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Original Research Article

DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHODS FOR THE ESTIMATION OF ASPIRIN AND ATORVASTATIN CALCIUM BY RP- HPLC METHOD

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Abstract: A new simple, specific, precise and accurate revere phase liquid chromatography method has been developed for estimation of atorvastatin calcium (AST) and ASPIRIN (ASP) simultaneously in a combined capsule dosage forms. The chromatographic separation was achieved on a 5 – micron C 18 column (250x 4.6mm) using a mobile phase consisting of a mixture of Orthophosphoric acid buffer: Acetonitrile (45:55) was used pH 4.5. The flow rate was maintained at 1.0 ml / min. The detection of the constituents was done using UV detector at 237 nm for AST and ASP. The retention time of ASP and AST were found be 3.8 min and 9.4 min respectively. The developed method was validated for accuracy, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) and robustness as per the ICH guidelines.

Key words: Development and new validation, Atorvastatin calcium, Aspirin, Capsule form and RP HPLC.

Introduction: Atorvastatin calcium is chemically a calcium salt of (β R, 8 R)-2-(4 – fluoro-phenyl) – α, δ di hydroxyl 5(1 methyl ethyl) 1, 3, phenyl, 4 (phenyl amino) carbonyl) - 1 H pyrroleheptanoic acid tri hydrate used as Antihyperlipidemic.

It is official in Indian pharmacopoeia 1450 SUMA. B.V. 1Aspirin is chemically 2-(Acetyloxy) benzoic acid, best known as anti-platelet drug. It is official both in I.P and B.P.2 Detailed survey of literature for atorvastatin calcium (AST) revealed several methods based on different techniques like extractive Spectrophotometry

3, HPLC 4-8, HPLC 9-11 for its determination in human serum, capillary electrophoresis 12, HPTLC for its determination in pharmaceutical. 13 Similarly literature survey for aspirin reveals several methods based on Spectrophotometry14, Raman spectroscopy 15 RP- HPLC 16. This paper describes a simple, precise and accurate RP HPLC method for the estimation of AST and ASP combination in a capsule dosage form.

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Material and methods Chemical and reagents AST & ASP was the generous gifts from Tri-Star pharma private limited pondy, Combination of these drugs was purchased from the local market (Ecosprin AV 75 containing Atorvastatin calcium 10 mg and aspirin 75 mg as per the label claim, marketed by USV limited, India). HPLC grade Acetonitrile, orthophosphoric acid,methanol was procured from Merck.

RP HPLC instrumentation and chromatographic conditions: The following chromatographic conditions were established for the separation of drug and maintained the same parameter throughout the method. System High performance liquid chromatograph10AT SHIMADZU- SPD10A detector Column SS Grace- C18, 250X 4.6 mm, 5 µm Detector UV detector Mobile phase Orthophosphoric acid buffers: Acetonitrile (45:55) was used pH 4.5. Detection wavelength 237 nm Mode gradient Sample Size 20 µl Temperature Room temperature Preparation of standard stock solution

Preparation of standard solution (A): Weigh accurately about 25mg of atorvastatin calcium working standard in a 100 ml volumetric flask. Add about 20 ml of methanol, sonicate to dissolve, and make up to the mark with mobile phase.

Preparation of standard solution (B): Weigh accurately about 75 mg of aspirin working standard into a 100 ml volumetric flask. Add about 20 ml of methanol, sonicate to dissolve, and make up to the mark with mobile phase.

Mixed standard preparation:

Take 2ml of standard solution A and 5ml of standard solution B to 50 ml with mobile phase.

Sample Preparation: Weigh and remove capsule and crush the content of 20 capsules, weigh accurately powder sample (10 mg equivalent of atorvastatin) and 75mg equvallent aspirin 392 mg into a 100 ml volumetric flask, add 20 ml of methanol. Sonicate for 10 minutes and dilute to the volume with mobile phase sonicate to dissolve too completely. Filter with o.45µ membrane filter. Dilute 5ml and 2ml of the above solution to 50 ml with mobile phase.

Preparation of Calibration Curve; In this method, the aliquots of stock solution of aspirin (0.5- 2.5ml) and atorvastatin (1-5ml) were transferred into a 25 ml of volumetric flask and made up to the mark with mobile phase. A solution contains 5, 10, 15, 20, 25µg / ml of aspirin and 10, 20, 30, 40, 50 µg /ml of atorvastatin in mobile phase were injected and the chromatograms were recorded at 237nm. It was found that the above concentration range was linear. The procedure was repeated for three times. The peak areas were plotted against concentration and the calibration curve was constructed.

Estimation of aspirin and atorvastatin in capsule formulation: Weigh remove the capsule and crush the content of 20 capsules, the average weight was found and powdered (10 mg equivalent of atorvastatin) and 75mg equvallent aspirin 392 mg into a 100 ml volumetric flask, add 20 ml of methanol. Sonicate for 10 minutes and dilute to the volume with mobile phase sonicate to dissolve completely. Filter with o.45 µ membrane filter. Dilute 5 ml of the above solution to 50 ml with mobile phase .Inject the solution and recorded the chromatogram. The concentration of each test solution was determined by using slope and intercept values from calibration graph.

Recovery studies: To ensure the reliability of the methods, recovery studies were carried out by mixing a known quantity of standard drug solution with the pre – analyzed sample formulation and the content were mixed and made to the volume with mobile phase and re-analyzed by the proposed method, the percentage recovery was calculated.

Limit of detection (lod) and limit of quantification (loq): Calibration of standard was repeated for three times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation of intercept.

System Suitability Studies: The system suitability studies carried out as specified in ICH guidelines and USP. The parameters like tailing factor, asymmetry factor, number of theoretical plates, capacity factor were calculated.

Robustness Robustness was checked by making a slight deliberate change in the experimental procedure like slight change in the temperature, flow rate and pH and the data are expressed in terms of relative standard deviation.

Conclusion The proposed HPLC method is simple and the total run time for the two components is less than 12min. The quantitation of each component was not affected by any of the possible interfering substances included during tablet manufacturing. The method is accurate and precise as indicated from the recovery study. It can be concluded that the proposed HPLC method has great promise for the simultaneous determination of two components in pharmaceutical formulations.

Results and Discussion: The methods were developed for the estimation of Aspirin and atorvastatin in pure form and in its Capsule dosage form .the method employed for analysis of Aspirin and atorvastatin are High performance liquid chromatography

RP- HPLC Method: An effort has been made to indentify simple, precise, specific and accurate methods for the estimation of aspirin and atorvastatin in bulk and in formulation by using RP- HPLC.

The solution of 10µg/ ml and 25 mg of aspirin and atorvastatin in mobile phase (buffer: acetonitrile) was prepared and the solution was scanned in the range of 200-400 nm. At 237nm, the drug showed maximum absorbance with 2 hours stability. Hence in this was selected as a detection wavelength. Quantification of aspirin and atorvastatin was done by external standard calibration method.

The optimization was done by various mobile phase such as acetonitrile; water ,acetonitil we ;methanol and only acetonitrile is used were employed and the chromatogram was recorded for aspirin and atorvastatin .These are shown in after considering suitability test parameters [buffer:acetonitrile] of 45:55 ratio was selected for analysis .The retention time of aspirin is 3.8 and atorvastatin is 9.4 minutes. The system suitability test parameters were calculated for optimized chromatograms and are shown in the table-2.

The linearity was done by using external standard calibration method with the optimized chromatographic conditions; stock solutions of aspirin were prepared by 20 ml of methanol and make up to mobile phase with 100ml. In that prepare various concentrations in the range of (1 TO 5) of aspirin and atorvastatin in mobile phase.20 µl of each solution were injected individually. The chromatogram was shown in figure part.

The calibration curve was plotted using concentration against peak area .The procedure was repeated for three times. The co-relation coefficient value was found to be 0.9999 indicates that the concentration of aspirin and atorvastatin has good linearity. The calibration graph is shown in figure part. The optical characteristics of aspirin and atorvastatin are shown in table part. The limit of detection and the limit of quantification were determined by using slope and standard deviation .The LOD AND LOQ was calculated.

The table formulation aspirin and atorvastatin was selected for analysis from the calibration curve, the nominal concentration aspirin and atorvastatin was prepared 20 µl of formulation was injected and the chromatograms were recorded. The percentage of aspirin and atorvastatin present in formulation was found to be tables the precision of the method was confirmed by repeatable injection of the formulation for six times and there chromatograms are shown in the figures. The percentage RSD value was found to be tables. This indicates that the method has good precision .The values are shown in tables

Accuracy was confirmed by recovery studies by adding known amount of pure drug to the

previously analyzed formulation and the mixture was re analyzed by the proposed method and there chromatograms were recorded as shown in the figures tables. The percentage recovery of aspirin and atorvastatin present in formulation was found to be tables. It was extremely low when compared to the normal value the high percentage recovery indicates that there is no inference produced due to the excipients used in formulation. Hence, the developed method was found to be accurate.

All the above parameters combined with simplicity and ease of operation ensures that the application of proposed method for the assay of drug in pharmaceutical dosage forms.

Summary and Conclution: The rapid simple RP-HPLC method has been developed; the mobile phase selected is Orthophosphoric acid buffers: Acetonitrile (45:55) was used pH 4.5. The flow rate was maintained at 1.0 ml / min. The detection of the constituents was done using UV detector at 237 nm for AST and ASP. The retention time of ASP and AST were found be 3.8 min and 9.4 min respectively the detection wavelength at 237 nm.

The formulation was selected for quantification and results were found to be satisfactory.

All the methods are validated as per ICH guidelines. The methods were found to be simple, accurate, precise and rapid.

Optimization of Chromatogram Using Opa: Acetonitrile (45:55)

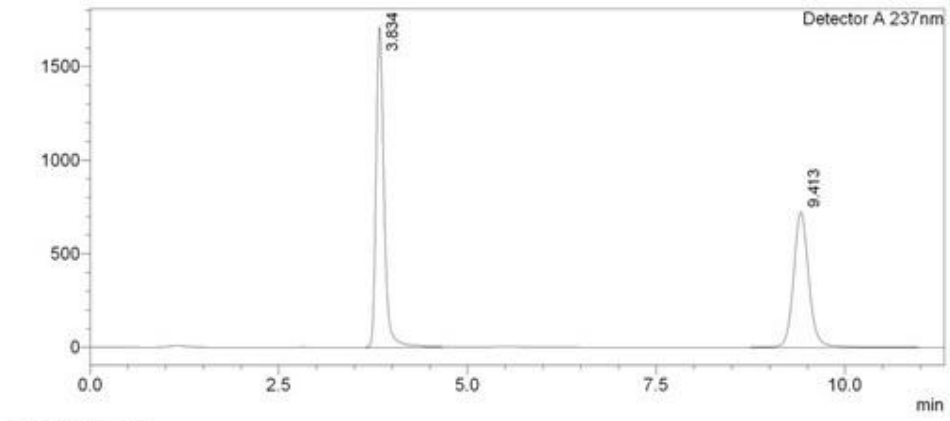
SHIMADZU LabSolutions

SURYA SCHOOL OF PHARMACY
VIKKIRAVANDI

<Sample Information>

Sample Name	: ATRAVASTATIN AND ASPIRIN		
Sample ID	: 07062016		
Data Filename	: ATRAVA AND ASP6062037.lcd		
Method Filename	: ASPIRIN AND ATRAVASTATIN.lcm		
Batch Filename	:		
Vial #	: -1	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 6/7/2016 11:42:39 AM	Acquired by	: Staff
Date Processed	: 6/7/2016 3:08:13 PM	Processed by	: Staff

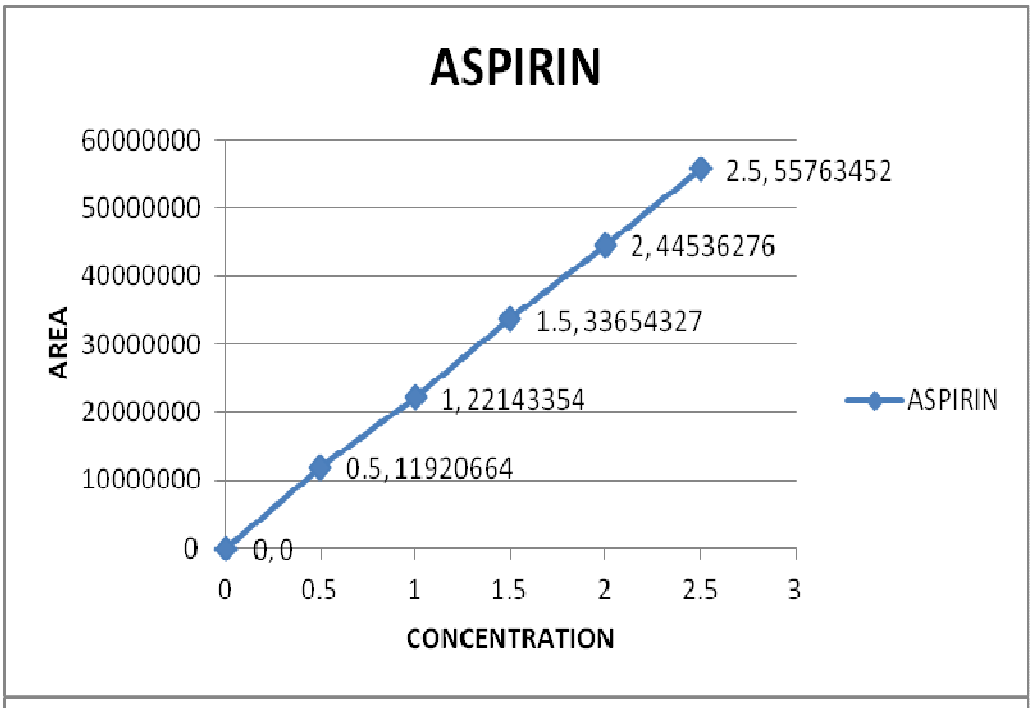
<Chromatogram>

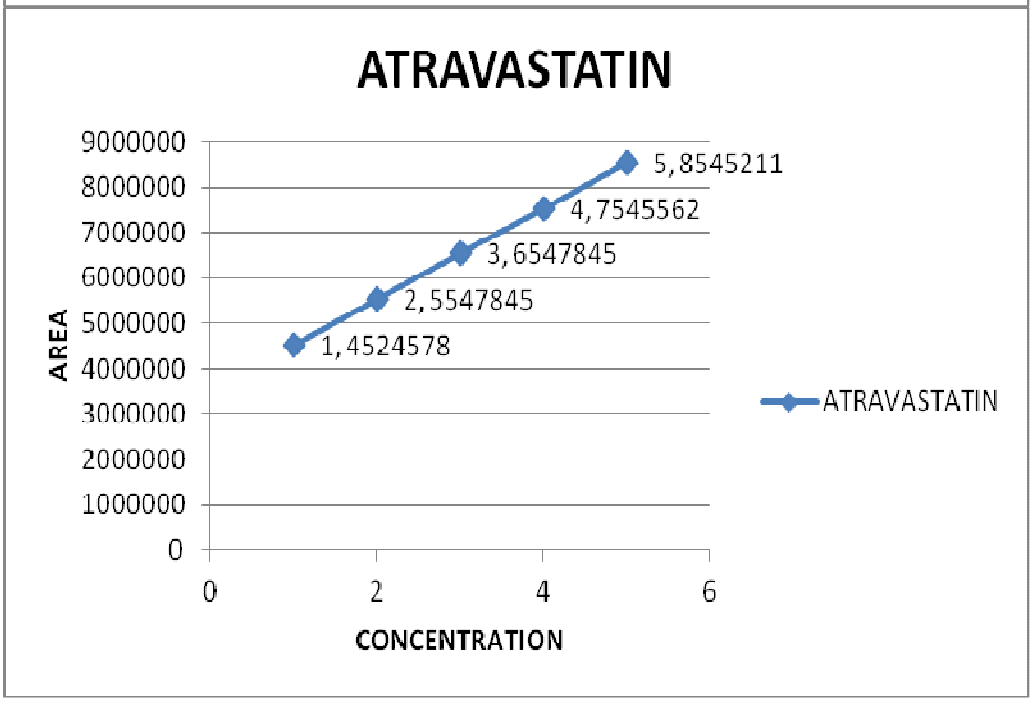


<Peak Table>

Detector A 237nm

Peak	Name	Retention time	Area	Height	Theoretical plates	Tailing factor
1	RT 3.853	3.834	452125462	3042745	36794	1.395
2	RT 9.483	9.413	11156584	362229	71070	1.132





Parameters table

PARAMETERS	ECOSPRIN-AV
Tailing factor	1.68
Asymmetrical factor	2.00
Capacity factor	0.34
Theoretical plate per unit Length	22.05

Assay of Commercial Formulation (Ecosprin-AV) By RP-HPLC (Atravastatin)

Sample No.	Labeled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained	Average (%)	S.D. (+/-)	% R.S.D.	S.E.
1	10	10.4	99				
2	10	10.5	102				
3	10	10.5	101	100.67%	0.1631	0.1615	
4	10	10.3	100				
5	10	10.1	101				
6	10	10.1	101				0.0665

Assay of Commercial Formulation (Ecosprin-AV) by RP-HPLC (Aspirin)

Sample No.	Labeled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained	Average (%)	S.D. (+/-)	% R.S.D.	S.E.
1	75	75.1	100.1				
2	75	75.0	100				
3	75	75.3	100.4	100.12%	0.1875	0.1254	
4	75	75.0	100				
5	75	75.0	100				
6	75	75.2	100.2				0.0458

Recovery Studies of Formulation – Ecosprin-AV By RP-HPLC Method (Atravastatin)

S.No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	Percentage Recovery (%)	S.D.	% R.S.D.	S.E.
1.	5.04	5	10.5435	5.5435	104.63			
2	5.04	10	15.5987	5.5987	105.31	1.6687	1.5876	0.9621
3	5.04	15	20.2532	5.2532	101.58			

Recovery Studies of Formulation – Ecosprin-AV BY RP-HPLC Method (Aspirin)

S.No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	Percentage Recovery (%)	S.D.	% R.S.D.	S.E.
1.	5.02	5	10.4632	5.4631	104.63			
2	5.02	10	15.5711	5.5711	105.31	1.5625	1.5253	0.8821
3	5.02	15	20.2734	5.2734	101.58			

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