hence, it can be suggested for use for the routine analysis of the cited phytochemicals, either in bulk form or in their combined dosage forms.

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References:

1. Mousmi Kar, Sujit Pillai, Gaurav Sharma, Nitin Deshmukh, Kunwar Nagrndra Krishna Singh, Method Development and Validation for the Estimation of Ramipril and Arorvastatin in Pharmaceutical Formulation by RP-HPLC, Manipal Journal of Pharmaceutical Sciences,2019,5:2.
2. Hari Krishna ,Niharanjandas, Development and Validation of RP-HPLC Method for Simaltaneous Estimation of Atorvastatin Calcium and Ramipril in Tabalet Dosage Forms, Research & Review in Pharmaceutical Sciences,2021.
3. Swati Patel, Bhakti J. Ladva, Sagar S. Patel, Dr .Bhavesh S. Nayak, and Heli R. Vyas Development of Stability Indicating HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Ramipril in Tablet Dosage Form, World Journal of Pharmacy and Pharmaceutical Sciences, 2015, vol 4.
4. Indian Pharmacopoeia, Indian Pharmacopoeia Commission, Government of India ministry of health and family welfare, Controller of India, Delhi, 2010; 6(3):2038-2039.
5. Pishawikar SA, Devolopment and Validtion of method for Simultneous Estimation of Atorvastatin calcium and Ramipril from Capsule Dosage Form by First Order Derivative Spectroscopy, Asian Journal Research Chem, 2009; 1:52-53.
6. https://youtu.be/ut8tF41GuTA
7. "Drug Bank Open Data and Drug Target Data Base", Ramipril,
8. "Drug Bank Open Data and Drug Target Data Base", Atorvastatin,
9. Potdar V.H, RP-HPLC method for Simaltaneous estimation of Atorvastatin Calcium and Ramipril from Plasma, Ars Pharma, 2011;2:14-19.
10. Indian Pharmacopoeia, Indian Pharmacopoeia Commission, Government of India ministry of health and family welfare, Controller of India, Delhi, 2010; 6(2): 849-850.
11. H.Panchal, B.Suhagia, N.Patel, Simultaneous HPTLC Analysis of Atorvastatin Calcium, Ramipril and Apirin in Capsule Dosage Form, Journal of Planar Chromatography-Modern TLC, 2009.
12. https://www.slideshare.net/SwatiSahani/high-performance-thin-layer-chromatographyhptlc
13. https://www.researchgate.net/publication/275533529_A_validated_HPTLC_method_for_the_quantification_of_podophyllotoxin_in_Podophyllum_hexandrum_and_etoposide_in_marketed_formulation
14. Shweta Havele and Sunil Dhaneshwar "Estimation of Metformin in Bulk Drug and in Formulation by HPTLC" J Nanomedic Nanotechnolo, 2010; 1: 102: 1-3.
15. Jyotshna, P. Srivastava, B. Killadi, K. Shanker, Uni-dimensional double development HPTLC-densitometry method for simultaneous analysis of mangiferin and lupeol content in mango(Mangifera indica) pulp and peel during storage, Food Chem.176 (2015) 91–98.
16. International Conference on Harmonization (2005) Q2 (R1) Validation of analytical procedures: text and methodology, International Conference on Harmonization, IFPMA, Geneva.
17. Indian pharmacopoeia, the Indian pharmacopoeia commission, Ghaziabad, govt. Of India ministry of health and family welfare, 2010, vol.II, pp 1349, 1705.

18. T. G. Altuntas and N. Erk, “Liquid chromatographic determination of Atorvastatin in bulk drug, tablets and human plasma”, Journal of Liquid Chromatography and Related Technologies, vol.27, no. 1, pp. 83–93, 2004.
19. “ICH Harmonized Tripartite Guidelines: Validation of Analytical Procedures: Text and Methodology,” Q2 (R1), pp. 1–13, 2005.
20. Deshpande PB, Shridharan G, Anandi L, Jadhav D, Damle MC, Gandhi SV, Validated method development for estimation of Atorvastatin Calcium and Fenofibrate in fixed dose combination by HPTLC,Pharma Review, May 2009,151-153.
21. Suvillan C, Sherma J, Determination of Salicylamide in pharmaceutical tablets by HPTLC with UV absorption densitometry, Acta Chromatographica, No.16,153-164.
22. Indian Pharmacopoeia 2007, Published by the Government of India, Ministry of Health and Family Welfare, the Indian Pharmacopoeia commission, New Delhi Volume I & II,131,1036.
23. Zambare YB, Karajgi SR, Simpi CC,Simultaneous estimation of Atorvastatin and Ramipril by First Derivative Spectrophotometric method, Journal of Pharmacy Research,2(5),2009,874-877.
24. Thamake SL, Jadhav SD, Pishawikar SA, Development and Validation of method for simultaneous estimation of Atorvastatin Calcium and Ramipril from capsule dosage form by First Order Derivative Spectroscopy, Asian J.Research Chem.2(1),Jan-Mar 2009,52-53.
25. Zahid Z, Farooqui MN, Mangle AA, Nikalje AG, Stability indicating HPLC determination of Atorvastatin Calcium in pharmaceutical dosage form, African Journal of Pharmacy and Pharmacology,Vol.2(10)Nov 2008,204-210.
26. Jaha S. M., Validation in Pharmaceutical Industry: Cleaning Validation - A Brief, Vol. 5, Issue 1, January 2017.
27. Chinmaya K. S, Muvvala S., Nalini k. S., Validation of Analytical Methods: A Review, International Journal of Chromatography and Separation Techniques, Volume 2018; Issue 01, 19 January, 2018.