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Research Article

Development and validation of a stability-indicating RP-HPLC for the simultaneous determination of Atorvastatin Calcium and Simvastatin in pharmaceutical solid dosage forms

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ABSTRACT

A reverse phase high performance liquid chromatographic (RP-HPLC) method was suitable for simultaneous determination of AT, ST and their degradation products in raw materials and pharmaceuticals formulations has been developed. The separation was accomplished on X-Bridge shield RP_{18} (50mm × 4.6mm, 3.5µm) column under isocratic mode. The mobile phase consisting of 0.02M ammonium acetate buffer with pH 4.0 and organic modifier mixture (acetonitrile-THF, 95:5) in the ratio of 50:50, *v/v* respectively. The flow rate was monitored at 0.8mL/min, with 5µL injection volume and a PDA detector set at 246nm used for detection. Forced degradation of AT and ST were carried out under thermal, acidic, alkaline, peroxide, sunlight and ultraviolet light conditions. Under stressed conditions AT and ST were degraded and the mass balance was found to be above 95%. The retention times of AT and ST were 2.5min and 10.5min, respectively. The total run time was 15min within which from the drug product and degradation processes. The linearity was established for atorvastatin calcium and simvastatin in the concentration range of 50% to 150% of target concentration and correlation found to be 0.999. A RP-HPLC method has been validated for specificity, precision, linearity, accuracy, ruggedness and robustness. The proposed analytical method demonstrated to be suitable for quantitation of all relevant degradants, as well as respective drug products.

Keywords: Atorvasatin Calcium*;* Simvasation*;* RP-HPLC; Forced degradation; Method delopment and validation.

INTRODUCTION

The IUPAC name of Simvastatin is (1S,3R,7S,8S,8aR)-8-{2-[(4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethyl butanoate and potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (hydroxyl methyl glutaryl COA reductases), which is the rate-limiting enzyme in cholesterol biosynthesis. It may also interfere with steroid hormone production. Due to the induction of hepatic LDL receptors, it increases breakdown of LDL cholesterol. The structural formula of Simvastatin was shown in Figure.1.

The IUPAC name of Atorvastatin is (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid and it is used for lowering cholesterol. Atorvastatin is a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-determining enzyme in cholesterol biosynthesis via the mevalonate pathway. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate. Atorvastatin acts primarily in the liver. Decreased hepatic cholesterol levels increases hepatic uptake of cholesterol and reduces plasma cholesterol levels. The structural formula of Simvastatin was shown in Figure.2.

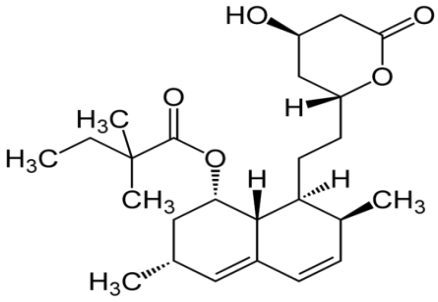

Figure 1: Simvastatin Structural formula

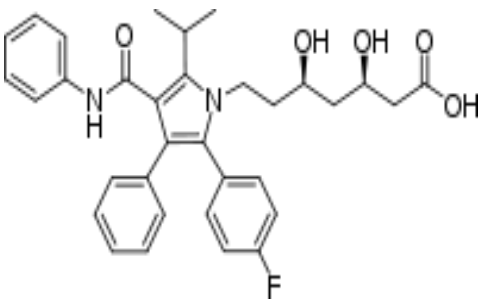

Figure 2: Atorvastatin Structural formula

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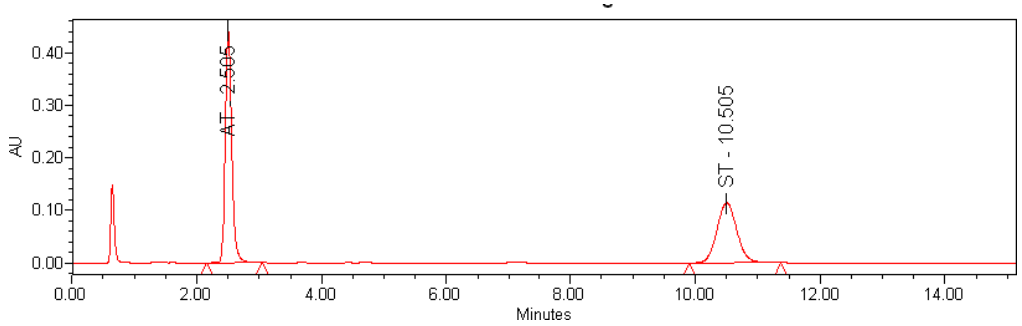

Figure 3A: A typical chromatogram of assay of AT-ST

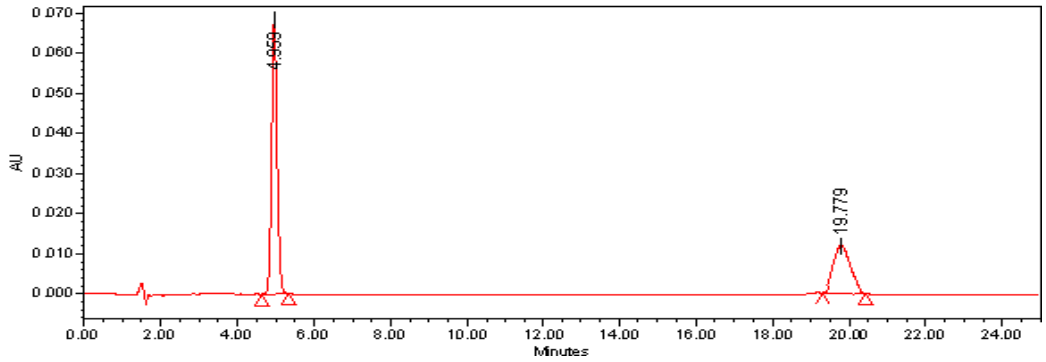

Figure 3B: A typical chromatogram of AT-ST- Hypersil BDS C18, 250mm × 4.6mm, 5µm

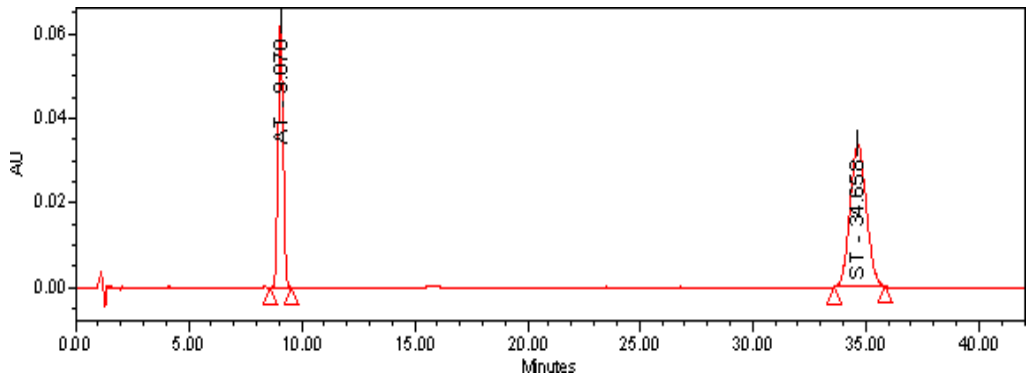

Figure 3C: A typical chromatogram of AT-ST- Inertsil ODS 3V, 250mm × 4.6mm, 5µm

Literature survey revealed that several HPLC and UV spectrophotometry methods for estimation of AT and ST from pharmaceutical dosage forms, biological system and it derivatives (Hermann. et.al, 2005; Vijaya,S.S.et.al,2007; Stanisz,B.et.al, 2008), raw materials, pharmaceutical formulation and human serum by HPLC and UV/Visible Spectrophotometer methods (Araye, M. S.et.al, 2008; Wang et.al,2000; Sultana.et.al, 2010; L. Guzik et.al, 2010. Suresh Kumar GV. et.al, 2010; Beata Stanisz.et.al, 2006; Lucie Nova´ Kova. et.al, 2008) have been reported.

The present work describes the development, validation and degradation pathway of ST and AT with simple, rapid isocratic mode of a RP-HPLC method for determination of AT and ST, degradation products and process related compounds in solid dosage forms in pharmaceutical preparations. Forced degradation of ST and AT were carried out under thermal, photo, acidic, alkaline and peroxide stress conditions.

Therefore aim of the present work was to develop simple, precise, accurate, linear, rugged and robust RP-HPLC method for simultaneous determination of AT and ST in tablets and application of the method for assay study. The developed method has been validated according to ICH Q2-R1 (Federal register, 1955) guidelines. The procedure based on the use of reverse phase high performance liquid chromatography is simple, rapid and provides accurate and precise results.

METHOD AND MATERIALS

Chemicals and reagents

All the reagents were of analytical reagents grade unless state otherwise. Glass-distilled and de-ionized water (Nanopure Barnsted, USA), HPLC grade acetonitrile, tetrahydrofuran, ammonium acetate, potassium dihydrogen phosphate, glacial acetic acid, sodium hydroxide hydrogen peroxide, hydrochloric acid (Merck, India) were used in the separation technique. Simvastatin

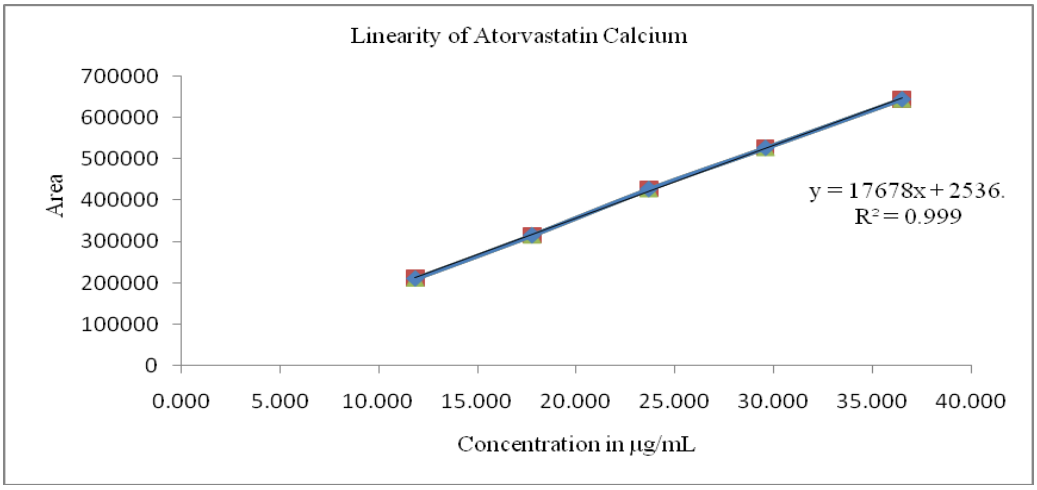

Figure 4A: Linearity graph of Atorvastatin Calcium

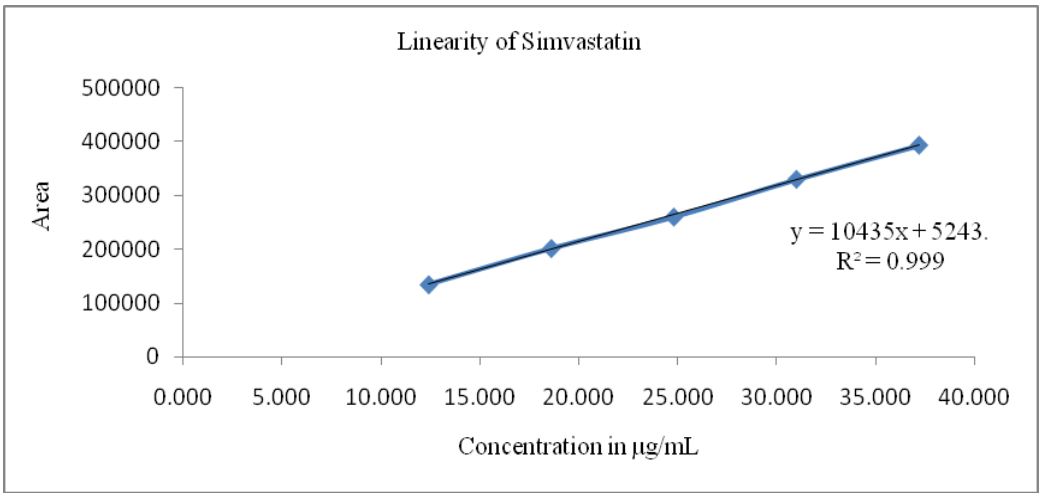

Figure 4B: Linearity graph of Simvastatin

and Atorvastatin Calcium tablets and APIs were manufactured by Dr.Reddy's Laboratories Limited (Hyderabad, India), Placebo mixtures were prepared in the laboratory using USP grade excipients.

CHROMATOGRAPHIC EQUIPMENT AND CONDITIONS

Reverse phase high performance liquid chromatography

The development work has been performed on a Waters Alliance HPLC with PDA detector and Agilent 1200 series HPLC quaternary pump system with UV detector. The chromatographic and the integration data were recorded by using Empower software. The chromatographic columns were used in the present work were -

1. Hypersil BDS C_{18}, 250mm × 4.6mm, 5µm (Thermo Electron Corporation, Runcorn, UK).
2. Inertsil ODS 3V, 250mm × 4.6mm, 5µm (G.L.Sciences Japan).
3. X-Bridge Shield RP_{18}, 50mm × 4.6mm, 3.5µm (Waters Corporation, USA).

Chromatographic conditions

The analysis carried out on X-Bridge Shield RP_{18}, 50mm × 4.6mm, 3.5µm. A HPLC column using the mobile phase consisting of 0.02M ammonium acetate buffer with pH 4.0 and organic modifier mixture (acetonitrile-tetrahydrofuran, 95:5) in the ratio of 50:50, *v/v* respectively. Before delivering the mobile phase in to the system, it was degassed and filtered through 0.45µm Nylon 66 filter using the vacuum pressure. Diluent: acetic acid buffer with pH 4.0 and acetonitrile in the ratio of 10:90, *v/v* respectively which was used for extraction of drug from sample. The injection volume was 5µL and the detector was performed at 246nm using a photodiode array and UV detector. The separation has been achieved by using flow rate with 0.8mL/min and with 25°C column oven temperature. The retention times of an Atorvastatin Calcium and Simvastatin were 2.5 and 10.5, respectively and the total run time was 15min.

Standard stock preparation

Standard stocks of AT (100µg/mL) and ST (100µg/mL) were prepared separately in diluent.

Standard preparation

The final working standard (25µg/mL) solution was prepared by transferring 5mL aliquots of standard

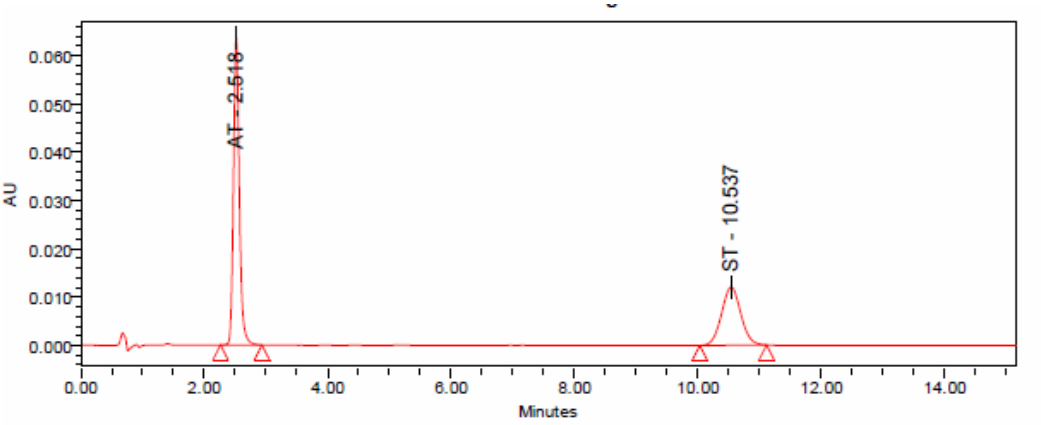

Figure 5: A typical chromatogram of Atorvastatin Calcium and Simvastatin

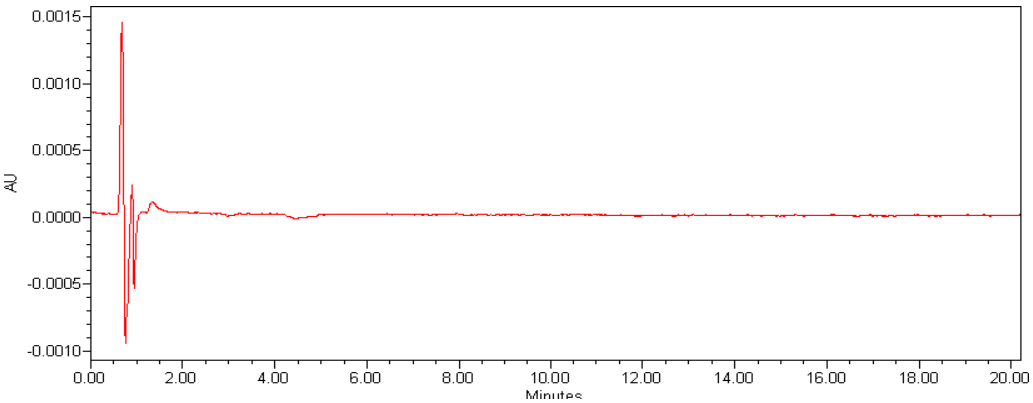

Figure 6: A typical chromatogram of placebo

stock solutions of AT and ST in 20mL volumetric flask and made upto mark with diluent.

Sample preparation

Crushed fine powder of ST and AT of 20mg tablets which were equivalent of 20mg and dissolved into 200mL of diluent and transferred 5mL aliquot of this solution into 20mL volumetric flask and made upto volume with diluent.

RESULTS AND DISCUSSION

Method development and optimization of assay method

The primary objective of this study was to develop a simple, rapid and novel analytical method for simultaneous determination of AT and ST under isocratic mode of elution RP-HPLC conditions. The mobile phase used was the mixture of acetonitrile with ammonium acetate buffer in different ratios. Finally, a mixture of acetonitrile-tetrahydrofuran and ammonium acetate buffer with pH4.0 in the ratio of 50:50, *v/v* respectively, proved to be effective mixture than the other mixture used for separation. Different HPLC columns (2.2.1) from different manufacturers were used during the method optimization. Then the flow rate tested includes 0.5, 0.8, 1.0 and 2.0 mL/min. Among the flow rates 0.8mL/min was selected for the assay because of better visibility, separation and resolution of the peaks. The final chromatographic conditions revealed to provide better resolution (more than 15) among AT and ST peaks. The tailing and plate count for AT and ST peaks were not more than 1.5 and less than 2000, respectively. The optimum wavelength for detection was 246nm, no indigenous interfering compounds eluted at the retention times of the drugs [Fig.3 (a-c)].

Selection of diluent (Extracting solvent):

The tablets of AT and ST are film coated, due to coating material solubility and extraction were found at lower side in following diluents;

a) Acetonitrile and water (50:50, 70:30, 90:10).

b) pH 4.0 Phosphate buffer and acetonitrile (50:50, 80:30).

c) pH 4.0 Ammonium acetate buffer and acetonitrile (10:90): The two actives are freely soluble in this selected diluent and the results were found within the limits.

Method validation

Method validation has been performed according to ICH guidelines of ICH Q2 (R1) for simultaneous determination of AT and ST in 20mg tablets of pharmaceutical preparations. The following validation characteristics were addressed: system suitability, linearity, precision, accuracy, robustness, ruggedness and specificity.

System suitability

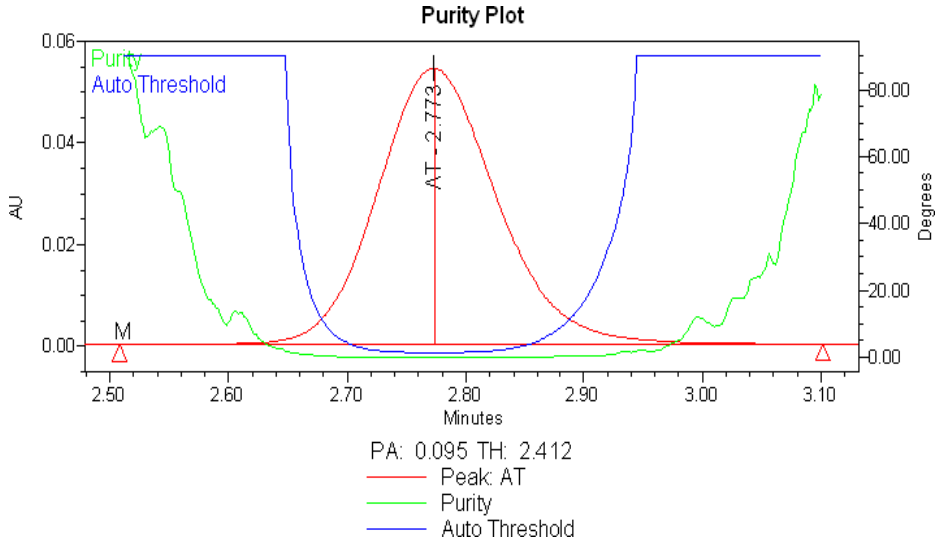

Figure 7A: A Purity chromatogram of Atorvastatin

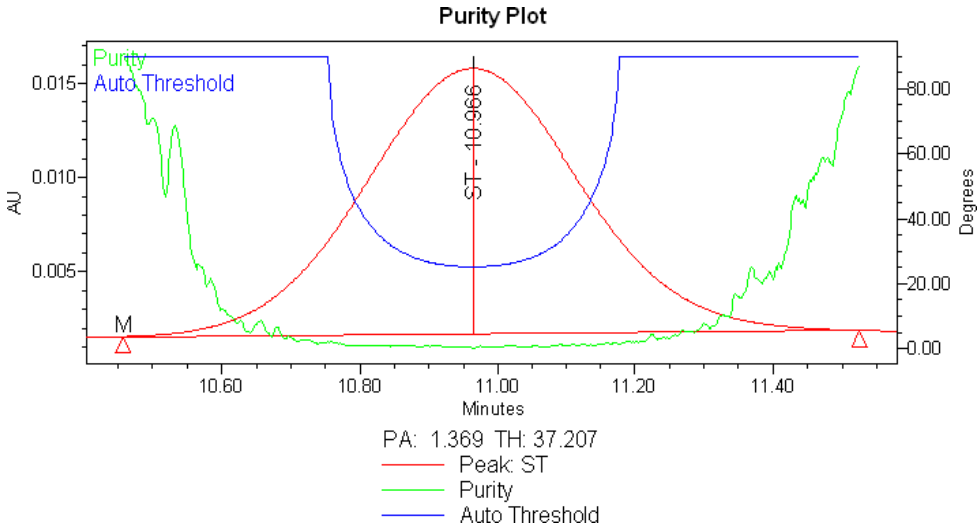

Figure 7B: A Purity chromatogram of Simvastatin

The system suitability parameters were evaluated by injecting standard solution of AT and ST. The tailing factor, column efficiency, resolution and %RSD for both peaks mentioned in Table.1.

Linearity

Linearity solutions were prepared from stock solutions at five concentration levels from 50% level to 150% of target concentration of AT and ST. The bias at 100% level, slope, y-intercept and correlation coefficient were calculated. The correlation coefficient were found 0.999 for AT and ST. The results show that an excellent correlation existed between peak area and concentration of an analyte. The mean regressions equations were found as "A = a C + b" (r^2 = 0.999, *n* = 5).Where, 'A' is the peak area ratio of the AT and ST, '*a' is* slope, '*b'* is intercept and '*C'* is concentration of the measured solution in µg.mL^{-1}. The results show that an excellent correlation existed between peak area and concentration of an analyte. The complete results are shown in Table.2a to Table.2b and the chromatograms are shown in Figure. 4a to Figure 4b.

Precision

The precision of the method was done by replicate preparations (n=6) of 20mg tablets analysis. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the results are given in Table. 3.

Accuracy

Accuracy can be describes about closeness of the test results obtained by the method to the true value. The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100% and 150% of target test concentration (25µg/mL of AT and ST) in solid dosage forms. The percentage of recoveries of AT and ST were calculated and an excellent recovery

Table 1: System suitability results

S. No.	System suitability parameters	AT	ST
1	%RSD*	1.9	2.1
2	USP Plate count	3731	5590
3	USP Tailing	1.1	1.1
4	USP Resolution	---	20.9

*Percentage relative standard deviation

Table 2A: Linearity results of Atorvastatin Calcium

Linearity of Atorvastatin Calcium		
Concentration in %	**Concentration in µg/mL**	**Area**
50%	11.838	209796
75%	17.756	314694
100%	23.675	426799
125%	29.594	526842
150%	36.513	644814
Correlation coefficient (r)		0.999781
Slope (a)		17678
Intercept (b)		2536.2
Bias at 100% Level		0.5

Table 2B: Linearity results of Simvastatin

Linearity of Simvastatin		
Concentration in %	**Concentration in µg/mL**	**Area**
50%	12.400	134433
75%	18.600	201650
100%	24.800	260190
125%	31.000	330230
150%	37.200	393619
Correlation coefficient (r)		0.999
Slope (a)		10434.71
Intercept (b)		5243.6
Bias at 100% Level		1.6

found at each added concentration and the results were shown in Table.4.

Solution stability

The solution stability and mobile phase stability experiment data confirms that sample solutions and mobile phase were stable upto 2days during the assay test.

Robustness

Robustness study is 'a measure of method's ability to remain unaffected by small but deliberate variations in method parameters'. For all changes of conditions the sample was assayed in triplicate. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. The USP tailing factor, plate counts and %RSD were calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust. The following method parameters deliberately verified during method validation:

1. Flow rate (+/- 0.2ml/minutes)
2. Mobile phase composition (+/- 10% of organic phase)
3. Column oven temperature (+/- 5°C)
4. pH of buffer in mobile phase (+/- 0.2 units)
5. Filter suitability (At least two filters)

Selectivity

Selectivity describes 'the ability of an analytical method to differentiate various substances in a sample'. This test method was more selective due to separation of AT and ST. The resolution in between two peaks was found more than 15.0. The chromatograms were shown in Figure.5.

Specificity

Specificity is 'an ability of an analytical method to measure the analyte free from interference due to other components'.

Placebo interference

The method was sufficiently specific to the drug products. The specificity of the method was established by

Table 3: Precision results

S. No.	Intra-precision		Inter-precision	
	AT	ST	AT	ST
1	100.4	101.0	99.8	100.3
2	100.1	100.2	99.8	100.3
3	100.9	100.7	99.2	99.8
4	99.9	99.6	99.6	100.1
5	99.3	98.8	99.1	99.7
6	99.0	98.7	99.6	100.2
Average	99.9	99.8	99.5	100.1
%RSD	0.7	1.0	0.3	0.3

Table 4: Accuracy results

% Spike level	AT			ST		
	µg/mL Added	µg/mL Found	Mean Recovery (n=3)	µg/mL Added	µg/mL Found	Mean recovery (n=3)
50	13.1230	12.9867	99.0	10.0192	10.0253	100.1
100	26.2461	26.2301	100.0	20.3211	20.3499	100.1
150	39.36916	39.5489	100.4	30.4817	30.6770	100.7

injecting duplicate sample preparations of placebo of AT and ST. No, interference found due to ingredients which are used in the formulation, this indicates that the method remains specific under tested conditions. The chromatograms were shown in Figure.6.

Forced degradation

The samples have been stressed to degradation such as refluxed with 0.1N HCl at 60°C/30min, refluxed with 0.1 N NaOH at 60°C/30min, refluxed with 1% peroxide at 60°C/30min, exposed to dry heat at 105°C/6hours in hot air vacuum oven, refluxed at 60°C/30min in water, exposed to visible light of 1.2 million lux-hours, UV light of 200 watt-hour/m^2 and exposed at 25°C/90%RH for 7days in humidity chamber.

Degradation was not observed in visible light, UV, humidity and water hydrolysis stress studies. Significant degradation was not shown in acid hydrolysis, base hydrolysis and oxidative conditions. However, thermal stress showed significant degradation. It is interesting to note that all the peaks due to degradation are well resolved from the peaks of AT &ST. Further the peak purity of AT & ST was found to be homogeneous based on the evaluation parameters such as purity angle and purity threshold using Waters Empower Software. The verification of peak purity indicates that there is no interference from degradants, facilitating error-free quantification of AT and ST. Thus, the method is considered to be "Stability-indicating". The chromatograms of purity plots were shown in Figure.7a to Figure.7b.

CONCLUSIONS

A simple and rapid stability indicating isocratic mode RP_HPLC method has been developed and validated for determination of AT and ST. The method was proved to be simple, linear, precise, accurate, robust, rugged and specific. The total run time was 15min within the drugs and their degradation products were separated. The method was completely validated showing satisfactory data for all the method validation parameters tested. Thus the developed method can be used for quantitative and simultaneous determination of the AT and ST in 20mg tablets of pharmaceutical preparations.

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