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Method development and validation for simultaneous estimation of aspirin, ramipril and atorvastatin by RP-HPLC method in its pure and tablet dosage form

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***Abstract*---**A simple, accurate, and precise method for estimating Aspirin, Atorvastatin, and Ramipril in Tablet dosage form was developed. Symmetry C18 (150 x 4.6 mm, 3.5) was used to run the chromatogram. At a flow rate of 1.0 ml/min, a mobile phase containing potassium dihydrogen ortho phosphate (pH adjusted to 3) and Methanol in the ratio 35:65 was pumped through the column. The temperature was kept at 30°C. The optimised wavelength chosen was 240.0 nm. Ramipril, Atorvastatin, and Aspirin had retention times of 1.803 minutes, 2.506 minutes, and 3.875 minutes, respectively. The percent RSD of Ramipril, Atorvastatin, and Aspirin were determined to be 0.99, 0.42, and 0.42, respectively. The method is linear for Ramipril concentrations ranging from 12.5 to 62.5 g/ml, Atorvastatin concentrations ranging from 160 to 800 g/ml, and Aspirin concentrations ranging from 5 to 25 g/ml. The method was accurate, with a percent recovery of 99.13 to 100 percent for Ramipril, 99.3 to 100.2 percent for Atorvastatin, and 99.3 to 100 percent for Ramipril, respectively. The developed method is robust after deliberate changes in flowrate and mobile phase composition with a percent RSD less than 2. Because the retention times were reduced and the run time was reduced, the method developed was simple and cost-effective, and it can be used for regular analysis of dosage forms containing Aspirin, Atorvastatin and Ramipril.

***Keywords*---**Aspirin, Atorvastatin and Ramipril, RP- HPLC, Validation.

Introduction

Analgesic aspirin (2-(acetyloxy) benzoic acid). Acetylsalicylic acid suppresses both COX-1 and COX-2 activity, reducing the production of precursors of prostaglandins and thromboxanes from arachidonic acid. Acetylsalicylic acid inhibits platelet aggregation by donating acetyl to cyclooxygenase. Atorvastatin (7-[2-(4-fluorophenyl)-3-(phenylcarbamoyl)-5(propan-2-yl)-1H-pyrrol-1-yl) Statins include -3,5-dihydroxyheptanoate. Cholesterol-lowering. Atorvastatin inhibits the rate-determining enzyme in cholesterol production via the mevalonate pathway. HMG-CoA reductase catalyses mevalonate formation. ((2S,3aS,6aS)-1-[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]aminopropanoyl] -octahydrocyclopenta[b]pyrrole-2-carboxylic acid) is an ACE inhibitor. Liver and kidneys metabolise it to ramiprilat. Ramiprilat inhibits ACE, the enzyme that converts angiotensin I (ATI) to angiotensin II (ATII).

Studies have shown that Ramipril, Atorvastatin, and Aspirin alone and in combination with other medicines may be measured using RP-HPLC. Few approaches exist for estimating medicines from formulation1-4. We plan to build a stability-indicating RP-HPLC method that is simple, speedy, sensitive, and fast.

Materials and Methods

Ramipril, Atorvastatin and Aspirin pure drugs are received as a gift sample from MSN pharma Ltd. Marketed tablet of Ramipril, Atorvastatin and Aspirin tabletswere purchased from local market. Potassium dihydrogen phosphate, Acetonitrile, Methanol, Ortho-phosphoric acid are from Merck.

Solutions:
Preparation of Standard solutions:
10 mg of aspirin, 320 mg of atorvastatin, and 25 mg of ramipril were accurately weighed and transferred into a 100 ml clean dry volumetric flask, 70 ml of diluent was added, sonicated to completely dissolve it, and the volume was made up to the mark with the same solvent to give a concentration of 100 g/ml, 3200 g/ml, and 250 g/ml. (Stock solution) Further, 1 ml of the above stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent to give concentrations of 10g/ml, 320g/ml, and 25 g/ml, respectively.

Samples Preparation
In a glass mortar, 10 tablets of contents were weighed and triturated. The amount of powder equivalent to 10 mg of aspirin present in 10 tablets (1754.5mg) was transferred into a 100 ml clean dry volumetric flask, 70 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 100 g/ml,320 g/ml (stock solution). 1 mL of filtered solution was transferred to a 10 mL volumetric flask and diluted up to the mark with diluent to give the required concentrations. Before injecting the solution into the HPLC system, it was filtered through a 0.45 m filter.

Preparation of placebo:
2.5 gm of potassium dihydrogen ortho phosphate was weighed and transferred in into 1000ml volumetric flask dissolved in hplc grade water and adjust ph upto 3 with ortho phosphoric acid. 350 ml (35%) of the prepared buffer and 650 ml (65%) of HPLC grade Methanol were mixed. The mobile phase is degassed using ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Diluent: Mobile phase is used as diluent.
Chromatographic conditions:
The new method was developed and validated using Symmetry C_{18} (4.6 x 150mm, 5µm). 65 volumes of HPLC grade Methanol and 35 volumes of Phosphate buffer adjusted to pH 3.0 (65: 35 % v/v) as mobile phase. Separation was done in an isocratic elution mode at a flow rate of 1.0 mL/min.

System suitability:
Sample solutions were injected into HPLC system in six replicates as per test procedure. The system suitability parameters were evaluated, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Method validation
The method validation was performed as per ICH guidelines

Linearity
Prepared standard dilutions of Ramipril, Atorvastatin and Aspirin were injected and chromatogram was recorded in duplicate. A calibration curve was constructed by taking concentration on X- axis and average peak area on Y- axis.

Accuracy
The recovery studies of the analyte determined accuracy. It is determined using the standard addition method, in which a known quantity test solution is spiked with standard solutions at three levels, 50 percent, 100 percent, and 150 percent in triplicate. The mean percentage recoveries at each level were computed.

Precision
The method's precision was established at two levels: repeatability and intermediate precision. In order to test repeatability, the working standard solution was injected. Six injections of the same standard solution were given, and the peak areas were calculated. For two drugs, the average area, standard deviation, and percent RSD were calculated. Multiple samples were taken from a sample stock solution prepared by two different analysts to achieve intermediate precision. Analysts 1 and 2 injected six working sample solutions of the same concentrations. For two drugs, the average peak area, standard deviation, and pooled percent RSD were calculated.

Robustness
Deliberate changes in chromatographic condition like Flow rate, mobile phase ratio were made. The conditions like Flow rate minus (0.9ml/min), Flow rate plus (1.1ml/min), mobile phase ration minus, mobile phase ratio plus, was considered

and samples were injected in six replicates. % RSD of Peak areas were calculated by making the deliberate changes.

Specificity:
The specificity was studied by establishing the interference of placebo with the drug. A sample of placebo was injected into the HPLC system as per the test procedure. Chromatogram of placebo should not show any peak at the retention time of analyte peak.

Results and Discussion

Assay of formulation:

The formulation is tested in accordance with the specified procedure. This was done three times. The amount of drug in the formulation was determined using a standard graph. The Ramipril, Atorvastatin, and Aspirin percent assays obtained were 99.83, 98.89, and 990.7 percent, respectively. Figures 4, and 5 show representative chromatograms for standard and test. Table 1 displays the results.

System suitability
ICH guidelines were used to determine system suitability parameters. The plate count exceeded 2000, the tailing factor was less than 2, and the resolution was greater than 2. All system-appropriate parameters were passed and were within the limits. Table 2 shows the results of the system suitability parameters.

Validation

Linearity
The linearity of Ramipril was determined at six concentrations ranging from 12.5 to 62.5 g/ ml, Atorvatatin from 160 to 800 g/ ml, and Aspirin from 5- 25 g/ ml. Peak areas were plotted against concentration, and a calibration curve was created. The calibration curve is shown in Figure 6,7 and 8. For all of the drugs tested, the correlation coefficient (r2) was greater than 0.99 within the concentration range. The linearity results are shown in table 3.

Accuracy
The method's accuracy was established using the standard addition method at three concentration levels. At each level of accuracy, triplicate injections were given, and percentage recoveries were calculated. The mean percent recovery for Ramipril, Atorvastatin, and Aspirin was 99.91 percent, 100.08 percent, and 99.8 percent, respectively. The accuracy results are shown in table 4.

Precision:
The method's precision was investigated by taking repeatability and intermediate precision into account. The repeatability was investigated by performing six replicate injections from the same homogeneous standard solution and measuring peak areas. For two drugs, the average area, standard deviation, and percent RSD were calculated. Analyst 1 and 2 determined analyst to analyst variation by preparing six test solutions of the same concentration and injecting once from each solution, and peak areas were determined. Ramipril's percent RSD for

repeatability and intermediate precision was found to be 0.237 and 0.24 for Ramipril, 0.52 and 0.28 for Atorvasatatin, and 0.26 and 0.76 for Aspirin. It is repeatable and has intermediate precision. The precision results are shown in table 5.

Robustness:
The method's robustness was investigated by making deliberate changes to the flow rate and mobile phase ratio. Chromatograms were recorded after each change in conditions by injecting the standard solutions in six replicates. At each level, system suitability parameters were checked. The system suitability parameters were not significantly affected, and all of them were passed. The percent RSD was within the acceptable range. Table 6 contains the results.

Specificity:
Standard and sample chromatograms are nearly identical, with nearly the same retention time. There was no interference from Placebo or Sample at the analyte retention time, indicating that the method was specific.

Conclusion

RP-HPLC was used to calculate Ramipril, Atorvastatin, and Aspirin. The method was optimised with a mobile phase composed of Methanol: Phosphate buffer mixed in a 65:35 percent v/ v ratio. As stationary phase, an Agilient C18 column (4.6 x 150 mm, 5m) or equivalent chemically bonded to porous silica particles was used. At a constant flow rate of 1 ml/min, the solutions were chromatographed. The linearity ranges of Ramipril, Atorvastatin, and Aspirin were discovered to be 12.5 to 62.5 g/ml for Ramipril, 160 to 800 g/ml for Ramipril Atorvastatin, and 5-25 g/ml for Aspirin. The linear regression coefficient was greater than or equal to 0.999. The percent RSD values are less than 2%, indicating that the method is accurate and precise. For Ramipril, Atorvastatin, and Aspirin, the percentage recovery ranges from 97 to 101 percent. The validation parameters' results met ICH and USP requirements. It deduced that the method discovered was simple, accurate, precise, and linear. The method was discovered to be suitable for routine laboratory analysis with a high degree of accuracy and precision.

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Table 1. Assay Data

S.no	Peak area of Ramipril	Peak area of Atorvastatin	Peak area of Aspirin
1	1250763	940627	932578
2	1247867	931161	934464
3	1255849	940306	920416
Avg	1251360	937364.7	929167.7
% Assay	99.83%	98. 89%	99.07

Table 2: System suitability parameters for Ramipril, Atorvastatin and Aspirin

S.No	Peak Name	R_t	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Ramipril	1.803	638986	835168	2487	1.62	
2	Atorvastatin	2.506	655764	103296	2281	1.51	3.04
3	Aspirin	6.459	119002	73286	2594	1.25	13.23

Table 3: Linearity data of Ramipril, Atorvastatin and Aspirin

Ramipril		Atorvastatin		Aspirin	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
12.5 ppm	626221	160 ppm	839286	5 ppm	631737
25ppm	778750	320ppm	1067774	10 ppm	753615
37.5ppm	931447	480 ppm	1246474	15 ppm	899796
50ppm	1070162	640 ppm	1439994	20 ppm	1035191
62.5ppm	1196060	800 ppm	1639065	25 ppm	1194356

Table 5: Accuracy data of Ramipril, Atorvastatin and Aspirin

% Level N=3	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery
Ramipril			
50 %	5	4.9	98.1
100%	10	9.91	99.1
150 %	15	14.86	99.69
Atorvastatin			
50 %	5	5.1	102
100%	10	9.95	99.5
150 %	15	14.99	99.79
Aspirin			
50 %	10	10.2	102
100%	20	20.2	101

150 %	30	29.8	99.33

Table 6: Precision data for repeatability

Injection No	Peak Area of Ramipril	Peak Area of Atorvastatin	Peak area of Aspirin
1	1247256	935035	954854
2	1248579	929353	937615
3	1243273	930459	950694
4	1243262	932389	940252
5	1249574	922057	922057
Avg	1246389	929858.6	945423.4
SD	2965.62	4865.16	7200.575
% RSD	0.23793	0.5232	0.761

Table 7: Robustness data of Ramipril, Atorvastatin and Aspirin

S.no	Condition	%RSD of Ramipril	%RSD of Atorvastatin	%RSD of Aspirin
1	Flow rate (-) 0.9ml/min	0.74	0.58	0.63
2	Flow rate (+) 1.1ml/min	0.94	0.74	0.58
3	Mobile phase (-) 60B:40A	0.45	0.65	0.61
4	Mobile phase (+) 50B:50A	0.52	0.39	0.84

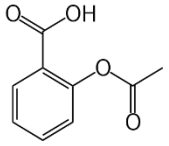

Fig 1: Structure of Aspirin

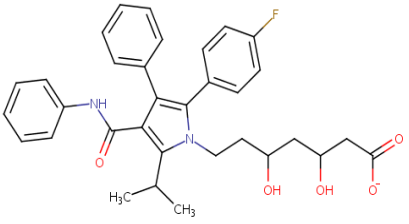

Fig.2: Structure of Atorvastatin

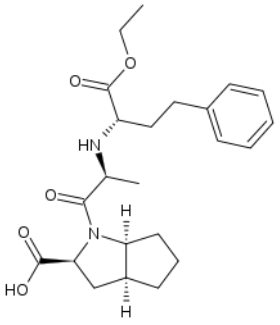

Fig.3: Structure of ramipril

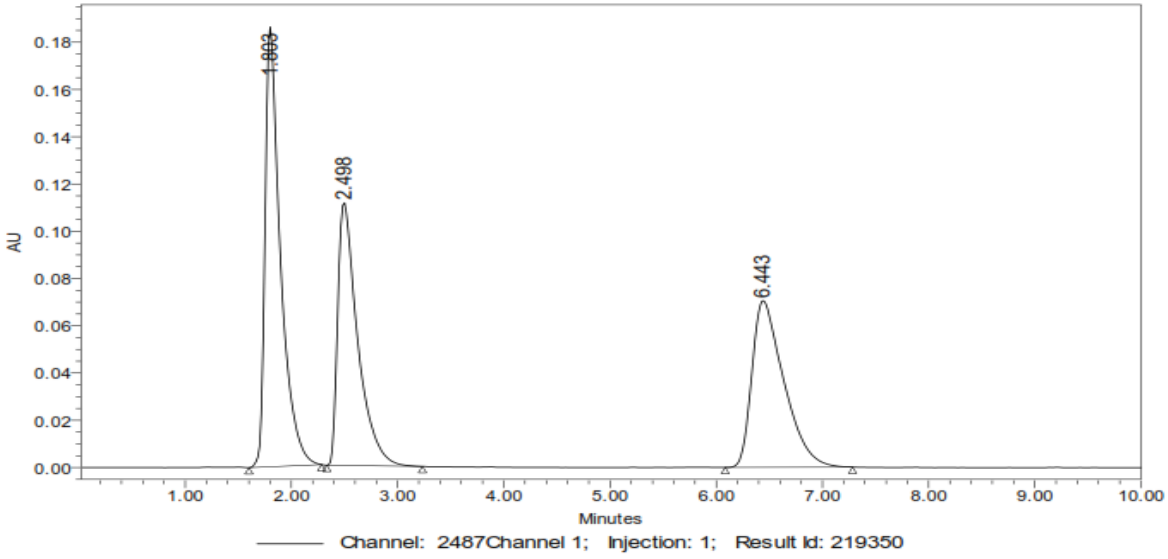

Fig 4: Representative Chromatogram of working standard solution

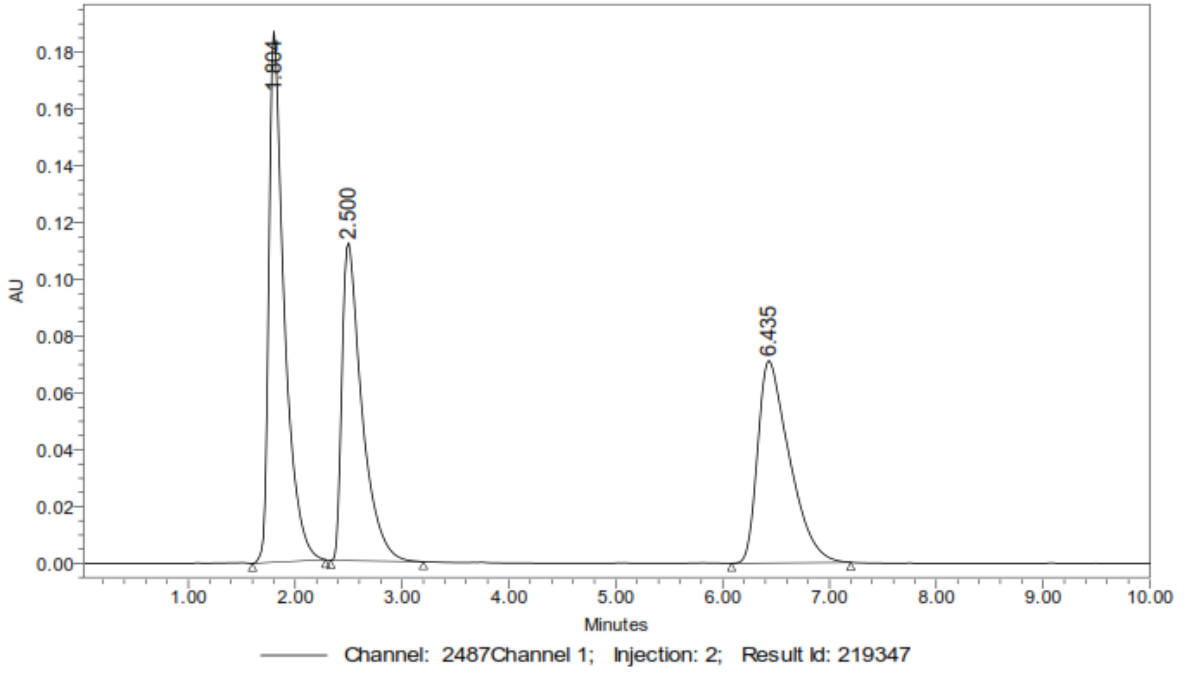

Fig 5: Representative Chromatogram of working sample solution

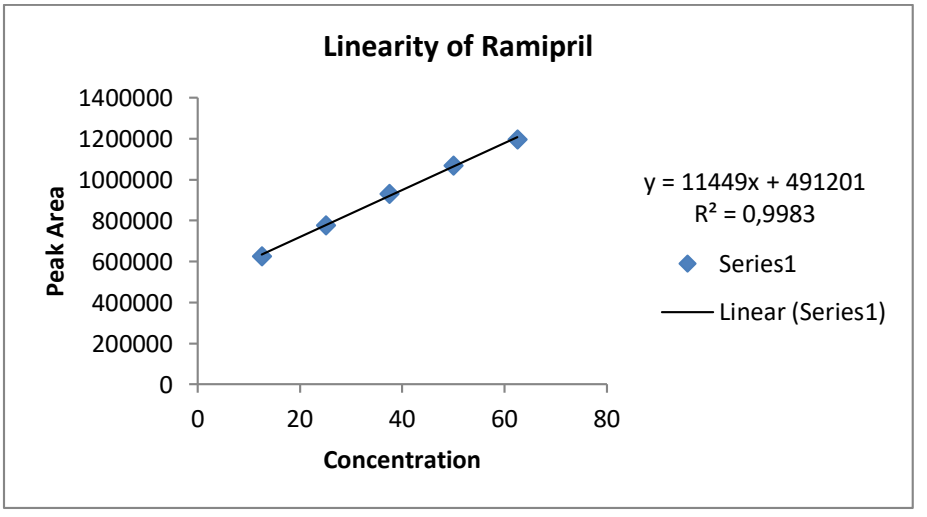

Fig 6: Calibration curve of Ramipril

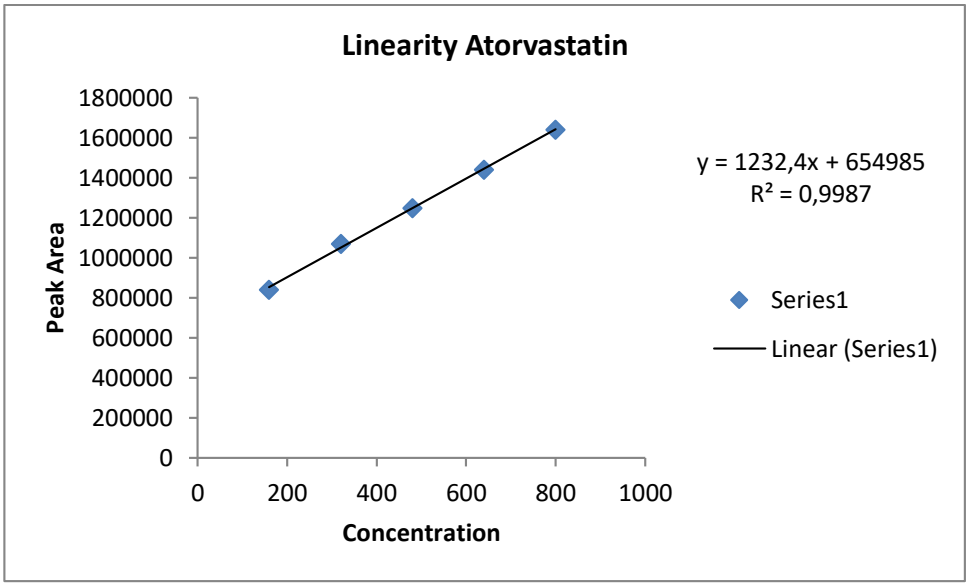

Fig 7: Calibration curve of Atorvastatin

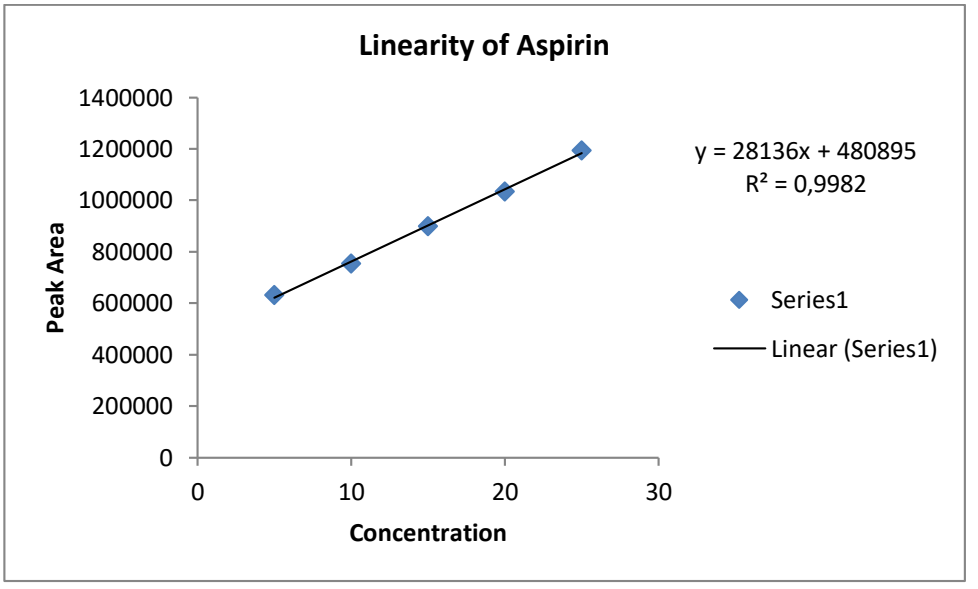

Fig 8: Calibration curve of Aspirin