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Original Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF ROSUVASTATIN CALCIUM AND ASPIRIN IN ITS PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Development and validation of simple, rapid, precise, accurate and sensitive HPTLC method for the simultaneous estimation of Rosuvastatin calcium and Aspirin in bulk and in capsule dosage form. The mobile phase consisting of Ethyl acetate:Toluene:Glacial acetic acid (6:3:1 v/v/v) and wavelength of detection 240nm was used . The linearity of the calibration curves for Rosuvastatin calcium and Aspirin in the desired concentration range is good (r^2 =0.998) by this method. The result of analysis has been validated statistically and recovery study confirmed the accuracy of proposed method. This method was successfully applied to the routine determination of these drugs in bulk and in its pharmaceutical dosage form.

Keywords*:* Rosuvastatin calcium; Aspirin*;* Simultaneous estimation; HPTLC.

INTRODUCTION

Rosuvastatin (RSV) is the calcium salt of (E)-7-[4-(4-fluorophenyl) -6-isopropyl-2-[methyl (methylsulfonyl) amino]pyrimidin-5-yl] (3R,5S)-3,5-dihydroxyhept-6-enoic acid. RSV is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol. RSV is a member of the class of statins, used to treat hypercholesterolemia and related conditions and to prevent cardiovascular disease. It increases the number of hepatic LDL (Low Density Lipoprotein) receptors on the cell surface to enhance uptake and catabolism of LDL. Secondly, RSV inhibits hepatic synthesis of VLDL (Very Low Density Lipoprotein), which reduces the total number of VLDL and LDL particles.[1]

Aspirin also known as acetylsalicylic acid, is a salicylate drug, often used as an analgesic, antipyretic, anti-inflammatory and also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecule together to create a patch over damage of the walls within blood vessels. Chemically it is 2-acetoxybenzoic acid and is a nonsteroidal anti-inflammatory drug (NSAIDs) and shows inhibition of the enzyme cyclooxygenase and it is official in Indian Pharmacopoeia, The United States Pharmacopeia and British Pharmacopoeia.[2-4]

A survey of literature has not revealed any HPTLC method for simultaneous determination of Rosuvastatin calcium and Aspirin. However few HPLC, capillary zone electrophoresis, spectrophotometric, HPTLC and GC have been reported for the drugs individually and in combination with other drugs. [5-17]

MATERIALS AND METHODS

Materials

The bulk drugs of RSV and ASP were obtained as gift samples from Glenmark Pharmaceutical Ltd. Mumbai and Cipla Pharmaceutical Ltd. Daund respectively. All analytical grade chemicals and solvents were purchased from Merck, India.

Equipment

Camag HPTLC system consisting Linomat 5 applicator, camag TLC scanner 3 and WinCATS software V-1.4.4 was used for chromatographic separation. Spotting of samples was done by using Hamilton microliter syringe.

Chromatographic condition

Methanol was used as a solvent for solution preparation. Stationary phase was aluminium HPTLC plate (20×10cm) precoated with silica gel F_{254}. Mobile phase consisting of Ethyl acetate:Toluene:Glacial acetic acid in the ratio 6:3:1 v/v/v was used. Linear ascending development was carried out in a 20×10cm twin trough glass chamber using mobile phase. 20 min saturation was required. The development distance was 8cm which was achieved in 10 min. The TLC plates were removed from chamber and dried at 35C for 5min. The wavelength of detection selected was 240nm since both drug showed optimum absorbance at that wavelength. The slit dimension of detection was kept 6.00×0.45 mm, scanning speed 20mm/sec and data resolution 100µm/step. The typical densitogram of working standard solutions is as shown in Fig 1.

Preparation of standard stock solutions

Stock solutions each of 100µg/ml of RSV and ASP were prepared by dissolving 10mg of each drug in 25ml methanol in separate volumetric flask and then the volume adjusted to 100ml with methanol separately.

Calibration curve

Varying concentrations of 400-1200ng/spot of RSV and ASP were prepared from their respective stock solutions and applied on the chromatographic plates. The plate was developed using mobile phase comprising of Ethyl acetate:Toluene:Glacial acetic acid in the ratio 6:3:1 v/v/v in twin trough chamber to a distance of 8cm. After removal from chamber, the plate was dried at 35C for 5min. The plate was scanned and quantified at 240nm. Peak area was recorded for RSV and ASP. A linear relationship between peak area and concentration was observed for both RSV and ASP in the range of 400-1200ng/spot. This range was selected as linear range for analytical method development of both the components.[2-4]

Table 1: Statistical parameters

Parameters	RSV	ASP
Slope	5.22	2.27
Correlation Co-efficient	0.998	0.995
Intraday Precision(% assay)	99.75	101.05
Intraday Precision (% R.S.D.)	0.00448	1.39
Interday Precision(% assay)	99.3	99.06
Interday Precision (% R.S.D.)	0.0179	1.028

RSV – Rosuvastatin, ASP-Aspirin

Analysis of capsule formulation

Marketed capsule containing 10mg of RSV and 75mg of ASP were used. Twenty capsules were weighed and finely powdered. A quantity of powder equivalent to 10mg of RSV and 75mg of ASP was weighed and transferred to a 100ml volumetric flask containing 50ml methanol, sonicated for 5min, and the volume was made up to 100ml with methanol. The solution was filtered using Whatmann filter paper No. 41. From the filtrate, 8µl was applied to an HPTLC plate to furnish final amount of 800ng per band for RSV and for ASP 1ml from stock solution was diluted by adding 2ml methanol and, from this solution 3.2µl was applied to HPTLC plate to furnish final amount of 800ng per band for ASP. After chromatographic development peak areas of the bands were measured at 240nm and amount of each drug present per capsule was estimated from the respective calibration plots and presented in Table No. 2. The procedure was repeated six times for analysis of homogeneous samples.

Table 2: Analysis of capsule formulation

Drug	Label claim mg/dose	Amount found mg/dose	%Recovery ± SD*
Rosuvastatin	10	9.94	99.49% ± 0.0581
Aspirin	75	74.94	99.93%±0.0178

Capsule formulation containing RSV 10 mg and ASP 75mg per dosage

*= Average of 6 determinations

Recovery studies

The accuracy of proposed methods was checked by recovery study by addition of standard drug solution to preanalysed sample solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for both the drugs. Each being analysed in a manner similar to as described for assay and the recovery of added standard was calculated. The result of recovery study is reported in Table 3.

Table 3: Result of recovery studies by the proposed method

Formulation used	Recovery level	Recovery of	% mean recovery*	%RSD
Capsule (Unistar*)	80%	RSV	98.42	0.0545
		ASP	99.21	0.00904
	100%	RSV	98.85	0.00904
		ASP	99.63	0.00448
	120%	RSV	99.17	0.0459
		ASP	99.103	0.4478

*= Average of 3 at each level of recovery

RESULT AND DISCUSSION

At the time of study literature survey revealed that not a single method was been reported for simultaneous analysis of the RSV and ASP by HPTLC method. So, the proposed methods for simultaneous estimation of RSV and ASP in combined dosage form were found to be new, simple, rapid, accurate and economic.

For the method, linearity was observed in the concentration range of 400-1200ng/spot for both RSV and ASP. Marketed brand of capsule was analysed and amount of drug determined by proposed method ranges from 98 to 102% as shown in table no 2. The proposed method was validated as per ICH guidelines. The accuracy of method was determined at 80, 100 and 120% level. The percentage recovery ranges from 98.07 to 99.83% for both methods. Precision was calculated as interday and intraday variations (% RSD is minimum) for both drugs. The method can be successfully used for simultaneous estimation of RSV and ASP in combined dosage form.

During this work, one HPTLC method has been published in the literature. So on comparison with the reported method the developed method has been found to be simpler, rapid, precise, accurate, sensitive and economic than the reported one (Table No.4).

Table 4: Comparison of developed HPTLC method with the reported HPTLC method

Parameters	Reported Method	Developed Method
Mobile Phase	n-Hexane: Acetone: Ethyl acetate: Formic acid (6:3:1:0.2 v/v)	Ethyl acetate: Toluene: Glacial acetic acid (6:3:1 v/v/v)
Linearity (ng/spot)		
Rosu	500-1000	400-1200
Asp	3750-7500	400-1200
Coefficient of correlation		
Rosu	0.995	0.998
Asp	0.995	0.995
Assay of capsules		
Rosu	100.39	99.49% ± 0.0581
Asp	99.71	99.93%±0.0178

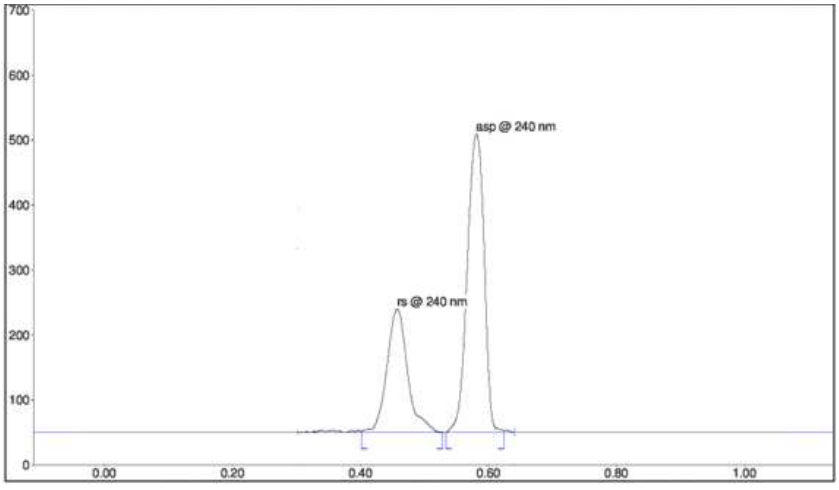

Fig. 1: Densitogram of marketed formulation containing RSV and ASP

CONCLUSION

The proposed method has been proved to be simple, rapid, precise, accurate sensitive and economical and is suitable for simultaneous quantification of RSV and ASP in bulk and in pharmaceutical dosage form.

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