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Research Article

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APPLICATION OF HPTLC DENSITOMETRIC METHOD FOR SIMULTANEOUS QUANTIFICATION OF ROSUVASTATIN CALCIUM AND ASPIRIN

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ABSTRACT

A simple and sensitive thin-layer chromatographic method has been established for analysis of rosuvastatin calcium and aspirin in bulk & capsule dosage form. Chromatography on silica gel 60 F_{254} plates using toluene:butanol:methanol (9:1:0.2 v/v/v) as the mobile phase furnished compact spots at R_f 0.32±0.02 and 0.67±0.03. Densitometric analysis of rosuvastatin calcium and aspirin was carried out in the absorbance mode at 234 nm. The minimum amount that could be authentically detected was found to be 22 and 156 ng for rosuvastatin calcium and aspirin respectively. Limit of Quantitation was found to be 68 and 445 ng for rosuvastatin calcium and aspirin respectively. The linear regression analysis data for the calibration plots showed good linear relationship with respect to peak area in the concentration range 200–1200 ng/spot for rosuvastatin calcium and 1500-9000 ng/spot for aspirin. The method was validated for precision, accuracy and robustness as per ICH guidelines. The proposed HPTLC method can be applied for identification and quantitative determination of rosuvastatin calcium and aspirin in bulk drug and capsule dosage form.

KEY WORDS

Rosuvastatin Calcium, Aspirin, HPTLC, Densitometric Analysis, Quantitative determination.

1. INTRODUCTION

Rosuvastatin calcium (**Figure 1**) is chemically (E)-(3R,5S)-7-{4-(4-flurophenyl)-6-isopropyl-2-{methyl(methylsulphonylamino)]pyrimidine-5-yl}-3,5-dihydroxyhepten-6-oicacid calcium.[1] Rosuvastatin belongs to statin class of drugs used to treat hypercholesterolemia both in patients with established cardiovascular disease as well as those who are at a high risk of developing atherosclerosis. These drugs inhibit the rate limiting key enzyme known as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase involved in cholesterol biosynthesis. Statins cause reduction in low density lipoproteins-C (LDL-C), total cholesterol (TC) and triglycerides (TG) and elevation in high-density lipoprotein-C (HDL-C). [2–6] Aspirin, 2-acetoxybenzoic acid (**Figure 2**) is one of the most widely used anti-inflammatory agents. The anti-inflammatory activity and anti-thrombotic activity are mainly due to its reversible inhibition of the cyclooxygenase. Cyclooxygenase catalyzes the formulation of thromboxane and prostacyclin which has opposite effects on aggregation and vasodilatation, at low doses (less than 100 mg) aspirin selectively inhibits the formation of thromboxane. [7] A combination of rosuvastatin calcium with aspirin (Unistar, Unichem laboratories, India) is commercially available in the market in capsule dosage form. Statins along with aspirin used for treatment of dyslipidemia associated with atherosclerotic arterial disease with risk of myocardial infarction, stroke or peripheral vascular disease. [8]

A number of analytical methods have been reported in the literature for the individual determination of rosuvastatin calcium or aspirin. These methods include: reversed phase liquid chromatography (LC),[9-13] high performance thin-layer chromatography (HPTLC)[14-17] and spectrophotometry.[18, 19] The simultaneous spectrophotometric determination of rosuvastatin calcium and aspirin were also reported.[20] Both drugs are official in Indian Pharmacopoeia. [1] However, no HPTLC method for the simultaneous analysis of rosuvastatin and aspirin in combined dosage forms has been reported so far. In this study, a normal phase HPTLC method for this purpose was developed and validated.

Figure 1: Chemical structure of Rosuvastatin Calcium

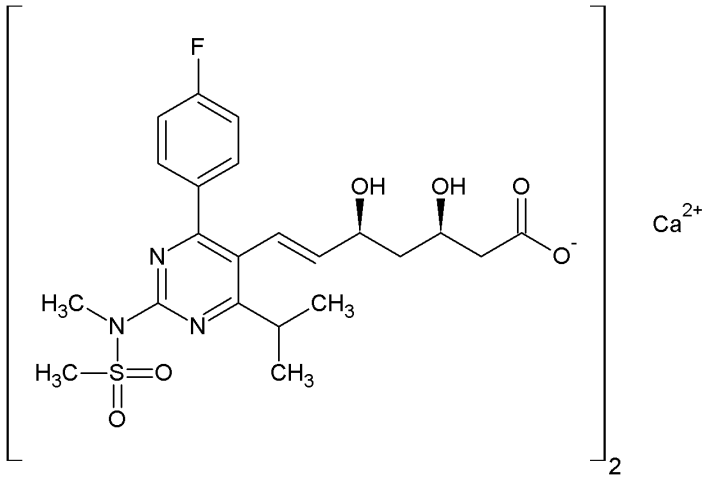
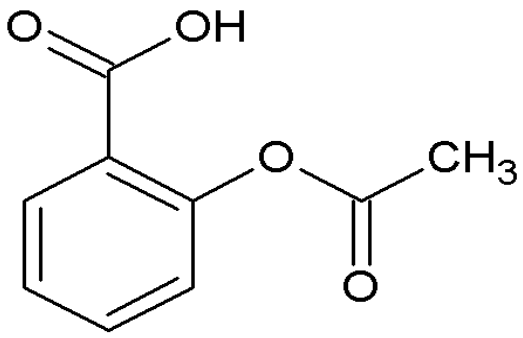


Figure 2: Chemical structure of Aspirin



2. EXPERIMENTAL

2.1 Material and Chemicals

Rosuvastatin calcium & aspirin were kindly supplied as a gift sample by Mepro Pharmaceuticals Pvt. Ltd. Surendranagar. Toluene, butanol & acetic acid were purchased from SD Fine Chemicals (Mumbai, India), all the solvents used are of AR grade without further modification.

2.2 Instrumentation and chromatographic condition:

The samples were spotted in the form of bands of width 6 mm with 100 µl sample syringe on aluminium plates precoated with silica gel $60F_{254}$, (E MERCK, Darmstadt, Germany) using a Camag Linomat 5 (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 80^0C for 10 min, prior to chromatography. The mobile phase consists of toluene:butanol:glacial acetic acid (9:1:0.2 *v/v/v*). Linear ascending development was carried out in 20 x 10 cm twin-through glass chamber. The optimized chamber saturation time for mobile phase was 20 min, at temperature (25^0C ± 2) and relative humidity (60 % ± 5 %); the TLC plates were air dried. Densitometric scanning was performed on Camag TLC Scanner 3 equipped with winCATS software version 1.3.0 at 234 nm. The source of radiation utilized was deuterium lamp. Evaluation was performed using peak area with linear regression.

2.3 Preparation of Standard solution

Rosuvastatin calcium equivalent to 10mg rosuvastatin and 75 mg of aspirin were accurately weighed and transferred to separate 10 mL volumetric flask and volume was adjusted up to mark with methanol (rosuvastatin 1000 µg/mL & aspirin 7500 µg/mL). 1ml of both stock solutions taken & diluted to 10 ml to obtain concentration 100µg/mL of rosuvastatin and 750 µg/mL of aspirin.

2.4 Analysis of Marketed Formulation

Content of twenty capsules were withdrawn and crushed in a glass mortar. An amount of powder equivalent to 10 mg of Rosuvastatin and 75 mg of Aspirin was transferred to 100 ml volumetric flask extract drugs with methanol by sonicating for 5 minutes. The solution was diluted up to mark with the same solvent and filtered. 4 ml of above solution diluted up to 10 ml. A sample solution of 10 µl was spotted on TLC plate followed by development and scanning. The concentration of drugs was determined from linear regression equations and % label claim was calculated.

3. METHOD VALIDATION

Validation was done with respect to various parameters required under ICH guidelines Q2 (R1). [21]

3.1 Linearity

Calibration curve was plotted over a concentration range from 200 to 1200 ng/band and 1500-9000 ng/band for rosuvastatin calcium & aspirin respectively. For the calibration curves, appropriate volumes of accurately prepared standard solution of rosuvastatin calcium and aspirin were applied on the plate. The plate was developed in a developing chamber previously saturated with the mobile phase for 20 min. Each reading was the average of three determinations.

3.2 Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of rosuvastatin calcium and aspirin by the standard addition method. Three different levels of standards (320, 400 and 480 ng/band for rosuvastatin calcium and 2400, 3000 and 3600 ng/band for aspirin) were added to the pre-analyzed tablet sample, each level was repeated three times, and the percentage recoveries were calculated.

3.3 Precision

Precision can be performed at two different levels- repeatability and intermediate precision. Repeatability is an indication of how easy it is for an operator in a laboratory to obtain the same results for the same batch of material using same method at different times using same equipment and reagent. Repeatability of sample application and measurement of peak area were carried out using six replicates of the same

standard (400 & 3000ng/band for rosuvastatin calcium & aspirin respectively). The intermediate precision results from the variations such as different days, analysts and equipment. The intra-day variation studies were performed by using three different concentrations from the linearity range within same day. The inter-day variations in the methods were assessed by studying three different concentrations for three different days over a period of week. The intra-day and inter-day variation for the determination of rosuvastatin calcium & aspirin was carried out at three different concentration levels 400, 600, 800 ng/band for rosuvastatin calcium and 3000, 4500, 6000 ng/band for aspirin

3.4 Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equations

LOD=3.3N/B and LOQ=10N/B

Where N is standard deviation of the peak area (n=3) and B is the slope of the corresponding calibration curve.

3.5 Specificity

Specificity of the method was tested by comparison of spectra of standard with sample which is prepared as mentioned in a previous section.

3.6 Robustness

Effects of small but deliberate changes in mobile-phase composition, total volume of mobile phase, time between application of sample and insertion into the development chamber, and time between development of the chromatograms and scanning the results were examined. Mobile phases with different compositions of toluene:butanol:glacial acetic acid (9:1:0.2, 9.2:0.8:0.2, 8.8:1.2:0.2) were used to develop chromatograms. The amount of mobile phase varied in the range of ±10%. Time variations (time from spotting to development and from development to scanning) varied in the range of ±20%. For each of the effects, three determinations performed at 400 ng/spot for rosuvastatin calcium and 3000 ng/spot for aspirin to study the separation and determination of robustness of the method. Relative standard deviations of the peak areas of the spots were used to evaluate the robustness of the method.

4. RESULTS AND DISCUSSION

4.1 Method Development

Several solvent mixtures in different ratios were tested to obtain a compact band of rosuvastatin calcium and aspirin. Toluene:butanol:glacial acetic acid (9:1:0.2 v/v/v) was found to give a compact well resolved bands for rosuvastatin calcium and aspirin with an R_f value of 0.32 & 0.67 respectively (Figure 3). UV-Vis spectra (Figure 4) of rosuvastatin calcium & aspirin were measured from 200 to 800 nm. A wavelength of 234nm selected for quantification of both drugs, good resolution of peaks with clear baseline separation obtained.

Optimal conditions used during validation

- Precoated silica-gel aluminium plate 60 F_{254} (20x10 cm, 250µm thick).
- Toluene: butanol: glacial acetic acid (9:1:0.2, v/v/v), 10 ml/run.
- Chamber saturation time, 20 min at room temperature (25°C±2°C) with relative humidity of 60 ±5%.
- Length of chromatogram run, 8 cm (approximately 18 min).
- Detection wavelengths, 234 nm

4.2 METHOD VALIDATION

4.2.1 Linearity

The relationship between the concentrations of rosuvastatin calcium & aspirin with peak area of

spot were investigated as shown in **Table 1 & 2**. It was found that rosuvastatin calcium and aspirin followed linearity in the concentration range of 200-1200 ng/spot and 1500-9000 ng/spot respectively with good correlation coefficients r^2= 0.997 & r^2= 0.997 for rosuvastatin calcium (**Figure 5**) and aspirin (**Figure 6**) respectively.

4.2.2 Accuracy

Recovery study was performed by spiking 80, 100 and 120 % to the pre-analyzed sample of rosuvastatin calcium and aspirin. Satisfactory recoveries ranging from 98.68 to 100.73 for rosuvastatin calcium and from 98.56 to 100.61 for aspirin were obtained using the proposed method. (**Table 3**)

4.2.3 Precision

The repeatability of sample application and measurement of peak area were expressed in terms of relative standard deviation and were found to be very low (0.63 and 0.18 for rosuvastatin calcium and 0.12 and 0.05 for aspirin, respectively) which in turn ensured reproducible performance of the instrument (**Table 4**). The coefficient of variation values showed that the proposed method provides acceptable intraday and interday variation of rosuvastatin calcium and aspirin, indicating the reproducible performance of the method (**Table 5**).

4.2.4 Limit of Detection & Limit of Quantification

The LOD and LOQ values were found to be 22 and 68 ng for rosuvastatin calcium and 156 and 445 ng for aspirin, respectively. The data shows that the method is sensitive for the determination of rosuvastatin calcium and aspirin.

4.2.5 Specificity

Specificity of the method was measured by comparison of spectra (**Figure 6 and 7**) of standard with sample that indicate there is no interference from the additives & so method is specific.

4.2.6 Robustness

Standard deviation of peak areas was calculated for each parameter and the coefficient of variation was less than 2%. The low values of coefficient of variation indicated robustness of the method (Table 8). Among the parameter chosen to evaluate robustness of experiment, very little variation was made on mobile phase composition, which is found to be very sensitive parameter during optimization step. When percentage change was considered, changing some parameter, such as amount of mobile phase, time from spotting to development and time from development to scanning caused a relatively small change in the result obtained . The changes caused by mobile phase composition, however, were relatively greater as compared to other parameters and this was expected because many HPTLC separations largely depend on percentage of Mobile phase composition.

4.3 Analysis of Marketed Formulation

The drug content of marketed capsule dosage form (Brand name: Unistar, label claim: 10 mg rosuvastatin and 75mg aspirin) were calculated using proposed method. The percentage recovery found to be 100% for both drugs which indicate that method can be suitable for analysis of marketed products (**Table 9**).

Table 1: Statistical analysis for the calibration curve of Rosuvastatin calcium

Sr. no.	Concentration of Rosuvastatin in [ng/spot]	Mean Peak area	% RSD
1	200	1537.05	0.67
2	400	2775.54	0.70
3	600	3943.52	0.70
4	800	5189.03	0.38
5	1000	6497.72	0.20
6	1200	8094.29	0.19

*** mean of three estimations at each level**

Table 2: Statistical analysis for the calibration curve of Aspirin

Sr. no.	Concentration of Aspirin in [ng/spot]	Mean Peak area	% RSD
1	1500	7912	0.22
2	3000	9313	0.24
3	4500	1044	0.75
4	6000	1168	0.77
5	7500	1310	0.43
6	9000	1399	0.54

*** mean of three estimations at each level**

Table 3: Recovery analysis of Rosuvastatin and Aspirin in Capsule

Drugs	Initial Amount [ng/spot]	Amount added [ng/spot]	Percentage Amount Recovered
Rosuvastatin	400	320	99.90
		400	100.14
		480	100.94
Aspirin	3000	2400	99.50
		3000	100.67
		3600	100.14

Table 4: Repeatability analysis of Rosuvastatin and Aspirin in Capsule

Sr. No.	Repeatability of Sample Application		Repeatability of Measurement of peak area	
	Area of Rosuvastatin	Area of Aspirin	Area of Rosuvastatin	Area of Aspirin
1	2764.5	9350.13	2764.5	9350.33
2	2798.71	9310.32	2758.23	9346.13
3	2750.32	9329.51	2762.71	9351.81
4	2756.45	9343.97	2755.93	9356.92
5	2780.37	9327.29	2762.67	9347.24
6	2767.91	9355.37	2763.83	9358.74
Mean Area	2769.71	9336.098	2761.312	9351.862
%RSD	0.63	0.18	0.12	0.05

Table 5: Results of precision studies (Intra-day and Inter-day)

Drug	Conc. [ng/spot]	Intra –day*		Inter –day*	
		Mean Amount found [ng]	%RSD	MeanAmount found [ng]	%RSD
Rosuvastatin	400	399.81	1.1	399.12	1.15
	600	597.39	0.57	596.80	1.13
	800	806.61	0.61	801.70	0.56
Aspirin	3000	3093.52	0.78	3050.76	0.71
	4500	4530.10	0.49	4515.01	0.49
	6000	5987.49	0.8	5990.23	0.25

*** mean of three estimations at each level**

Table 6: Robustness of the method

Parameters	%RSD for Peak Area of Rosuvastatin*	%RSD for Peak Area of Aspirin*
Mobile phase composition (± 0.2mL)	1.2	1.3
Mobile phase volume (± 2mL)	0.94	0.81
Development distance (± 0.5 cm)	0.79	0.67
Duration of saturation (± 2 min)	0.81	0.92
Time from spotting to chromatography (± 10 min)	0.43	0.52
Time from chromatography to scanning (± 10 min)	0.82	0.63

*** mean of three estimations**

Table 7: Results obtained in the determination of Rosuvastatin Calcium and Aspirin in Capsule formulation

Drugs	Label claim[mg]	Amount found[mg]	Amount found [%]
Rosuvastatin	10	9.96	99.6
		10.19	101.9
		10.07	100.7
		9.89	98.9
		9.90	99.0
		10.07	100.7
	Mean ± SD	10.018± 0.117	100.18 ± 1.17
	% RSD	1.17	1.17
Aspirin	75	75.22	100.30
		75.79	101.06
		75.33	100.44
		75.18	100.25
		75.35	100.46
		75.16	100.22
	Mean ± SD	75.03 ±0.23	100.46 ± 0.31
	% RSD	0.31	0.31

Figure 3: Typical HPTLC chromatogram of Rosuvastatin Calcium (R_f = 0.32) and Aspirin (R_f = 0.67)

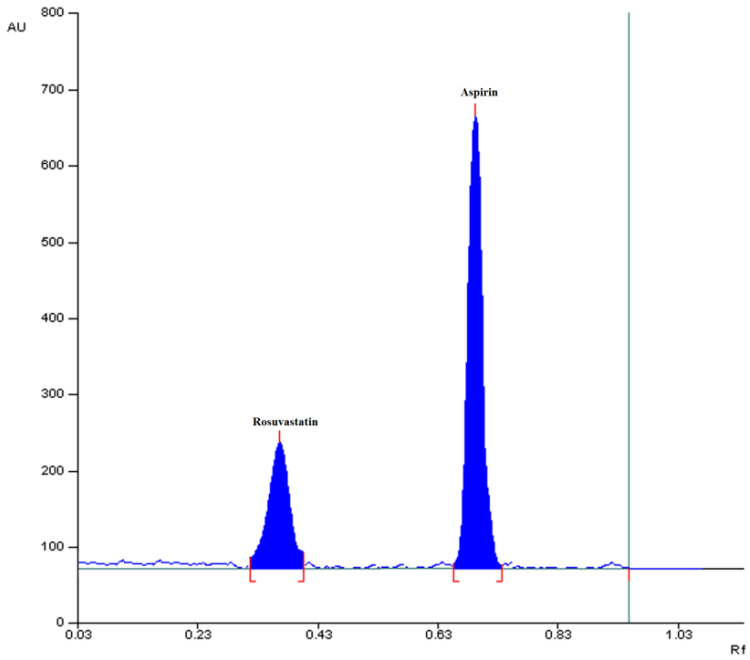


Figure 4: Overlay spectra of Rosuvastatin Calcium & Aspirin standard

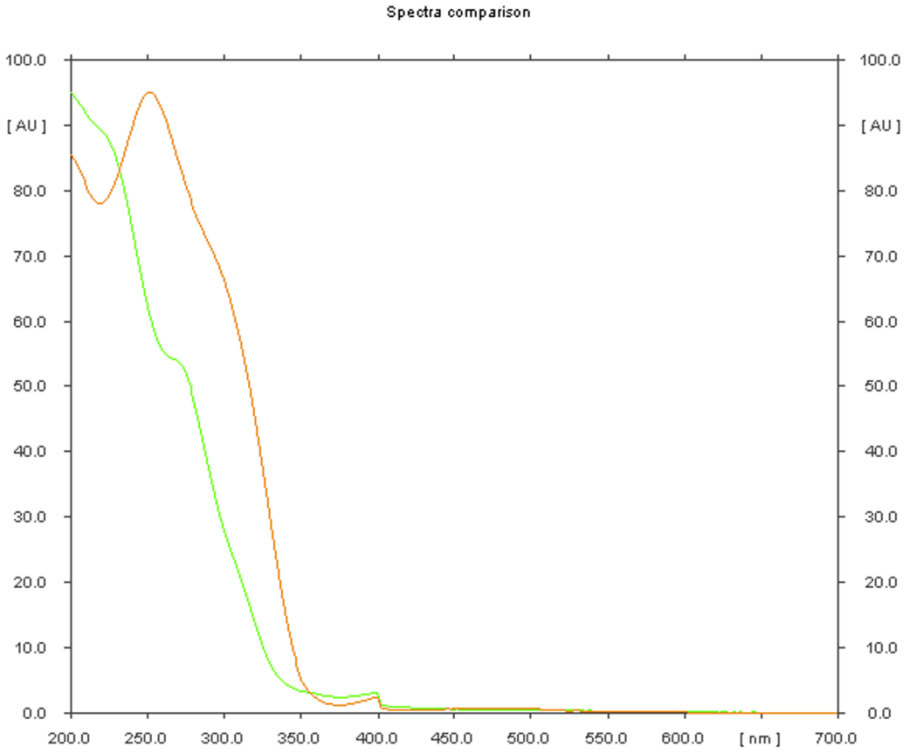


Figure 5: 3D Densitogram for the Linearity of Rosuvastatin Calcium & Aspirin

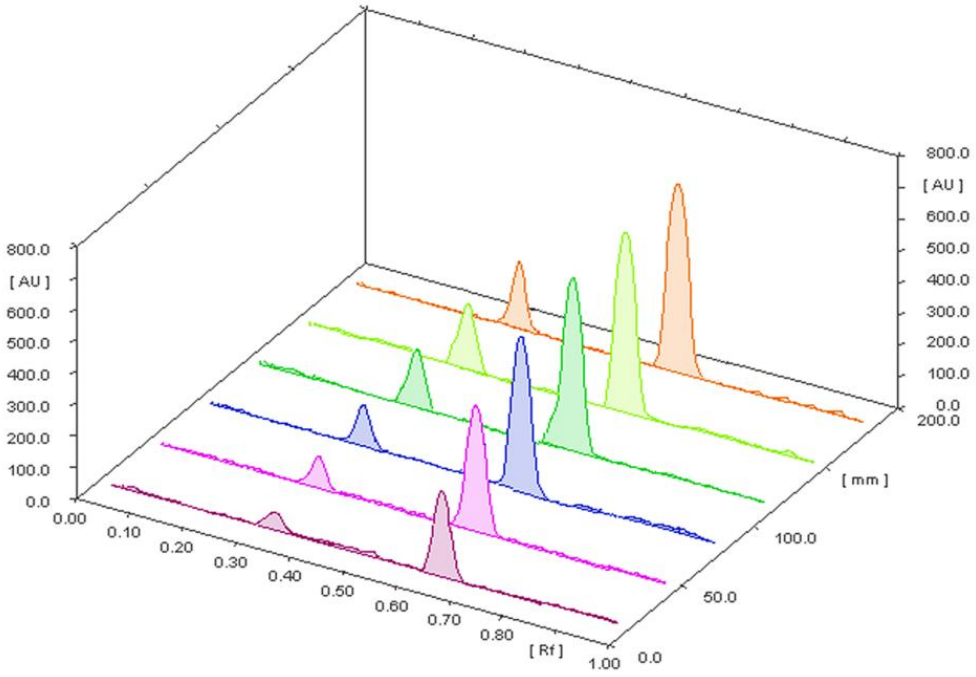


Figure 6: Peak purity spectra of Rosuvastatin Calcium

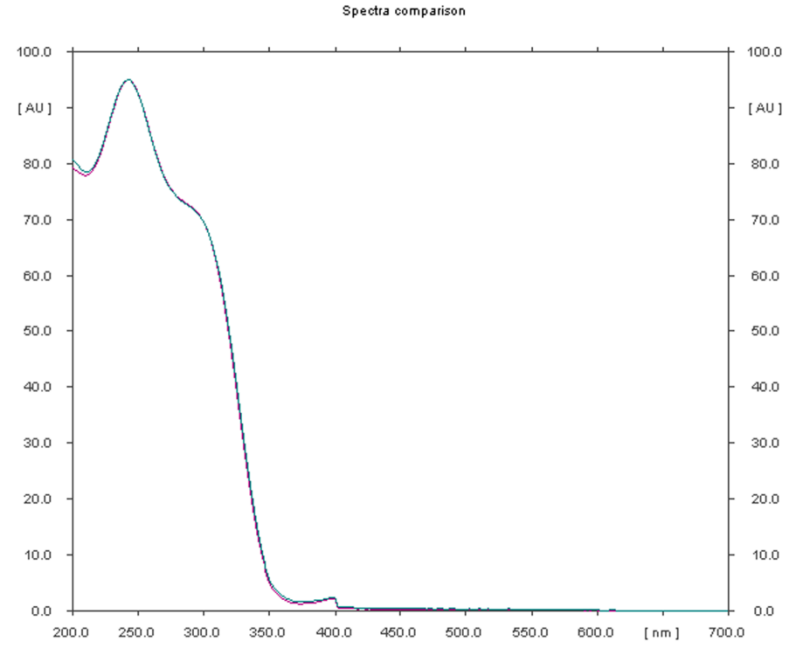
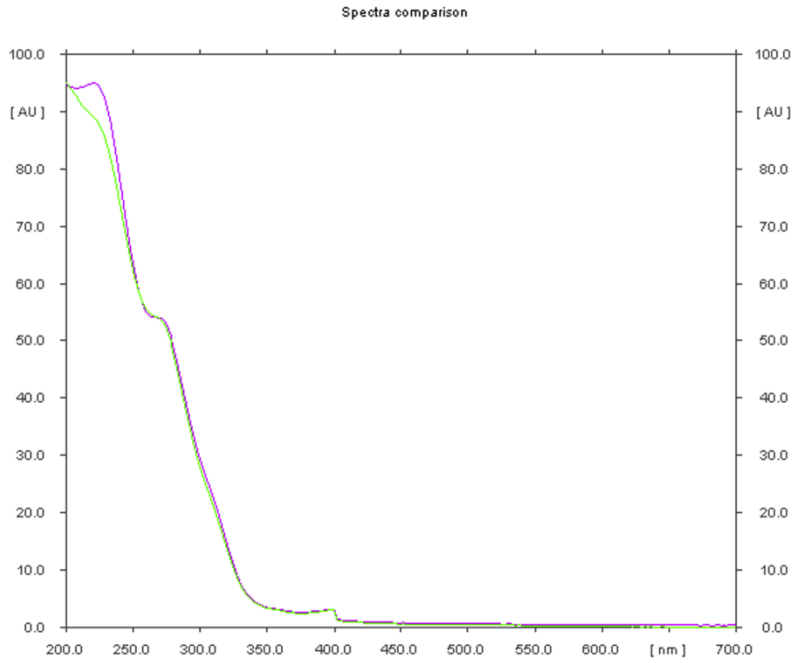


Figure 7: Peak purity spectra of Aspirin



5. CONCLUSION

A simple, specific, precise and accurate HPTLC method has been developed for quantitative determination of rosuvastatin calcium & aspirin in capsule formulation. The developed method was validated based on ICH guidelines. Statistical analysis proves that the method is reproducible and selective for the analysis of rosuvastatin calcium and aspirin as bulk drug and in pharmaceutical formulations. The advantages of the proposed methods involve a simple procedure for sample preparation and relatively

short time of analysis. The proposed HPTLC method is suitable for the analysis of rosuvastatin calcium and aspirin in commercial tablets.

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