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A NOVEL RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CARDIOVASCULAR DRUGS IN A POLYCAP FORMULATION

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ABSTRACT

Cardiovascular disease continues to occur in epidemic proportions globally and often requires multiple strategies for control and reversal, so as to reduce mortality and morbidity. A Polycap containing low dose Aspirin, a potent Statin (Simvastatin) and three BP lowering drugs at reduced standard dose (HCTZ –thiazide diuretic, Ramipril-ACE inhibitor and Atenolol- beta blocker) is available for use in primary and secondary prevention of cardiovascular events. Developing an analytical method for simultaneous estimation of components of FDC is challenging due to complexity caused by drug-drug and drug-excipient interference. A new, simple and rapid RP-HPLC method has been developed for the simultaneous estimation of Aspirin, Simvastatin, Ramipril, Atenolol and Hydrochlorothiazide in a polycap formulation. A planar C18 (250 X 4.6 mm, 5 µ) chromatographic column was used for the separation. The mobile phase employed for the analysis was Methanol-Water (80:20, v/v) adjusted to pH 3.00 with dilute orthophosphoric acid, at a flow rate of 1.0 ml/min. The eluent was monitored at wavelength of 220 nm. The developed method was validated following ICH guidelines. The Beer Lambert's range for each drug was established. The method was found to be accurate, specific, precise and robust. The developed method was applied in analysis of a Polycap formulation. The mean assay results for the drugs were within acceptance limits. The developed method is thus a valuable quality control tool for the routine analysis of the drugs by HPLC in bulk and in their polycap formulation without the need for their prior separation.

KEYWORDS: Aspirin, Atenolol, Hydrochlorothiazide, Ramipril, Simvastatin, RP-HPLC, Polycap.

INTRODUCTION

Cardiovascular diseases continue to occur in epidemic proportions globally. Multiple strategies are needed for control and reversal, so as to reduce mortality and morbidity.[1] One such is to promote the ABC i.e. Aspirin when needed, Blood Pressure control and Cholesterol Management. A Polycap containing low dose Aspirin, a potent Statin (Simvastatin) and three BP lowering drugs at reduced standard dose (HCTZ –thiazide diuretic, Ramipril- ACE inhibitor and Atenolol- beta blocker) is available for use in primary and secondary prevention of cardiovascular events.[2]

Fixed Dose Combinations (Poly pill or Poly cap) are valuable therapeutic alternatives due to improved antihypertensive efficacy brought about by different mechanistic action of components targeting different effector mechanisms. FDCs improve medication compliance and reduces pill burden, thus improving cardiovascular events. Also, due to use of generic components, cost of FDCs is lower than total cost of individual drugs.

Simultaneous estimation of Aspirin, Atenolol, Hydrochlorothiazide, Ramipril and Simvastatin has been attempted previously.[3-9] However; the methods employ buffer which decreases column life, and use of increased quantity of HPLC grade solvents for washing procedure. Further, longer run time leads to decreased output, thus decreasing cost benefits. Hence, there is a need to have newer HPLC methods with shorter run time and use of simpler solvents so as to increase column life and offer cost benefits. The objective of the present work was to develop and validate a new cost effective Rp-HPLC method for the simultaneous analysis of Aspirin, Atenolol, Hydrochlorothiazide, Ramipril and Simvastatin and to apply the developed method in the analysis of a polycap formulation containing the cardiovascular drugs.

MATERIALS AND METHODS

All chemicals used in analysis were of HPLC/AR grade. The APIs used in analysis were obtained as gift samples from Indoco Remedies Limited, Verna, Goa. The Polycap formulation containing Aspirin 100 mg, Atenolol 50 mg, Hydrochlorothiazide 12.5 mg, Ramipril 5 mg and Simvastatin 20 mg was prepared inhouse at Indoco Remedies Limited, Verna, Goa. An HPLC of Agilent Technologies bearing model number 1260 Infinity with a

UV detector was used for analysis. A planar C18 column, 5 µ (250 X 4.6 mm) was used for analysis at a column temperature of 25°C. The Mobile phase used was Methanol: Water (80:20, v/v) adjusted to pH 3.00 with dilute orthophosphoric acid. An Injection volume of 20 µL was used with autosampler temperature of 25°C, at Flow Rate of 1.0 ml/min. The eluate was monitored at detector wavelength of 220 nm.

Mixed Drug Standard solution

Stock solutions of the five drugs were prepared separately by accurately weighing about 50 mg each of Aspirin, Atenolol, HCTZ, Ramipril and Simvastatin working standard into a 50 ml volumetric flask and dissolving in 50 ml of Mobile Phase. Further dilutions were prepared to obtain a mixed working standard solution containing 0.5 mg/ml, 1 mg/ml, 0.12 mg/ml, 0.05 mg/ml and 0.2 mg/ml of the drugs respectively. The solution was filtered through 0.45 µm PVDF filter and analyzed. The HPLC system was subjected to system suitability testing.

Method Validation

The developed HPLC method was validated for specificity, precision, linear range, accuracy, robustness, filter compatibility and forced degradation study.[10]

Specificity

Specificity of the analytical method was checked for blank/placebo interference. Blank and placebo solution was injected along with analyte mixture into the HPLC system as per the test procedure and chromatograms recorded. There should not be any interference due to blank, placebo or any impurity with the peak of Aspirin, Atenolol, Hydrochlorothiazide, Ramipril and Simvastatin.

Linearity

The linearity was performed at five levels over the range of 80% to 120% for Aspirin, Atenolol, Hydrochlorothiazide, Ramipril and Simvastatin with respect to standard concentration. The standard stock solution was diluted to achieve the concentration at about 80%, 90%, 100%, 110% and 120% for the drugs (table 1). A calibration curve was plotted and correlation coefficient (r^2) was obtained which should not be less than 0.99.

Table 1: Concentrations of drugs in diluted standard solution.

ATN mg/ml	ASP mg/ml	HCTZ mg/ml	RAM mg/ml	SIM mg/ml
0.4	0.8	0.096	0.04	0.16
0.45	0.9	0.108	0.045	0.18
0.5	1	0.12	0.05	0.2
0.55	1.1	0.132	0.055	0.22
0.6	1.2	0.144	0.06	0.24

Precision

Intraday and interday precision analysis was performed by analyzing sample for assay at test concentration as per methodology. The individual assay value and %RSD was calculated, which should be within acceptance criteria of less than 2%.

Accuracy

Accuracy was performed at three levels (80%, 100% and 120%) in triplicate by adding known amount of API in placebo and calculating the % recovery which should be between 90% to 110%.

Robustness

Robustness of the method was evaluated by analyzing the Sample solution using chromatographic conditions recommended in test procedure and adopting minor changes in method variables such as change in column oven temperature 50°C, Buffer pH 2.8 and Flow rate 1.2 ml/min. System suitability parameters were evaluated for each variable condition.

Stability of Standard Solution

Stability of solution is a time interval within which drug solution is observed to give reliable and reproducible results without degradation products under normal laboratory conditions. Mixed Standard solution was prepared as per methodology and stored upto about 24 hours on bench top at room temperature and in autosampler. The solutions were injected and the average peak area of the drugs as compared with freshly prepared average peak area was determined. The average peak area of the drugs in mixed standard (autosampler and bench top) at different time intervals should not differ by $\pm$ 2% from the average peak area in freshly prepared mixed standard solution.

Forced Degradation study

Forced degradation study was attempted with an aim to get information about the impurities / degradation products which may generate over a period of time on storage under different conditions. Following stress conditions were employed: 1N HCl for 30 minutes, 10N NaOH for 30 minutes, 5% Hydrogen peroxide for 30 minutes and exposure to heat at 80°C. Target degradation was aimed between 10% and 15%. After degradation, the samples were neutralized and analyzed as per the test procedure. Peak shape/area distortion with respect to mixed standard of precision experiment was checked.

Analysis of Polycap formulation

The content of an intact capsule was transferred into a 200 ml volumetric flask. About 100 ml of mobile phase was added and allowed to stand for 5 minutes. The solution was sonicated for 15 minutes with intermittent shaking, allowed to equilibrate to room temperature and diluted to volume with mobile phase. The solution was filtered through 0.45 µm PVDF filter and used for analysis after discarding first 4-5 ml of the filtrate.

RESULTS AND DISCUSSION

A mobile phase of Methanol: Water (80:20, v/v) adjusted to pH 3.0 with Orthophosphoric acid was optimized for use on a planar C18 (250 X 4.6 mm, 5 µ) chromatographic column at a flow rate of 1.0 ml/min. The eluent was monitored at wavelength of 220 nm. System Suitability was verified by injecting the mixed drug standard solution six times in HPLC system. A representative chromatogram is depicted in Figure 1.

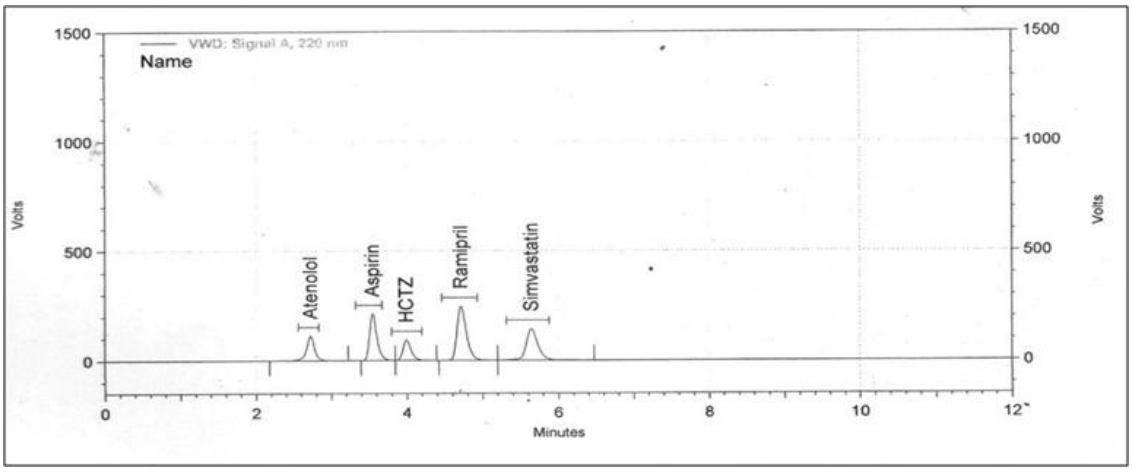


Figure 1: A representative chromatogram of mixed drug standard for system suitability.

The Retention time for the drugs were found to be: Atenolol 2.7 min, Aspirin 3.5 min, Hydrochlorothiazide 4.0 min, Ramipril 4.7 min and Simvastatin 5.6 min. The system complied with the requirements for Column Efficiency (more than 2000 plates), Tailing Factor (not more than 2.0), Relative Standard Deviation (not more than 2.0 % RSD) and Resolution (more than 1.5 between adjacent peaks).

The method was found to be specific as there was no interference from the mobile phase and placebo at the retention time of the drugs (fig 2).

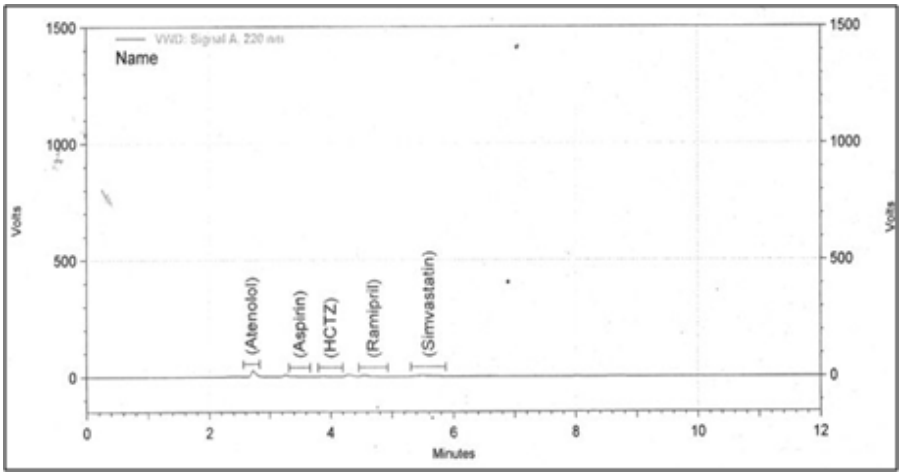
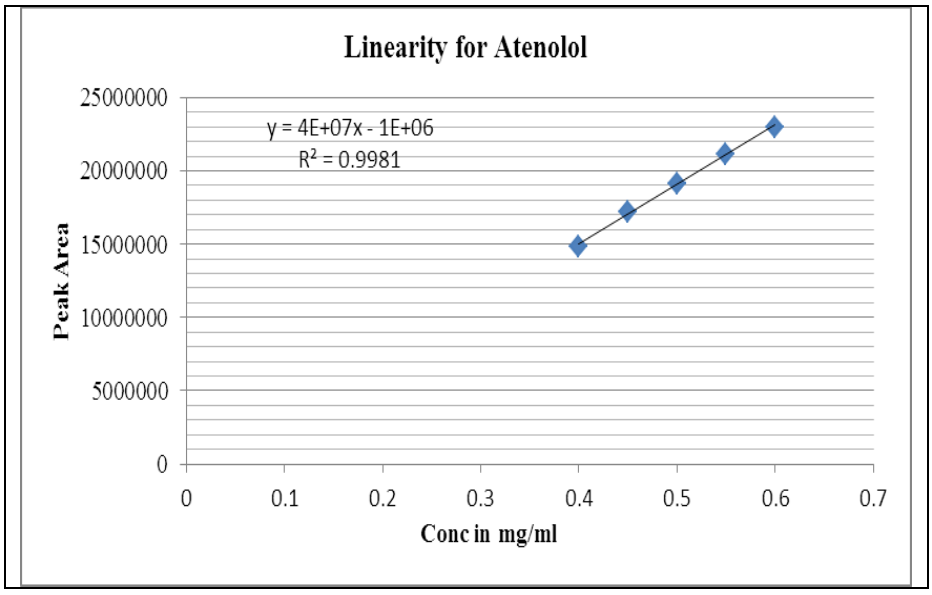
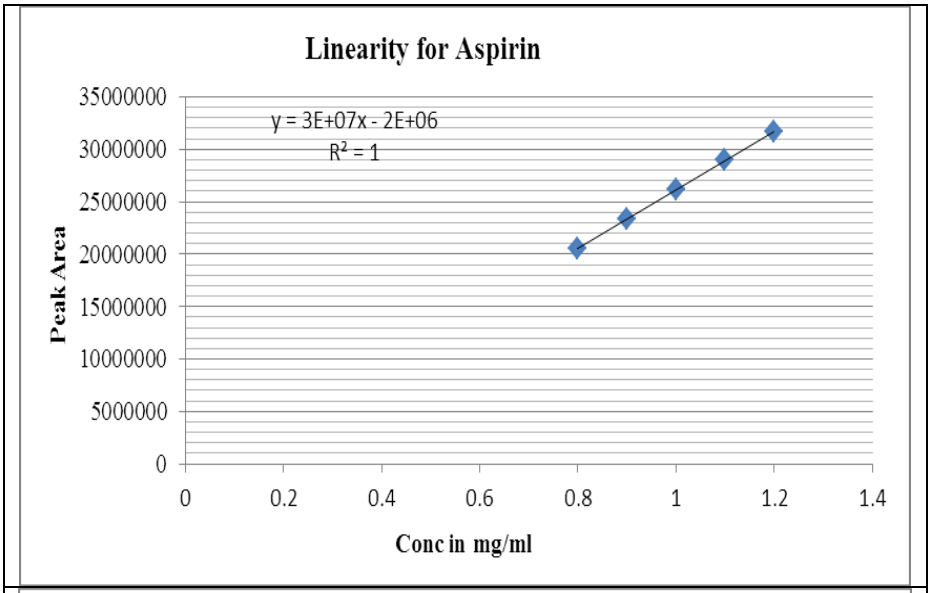
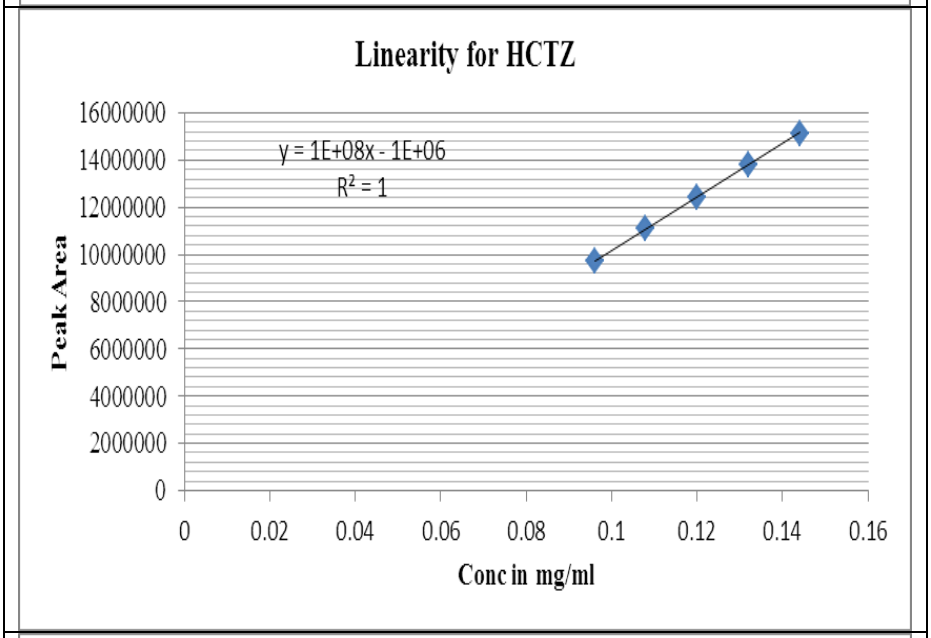


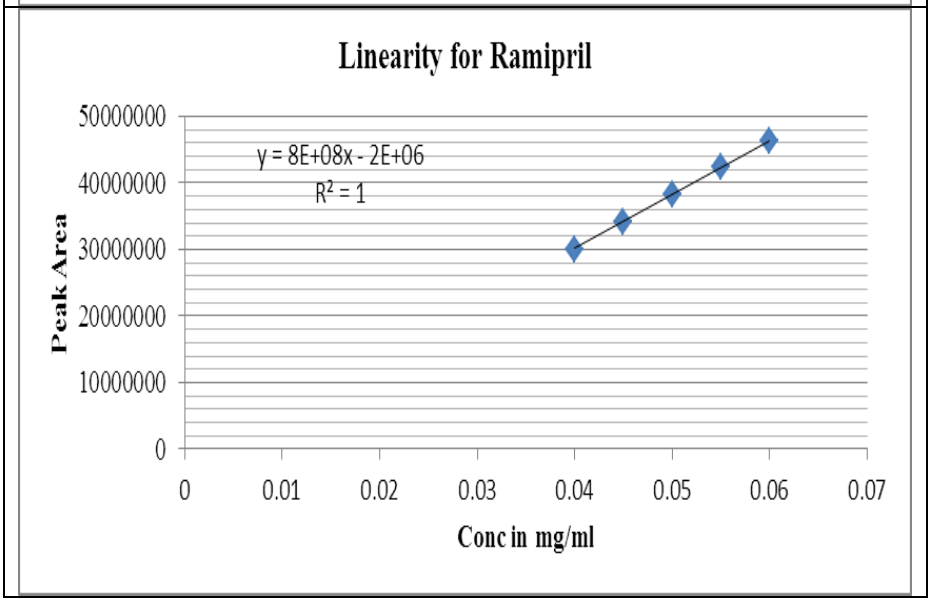
Figure 2: Chromatogram for placebo.

Linearity study performed at five levels over the concentration range of 80% to 120% yielded calibration curves for the analytes as seen in Figure 3.




Linearity for Aspirin
y = 3E+07x - 2E+06
R² = 1
Peak Area
35000000
30000000
25000000
20000000
15000000
10000000
5000000
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Conc in mg/ml


Linearity for HCTZ
y = 1E+08x - 1E+06
R² = 1
Peak Area
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
Conc in mg/ml


Linearity for Ramipril
y = 8E+08x - 2E+06
R² = 1
Peak Area
50000000
40000000
30000000
20000000
10000000
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
Conc in mg/ml

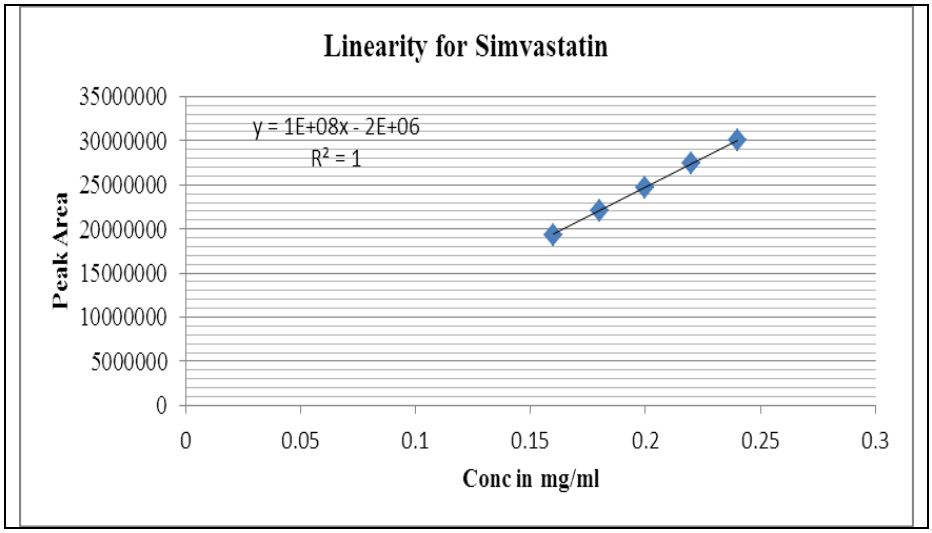


Figure 3: Calibration curves for drugs.

The linearity data is presented in table 2, and as seen from the linearity data there was good correlation.

Table 2: Linearity data for drugs.

Drugs	Linearity Range (mg/ml)	Regression Equation	Correlation coefficient (r^2)
Atenolol	0.4 – 0.6	y = 4E+07x - 1E+06	r^2= 0.9981
Aspirin	0.8 – 1.2	y = 3E+07x - 2E+06	r^2= 1
HCTZ	0.096 – 0.144	y = 1E+08x - 1E+06	r^2= 1
Ramipril	0.04 – 0.06	y = 8E+08x - 2E+06	r^2 = 1
Simvastatin	0.16 – 0.24	y = 1E+08x - 2E+06	r^2 = 1

The precision of the system was established by performing intraday and interday precision by analyzing sample for assay at test concentration as per methodology. The %RSD for individual assay values was calculated, and found to be within acceptance criteria (table 3).

Table 3: Intraday and Interday precision study.

Drug	Atenolol	Aspirin	HCTZ	Ramipril	Simvastatin
Intraday Precision %RSD(n=6)	0.340145	0.129562	0.133667	0.117235	0.101128931
Intermediate Precision %RSD(n=6)	0.072206	0.101473	0.10701	0.121243	0.287127198

The results of accuracy studies (% Recovery) at the three levels is depicted in table 4. The individual recovery of the known amount added was found to be in between 90% to 110% which proved that the method was accurate for the analysis performed.

Table 4: Results of %Recovery at 80 %, 100% and 120%.

Drug	Atenolol	Aspirin	HCTZ	Ramipril	Simvastatin
% Recovery 80%	99.45709	92.47249	92.40255	92.70729	92.9286443
% Recovery 100%	95.28774	95.4584	95.35184	95.43183	95.3062159
% Recovery 120%	92.16348	91.3751	91.1694	91.38796	91.2335631

The method was found robust (table 5) as the system suitability parameters complied with for all the variables employed.

Table 5: System suitability for robustness studies.

Drug	Atenolol	Aspirin	HCTZ	Ramipril	Simvastatin
COT 50°C %RSD	1.082587	1.096628	1.091084	1.162544	1.087467658
Buffer pH 2.8 %RSD	0.548292	0.014512	0.015496	0.032778	0.025239488
Flow rate 1.2 ml/min %RSD	0.127895	0.101747	0.110656	0.107316	0.097183917

The results of stability studies (table 6) show that the area difference for mix standard in autosampler was within limits of 2% in comparison to that of freshly prepared bench top solution. The bench top solution however did not comply. Hence autosampler solution is stable, while bench top solutions kept for 24 hours are not stable.

Table 6: % Recovery for Mixed Standard Autosampler and Bench Top.

Drug	Atenolol	Aspirin	HCTZ	Ramipril	Simvastatin
% Recovery Autosampler	99.7581726	99.30293652	99.06851231	98.9341693	98.71948553
% Recovery Bench Top	55.7535243	52.82235805	52.17683153	52.9199761	52.14728043

In forced degradation studies, peak shape and peak area distortion was observed in all degradation samples (table 7) with respect to mixed standard of precision experiment.

Table 7: Peak area distortion by forced degradation methods.

Drug	Normal Area	HCl	NaOH	H_2O_2	Thermal
Atenolol	14188035	83241217	83057597	1063125100	285274228
Aspirin	24479051	20825878	20625994	21127852	20819757
HCTZ	11600124	5881219	5827644	5996247	5886893
Ramipril	36049390	21281288	21848970	21828015	21859908
Simvastatin	25405073	15214410	17415800	21381936	15199994

The assay of the formulation was performed and the individual assay value obtained (table 8) were within acceptance criteria.

Table 8: Assay for components of Polycap formulation.

Drug	Atenolol	Aspirin	HCTZ	Ramipril	Simvastatin
Assay	104.4122	100.3068	99.8257	104.5036	103.8706406

CONCLUSION

A new, simple and rapid RP-HPLC method has been developed and validated for the simultaneous estimation of Aspirin, Atenolol, HCTZ, Ramipril and Simvastatin. The method is accurate, specific, precise and robust. The method has the advantage of having a shorter run time along with use of a simple mobile phase and column. This leads to savings in cost and time of analysis. Thus the new, developed method offers a cost effective alternative to RP-HPLC separation of the cardiovascular drugs either in bulk or in combination without the need for prior separation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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