Journal of Applied Pharmaceutical Science Vol. 7 (03), pp. 048-056, March, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.70308
ISSN 2231-3354

Stability Indicating HPLC Method for the Simultaneous Quantification of Aspirin and Pravastatin in bulk and Tablets: Method Development and Validation

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ARTICLE INFO

Article history:
Received on: 09/12/2016
Accepted on: 07/01/2017
Available online: 30/03/2017

Key words:
Aspirin, Pravastatin, Stability indicating, Simultaneous, Analysis.

ABSTRACT

The aim of the present study was to develop and validate a stability indicating HPLC method for the simultaneous determination of aspirin and pravastatin in bulk and pharmaceutical dosage forms. The chromatographic separation of aspirin, pravastatin and their degradation products was achieved on Phenomex C18 column with mixture of water, acetonitrile and acetic acid (40:59:01, *v/v/v*) as mobile phase in an isocratic elution mode at a flow rate of 1.5 mL/min. The method exhibited linearity in the concentration range of 20.5-61.5 µg/mL and 10-30 µg/mL for aspirin and pravastatin, respectively. The limits of detection and quantification were 0.204µg/mL and 0.680 µg/mL for aspirin and 0.077 µg/mL and 0.256 µg/mL for pravastatin, respectively. The developed method was validated with respect to accuracy, precision, selectivity, specificity and robustness. All the parameters examined were within the acceptance limits. The stability-indicating power of the proposed method was proved by subjecting the drugs to hydrolytic (acid and base), oxidative, photolytic and dry heat stress conditions. The developed method was found to be suitable for routine analysis of aspirin and pravastatin simultaneous in the presence of its stress degradation products.

INTRODUCTION

Pravastatin, chemically known as (3R,5R)-7-[(1S,2S,6S,8S,8aR) -6-hydroxy-2-methyl-8- [(2S)-2-methyl butanoyl] oxy-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid (Figure 1), is a synthetic lipid lowering agent (Akira, 2010). Pravastatin lowers cholesterol synthesis by acting as an inhibitor of hydroxymethylglutaryl-CoA reductase enzyme. Pravastatin is used for lowering plasma cholesterol levels and prevention of cardiovascular disease (Nakamura *et al.*, 2006; Toshio *et al.*, 2009). Literature survey reveals that pravastatin is official in British Pharmacopoeia (British Pharmacopoeia, 2007a). Several analytical methods are available for the estimation of pravastatin in bulk, pharmaceutical dosage forms and biological matrices, these include UV spectrophotometry (Balaji and Suman, 2009; Kunjan *et al.*, 2014), visible spectrophotometry (Kalvikkarasi *et al.*, 2009; Safwan and Mouhammed, 2011), HPLC (Bauer *et al.*, 2005; Önal and Sagirli, 2006; Brain-Isasi *et al.*, 2008; Safwan *et al.*, 2008; Raj *et al.*, 2010; Jane and Janivishal, 2013; Vania, 2014), LC-MS (Mulvana *et al.*, 2000; Zhu and Neirinck, 2003; Kawabata *et al.*, 2005; Sampath *et al.*, 2011), capillary electrophoresis (Kircali *et al.*, 2004; Nigovic and Vegar, 2008) and voltammetry (Nigović, 2006).

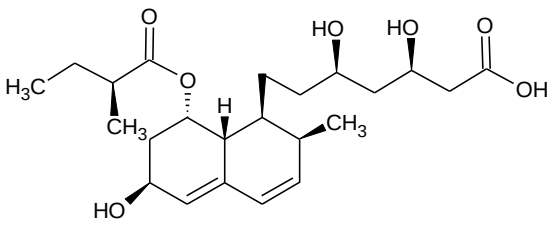

Fig. 1: Structure of pravastatin.

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Aspirin, chemically known as 2-acetyloxybenzoic acid (Figure 2), is a nonsteroidal anti-inflammatory drug used in the temporary relief of various forms of pain and inflammation associated with various conditions (Valentin and Joseph, 2011). Aspirin is also commonly known as acetylsalicylic acid. Aspirin shows antipyretic, antirheumatic, analgesic and anticoagulant properties. Aspirin inhibits the biosynthesis of prostaglandins by inhibiting the activity of cyclooxygenase (Rod, 2003). Aspirin also prevents arterial and venous thrombosis by inhibiting platelet aggregation (Enma *et al.*, 2012).

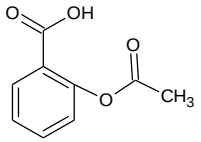

Fig. 2: Structure of aspirin.

Aspirin is official in United States Pharmacopoeia (United States Pharmacopoeia, 2000), British Pharmacopoeia (British Pharmacopoeia, 2007b) and Indian Pharmacopoeia (The Indian Pharmacopoeia, 2007). Different methods have been cited in the literature for determination of aspirin involving UV spectrophotometry (Lei, 2014), visible spectrophotometry (Maruf *et al.*, 2001; Sahar, 2013), kinetic spectrophotometry (Senzana *et al.*, 2008), FT-IR spectrophotometry (Andrei *et al.*, 2006), surface-enhanced raman scattering spectroscopy (Sallum *et al.*, 2014), fluorescence spectroscopy (Moreira *et al.*, 2004), HPLC (Kokot and Burda, 1998; Kumar *et al.*, 2010; Ramjith *et al.*, 2013), LC-MS (Bae *et al.*, 2008; Xu *et al.*, 2009), potentiometry (Fernandes *et al.*, 1998), differential scanning calorimetry (Luigi *et al.*, 2010), voltametry (Majdi *et al.*, 2007; Sartori *et al.*, 2009; Codruţa and Ciprian, 2011) and capillary electrophoresis (Sekar *et al.*, 2003). The aspirin and pravastatin combination is used to treat high cholesterol, lower the risk of stroke and heart attack in people with coronary heart disease (Charles *et al.*, 2004). So far only liquid chromatography–tandem mass spectrometric (LC–MS/MS) assay method has been developed for the simultaneous quantification of pravastatin and aspirin using furosemide as internal standard in human plasma (Srinivasarao *et al.*, 2012). The analytes and internal standard samples were chromatographed on a Zorbax SB-C18 column by using a mixture of 5 mM ammonium acetate buffer and acetonitrile (20:80, *v/v*) as the mobile phase at a flow rate of 0.8 mL/min. To the best of our knowledge, no reports could be found for the simultaneous determination of aspirin and pravastatin using HPLC with photodiode-array (PDA) detector. The present investigation describes the development of a stability indicating HPLC method applicable for the routine quality control analysis of aspirin and pravastatin simultaneously in their pure and tablets dosage forms.

EXPERIMENTAL

Chemicals and solvents

HPLC grade acetonitrile was purchased from Merck Pvt Ltd., Mumbai, India. Analytical reagent grade glacial acetic acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide were obtained from Sd Fine Chemicals Ltd., Mumbai, India. Purified water was obtained from Milli-Q system. Aspirin and pravastatin were kindly provided by Lara Drugs Private Limited (Telangana, India). Pravigard tablets labeled to contain 81 mg and 40 mg of aspirin and pravastatin, respectively are manufactured by Bristol-Myers squibb company, Princeton, New Jersey.

Instrumentation and chromatographic conditions

The Waters HPLC system, consisted of a binary HPLC pump model 2695, PDA detector model 2998 and a vacuum degasser, all controlled by a Waters Empower 2 software, was used. HPLC analysis was performed isocratically at 25 ^{o}C temperature using a Phenomex C18 (200 mm × 4.6 mm, 5 µm) column. The mobile phase consisted of a mixture of water, acetonitrile and acetic acid (40:59:01, *v/v/v*). The flow rate was 1.5 mL/min and injection volume was 20 µL. The eluent was monitored with a detector set at 260 nm.

General procedure

Stock standard solution of aspirin and pravastatin was prepared with mobile phase at a concentration of 410 µg/mL and 200 µg/mL, respectively. Stock solution was kept refrigerated at 4 °C until further use. For the construction of calibration curves, serial portions of the stock solution were separately transferred into 5 sets of 10 mL volumetric flasks to produce working standard solutions of concentrations 20.50 µg/mL, 30.75 µg/mL, 41.0 µg/mL, 51.25 µg/mL and 61.50 µg/mL for aspirin and 10 µg/mL, 15 µg/mL, 20 µg/mL, 25 µg/mL and 30 µg/mL for pravastatin. All the flasks were completed to volume with mobile phase. From these working standard solutions, 20 µL portions of each standard solution were injected into the HPLC column. The peak areas of aspirin and pravastatin were determined by following the operating chromatographic conditions. For each drug, the obtained peak areas were correlated to the corresponding concentrations to construct calibration curves. The regression equation was developed.

Analysis of tablets

For the analysis of aspirin and pravastatin in its pharmaceutical dosage form, twenty tablets were accurately weighed, finely powdered and thoroughly mixed. A quantity of the powdered tablets was precisely weighed so as to contain 41 mg of aspirin and 20 mg of pravastatin. The measured weight was extracted with 50 mL of mobile phase with the aid of sonication for 10 min. The obtained extract was filtered into a 100 mL volumetric flask and the volume was completed to mark with mobile phase. The produced tablet extract solution was labeled to contain 410 µg/mL aspirin and 200 µg/mL pravastatin. 5 mL from the aforementioned extract solution was transferred into 50 mL volumetric flask to prepare working sample solution (aspirin - 41 mg and pravastatin - 20 mg) and the flasks were completed to volume with mobile phase. 20 µL of working tablet sample solution was injected into the HPLC system. The peak areas of

aspirin and pravastatin were determined by following the optimized chromatographic conditions. The concentration of aspirin and pravastatin in tablet was calculated either from the corresponding calibration curve or regression equation.

Degradation studies

This study was performed by using different ICH prescribed (acidic, basic, oxidative, thermal and photolytic) stress conditions (Q1A (R2) International Conference on Harmonisation, 2003).

Acid degradation studies

For acid degradation study, 5 mL of tablet sample solution (410 µg/mL aspirin and 200 µg/mL pravastatin) was taken and placed in a 50 mL volumetric flask. 10 mL of 0.1 N HCl was added to the flask and sonicated for 30 min. After completion of the stress, the solution was neutralized by using 0.1 N NaOH and completed up to the mark with mobile phase.

Base degradation studies

Base degradation study was performed by transferring 5 mL of tablet sample solution (410 µg/mL aspirin and 200 µg/mL pravastatin) to a 50 mL volumetric flask. 10 mL of 0.1 N NaOH was added to the flask and sonicated for 30 min. After completion of the stress, the solution was neutralized by using 0.1 N HCl and completed up to the mark with mobile phase.

Oxidative degradation studies

For this study, 5 mL of tablet sample solution (410 µg/mL aspirin and 200 µg/mL pravastatin) was transferred to a 50 mL volumetric flask followed by addition of 10 mL of 30% H_2O_2. The contents of the flask were sonicated for 30 min. After completion of stress, the flask was completed up to the mark with mobile phase.

Thermal degradation studies

The thermal degradation study was performed at 105 ^oC for 30 min in oven. For this study, a quantity of the powdered tablets equivalent to 41 mg aspirin and 20 mg pravastatin was placed in petric disc and exposed to 105 ^oC for 30 min in oven. After the specified time, the tablet powder was cooled and transferred to a 100 mL volumetric flask containing 50 mL of mobile phase. After sonication for 10 min, the flask was made up to volume with mobile phase. This solution was diluted appropriately with the same solvent to get a final concentration of 41 µg/mL (aspirin) and 20 µg/mL (pravastatin).

Photolytic degradation studies

For photolytic degradation, a quantity of the powdered tablets equivalent to 41 mg aspirin and 20 mg pravastatin was placed in petric disc and exposed to sun light for 24 hrs. After the specified time, the solution was prepared as described in section '*Thermal degradation studies'*. All the stress degraded samples were filtered before injection into the HPLC system. The peak areas of aspirin and pravastatin were recorded from the respective chromatogram.

RESULTS AND DISCUSSION

Method development

The study was intended for the effective separation of aspirin, pravastatin and its stress induced degradation products. Various mobile phase combinations of acetonitrile with water, dipotsssium hydrogen phosphate, disodium hydrogen phosphate and glacial acetic acid were investigated to minimize peak tailing, improve peak symmetry, improve column efficiency, improve resolution and minimize total analysis time. Finally a mobile phase consisting of a mixture of water, acetonitrile and glacial acetic acid in the ratio of 40:59:01 (*v/v/v*) was selected as mobile phase to achieve maximum separation and sensitivity. Different flow rates of mobile phase were studied. A flow rate of 1.5 mL/min gave better results. Different temperatures (15°C - 35°C) were evaluated. It was found that the temperature had a negligible influence on resolution and tailing factors. Therefore, room temperature was chosen. Using a reversed phase Phenomex C18 (200 × 4.6 mm, 5 µm) column and optimized HPLC conditions (mobile phase combination, flow rate and column temperature), the retention times for aspirin and pravastatin were observed to be 3.654 min and 4.664 min, respectively. Total time of analysis was <7 min. The chromatogram at 260 nm showed a complete resolution of aspirin and pravastatin peaks (Figure 3). The method was validated as per ICH guidelines (International Conference on Harmonization, 2005).

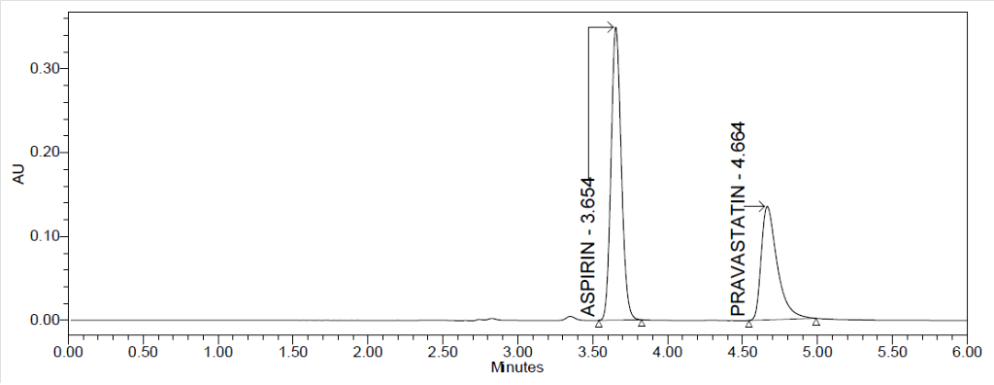

Fig. 3: Typical chromatogram of aspirin and pravastatin Method validation.

System suitability

Prior to analysis, the chromatographic system must satisfy system suitability test requirements. The system suitability test was performed by injecting working standard solution containing 41 µg/mL (aspirin) and 20 µg/mL (pravastatin) in five replicates. The percent relative standard deviation (RSD) of the peak area responses and retention times of analytes were determined. Also, USP tailing factor, USP plate count and USP resolution factor were calculated. The results of system suitability in comparison with the required limits are shown in Table 1. The system suitability parameters of the developed method are found to be within the recommended limits.

Linearity

The linearity of the method was evaluated by analyzing standard solutions of aspirin and pravastatin in the range of 20.5-61.5 µg/mL and 10-30 µg/mL, respectively. Calibration curve was constructed by plotting peak area *versus* concentration of analytes (Figures 4 and 5). The regression equation was calculated. The linear equations for the calibration curves were:

Y = 39148 *x*+17786, R^2 = 0.9999 for aspirin

Y = 50402 *x* + 944.2, R^2 = 0.9998 for pravastatin

Where Y = peak area of drug; *x* = Concentration of drug in µg/mL; R^2 = regression coefficient.

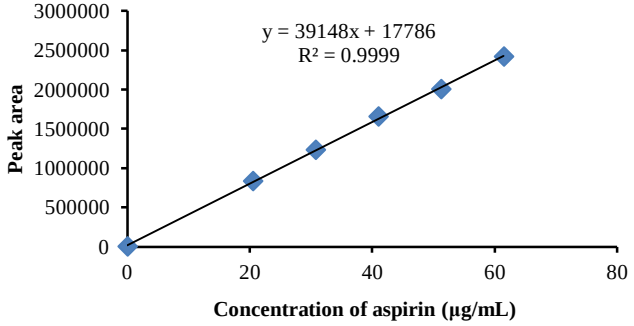

Fig. 4: Aspirin linearity curve.

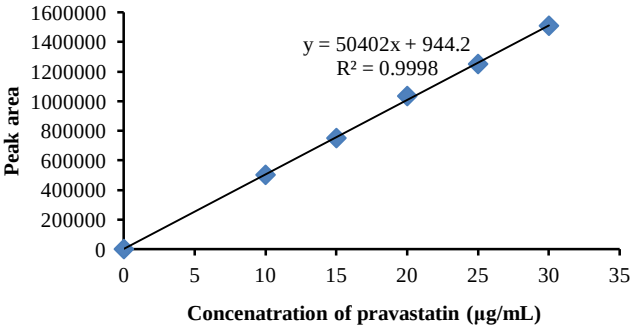

Fig. 5: Pravastatin linearity curve.

Limits of detection (LOD) and quantitation (LOQ)

LOD and LOQ were calculated as signal-to-noise ratio of 3:1 and 10:1, respectively. LOD and LOQ, respectively, were found to be 0.204 µg/mL and 0.680 µg/mL for aspirin and 0.077 µg/mL and 0.256 µg/mL for pravastatin. The low values of LOD and LOQ indicate the adequate sensitivity of the proposed method.

Selectivity

The chromatograms of mobile phase blank, placebo blank and tablet sample solution were checked with the standard solution for the appearance of any extra peaks. Placebo blank solution was prepared in the same way of the tablet sample solution by common excipients (starch-40 mg, acacia-30 mg, sodium citrate-35 mg, hydroxyl cellulose-40 mg, magnesium stearate-40 mg, talc-30 mg and sodium alginate-30 mg) of the tablet dosage form but without aspirin and pravastatin. In the chromatogram of tablet sample, single peak for aspirin (retention time-3.653 min) and pravastatin (retention time-4.624 min) was obtained under optimized conditions (Figure 6d). The retention times of aspirin and pravastatin in the chromatograms of standard and tablet sample solutions are same (Figures 6c and 6d). No peaks were observed in the chromatograms of mobile phase blank and placebo blank solutions (Figures 6a and 6b), showing no interference from common tablet excipients and components of mobile phase.

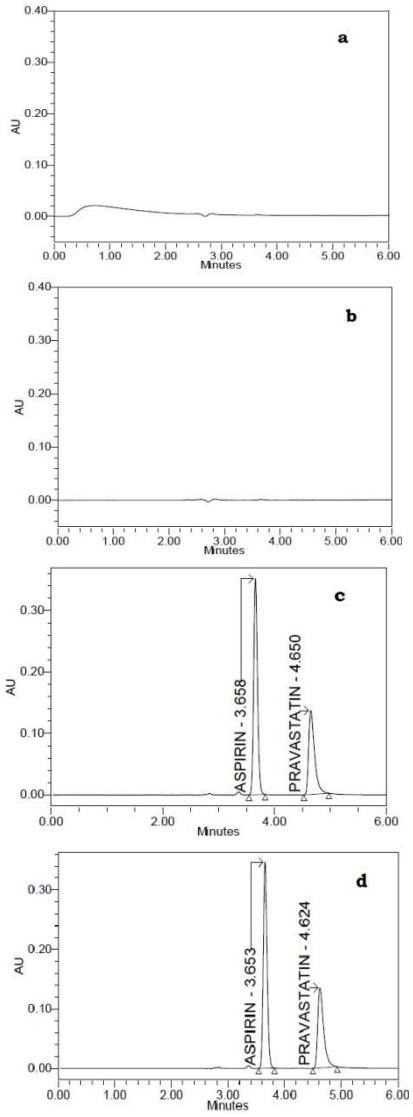

Fig. 6: Chromatogram of [a] Mobile phase blank [b] Placebo blank [c] Standard solution (aspirin-41µg/mL and pravastatin-20 mgµg/mL) [d] Tablet sample solution (aspirin-41 µg/mL and pravastatin-20 µg/mL)

Precision

Precision of the proposed method was studied by making six repeated injections of the standard solution (41 µg/mL-aspirin and 20 µg/mL-pravastatin). Relative standard deviation of aspirin and pravastatin peaks was then calculated to represent precision. The relative standard deviation after six determinations was 0.385% at 41 µg/mL concentration of aspirin and 0.347% at 20 µg/mL concentration of pravastatin (Table 2). The low relative standard deviation (<0.5%) values indicate that the developed method is precise.

Accuracy

Accuracy of the proposed method was established by making six repeated injections of the standard solution (41 µg/mL-aspirin and 20 µg/mL-pravastatin). The mean percent recovery of aspirin and pravastatin was calculated to represent the accuracy of the method. The mean percent recovery was 99.58% for aspirin and 99.16% for pravastatin (Table 2). The high percent recovery values indicate good accuracy of the developed method. The accuracy of the proposed method was further assessed by recovery studies at three different concentration levels by standard addition method. For recovery study, fixed amount of standard aspirin and pravastatin was spiked into preanalyzed sample at three different concentration levels (50%, 100% and 150%). The samples were injected into the HPLC system in triplicate and their percent recovery was determined.

The mean percent recoveries obtained for aspirin and pravastatin were in the range of 98.43%-100.16% and 100.06%-100.81%, respectively (Table 3). The results indicated that there was no interference from tablet excipients.

Robustness

Influence of small changes in chromatographic conditions, such as change in flow rate of the mobile phase (±0.1 mL/min) and column temperature (± 5°C), on the method performance was studied to verify the robustness of the developed HPLC method. The method robustness was determined at a concentration of 41 µg/mL and 20 µg/mL of aspirin and pravastatin, respectively. In all deliberately varied chromatographic conditions, the system suitability parameters were found to be well within the acceptable limit (Table 4) indicating the method robustness.

Table 2: Precision and accuracy of the method.

Injection No.	Aspirin		Pravastatin	
	Peak area	Recovery (%)	Peak area	Recovery (%)
1	1668363	99.92	1001293	98.68
2	1664183	99.67	1011739	99.71
3	1664920	99.72	1006305	99.17
4	1650119	98.83	1003973	98.94
5	1665633	99.76	1005997	99.14
6	1662800	99.59	1007472	99.29
Mean	**1662670**	**99.58**	**1006130**	**99.16**
RSD	**0.385**	**0.386**	**0.347**	**0.348**

Table 3: Recovery of aspirin and pravastatin.

Spiked Level (%)	Concentration of drug (µg/mL)		Recovery (%)	Mean (%)	Concentration of drug (µg/mL)		Recovery (%)	Mean (%)
	added	found			added	found		
	Aspirin				Pravastatin			
50	20.250	20.34	100.45	100.16	9.900	9.92	100.18	100.26
	20.250	20.29	100.21		9.900	9.93	100.34	
	20.250	20.21	99.82		9.900	9.93	100.27	
100	40.500	39.95	98.64	99.14	19.800	19.76	99.79	100.06
	40.500	40.60	100.25		19.800	19.83	100.14	
	40.500	39.90	98.53		19.800	19.85	100.25	
150	60.171	59.81	99.40	98.43	29.417	29.59	100.58	100.81
	60.171	59.19	98.37		29.417	29.63	100.72	
	60.171	58.68	97.52		29.417	29.75	101.14	

Table 4: Robustness of the method.

Parameter	Investigated value	Peak Area	USP Plate Count	USP Tailing	USP resolution
Aspirin					
Temperature (°C)	25 - 2	1839785	14233	1.17	-
	25 + 2	1493451	12777	1.16	-
Flow rate (mL/min)	1.0 - 0.1	1846468	14230	1.17	-
	1.0 + 0.1	1502076	12452	1.16	-
Pravastatin					
Temperature (°C)	25 - 2	1130638	9345	1.67	5.96
	25 + 2	904102	8870	1.59	5.75
Flow rate (mL/min)	1.0 - 0.1	1097569	9995	1.67	6.03
	1.0 + 0.1	919782	8544	1.62	5.76

Forced degradation studies/specificity

The forced degradation study was intended to make sure the effective separation of aspirin, pravastatin and their degradation peaks. Forced degradation studies were performed to assess the stability indicating properties and specificity of the proposed method. Tablet sample solutions for use in stress studies were prepared at a concentration of 41 µg/mL (aspirin) and 20 µg/mL (pravastatin). Forced degradation study was performed by subjecting the analytes to acid hydrolysis, base hydrolysis, hydrogen peroxide mediated hydrolysis, photolytic and dry heat degradation conditions. The results (percent assay, percent degradation, peak purity angle and purity threshold) of degradation studies are summarized in Table 5. The chromatograms of the degraded samples are shown in Figures 7a-7e. The percentage degradation of aspirin is more in hydrogen peroxide mediated oxidative degradation condition and less in alkali hydrolysis. The pravastatin is degraded more in acid hydrolysis and less in hydrogen peroxide mediated oxidative degradation condition. The determination of the peak purity angle and peak purity threshold values demonstrated that the aspirin and pravastatin peaks were pure in all situations. The stability indicating nature and specificity of the method was demonstrated as no degradation products from different stress conditions affected the detection and quantification of aspirin and pravastatin.

Table 5: Forced degradation studies.

Type of degradation	Peak area	Assay (%)	Degradation (%)	Purity Angle	Purity Threshold
		Aspirin			
Acid	1534936	91.93	8.07	0.357	0.520
Base	1539578	92.21	7.79	0.347	0.528
Oxidative	1506459	90.23	9.77	0.363	0.524
Heat	1523013	91.22	8.78	0.328	0.519
Sunlight	1511065	90.50	9.50	0.360	0.531
		Pravastatin			
Acid	970526	95.65	4.35	0.503	0.664
Base	972016	95.79	4.21	0.479	0.657
Oxidative	992323	97.80	2.20	0.487	0.567
Heat	981560	96.73	3.27	0.484	0.660
Sunlight	985498	97.12	2.88	0.508	0.667

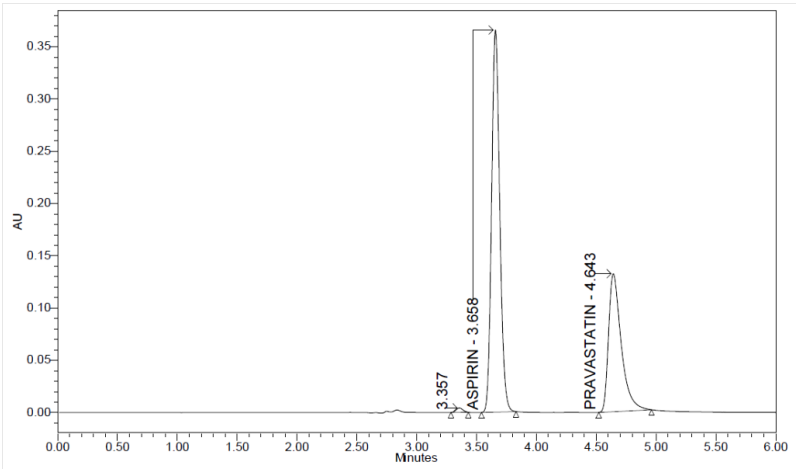

Fig.7a: Chromatogram of acid degraded sample.

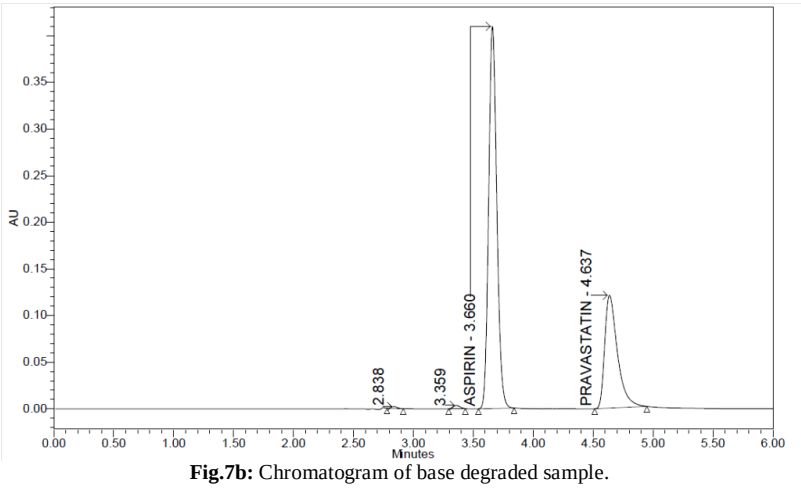

Fig.7b: Chromatogram of base degraded sample.

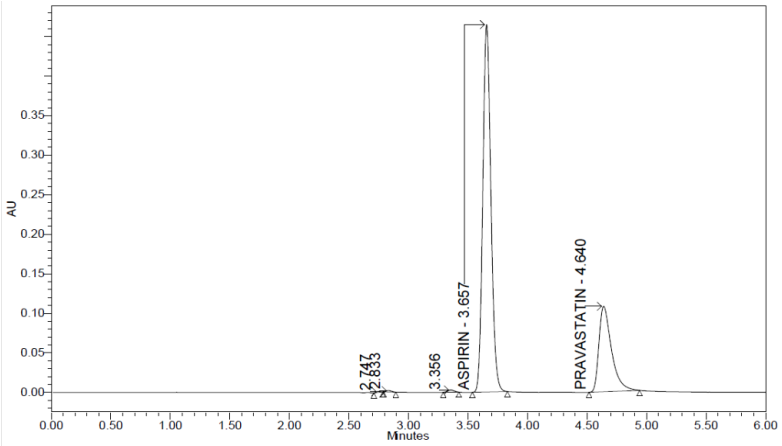

Fig.7c: Chromatogram of hydrogen peroxide degraded sample.

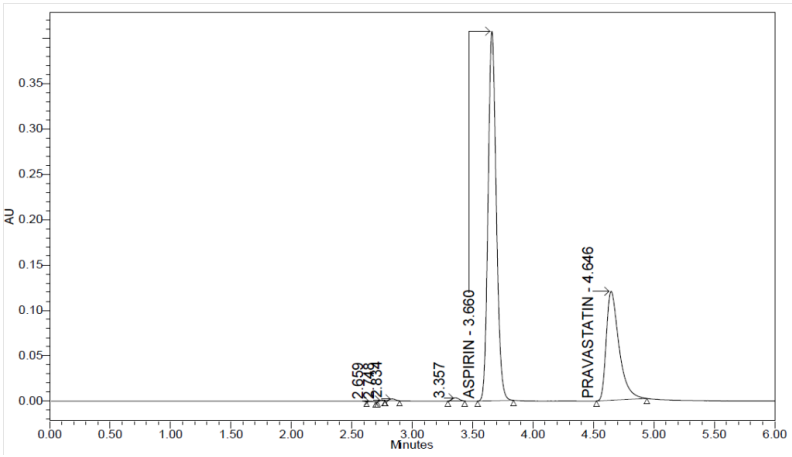

Fig.7d: Chromatogram of dry heat degraded sample.

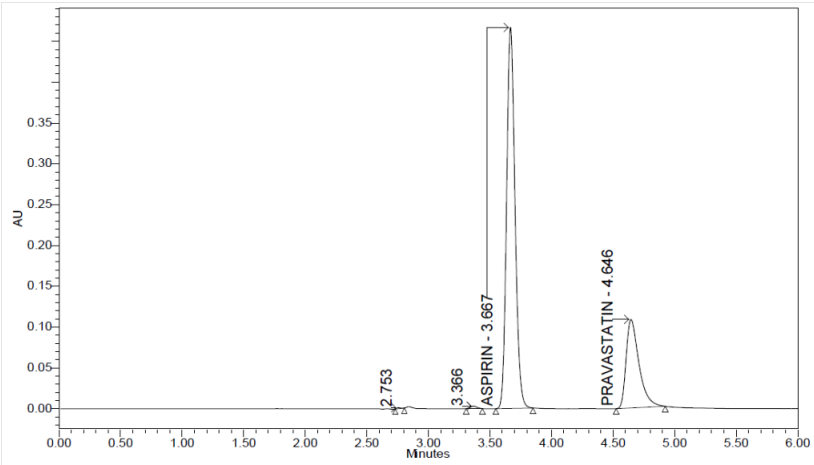

Fig.7e: Chromatogram of photolytic degraded sample.

CONCLUSION

A simple stability indicating HPLC method with PDA detection for the simultaneous determination of aspirin and pravastatin in bulk and in its pharmaceutical dosage forms has been described for the first time. The proposed method was successfully validated in terms of system suitability, linearity, precision, accuracy, selectivity, specificity, LOD, LOQ and robustness as per the guidelines of ICH. The results of validation parameters were found to be within the acceptable limits. The short run time (≤ 6 min) make the method suitable for quick and routine analysis. The results of forced degradation studies showed that the proposed method is stability indicating and capable of simultaneous quantification of aspirin and pravastatin in the presence of their degradation products.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. K. Ravindra Nath (RPAC Chairman, Department of chemistry, KL University) for giving valuable suggestions in completion of this present work and also to the Rainbow Pharma Training Laboratory, Kukatpally Hyderabad for providing the instruments.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Athota RV, Jagarlapudi SK, Singampalli MR. Stability Indicating Hplc Method for the Simultaneous Quantification of Aspirin and Pravastatin in bulk and Tablets: Method Development and Validation. J App Pharm Sci, 2017; 7 (03): 048-056.