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VALIDATED STABILITY-INDICATING RP-HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE BESYLATE AND ROSUVASTATIN CALCIUM IN COMBINED TABLET DOSAGE FORM

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ABSTRACT
A new simple, accurate, precise and selective stability-indicating reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous estimation of Amlodepine besylate and Rosuvastatin calcium in combined tablet dosage form. An isocratic, reverse phase HPLC method was developed on Jasco HPLC system equipped with Grace C_{18} column (150 x 4.6 mm i.d.) using acetonitrile: 50mM sodium acetate (pH adjusted to 3.1 with ortho phosphoric acid) (60: 40, v/v) as mobile phase and detection was carried out at 250 nm. Both the drugs were subjected to stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Results were linear in the range of 10-50 µg mL^{-1} for both the drugs. The retention times for Amlodepine besylate and Rosuvastatin calcium were 3.6 min and 6.1 min, repectively.

KEYWORDS: RP-HPLC, Amlodipine besylate, Rosuvastatin calcium, Forced degradation.

INTRODUCTION
Amlodipine Besylate, chemically, 3-*O*-ethyl 5-*O*-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate is used as antihypertensive and antianginal drug.[1] Rosuvastatin calcium is the calcium salt form of rosuvastatin with antilipidemic activity and chemically is calcium (*E*, 3R, 5S)-7-[4-(4-fluorophenyl)-2-[methyl (methylsulfonyl) amino]-6-propan-2-ylpyrimidin-5-yl]-3, 5 dihydroxyhept-6-enoate.[2]

Extensive literature survey revealed that different analytical methods such as spectrophotometry,[3] high performance liquid chromatography (HPLC)[4-9] and high performance thin layer chromatography (HPTLC)[10] has been reported for the determination of amlodipine besylate either as single drug or in combination with other drugs. Analytical methods reported for rosuvastatin calcium includes spectrophotometry,[11] high performance liquid chromatography (HPLC)[12-16] either as single drug or in combination with other drugs.

To best of our knowledge, no reports were found in literature for simultaneous determination of Amlodipine besylate and Rosuvastatin calcium in combined tablet formulation by stability indicating RP-HPLC method. Therefore, the present study is aimed at development of suitable stability indicating RP-HPLC method for this combination by degrading the drugs below different stress conditions such as hydrolysis, oxidation, thermal and photolytic stress which is suggested by ICH guidelines.[17,18]

MATERIALS AND METHODS
Chemical and reagents
Working standards Amlodipine besylate and Rosuvastatin calcium were obtained as gift samples from Pfizer Ltd., Thane (Mumbai, India). The pharmaceutical tablet dosage form containing 10 mg of Amlodipine besylate and 10 mg of rosuvastatin calcium was procured from local pharmacy. Acetonitrile (HPLC grade) and AR grade sodium acetate were obtained from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions
JASCO HPLC system equipped with pump (Model PU 2080 Plus), Rheodyne sample injector of 20 µL capacity, PDA detector (MD 2010) operated with Borwin- PDA software (version 1.5). Grace C18 column with dimension 150 x 4.6 mm was utilized. Flow rate of 1 mL min^{-1} was maintained and detection was monitored at 250 nm.

Preparation of standard solution

Individual stock solutions of Amlodipine besylate and Rosuvastatin calcium having concentration 10 µg mL^{-1} were prepared, followed by preparation of Mixed Standard Solutions wherein the ratio of amlodipine and rosuvastatin was 10: 10, similar to the one found in marketed formulation.

Selection of analytical wavelength

Stock solutions for drugs were prepared in acetonitrile. UV spectra of each drug were recorded and obtained spectra were overlain. Considerable absorbance was shown by drugs at 250 nm i.e. isobestic point and was chosen as wavelength for detection.

Analysis of tablet formulation

Tablet formulation containing 10 mg of amlodipine and 10 mg of rosuvastatin was used for analysis. Powder quantity equal to 10 mg amlodipine was transferred to volumetric flask (100 mL) having 60 mL mobile phase. Sonication was made for 10 min to dissolve contents, filtered and volume made to 100 mL to obtain the concentration 100 µg mL^{-1} for both drugs. Further dilutions were made using mobile phase to acquire finishing concentration of 20 µg mL^{-1} of amlodipine and rosuvastatin. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, and chromatogram was obtained. The injections were repeated six times. The peak areas were determined. The amount of each drug present in tablet was calculated from the respective calibration curves.

Stability studies

The stability studies were performed by subjecting the mixed standard solution of bulk drugs to the physical stress (hydrolysis, peroxide, heat and light) and stability was accessed. The stress degradation studies were carried out at initial drug concentration of 100 µg mL^{-1} for both drugs in mobile phase. The hydrolytic studies were carried out by mixing the drug solutions of amlodipine and rosuvastatin with 0.1 N HCl and 0.1 N NaOH and the resulting solutions were refluxed at 80ºC for 1 h and 4 h separately to achieve degradation within the acceptable limit. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 50 µg mL^{-1} for both drugs. The oxidative degradation was carried out in 15 % H_2O_2 and the sample was diluted with methanol to obtain solution having concentration 50 µg mL^{-1} for both drugs. Thermal stress degradation was performed by keeping the solid drugs individually in oven at 60ºC for a period of 24 h. Photolytic degradation studies were carried out by exposing both drugs individually to UV light up to 200-watt h square $meter^{-1}$ for 72 h.

RESULTS AND DISSCUSSION

Method optimization

The main aim in developing this stability indicating HPTLC method is to achieve the satisfactory resolution of drugs from each other and also from their degradation products. Initially, many method trials were performed using different mobile phases in order to obtain better separation. Finally the mobile phase composing acetonitrile: 50 mM sodium acetate (pH adjusted to 3.1 with ortho phosphoric acid) (60: 40, v/v) was selected as optimal for obtaining well defined and resolved peaks for the drugs. The mobile phase utilized offered excellent resolution along with sharp and well resolved peaks without any tailing. Densitometric evaluation was carried out at 250 nm. The retention times for amlodipine and rosuvastatin were 6.1 min and 3.6 min, respectively by the use of the proposed method. Representative densitogram of mixed standard solution of both drugs is shown in Figure 1.

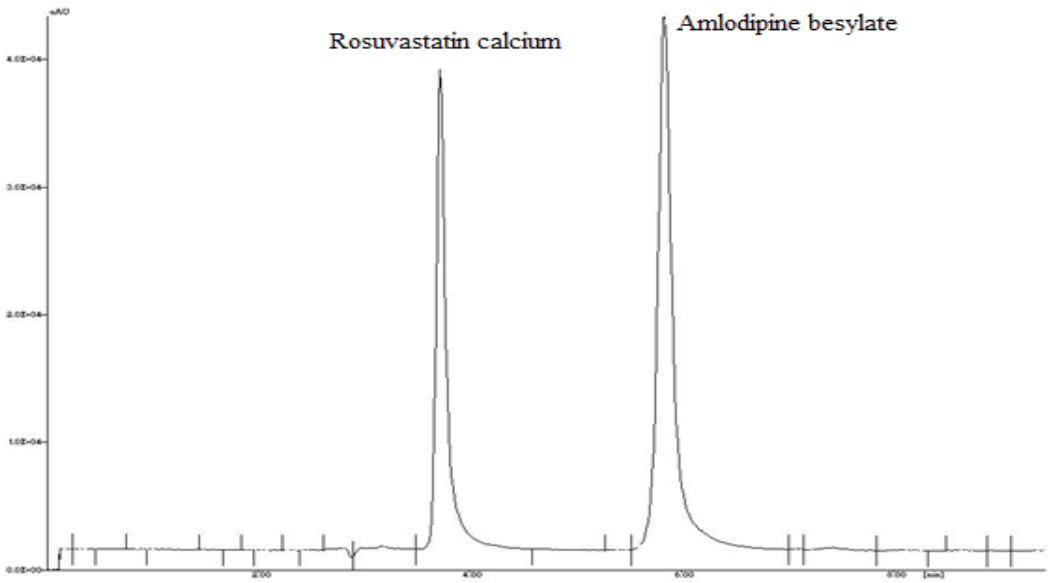
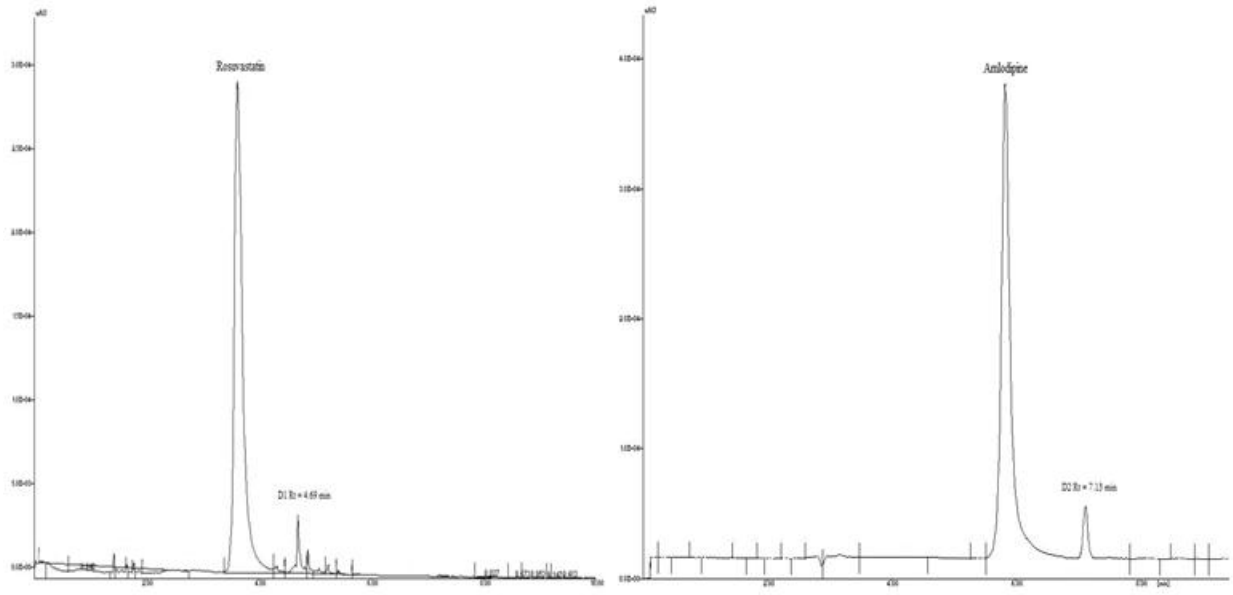


Figure 2: Representative chromatogram of standard mixture containing rosuvastatin calcium (40 µg mL^{-1}, RT = 3.6 min) and amlodipine besylate (40 µg mL^{-1}, RT = 6.1 min).

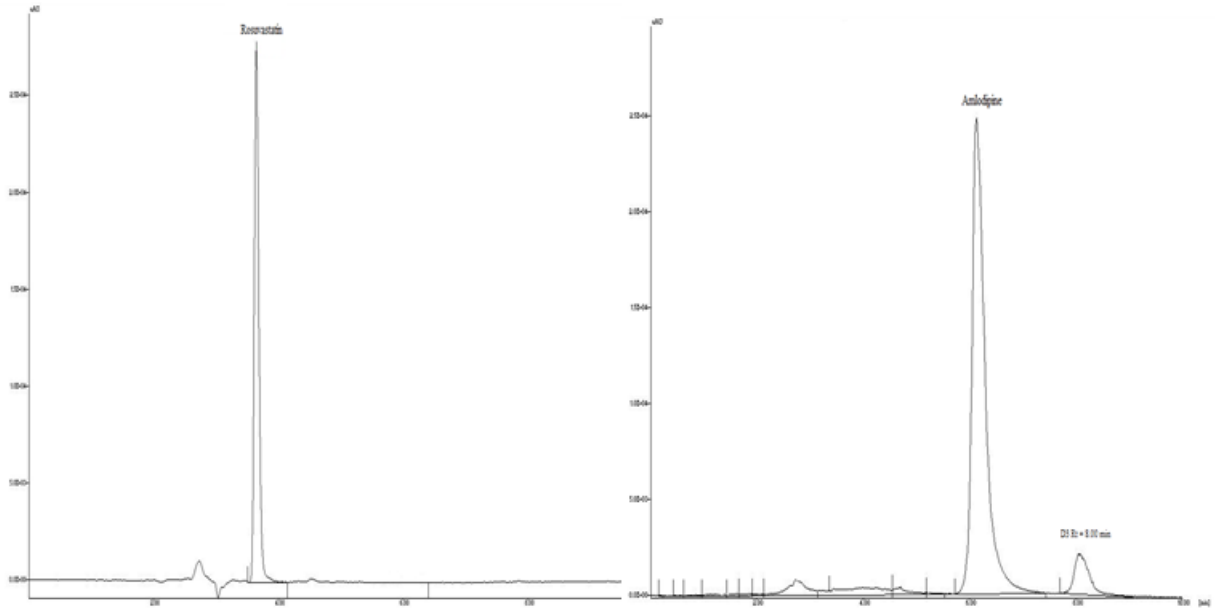
Results of stress degradation studies

The stress degradation results revealed the susceptibility of both the drugs to hydrolytic, oxidative stress conditions and stability under thermal and photolytic stress conditions. Marked degradation in the densitograms was observed with appearance of degradation products for amlodipine and rosuvastatin under hydrolytic and oxidative conditions. Figures 2 and 3 shows the chromatograms of acid and alkali hydrolytic degradation, while Figure 4 shows the chromatogram of oxidative degradation. Peak purity results greater than 991 indicate that peaks for both drugs are homogeneous in all stress conditions tested. The unaffected assay of tablet formulation confirmed the stability indicating power of the method. The findings of degradation studies are represented in Table 1.



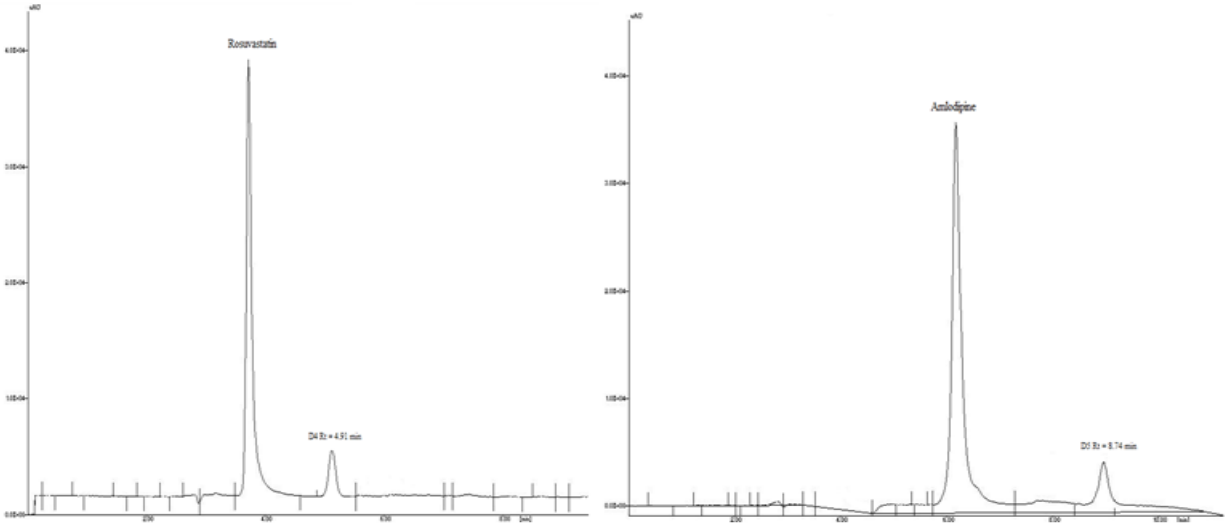
(a) (b)

Figure 2: Chromatogram of (a) Rosuvastatin (D1, RT= 4.69 min) and Amlodipine (D2, RT= 7.13 min) after acid degradation.



(a) (b)

Figure 3: Chromatogram of (a) Rosuvastatin and Amlodipine (D3, RT= 8.00 min) after alkali degradation.



(a) (b)

Figure 4: Chromatogram of (a) Rosuvastatin (D4, RT= 4.91 min) and Amlodipine (D5, RT= 8.74 min) after peroxide degradation.

Table 1: Results of stability studies by proposed RP-HPLC method.

Degradation condition	% recovery		% degradation	
	AMLODIPINE	ROSUVASTATIN	AMLODIPINE	ROSUVASTATIN
Acid (0.1 N HCl Reflux at 80°C for 1 h)	82.76	86.60	17.24	13.40
Alkali (0.1N NaOH Reflux at 80°C for 4 h)	81.38	89.36	18.62	10.64
Oxidation (15 % H_2O_2 80°C for 4 h)	84.16	85.28	15.84	14.72
Photolysis (UV, 72 h)	98.70	99.00	---	---
Dry heat (60°C 24 h)	98.54	99.22	---	---

Validation of analytical procedure

The optimized method was validated in accordance with ICH guidelines with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness.[17, 18]

System suitability

Reproducibility of developed procedure was checked by carrying out system suitability tests. The parameters such as capacity factor, number of theoretical plates and tailing factor for active substances were in limits suggesting the system suitability. The achieved values for various parameters confirmed appropriateness of the system for the analyzing these drugs in combination.

Table 2: System suitability parameters.

Drug	Retention Time (min)	Area (µV. Sec)	Theoretical plates	Resolution	Asymmetry
Rosuvastatin	3.6	541326	14215	2.01	1.08
Amlodipine	6.1	606423	15223	2.06	1.03

Linearity and range

The proposed RP-HPLC method was found to be linear in the concentration range 10-50 µg mL^{-1} for both the drugs. Linear response was shown by both drugs within specified range of concentration. Calibration curves were constructed by plotting obtained peak area against drug concentrations to prove the linearity. The regression equation for amlodipine was y = 14398x + 19532 with correlation coefficient 0.994 and for rosuvastatin, it was y = 12983x + 9635.5 with correlation coefficient 0.995.

Precision

The % R.S.D. values calculated after intraday studies were in the range of 0.90-1.06 for amlodipine and 0.21-0.72 for rosuvastatin. The % R.S.D. values calculated after analysis on three consecutive days were in the range of 0.34-1.01 for amlodipine and 0.21-1.09 for rosuvastatin. The calculated % relative standard deviations were within satisfactory limits (below 2 %) demonstrating reproducibility of proposed method.

Sensitivity

Sensitivity was evaluated in the form of detection and quantitation limit. The values of detection limit estimated through method were 0.44 and 0.49 µg mL^{-1} whereas

quantitation limit values were 1.33 and 1.49 µg mL^{-1} for amlodipine and rosuvastatin, respectively.

Specificity

The chromatogram for sample solution was recorded to check emergence of any extra peaks. The interference from excipients was not observed at the retention times of the tested compounds which indicated specificity of the method.

Assay of tablet formulation

The peak areas of the sample were compared with area of standard to estimate amount of each drug present within formulation. The percentage drug content (Mean± S.D.) was 99.76 ± 1.03 and 99.80± 0.16 for amlodipine and rosuvastatin, respectively.

Accuracy

The average recovery obtained after standard addition method was found to be 99.35±0.58 and 100.01 ± 0.71 for amlodipine and rosuvastatin, respectively. Satisfactory recoveries achieved for both drugs indicated accuracy of method for their estimation in combined tablet dosage form.

Table 3: Accuracy data.

Drug	Basic sample concentration (µg mL^{-1})	Concentration added (µg mL^{-1})	Total concentration recovered (µg mL^{-1})	% Recovery ± R.S.D.*
Amlodipine	20	16	35.77	99.35 ± 0.52
	20	20	40.34	100.85 ± 0.90
	20	24	43.97	99.92 ± 0.32
Rosuvastatin	20	16	35.92	99.77 ± 1.39
	20	20	40.25	100.55 ± 0.54
	20	24	43.87	99.71 ± 0.21

*Average of three determinations.

Robustness

The deliberate variations made in the flow rate and wavelength does not produced a marked change in chromatograms as well as peak areas of drugs which demonstrated robustness of the developed method.

CONCLUSION

Stability indicating reverse phase HPLC method for simultaneous estimation of amlodipine besylate and rosuvastatin calcium in combined tablet formulation has been developed and validated as per ICH guidelines. The developed method is simple, precise and accurate. Stability indicating nature of method was confirmed by performing stress degradation of drugs under different conditions viz. hydrolysis, oxidation, thermal and photolysis. The peaks obtained for degradation products did not merge with drug peaks which denote the specificity of developed method. The proposed methods may be used for routine analysis of these drugs in quality control laboratories and can also be beneficial for monitoring the potency of this combination during shelf life.

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