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Article

Simultaneous estimation of rosuvastatin and amlodipine in pharmaceutical formulations using stability indicating HPLC method

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A viable cost-effective and isocratic approach employing C-18 column (250 mm × 4.6 mm, 5 µm) based HPLC has been utilized to separate and estimate the drugs, rosuvastatin, amlodipine and their stress induced degradation products, simultaneously in pharmaceutical formulations. Focused on ICH guideline parameters, the efficient separation of both drugs and their degradation products was achieved by optimizing a 30:70 (v/v) solvent mixture of acetonitrile and 0.1 M ammonium acetate buffer (pH 5) as mobile phase. The flow rate of the mobile phase was 1.5 mL/min and all the detections were carried out at 240 nm using UV detector. The method was linear in the concentration range of 1-200 µg/mL for rosuvastatin with 0.996 coefficient of determination value. For amlodipine, linearity was in the range of 0.5-100 µg/ml with 0.994 coefficient of determination value. Both the drugs along with their degradation products were separated in less than twenty minutes. The results of within-day and between-day precision were varied from 0.72 to 1.81% for rosuvastatin and 0.83 to 1.88% for amlodipine. The results show that this ICH validated method can be employed successfully for the routine as well as stability quantification of both the active ingredients simultaneously in pharmaceutical formulations.

Uniterms: High Performance Liquid Chromatography/quantitative analysis. Rosuvastatin/quantitative determination. Amlodipine/quantitative determination. Pharmaceutical formulations/quantitative analyis. Acetonitrile. UV Detector.

Utilizou-se abordagem de viabilidade custo-efetividade e isocrática, baseada em CLAE, empregando coluna C-18 (250 mm x 4,6 mm, 5 µm) para separar e avaliar os fármacos, rosuvastatina, anlodipino e seus produtos de degradação induzida por estresse, simultaneamente, em formulações farmacêuticas. Focada nos parâmetros das diretrizes da ICH, a separação eficiente de ambos os fármacos e de seus produtos de degradação foi obtida por meio da otimização da fase móvel com mistura de solventes 30:70 (v/v), respectivamente, acetonitrila e tampão acetato de amônio O,1 M (pH 5). A velocidade de fluxo da fase móvel foi de 1,5 mL/min e todas as detecções foram realizadas em 240 nm, utilizando detector de UV. O método foi linear no intervalo de concentração de 1-200 µg/mL para a rosuvastatina com coeficiente de determinação 0,996. Para o anlodipino, a linearidade ficou na faixa de 0.5-100 µg/mL, com coeficiente de determinação 0,994. Ambos os fármacos, junto com seus produtos de degradação, foram separados em menos de vinte minutos. Os resultados de precisão intra-dia e inter-dia variaram de 0,72 a 1,81% para a rosuvastatina e de 0,83 a 1,88%, para o anlodipino. Os resultados mostram que este método validado pelo ICH pode ser empregado com sucesso tanto para a rotina quanto para a quantificação simultânea da estabilidade de ambos os ingredientes ativos em formulações farmacêuticas.

Unitermos: Cromatografia Líquida de Alto Desempenho/análise quantitative. Rosuvastatina/determinação quantitativa. Anlodipino/determinação quantitativa. Formulações farmacêuticas/análise quantitativa.

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INTRODUCTION

Rosuvastatin [Figure 1A] 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. It is a competitive inhibitor of HMG-CoA reductase (Lennernas, Fager, 1997; Nissen *et al*., 2006), used in the treatment of dyslipidemia and hypercholesterolemia (McCormick *et al*., 2000; Olsson, McTaggart, Raza, 2002). Rosuvastatin is very effective in reducing low density lipoprotein cholesterol and many studies have demonstrated its superiority nature over other drugs of its class such as atorvastatin, simvastatin and pravastatin (Olsson *et al*., 2001; Paoletti *et al*., 2001; Davidson *et al*., 2002). Literature revealed many HPLC methods, including stability indicating methods, for the separation of rosuvastatin (Mehta *et al*., 2005; Pandya *et al*., 2010; Gomes *et al*., 2009; Kaila *et al*., 2010; Reddy *et al*., 2011; Mostafa *et al*., 2012; Trivedi, Patel, 2012).

Amlodipine besylate [Figure 1B], chemically designated as 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylic acid-3 ethyl-5 methyl ester, is a calcium channel blocker used to treat hypertension and angina (Arrowsmith *et al*., 1986; O` Neil *et al*., 2006). The drug is found to metabolize in the liver and the produced metabolites are excreted via urine along with some unchanged drug (Abernethy, 1989; Meredith, Elliott, 1992). Many stability indicating HPLC methods are reported in the literature for amlodipine either alone or in combination with other active pharmaceutical ingredients (Naidu, Kale, Shingare, 2005; Chaudhari, Patel, Shah, 2007; Mohammadi *et al*., 2007; Shah *et al*., 2008; Zaazaa *et al*., 2013; Gumustas, Ozkan, 2013).

The combination of antihyperlipidemic and antihypertensive agents is very proficient, as majority of hyperlipdemic patients may also have the hypertension problems. In order to cope with both the diseases simultaneously, a combination comprising of rosuvastatin and amlodipine was approved and marketed. This combination is available in the commercial market on prescription. Literature revealed only two HPLC methods for the simultaneous determination of both the drugs (Tajane *et al*., 2012; Banerjee, Vasava, 2013). The reported methods although utilized isocratic elution with low retention times of both the analytes but they lacks stress testing on the drugs and therefore unable to separate degradation products. ICH guidelines stress to include forced degradation studies in all the developed methods including HPLC methods for pharmaceutical formulations and they must have stability indicating properties. Keeping in view the problem, we focused our attention to develop and validate a simple and precise stability indicating HPLC method for the concurrent determination of rosuvastatin and amlodipine. We are currently engaged in binary combination analysis of different classes of drugs in pharmaceutical formulations and human plasma (Qutab *et al*., 2007a,b; Qutab *et al.*, 2009; Ashfaq *et al.,* 2007; Ashfaq, Khan, Asghar, 2008; Khan *et al*., 2008, Khan *et al*., 2010; Khan *et al*., 2013; Khan, Jilani, Ashfaq, 2010; Sharif *et al*., 2010.; Razzaq *et al*., 2012a,b,c,d; Razzaq *et al*., 2013). The present work is therefore aimed to attain the optimum chromatographic conditions for the simultaneous determination of rosuvastatin and amlodipine in pharmaceutical formulations. The developed method is capable to separate both the pharmaceutical components in less than twenty minutes and therefore can be proficiently utilized in quality control analysis of both drugs and for other analytical purposes.

MATERIAL AND METHODS

Chemicals and reagents

Reference standards of both rosuvastatin calcium and amlodipine with claimed purity of 99.55% and 99.06% respectively, were kindly donated by Schazoo Zaka Laboratories (Lahore, Pakistan). The combination

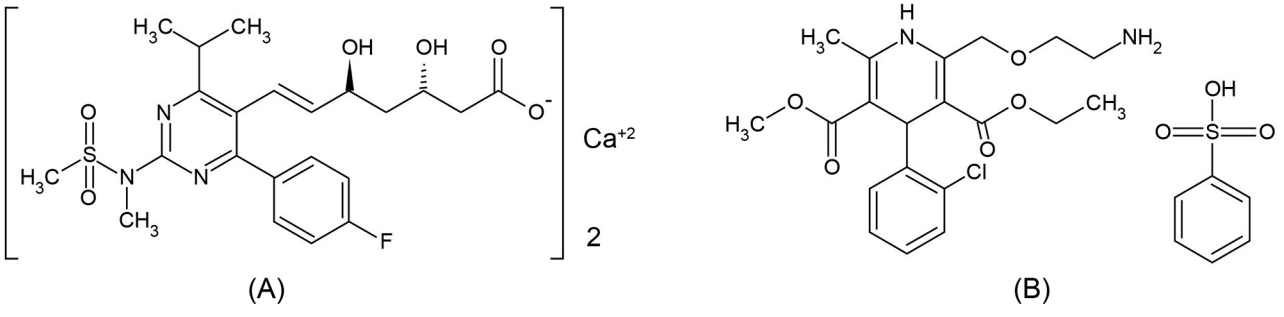

FIGURE 1 - Chemical structures of rosuvastatin calcium (A) and amlodipine besylate (B).

drug product claimed to contain 10 mg rosuvastatin and 5 mg amlodipine were purchased from the local market. HPLC grade acetonitrile and analytical reagent grade other chemicals were purchased from Fluka and were used without further purification. Throughout the analysis, double distilled water was used. Nylon filters (0.45 µm) for mobile phase filtration were from Millipore, USA.

Equipment and chromatographic conditions

Shimadzu LC-20A system (Shimadzu, Japan) was used for performing all the experiments including development and its subsequent validation. The system was equipped with online degasser and auto sampler. Detections of both components were performed at 240 nm and Class VP HPLC software was used for peak areas calculations. Reversed phase discovery C18 column (250 × 4.6 mm, 5 µm particle size) was used at room temperature. Mobile phase was prepared by mixing acetonitrile and 0.1 M ammonium acetate (pH 5.0) in the ratio of 30:70 (v/v). Isocratic elution was carried out with 1.5 mL/min as flow rate of the mobile phase.

Preparation of standard solution

The standard stock solution of rosuvastatin and amlodipine was prepared by accurately weighing 50 mg of rosuvastatin and 25 mg amlodipine in 50 ml volumetric flask, sonicated with few ml of the mobile phase and then marked up to point with mobile phase. This provides the concentration of rosuvastatin and amlodipine equal to 1000 µg/mL and 500 µg/mL, respectively. The solutions of different concentrations were then prepared by diluting the standard stock solution with mobile phase by using the dilution formulae.

Preparation of sample solution

Average weight of twenty tablets were taken and then ground manually with mortar and pestle. A weighed portion of the ground tablets, which was equal to 50 mg rosuvastatin and 25 mg amlodipine, was dissolved in 50 mL of mobile phase. The solution obtained above was then further diluted to get desired concentrations.

Linearity

To carry out the linearity, six different concentrations of rosuvastatin and amlodipine ranging from 1 µg/mL to 200 µg/mL (1, 5, 10, 50, 100 and 200 µg/mL) and 0.5 µg/mL to 100 µg/mL (0.5, 2.5, 5, 25, 50 and 100 µg/mL), respectively, were prepared and analyzed. Triplicate analysis of each solution was performed.

Limit of detection (LOD) and limit of quantification (LOQ)

For calculating LOQ and LOD of the developed method, blank solution and a solution spiked with known concentrations of each analyte in decreasing concentration were prepared and injected into HPLC system. The LOD and LOQ were calculated for both the drugs by measuring the minimum level of analytes that can be detected (Signal to noise ratio of 3:1) and quantified (Signal to noise ratio of 10:1) with accuracy and precision by the system.

Accuracy

For demonstrating accuracy, known amounts of rosuvastatin and amlodipine were added into the pre-analyzed solutions. Theoretical and experimental results were then compared. The solutions corresponding to 50, 100 and 150% of the nominal analytical concentration were prepared for this purpose i.e. 50 µg/mL for rosuvastatin and 25 µg/mLfor amlodipine.

Precision

For intraday precision, three set of concentrations of rosuvastatin and amlodipine were tested five times within the same day. From these replicates, percentage RSD was calculated. For inter day precision, same samples which were used for intraday precision are tested for three consecutive days. RSD was then calculated from those.

Robustness

Pre-meditate variations were carried out in the experimental conditions of the proposed method to assess the method robustness. For this, small variations were made in the operating conditions of the method like mobile phase composition, flow rate and pH of buffer solution. The effect of these changes on chromatographic results was then measured.

Forced degradation studies

To carry on forced degradation studies, 2 mL each of standard solution was taken in four different 25 mL volumetric flasks. All the four flasks were marked as acid, base, oxidative and thermal. 2 mL of 5 M HCl was added in acid flask, 2 mL 5 M NaOH in basic flask and 1 mL

of 6% hydrogen peroxide in oxidative flask were added. The thermal flask was remained as such. All the flasks except oxidative flask were placed at 60 °C for four hours. After completion of stress time, all the three flasks were cooled, and acid and base flasks were neutralized using appropriate amount of either 5 M HCl or 5 M NaOH. The oxidative flask was kept at room temperature for 24 h. All the stressed samples were then completed up to the mark and analyzed by the proposed method.

RESULTS AND DISCUSSION

The aim of the present research work was to develop a sensitive, accurate, isocratic and simple HPLC method for the separation and estimation of rosuvastatin and amlodipine along with their stress induced degradation products. Initially various mobile and stationary phases were attempted to accomplish the best separation conditions and resolution between rosuvastatin, amlodipine and their stress induced degradation products. Among all the stationary phases, C-18 stationary phase (250 mm × 4.6 mm) was found best for the resolution of both the drugs. For the selection of appropriate mobile phase composition, acetonitrile was selected along with water in different proportions as the start-up mobile phase. Different proportions of these two solvents were tried but all found unsuitable as good peaks were not obtained. The poor peak shapes were expected to be due to the lesser polarity of the mobile phase. So in the next phase, polarity of the mobile phase was increased by replacing the water with ammonium acetate buffer. At 60:40 v/v ratio of acetonitrile and ammonium acetate, better peaks for both the drugs were obtained but with certain tailing. In order to reduce tailing, pH of the ammonium acetate solution was varied. For this purpose, 0.1 M ammonium acetate with pH equal to 3.0, 5.0 and 7.0 were tried along with acetonitrile in 40:60 v/v ratios. Among the three buffer solution, pH 5.0 was proved to be the best where sharp peaks were obtained with acceptable tailing and resolution. When this mobile phase composition was applied to analyze samples, stressed through acid, base, hydrogen peroxide and heat, degradation products produced during the stress, interfered the peaks of the active product ingredients. Here both the acid and base stressed samples produced co-eluting peak of rosuvastatin with the degradation products. Increase of organic modifier results either with same results or peak of rosuvastatin with some degree of fronting. It was then decided to decrease the concentration of organic modifier so as to completely separate the degradation products from the main peaks. The percentage of organic modifier was varied from 60 to 28 percent by decrease of 2% each time. The main problem was associated with the separation of rosuvastatin from degradation product 5 (Deg 5 in chromatograms). At 72:28 v/v ratio of buffer and acetonitrile, all the degradation products were well separated from the main peaks with resolution greater than 3 between rosuvastatin and degradation product 5. However, the total run time was greater than 30 minutes. At 68:32 buffer acetonitrile ratios, separation between the above two was not complete and merging of both the components was very clear. Finally, 70:30 buffer and acetonitrile were chosen as the mobile phase where resolution between rosuvastatin and degradation product 5 was exactly equal to 1.5 with reasonable run time of about 20 minutes. By employing the above mentioned chromatographic conditions, rosuvastatin and amlodipine were separated at retention times of 13.9 and 19.3 min respectively [Figure 2].

The developed method was validated using ICH prescribed validation parameters such as linearity, accuracy, precision, limit of detection and quantification, specificity, stability of solutions and robustness. These parameters were performed to check the validity of the proposed method.

To carry out the linearity, six different concentrations of rosuvastatin and amlodipine ranging from 1 µg/mL to 200 µg/mL (1, 5, 10, 50, 100 and 200 µg/mL) and 0.5 µg/mL to 100 µg/mL (0.5, 2.5, 5, 25, 50 and 100 µg/mL), respectively, were prepared and analyzed. The calibration curves were constructed between concentration on X-axis and peak area on Y-axis. The regression equation for the rosuvastatin was Y=21604 X + 56856 with value of the regression constant or coefficient of determination equal to 0.996. The regression equation for amlodipine was Y=29444 X + 29461 with value of the regression constant or coefficient of determination equal to 0.994. Results indicated very good correlation between the concentrations and their chromatographic response. The limits of detection of an analytical method is the concentration of analyte that corresponds to S/N ratio (signal to noise ratio) of 3:1. For rosuvastatin and amlodipine that concentration corresponds to 0.15 µg/mL and 0.30 µg/mL, respectively, where detector gives the desired response with S/N ratio 3:1. The quantification limit is the lowest concentration detected accurately and generally corresponds to the detector response with S/N ratio of 10:1 with acceptable precision. For rosuvastatin, 1 µg/mL and for amlodipine 0.5 µg/mL were the concentrations that produce the desired response with acceptable accuracy and precision. Therefore, LOD was 0.30 µg/mL and 0.15 µg/mL, whereas LOQ was 0.5 µg/mL and 1 µg/mLfor rosuvastatin and amlodipine, respectively.

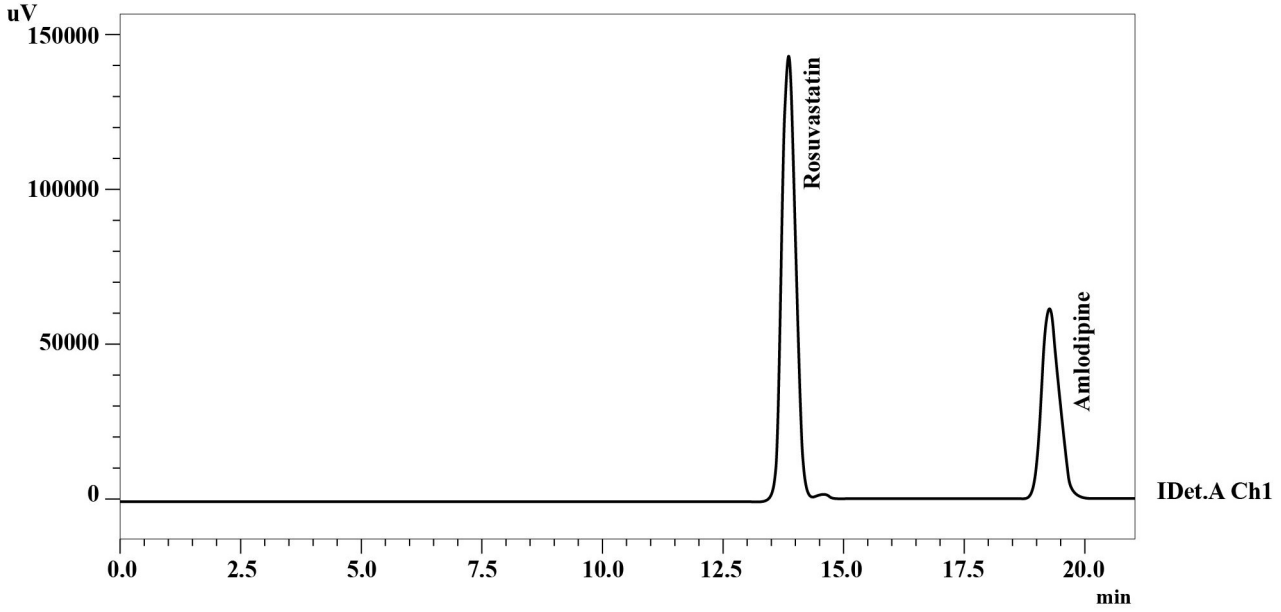

FIGURE - 2 Chromatogram of rosuvastatin and amlodipine reference standard.

For demonstrating accuracy, known amounts of rosuvastatin and amlodipine were added into the pre-analyzed solutions. Theoretical and experimental results were then compared. The solutions corresponding to 50, 100 and 150% of the nominal analytical concentrations were prepared for this purpose i.e. 50 µg/mL for rosuvastatin and 25 µg/mL for amlodipine. From these solutions, percentage recovery along with RSD was calculated. The results of accuracy are given in Table I.

For intraday precision, three sets of concentrations of rosuvastatin and amlodipine were tested five times within the same day. For inter day precision, same samples which were used for intraday precision were tested for three consecutive days. RSD was then calculated from both types of data which was less than 2% as shown in Table II.

TABLE I - Accuracy of the method

Drug	n	Level (%)	Concentration (µg/mL)	Amount Recovered (µg/mL)	Recovery (%)	RSD (%)
	5	50	25.0	24.59	98.36	1.21
Rosuvastatin	5	100	50.0	50.02	100.04	0.99
	5	150	75.0	74.05	98.73	0.35
	5	50	12.5	12.39	99.12	1.39
Amlodipine	5	100	25.0	24.77	99.08	1.05
	5	150	37.5	37.11	98.96	0.67

TABLE II - Intra-day and Inter-day precision of the method

Compound	Concentration (µg/mL)	Intra-day Precision RSD (%)	Inter-day Precision RSD (%)
	1.0	1.57	1.81
Rosuvastatin	10.0	1.18	1.59
	100.0	0.72	1.13
	0.5	1.49	1.88
Amlodipine	5.0	1.12	1.63
	50.0	0.83	1.23

TABLE III - Robustness study of rosuvastatin

Conditions	Assay (%)	t_R (min)	Theoretical Plates	Tailing
Acetonitrile: buffer (30:70)	98.89	13.87	12884	1.12
Acetonitrile: buffer (28:72)	99.34	17.23	14114	1.09
Acetonitrile: buffer (32:68)	100.15	10.69	12165	1.15
Flow rate (1.4 mL/min)	100.53	14.86	13009	1.10
Flow rate (1.6 mL/min)	99.29	12.99	12264	1.16
Buffer (pH 5.1)	100.33	13.85	12896	1.13
Buffer (pH 4.9)	100.85	13.88	12784	1.13

TABLE IV - Robustness study of amlodipine

Conditions	Assay (%)	t_R (min)	Theoretical Plates	Tailing
Acetonitrile: buffer (30:70)	99.78	19.28	16919	1.22
Acetonitrile: buffer (28:72)	100.38	27.56	18612	1.17
Acetonitrile: buffer (32:68)	99.08	15.43	15006	1.30
Flow rate (1.4 mL/min)	100.64	20.66	16812	1.20
Flow rate (1.6 mL/min)	98.79	18.08	17016	1.21
Buffer (pH 5.1)	99.25	19.33	16716	1.22
Buffer (pH 4.9)	99.05	19.30	16707	1.22

Pre-meditate variations were carried out in the experimental conditions of the proposed method to assess the method robustness. For this, faint modifications were made in the operating conditions of the method like mobile phase composition, flow rate and pH of buffer solution. The results showed that slight variations in chromatographic conditions had negligible effect on the chromatographic parameters. The results of robustness are given in Table III and IV.

Forced degradation study was performed on both the drugs in order to judge the specificity of the described method. Different ICH prescribed stresses like acid, base, oxidative and thermal stresses were provided to both rosuvastatin and amlodipine in combined form. The chromatograms under different stress conditions are shown in [Figures 3-5]. Out of the above mentioned

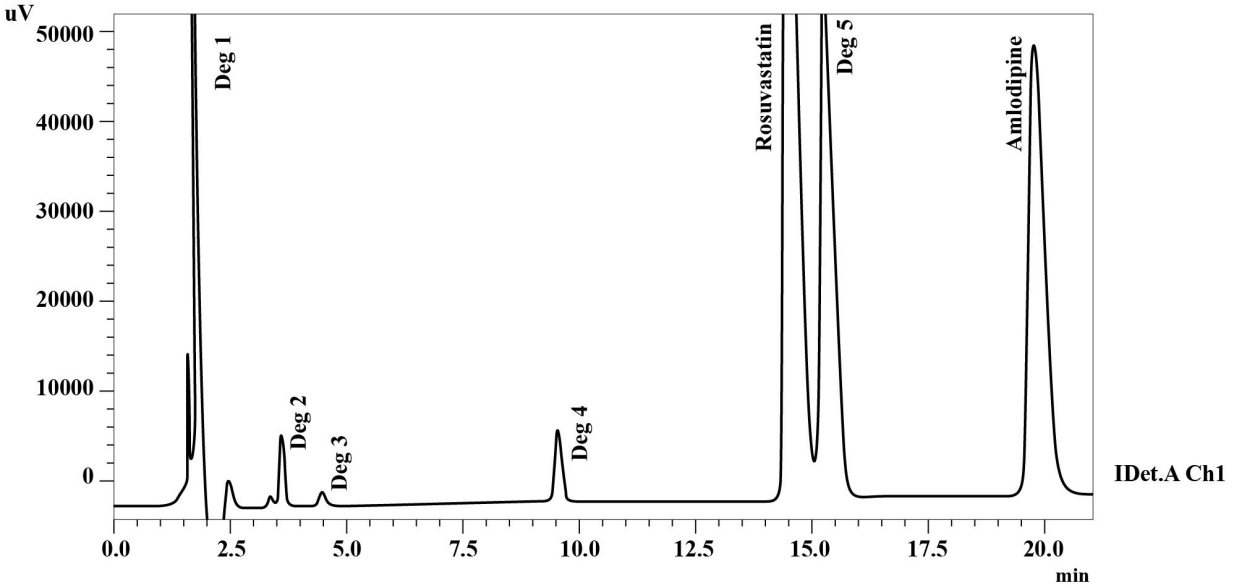

FIGURE 3 - Chromatogram of rosuvastatin and amlodipine under acidic stress.

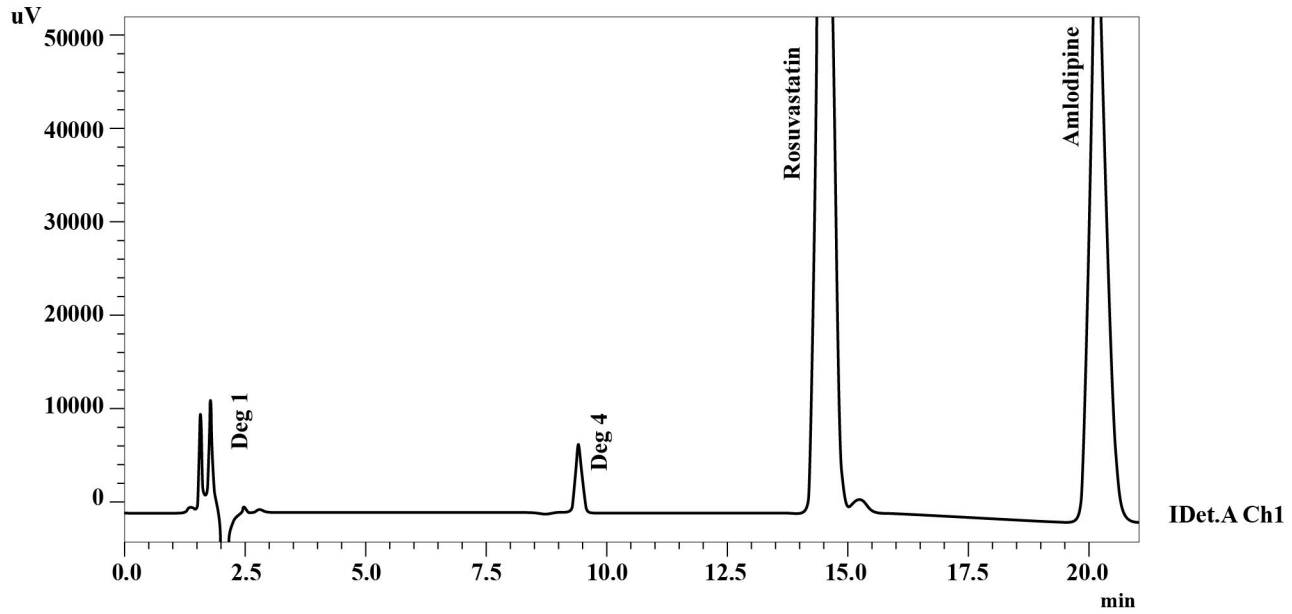

FIGURE 4 - Chromatogram of rosuvastatin and amlodipine under basic stress.

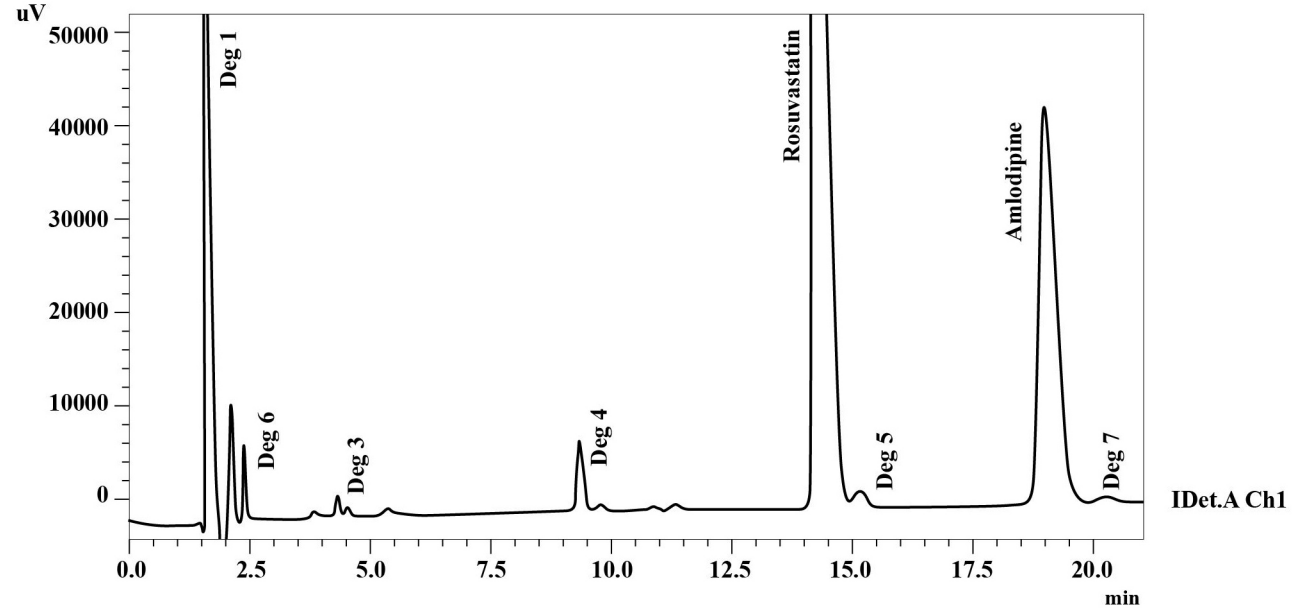

FIGURE 5 - Chromatogram of rosuvastatin and amlodipine under oxidative stress.

stresses, acidic, basic and oxidative stresses degrade both the drugs to some extent whereas thermal stress could not produce any degradability to them. Acidic conditions were proved to be more damaging where 40% of rosuvastatin and about 35% of the amlodipine were degraded. Under oxidative stress, the degradation of amlodipine was fast compared to rosuvastatin and about 40% of it was decomposed in contrast to only 6% of rosuvastatin. Basic stress degrades rosuvastatin to about 5% in contrast to 14% for amlodipine. Thermal stress had no effect on the degradability of both the drugs. A number of degradation products were also produced during stress testing whose chromatographic parameters are shown in Table V.

Application of the proposed method was checked by analyzing the rosuvastatin and amlodipine in commercially available pharmaceutical products. The results are provided in Table VI, which showed high percentage recoveries and low RSD (%) values for both analytes.

To check the stability of both components, the solution of these components was placed in tight containers at room temperature for 48h and their stability was checked after each 12 hours period. Results indicate

TABLE V - Chromatographic parameters of stress induced degradation products

Nature of Stress	Retention times (min)	Number of theoretical plates	Tailing factors
Acidic	1.70, 3.40, 4.20, 9.01, 14.55	3448, 4895, 7914, 20281, 13200	1.58, 1.18, 1.23, 1.08, 1.25
Basic	1.52, 2.04, 9.01	3564, 6138, 20333	1.58, 0.75, 1.09
Oxidative	1.71, 2.42, 4.36, 9.41, 15.32	3213, 7503, 10859, 21102, 13409	2.45, 1.38, 1.07, 1.11, 1.25
Thermal	----------	----------	----------

*n=Average of 3 determinations

TABLE VI - Application of the method in commercial formulations

Ingredient	Label Value (mg/tablet)	n	Found (mg/tablet)	Recovery (%) ± RSD (%)
Rosuvastatin	10.00	10	9.87	98.70 ± 0.85
Amlodipine	5.00	10	5.02	100.40 ± 0.98

that their percentage recovery remained within the acceptable range and no degradation occurred during the said period.

CONCLUSION

The present work demonstrates a sensitive and reproducible HPLC method for a pharmaceutical combination comprising of an anti-hyperlipidemic agent along with an anti hypertensive drug. The method is highly selective and there was no interference caused by any kind of other particles including the degradation products produced through forced degradation study. Both the drugs and their degradation products were separated within twenty minutes. The results of accuracy, linearity, precision, LOD, LOQ and specificity indicate that method can be used not only for routine analytical analysis but also for stability studies for the separation and quantification of both rosuvastatin and amlodipine either alone or in combination with each other.

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