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A NOVEL STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF ASPRIN, ATORVASTATIN AND CLOPIDOGREL IN CAPSULES

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Abstract:

A novel stability-indicating, RP-HPLC method was developed for the simultaneous determination of the Aspirin, Atorvastatin and Clopidogrel in capsules. Chromatogram was run through BDS (150 mm X 4.6mm, 5µ). Mobile phase containing phosphate buffer pH 4.5 and acetonitrile in the ratio of (50:50, v/v) was pumped through column at a flow rate of 1ml/min. Optimized wavelength for Aspirin and Atorvastatin and Clopidogrel was 249 nm. Retention time of Aspirin, Atorvastatin and Clopidogrel were found to be 2.367 min, 5.463 min and 4.658 min, respectively. % RSD of the Aspirin, Atorvastatin and Clopidogrel were and found to be 1.01, 0.89 and 0.77 respectively. % recover was obtained as 99.79 %, 99.73 % and 100.57 % for Aspirin, Atorvastatin and Clopidogril, respectively. Limit of detection and quantification values are obtained from regression equations of Aspirin (0.8 ppm, 0.25 ppm), Atorvastatin (0.14 ppm, 0.41 ppm) and Clopidogrel (0.25 ppm, 0.76 ppm). Regression equation of Aspirin is y = 2433.x + 706.4, and of Atorvastatin is y = 17432x + 872.7and Clopidogrel is y = 2403.x + 412.3. The present method was performed the degradation studies like acid, base, oxidation, UV, thermal and neutral, results founds to be negligible.

Key words: *Aspirin, Atorvastatin, Clopidogrel, RP-HPLC and Estimation*

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INTRODUCTION:

Aspirin is chemically called as **(Figure 1)** 2-(acetyloxy) benzoic acid and it is an anti-inflammatory compound that inhibits NFKB**,** cylooxygenase-1 (Cox-1), and cyclooxygenase-2 (Cox-2). Aspirin is used for the analgesic, anti-inflammatory and anti-pyritic effect. Atorvastatin is chemically called as **(Figure 2)** 7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3, 5-dihydroxyheptanoate and it is a selective, competitive HMGCR inhibitor by inhibition of HMG-COA reductase results in a drastic reduction in the production of cholesterol and other isoprenoids. Clopidogrel is chemically called as **(Figure 3)** Methyl (2S)-2-(2-chlorophenyl)-2-{4H, 5H, 6H, 7H-thieno [3, 2-c] pyridin-5-yl} acetate and it is a prodrug, it must be metabolized by CYP450 enzymes to produce the active metabolite that inhibits platelet aggregation. This active metabolite selectively inhibits adenosine diphosphate (ADP) binding to its platelet P2Y12 receptor and subsequently the ADP-mediated activation of the glycoprotein gpiib/iiia complex, thereby inhibiting platelet aggregation. Few analytical methods were reported for the determination of Aspirin, Atorvastatin and Clopidogrel as individually or other combination of drugs by UV [1-2] and HPLC [3-4] methods in its pharmaceutical dosage form. The present RP-HPLC method was validated and performed the stability-indicating studies as per ICH guidelines [5-6].

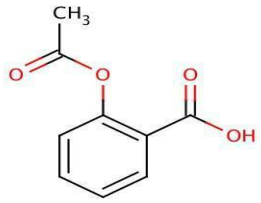

Fig. 1: Structure of Aspirin

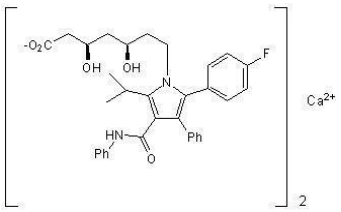

Fig. 2: Structure of Atorvastatin

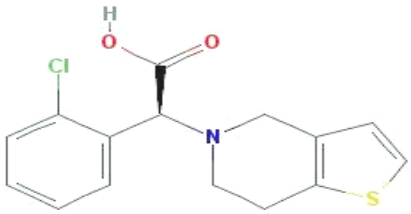

Fig. 3: Structure of Clopidogrel

MATERIALS AND METHODS:

Equipments and Conditions:

The chromatographic separation was conducted on a water liquid chromatographic system equipped with a Water 2695 isocratic solvent delivery system (pump), Water 2996 photo diode detector. A BDS column (250 cm X 4.6 mm, 5 µm) was used for the separation. The mobile phase consisted of a mixture of ammonium acetate buffer pH 4.5 adjusted with ortho phosphoric acid and acetonitrile (50:50, v/v). The mobile phase was prepared daily, filtered, sonicated before use and delivered at a flow rate of 1.0 ml/min at the detection wavelength of 249 nm.

Chemicals and Reagents:

The pure samples of Aspirin, Atorvastatin and Clopidogrel obtained from Corpuscle Research Solutions, Hyderabad. HPLC grade methanol, glacial acetic acid, ortho phosphoric acid, acetonitrile, tri ethyl amine, ammonium acetate buffer, dihydrogen phosphate buffer were used; all analytical grade chemicals and solvents were obtained from E Merck (India) Ltd, Mumbai. The water HPLC grade was obtained from a Milli-QRO water purification system.

Standard solution preparation:

Accurately weighed and transferred 60 mg of Aspirin, 60 mg of Clopidogrel and 8 mg of Atorvastatin working standards in to a 50 ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above second stock solutions was taken into a 10 ml volumetric flask and made up to 10 ml. The detection wave length was 249 nm and the retention time of Aspirin, Clopidogrel, Atorvastatin were found to be 2.367 min, 4.620 min, 5.445 min, respectively as shown in the **Figure 4**.

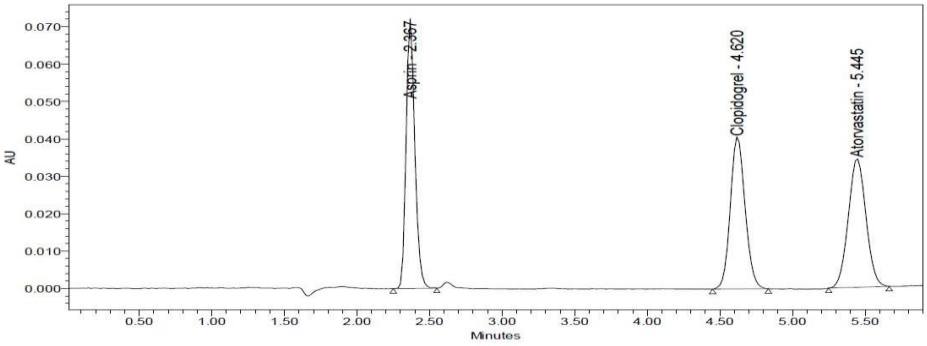

Fig. 4: Standard chromatogram of Aspirin, Clopidogrel and Atorvastatin

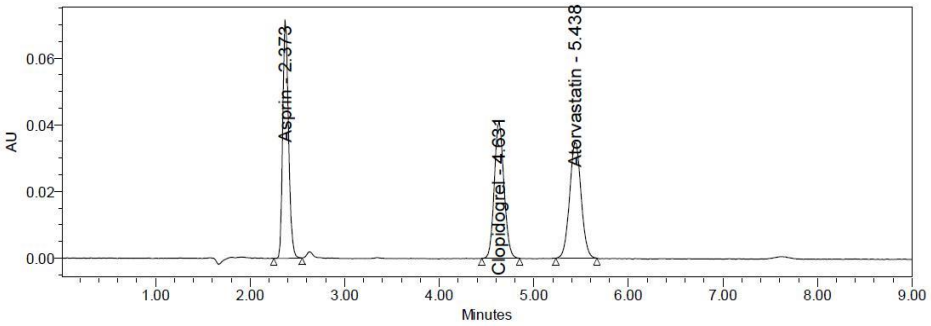

Fig. 5: Sample chromatogram of Aspirin, Clopidogrel and Atorvastatin

Sample preparation:
20 tablets were weighed, powdered and then the weight 303.2 mg (equivalent to 60 mg of Aspirin and 60 mg of Clopidogrel and 8 mg of Atorvastatin) was transferred into a 50 ml volumetric flask, 30 ml of diluent added and sonicate for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent. The detection wave length was 249 nm and the retention time of Aspirin, Clopidogrel, Atorvastatin were found to be 2.373 min, 4.634 min, 5.438 min, respectively as shown in the **Figure 5**.

Method Validation:
Linearity: Linearity solutions were prepared such that 0.25 ml, 0.5 ml, 0.75 ml, 1 ml, 1.25 ml and 1.5 ml from the stock solutions of Aspirin, Clopidogrel and Atorvastatin are taken in to 6 different volumetric flasks and diluted to 10 ml with diluents to get 30 ppm, 60 ppm, 90 ppm, 120 ppm, 150 ppm, 180 ppm of Aspirin and 4 ppm, 8 ppm, 12 ppm 16 ppm, 20 ppm, 24 ppm of Atorvastatin and 30 ppm, 60 ppm, 90 ppm, 120 ppm, 150 ppm, 180 ppm of Clopidogrel, respectively.
Precision: The precision of RP-HPLC method was obtained by analyzing on the same day (intra-day) and analyzing on the different day by triplicate analysis (inter-day) and expressed as percentage relative standard deviation (%.R.S.D).
Accuracy: Accurately weighed and transferred 60 mg of Aspirin and 60 mg of Clopidogrel and 8 mg of Atorvastatin working standards in to a 50 ml clean and dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1 ml from the above two stock solutions was taken in to a 10 ml volumetric flask and made up to 10 ml.
LOD and LOQ: The sensitivity of Aspirin, Clopidogrel and Atorvastatin was determined as limit of detection (LOD) and limit of quantification (LOQ), they were calculated by use of the equations LOD = 3.3 x N/S and LOQ = 10 x N/S, where N is the standard deviation of the drug (n=3), taken as a measure of the noise and S is the slope of the corresponding calibration plot.
Degradation studies:
Acid degradation: To 1 ml of stock solution Aspirin and Clopidogrel, Atorvastatin 1 ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60^{0}C .The resultant solution was diluted to obtain 120 µg/ml, 120 µg/ml and 16µg/ml solution and 10 µl solutions were injected in to the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation: To 1 ml of stock solution Aspirin and Clopidogrel and Atorvastatin 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins at 60^0C. The resultant solution was diluted to obtain 120 µg/ml, 120 µg/ml and 16 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidation degradation: To 1 ml of stock solution of Aspirin and Clopidogrel and Atorvastatin1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 120 µg/ml, 120 µg/ml and 16 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry heat degradation: The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 120 µg/ml, 120 µg/ml and 16 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo degradation: The photochemical stability of the drug was also studied by exposing the 120 µg/ml, 120 µg/ml and 16 µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 120 µg/ml, 120 µg/ml**,** 16 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation: Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 120 µg/ml, 120 µg/ml and 16 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION:

Several attempts were performed in order to get satisfactory resolution of Aspirin, Clopidogrel and Atorvastatin in different mobile phases with various ratios of organic phase and buffer by using BDS column. Initially the mobile phase was mixture of water and methanol fallowed by water and acetonitrile in different ratios. Another mobile phase tried was ammonium sulphate buffer pH 5.8 and acetonitrile (70:30, v/v) by isocratic elution did not give satisfactory resolution. Ammonium acetate buffer pH 4.5 and acetonitrile (50:50, v/v) mobile phase was used by isocratic elution to obtain satisfactory and good resolution. The resolution of standard and sample solution of Aspirin, Clopidogrel and Atorvastatin found reproducible and satisfactory.

Method validation:

Linearity: Six linear concentrations of Aspirin (30-180 ppm), Atorvastatin (4-24 ppm) and Clopidogrel (30-180 ppm) were prepared and injected. Regression equation of the Aspirin, Atorvastatin and Clopidogrel were found to be, y = 2433.x + 706.4, y = 17432x + 872.7 and y = 2403.x + 412.3. Regression co-efficient was 0.999 were as shown in **Figure 6, 7, 8** and **Table 1.**

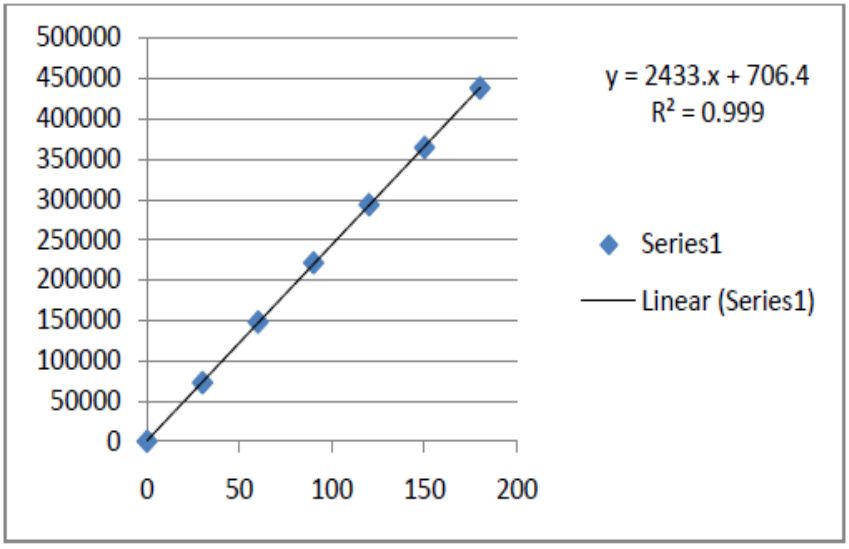

Fig. 6: Calibration curve of Aspirin

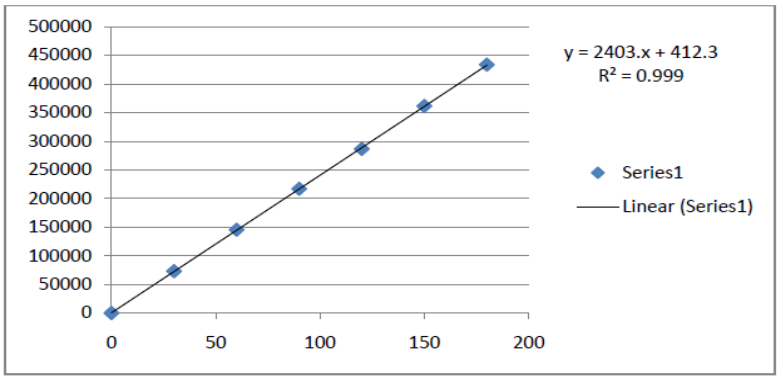

Fig. 7: Calibration curve of Clopidogrel

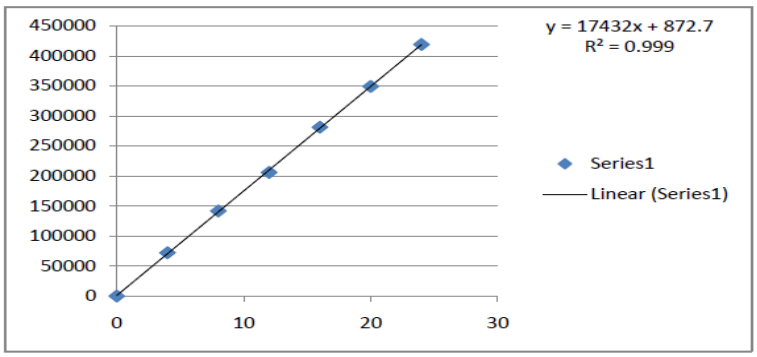

Fig. 8: Calibration curve of Atorvastatin

Table 1: Calibration data of Aspirin, Atorvastatin and Clopidogrel

S. No	Concentration Aspirin (µg/ml)	Peak area	Concentration of Clopidogrel (µg/ml)	Peak area	Concentration of Atorvastatin (µg/ml)	Peak area.
1	0	0	0	0	0	0
2	30	72594	30	73074	4	72243
3	60	147844	60	145371	8	141759
4	90	221462	90	216940	12	206117
5	120	293448	120	286475	16	281376
6	150	364482	150	361538	20	349409
7	180	438279	180	433589	24	419466

Precision: Intra-day precision was performed and % RSD for Aspirin, Atorvastatin and Clopidogrel were found to be 1.01 % and 0.77 % and 0.89 %, respectively. Inter-day precision was performed with 24 hrs time lag and the % RSD obtained for Aspirin, Atorvastatin and Clopidogrel were found to be 0.35 %, 0.4 % and 0.4% shown in **Table 2** and **Table 3.**

Table 2: Intra-day precision data of Aspirin, Atorvastatin and Clopidogrel

S.No	Aspirin	Atorvastatin	Clopidogrel
1	299583	295537	288319
2	305729	291200	288768
3	299218	296317	288240
4	299345	296835	294360
5	304680	295864	288201
6	299302	297637	291977
Mean	301310	295565	289978
Std. Dev	3037.7	2263.4	2592.0
%RSD	1.01	0.77	0.89

Table 3: Inter-day precision data of Aspirin, Atorvastatin and Clopidogrel

S.No	Aspirin	Atorvastatin	Clopidogrel
1	299839	292412	291145
2	302607	291400	288852
3	300304	293985	288211
4	300304	293688	289449
5	300721	293079	290490
Mean	300746	292724	289593
Std. Dev	1059.2	1038.5	1070.3
%RSD	0.35	0.40	0.4

Accuracy: Three concentrations 60 %, 120 %, 180 % of Aspirin, 8 %, 16 % and 24 % concentrations of Atorvastatin and 60 %, 120 %, 180 % concentrations of Clopidogrel were injected in a triplicate manner and amount recovered and % recovery was reported in **Table 4**.

Table 4: Accuracy data of Aspirin, Atorvastatin and Clopidogrel

Sample	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	% RSD
Aspirin	60	60.73	101.23	0.63
	120	120.07	100.14	0.62
	180	179.20	99.51	0.61
Atorvastatin	8	8.09	101.73	0.91
	16	15.37	99.28	0.90
	24	24.11	100.23	0.91
Clopidogrel	60	60.37	100.73	0.70
	120	119.40	99.39	0.80
	180	180.27	100.57	0.50

LOD and LOQ: LOD and LOQ were indicating sensitivity of the method and the LOD and LOQ value were reported in **Table 5**.

Table 5: LOD and LOQ data of Aspirin, Atorvastatin and Clopidogrel

S. No	Parameter	Aspirin	Atorvastatin	Clopidogrel
1	LOD (µg/ml)	0.08	0.01	0.25
2	LOQ (µg/ml)	0.25	0.02	0.76

Stability-indicating studies:

Stability-indicating studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. The percentage of degradation of Aspirin, Atorvastatin and Clopidogrel shown in **Table 6 to Table 8.**

Table 6: Degradation Data of Aspirin

S.No	Degradation condition	% Drug degraded	Purity angle	Purity threshold
1	Acid	4.69	0.159	0.291
2	Alkali	1.3	0.163	0.295
3	Oxidation	2.1	0.168	0.297
4	Thermal	1.3	0.166	0.303
5	UV	0.9	0.153	0.297
6	Water	0.75	0.162	0.300

Table 7: Degradation Data of Atorvastatin

S.No	Degradation condition	% Drug degraded	Purity angle	Purity threshold
1	Acid	4.9	0.478	0.667
2	Alkali	2.5	0.530	0.669
3	Oxidation	4.8	0.494	0.652
4	Thermal	1.5	0.512	0.762
5	UV	1.2	0.471	0.690
6	Water	0.58	0.472	0.701

Table 8: Degradation Data of Clopidogrel

S.No	Degradation condition	% Drug degraded	Purity angle	Purity threshold
1	Acid	5.7	0.134	0.345
2	Alkali	1.9	0.139	0.344
3	Oxidation	4.7	0.144	0.349
4	Thermal	1.3	0.193	0.374
5	UV	1.2	0.471	0.690
6	Water	0.7	0.174	0.385

CONCLUSION:

A simple, accurate, precise method was developed for the stability-indicating, simultaneous determination of the Aspirin, Atorvastatin and Clopidogrel in capsule dosage form. Retention time of Aspirin, Atorvastatin and Clopidogrel were found to be 2.367 min, 5.463 min and 4.658 min, respectively. % RSD of the Aspirin, Atorvastatin and Clopidogrel were and found to be 1.01, 0.89 and 0.77, respectively. % Recover was obtained as 99.79 %, 99.73 % and 100.57 % for Aspirin, Atorvastatin and Clopidogrel respectively. LOD, LOQ values are obtained from regression equations of Aspirin (0.8 ppm, 0.25 ppm), Atorvastatin (0.14 ppm, 0.41 ppm) and Clopidogrel (0.25 ppm, 0.76 ppm). Regression equation of Aspirin is y = 2433.x + 706.4, and of Atorvastatin is y = 17432x + 872.7and Clopidogrel is y = 2403.x + 412.3. The stability-indicating nature of the proposed method was established by performing forced degradation, which provided degradation behaviour of Aspirin, Atorvastatin and Clopidogrel under various conditions. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular quality control test in industries.

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