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A NOVEL RP-HPLC METHOD FOR THE DETERMINATION OF ASPIRIN, ATENOLOL, ATORVASTATIN AND LOSARTAN IN PHARMACEUTICAL DRUG PRODUCTS

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ABSTRACT

High resolution RP-HPLC method has been developed for the simultaneous determination of aspirin, Atenolol, Losartan Potassium and Atorvastatin in pharmaceutical dosage forms. HPLC analysis was carried out by using a X-Terra RP-18, 150x4.6mm, 5micron column with the gradient mobile phase composed of Sol-A: ammonium acetate buffer (1.4g of ammonium acetate in to 1000ml of HPLC water) and sol-B: acetonitrile with simple gradient program (0-4min, sol-A:98-92; 4-8min- sol-A:92-60; 8-13min- sol-A:60-65; 13-15min- sol-A:65-98 and 15-20min- sol-A:98-98). 1.0ml per min flow rate, 40°C column oven temperature and 230nm was selected for this study. The retention times of Aspirin, Atenolol, Losartan and Atorvastatin were 2.0min, 4.7min, 10.0min and 11.8min, respectively. Percent relative standard deviation for five replicate standard injections area is below 1.5percent. Validated the method with specificity, precision, linearity, accuracy, ruggedness and robustness. The response was linear over the concentration range of 10 to 60 µg per mL for each ingredient, with correlation coefficients value is greater than 0.999. Recovery results were between 98.0% to 102.0%. The developed method has applicable for regular analysis.

KEYWORDS : Aspirin, Atenolol, Losartan Potassium, Atorvastatin and RP-HPLC method.

INTRODUCTION

Aspirin [1-3] (acetylsalicylic acid) is a salicylate drug, often used as an analgesic to relieve minor aches and pains, antipyretic to reduce fever and anti-inflammatory medication. There are two distinct uses of aspirin for prophylaxis of cardiovascular events: primary prevention (decreasing strokes and heart attacks in the general population of those who have no diagnosed heart or vascular problems) and secondary prevention (patients with known cardiovascular disease). The main undesirable side effects of aspirin are gastrointestinal ulcers, stomach bleeding and tinnitus [4-9].

Atenolol [10-12] is a β_1 receptor antagonist (beta blockers sometimes written β-blockers) and used for high blood pressure drug (hypertension), chest pain (angina pectoris) related to coronary artery disease , slowing and regulating certain types of abnormally rapid heart rates (tachycardias), acute myocardial infarction (heart attack). Atenolol should be taken at fasted conditions and side effects are mild and transient. Rare side effects include abdominal cramps, diarrhea, constipation, fatigue, insomnia, nausea, depression, dreaming, memory loss, fever, impotence, lightheadedness, slow heart rate, low blood pressure, numbness, tingling, cold extremities and sore throat.

Atorvastatin [13-16] is a statins class of drug and used for lowering blood cholesterol. It prevents strokes through anti-inflammatory and other mechanisms. Atorvastatin has administrated during fast and fed conditions. Side effects are diarrhea, constipation, gas, headache and joint pain.

Losartan Potassium [17-21] is an angiotensin II receptor antagonist and used for the treatment of high blood pressure (hypertension). The recommended dosage of Losartan Potassium for people with high blood pressure (hypertension) is 50 mg and 100 mg once a day.

The chemical structures of the all active ingredients were represented in figure-1. All four active ingredients are available in individual and combination dosage forms and have methods [22 to 26]. In the present study developed a single RP-HPLC method for the simultaneous determination of four active ingredients and validated the method as per ICH and FDA guidelines with specificity, linearity, accuracy and reproducibility.

MATERIALS AND METHOD

Apparatus and Chromatographic conditions:

Chromatographic separation was achieved on a Waters make chromatographic system equipped with an Alliance 2695 module and Agilent waters HPLC module 1200, variable wavelength programmable UV/Visible detector equipped with Empower-2 software and Mettler Toledo analytical balance, Waters X-Terra RP-18 HPLC column (150 × 4.6 mm, 5micron) was used for separation. Injection volume is 20µL and UV absorbance measured at 230nm. Mobile phase consisting of Sol-A: 1.4g of ammonium acetate in 1000ml of HPLC water, Sol-B acetonitrile with simple gradient elution (0-4min- sol-A:98-92; 4-8min- sol-A:92-60; 8-13min- sol-A:60-65; 13-15min- sol-A:65-98 and 15-20min- sol-A:98-98) was delivered at a flow rate of 1.0ml per min. The mobile phase was filtered through a 0.45µ membrane filter and sonicated for 15min. Column oven temperature maintained at 40°C. A mixture of water and acetonitrile (1:1) used as diluent for this study.

Reagents and Solutions:

Pure (not less than 98.5%) standards of all active ingredients, HPLC grade acetonitrile and water were used for this study. All other reagents used in this study were of AR grade.

Standard solution:

Weighed accurately 40mg of all pure standards and transferred into 100 ml of volumetric flask, dissolved the contents with 50ml of diluent, sonicated for 15min and diluted to 100ml volume with diluent. The above resulting solution diluted in to a suitable volumetric flask (40ppm for each active ingredient).

Sample solution:

All market available dosage forms were analyzed with a concentration of 40ppm for each ingredient.

Calculation: All active ingredients were quantified with the following calculation.

$$\frac{\text{Sample area x standard concentration x Potency of standard}}{\text{Standard area x sample concentration x 100}} \times 100$$

RESULTS AND DISCUSSIONS

Method development:

Method development trials were performed with different buffer salts, organic modifiers and columns. Finally the separation was achieved with ammonium acetate buffer and acetonitrile with simple gradient program (reported in materials and methods). Diluent and standard solution represented in figure-2 and 3. All the active ingredients were well separated and the peak shape, resolution (not less than 5.0) and tailing factor (not less than 1.5) were also within the limit.

System suitability

System suitability parameters were established by injecting the freshly prepared standard solution (each active 40ppm/five replicate injections) in to the chromatographic system. Calculated the percent relative standard deviation for peak area and retention time and results found to be satisfactory. System suitability chromatograms represented in figure-4 and tabulated the results in table-1 and 2.

Method validation:

Validated the finalized method as per ICH and FDA [27- 30] guidelines with parameters like specificity, precision, accuracy, linearity and range, ruggedness, robustness etc.

Specificity

Different forced degradation studies were performed with acid, alkali, peroxide, UV and photo degradation conditions. All samples were passed the purity test. The purity angles for drug components in all stress

conditions were found to be less than the threshold angle and no interference was observed with diluent and placebo.

Precision: Precision was evaluated by carrying out six different sample preparations for all individual and combination products. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that the developed method is precise. Results were tabulated in Table-3.

Linearity:

The linearity of method was evaluated by analyzing different concentrations (10ppm to 60ppm for each ingredient) of the standard solution. Calibration graph was plotted against peak area and concentration of solution. The correlation coefficient value found to be within the limit 0.999. The linearity chromatograms shown in figure-5 and linearity results tabulated in table-4 and linearity plots were represented in graph-1.

Accuracy:

Accuracy of the method was carried out with a known quantity of the pure drug was added to the placebo sample at the levels of 25% and 150% of the test concentration. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The mean recoveries were in range of 98.0-102.0 % which shows that there is no interference from excipients. Table-5 represents the recovery results.

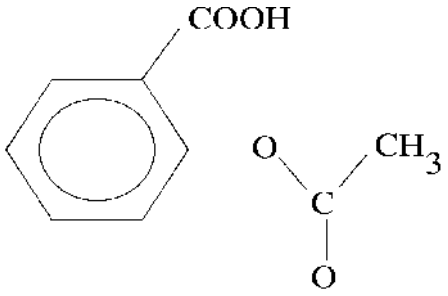
Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different instruments like waters HPLC and Agilent HPLC by different analysts using different columns of similar types. The percent RSD of six different preparations assay values with two different instruments, analysts and columns were 1.6- 0.9, 1.8- 1.3 and 0.4- 1.5% respectively.

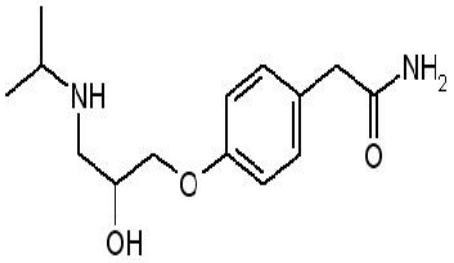
Robustness of the method was determined by making slight changes in the chromatographic conditions, such as flow rate and column temperature. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The robustness limit for mobile phase variation, flow rate variation and temperature variation were well within the limit, which shows that the method is having good repeatability under given set of conditions and results were within the limit. Robustness results were tabulated in table-6.

CONCLUSION

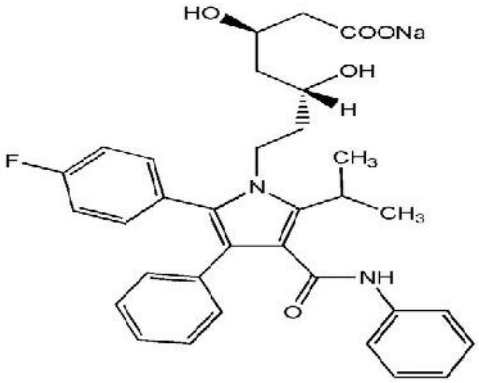
The complete study results reveals that the developed and validated method has applicable for the determination of Aspirin, Atenolol, Losartan Potassium and Atorvastatin in pharmaceutical drug products. The developed method has potential application for all ingredients and applicable for routine quality control analysis.



Aspirin



Atenolol



Atorvastatin

Losartan Potassium

Figure-1: Chemical structures of active ingredients

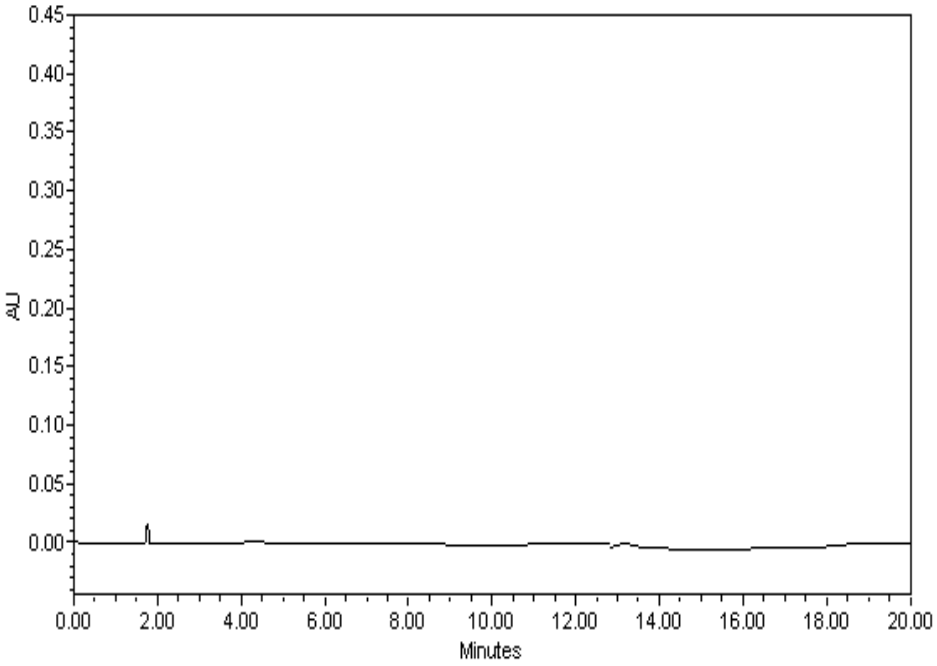


Figure-2: Diluent chromatogram

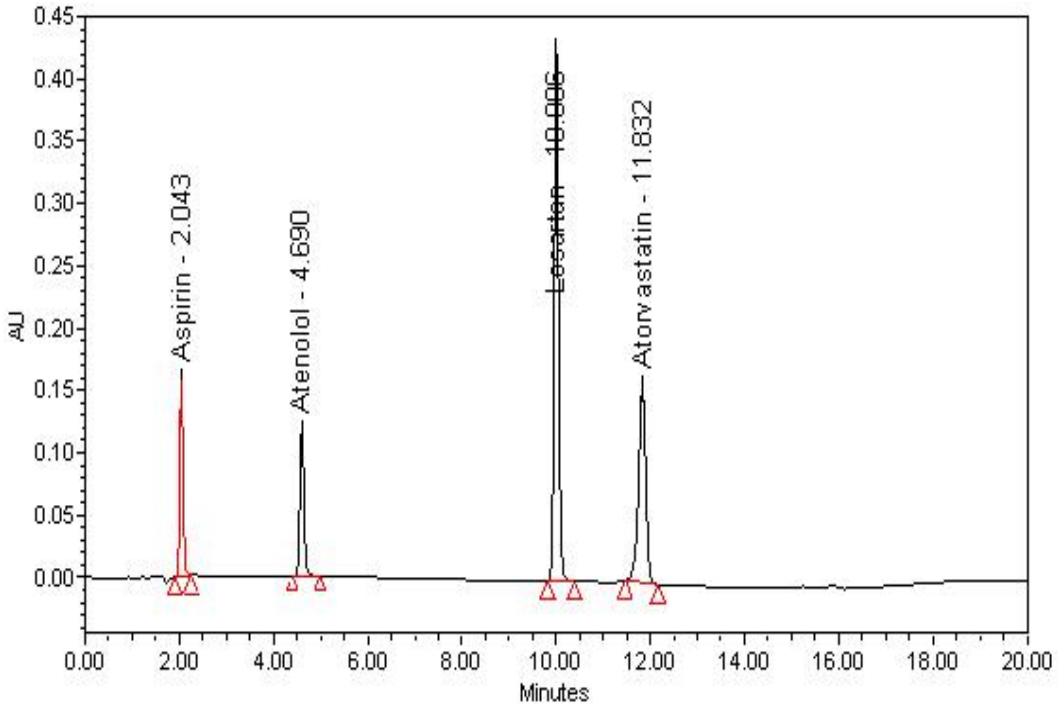


Figure-3: Standard chromatogram

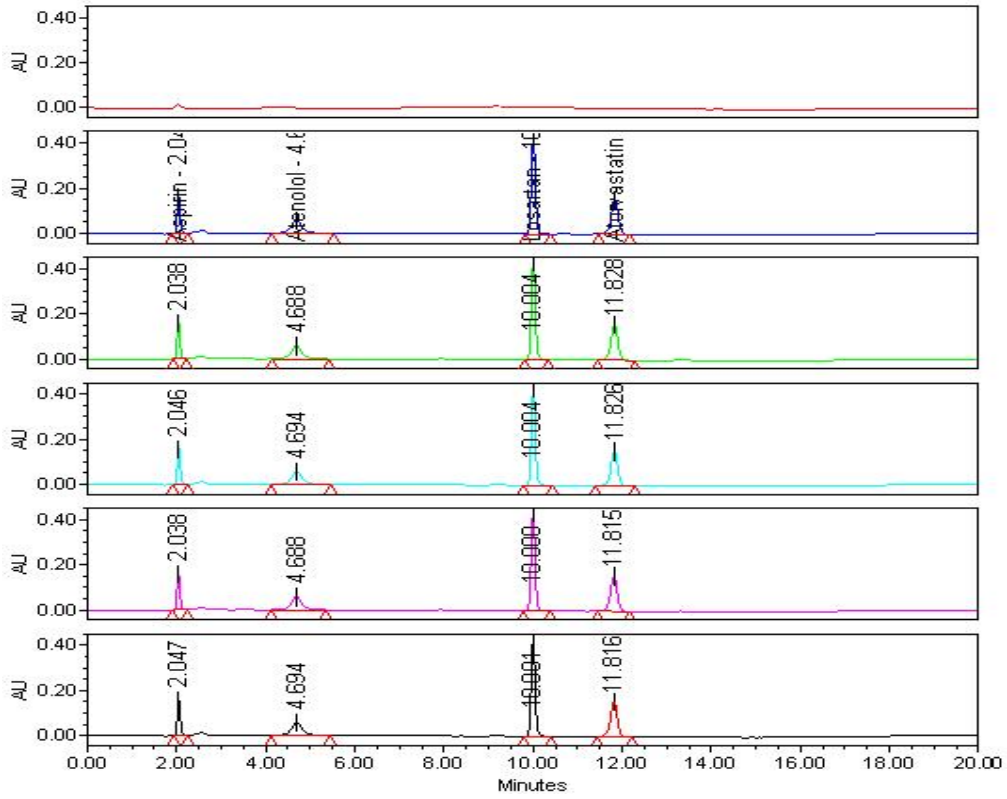


Figure-4: System suitability chromatograms

Table-1: System suitability (Area %RSD)

Active Ingredient Name	Standard solution Area						
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average	%RSD
Aspirin	774011	771962	762894	769608	746250	764945	1.47
Atenolol	1030759	1021333	1027598	1005793	1026010	1022299	0.96
Losartan Potassium	2728535	2734324	2706716	2700782	2682754	2710622	0.78
Atorvastatin	1681235	1712515	1692515	1679637	1676856	1688552	0.87

Table-2: System suitability (Retention time %RSD)

Active Ingredient Name	Standard solution Retention time (min)						
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average	%RSD
Aspirin	2.04	2.03	2.04	2.03	2.04	2.036	0.27
Atenolol	4.69	4.68	4.69	4.68	4.69	4.686	0.12
Losartan Potassium	10.00	10.00	10.00	10.00	10.00	10.0	0.00
Atorvastatin	11.83	11.82	11.82	11.81	11.81	11.818	0.07

Table-3: Precision Results.

Active Ingredient Name	Sample preparations						Average (%)
	Prep-1	Prep-2	Prep-3	Prep-4	Prep-5	Prep-6	
Aspirin	99.41	99.80	97.90	98.70	98.85	99.12	98.96
Atenolol	99.6	97.90	100.50	99.09	100.40	99.10	99.43
Losartan Potassium	100.4	99.63	98.90	99.18	99.81	100.80	99.79
Atorvastatin	99.78	99.92	100.50	99.16	99.61	99.26	99.71

Table-4: Linearity Results.

Active Ingredient Name	Linearity solutions area						
	Level-1 (10ppm)	Level-2 (20ppm)	Level-3 (30ppm)	Level-4 (40ppm)	Level-5 (50ppm)	Level-6 (60ppm)	Co-relation Coefficient
Aspirin	184577	360855	574024	749122	931512	1143821	0.99960
Atenolol	248950	499458	753049	997759	1242585	1498543	0.99998
Losartan Potassium	679675	1312431	2030077	2681904	3338764	4012457	0.99991
Atorvastatin	409573	811037	1266873	1687331	2110843	2547585	0.99992

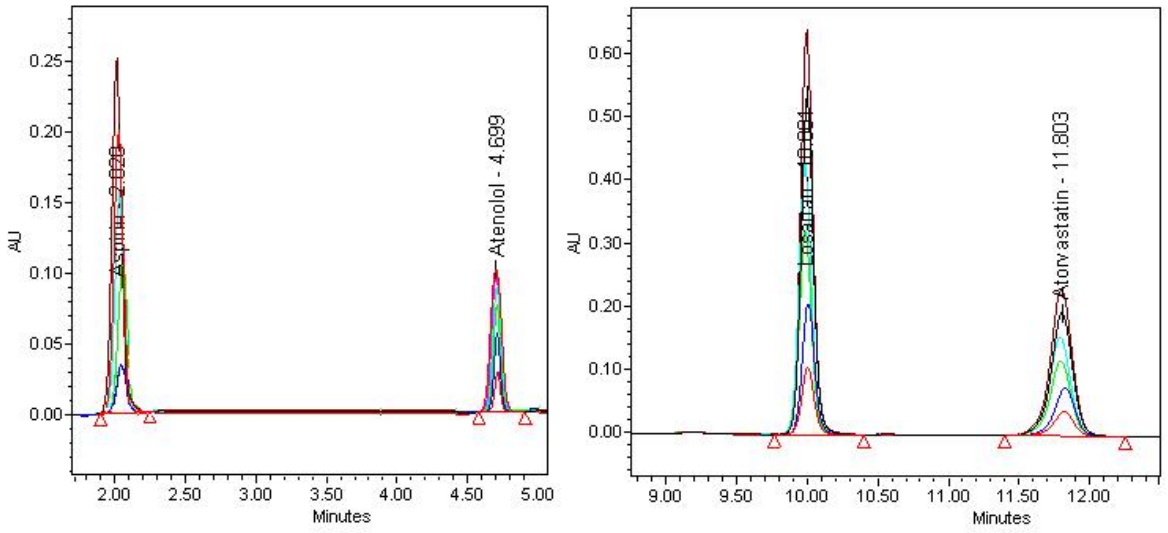
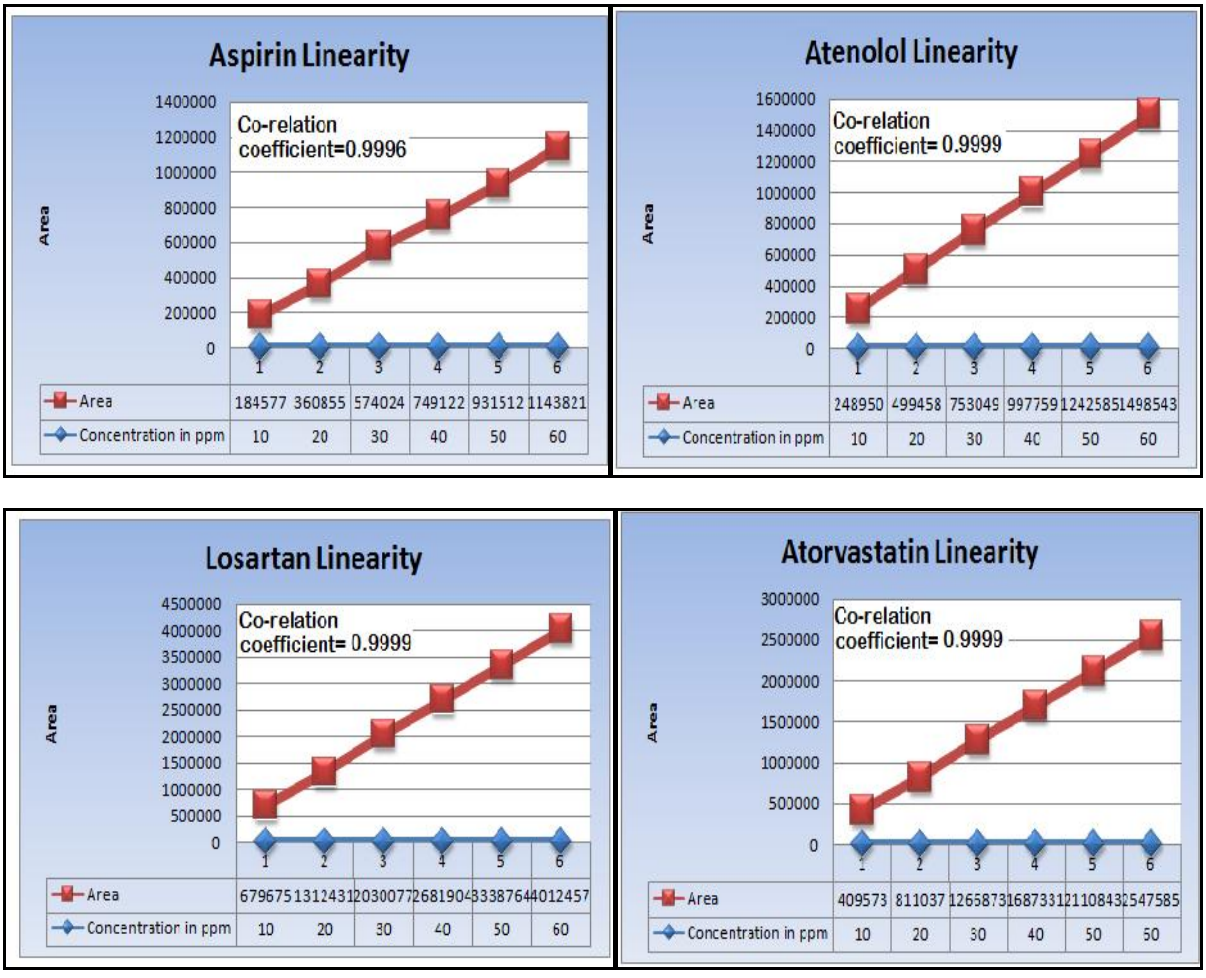


Figure-5: Linearity chromatograms



Graph-1: All active ingredients linearity graph.

Table-5: Accuracy (recovery) Results.

Active Ingredient Name	Spike level						Average % Recovery
	25%	50%	75%	100%	125%	150%	
Aspirin	98.49	99.7	99.36	98.7	100.5	100.6	99.56
Atenolol	99.51	99.01	100.34	99.09	99.25	99.8	99.50
Losartan Potassium	101	100.5	100.58	99.18	98.95	99.23	99.91
Atorvastatin	99.25	100.34	99.63	99.24	99.89	100.82	99.86

Table-6: Robustness Results.

Parameter	System suitability	
	Tailing factor	Percent (%) RSD
Standard solution	1.0-1.4	1.2-1.0
Flow Rate		
+0.1mL per min	1.2-1.0	1.3-1.2
-0.1mL per min	0.9-1.5	0.9-1.2
Column Oven Temperature		
+5°C	1.1-1.0	1.0-0.9
-5°C	1.2-1.1	1.1-0.7

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