Simultaneous Estimation and Validation of Atorvastatin Calcium and Aspirin in Combined Capsule Dosage Form by RP HPLC Method

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Abstract: A new simple, specific, precise and accurate reverse 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 Acetonitrile: Ammonium Acetate buffer 0.02M (68:32) pH 4.5. The flow rate was maintained at 0.8 ml / min. The detection of the constituents was done using UV detector at 245 nm for AST and ASP. The retention time of AST and ASP were found to be 4.5915 ± 0.0031 min and 3.282 ± 0.0024 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: Simultaneous estimation, 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 pyrrole-heptanoic acid tri hydrate used as antihyperlipidaemic. It is official in Indian pharmacopoeia

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 Spectrophotometry³, HPLC⁴⁻⁸, HPLC⁹⁻¹¹ for its determination in human serum, capillary electrophoresis¹², HPTLC for its determination in pharmaceutical.¹³

Similarly literature survey for aspirin reveals several methods based on Spectrophotometry¹⁴, Raman spectroscopy¹⁵ RP- HPLC¹⁶. This paper describes a simple, precise and accurate RP HPLC method for the estimation of AST and ASP combination in a capsule dosage form.

Material and methods

Chemical and reagents

AST was the generous gifts from Biocon Limited Bangalore, and aspirin was procured from Qualigens Fine Chemicals (Glaxo Ltd). 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, ammonia, glacial acetic acid 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 chromatograph 10AT SHIMADZU- SPD10A detector
Column	SS Grace- C18, 250X 4.6 mm, 5 µm
Detector	UV detector
Mobile phase	Acetonitrile: Ammonium acetate 0.02M (68:32) pH 4.5.
Detection wavelength	245 nm
Mode	Isocratic
Sample Size	20 µl
Temperature	Room temperature

Preparation of standard stock solution

- Preparation of standard stock solution of AST: An accurately weighed quantity equivalent to 10 mg AST was dissolved in 25 ml of methanol and then the volume was made up to 50 ml with same solvent.
- Preparation of standard stock solution of AST: An accurately weighed quantity equivalent to 75 mg ASP was dissolved in 25 ml of methanol and then the volume was made up to 50 ml with same solvent.
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Validation of the method¹⁷

The method was validated as per ICH guide line. The parameters checked were linearity, accuracy, precision, limit of detection, limit of quantification, robustness and specificity.

System suitability testing

A standard solution was prepared using AST and NA working standard as per the test method and was injected nine times into the HPLC system. The parameters like theoretical plates, tailing factor and resolution for the standard solution were calculated.

Calibration curve

From the working standard solution of AST (200 µg/ml) and AST (1500 µg/ml), appropriate aliquots of AST and ASP stock solution were diluted in methanol to obtain final stock solution of 2, 4, 6, 8 10 µg/ml of AST and 15, 30, 45, 60, 75 µg/ml of ASP. The solutions were injected using a 20 µl fixed loop system and chromatograms were recorded. Calibration curve were constructed and regression equation were computed for AST and AST.

Specificity

A blank solution (mobile phase) was injected and the chromatogram showed no inferring peaks at retention time of the two drugs. The chromatogram of AST and ASP extracted from the tablet dosage form were compared with those acquired from AST and ASP standards, correlation was good (in terms of retention time and area) indicates specificity of method.

Precision: (Reproducibility)

The precision of the method was verified by performing the intraday and interday precision. The intraday and interday precision of the proposed method was determined by estimating the corresponding response three times on the same day and on three different days.

Accuracy: (% Recovery)

For accuracy of method, recovery studies were carried out by applying a known amount of standard AST and ASP at a level of 80,100,120 % to the sample solution (standard addition method). Three determinations were performed at each level, using same chromatographic condition as describe above.

Limit of Detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ were calculated using following equations as per International conference on Harmonization guide line

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where σ is standard deviation of the response and S is the standard deviation of y intercept of regression lines.

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.

Analysis of the marketed products

To find the content of the marketed formulation, (Ecosprin AV 75 containing Atorvastatin calcium 10 mg and aspirin 75 mg as per the label claim, marketed by USV limited, India), twenty tablets were weighed and average weight was determined, powdered, from this equivalent weight of 10 mg for AST and 75 mg of ASP was transferred into a 50 ml volumetric flask, containing 15 ml of methanol and sonicated for 30 minutes, filtered through Whatmann filter paper No.41 and then volume was made up to 50 ml with methanol.

Results and Discussion

Validation

To optimize the HPLC parameters, several mobile phases were tried and satisfactory results were obtained by using the mobile phase consisting of Acetonitrile: ammonium acetate

0.02M (68:32) at of P^H of 4.5, with a flow rate of 0.8ml / min. UV detection was carried out at 245nm and run time was 15min. The selection of the mobile phase was made on the basis of resolution, asymmetric factor, and theoretical plates. The retention time of AST and ASP was found to 4.5915 ± 0.0031 min and 3.282 ± 0.0024 min respectively. A typical chromatogram of the test is shown in Figure 1.

Figure 1. A Representative Chromatogram of Atorvastatin Calcium (AST) and Aspirin (ASP) at 245 nm.

System suitability parameters

System suitability parameters proved that the proposed method suits for the simultaneous estimation of AST and ASP. After various trials performed, chromatogram for AST and ASP was found satisfactory on, using mobile phase composition of Acetonitrile: Ammonium acetate 0.02M (68:32) pH 4.5. Drug peak was found to be symmetrical as observed from asymmetry factor of 1.4315 for AST and 1.4455 for ASP. Resolution of the proposed method was found to be satisfactory and the results are given in table 1.

Table: 1. Study of System Suitability Parameters.

Sl.No.	Parameters	AST	ASP
1	AUC(Area under the curve) *	112515.9401	165139.9916
	±S.D	277.8376	848.5548
	% RSD	0.058	0.513
2	Resolution	4.4400	0.7935
3	Theoretical levels	9865.4064	8070.8288
4	Tailing factor	1.2601	1.2557
5	Asymmetry	1.4315	1.4455

* Mean of nine trials.

Linearity

The linear regression data revealed a good linear relationship over the concentration range of 2-10 $\mu\text{g/ml}$ for AST and 15-75 $\mu\text{g/ml}$ for ASP with correlation coefficient ($r^2 = 0.9998$) for AST and correlation coefficient ($r^2 = 0.9994$) for ASP respectively. The results are showed in table 2 and Figure 2 and Figure 3.

Mean of six trials

Figure 2. Linearity graph of Atorvastatin calcium (AST).

Mean of six trials

Figure 3. Linearity graph of Aspirin (ASP).

Specificity

The method was found to be specific since no interferences chromatograms were seen when carried out in presence of additives, when only mobile phase was injected.

Table 2. Linear regression data of AST and NA.

Sl.No	Parameters	AST	ASP
1	Detection Wavelength	254 nm	254 nm
2	Linearity Range $\mu\text{g}/\text{ml}$	2-10	15-75
3	Correlation Coefficient(r^2)	0.9998	0.9994
4	Linearity Regression equation ($y=mx+c$)	$Y=16617x+13440$	$Y=3266.8x+20108$
5.	LOD	0.088 ng/ml	1.31 ng/ml
6	LOQ	0.266 ng/ml	3.98 ng/ml
7.	Precision(%RSD)		
	Intraday	0.37	0.54
	Interday	0.45	0.975

Precision

The proposed method was found to be precise as indicated by percent RSD not more than 2% as per ICH guidelines for interday and intra day determination. The results are shown in the table 2.

Accuracy

The proposed method when used for the estimation of AST and ASP from pharmaceutical dosage form after spiking with the standard, afforded recovery of 100.40(80%), 99.23 %(100%), 100.61 %(120%) at different levels were found for AST and 99.82 %(80%), 100.23% (100%), 99.14 %(120%) for ASP respectively, as shown in table 3 and table 4.

Table 3. Recovery studies of Atorvastatin Calcium Excepted concentration.

	Level of recovery		
	80%	100%	120%
	8 $\mu\text{g}/\text{ml}$	10 $\mu\text{g}/\text{ml}$	12 $\mu\text{g}/\text{ml}$
Peak Area*	90248.359	111541.049	135718.00
%RSD	0.07918	0.3414	0.06588
Mean % recovery	100.40	99.23	100.61

* Mean of three trials.

Table 4. Recovery studies of Aspirin Excepted concentration.

	Level of recovery		
	80%	100%	120%
	60 $\mu\text{g}/\text{ml}$	75 $\mu\text{g}/\text{ml}$	90 $\mu\text{g}/\text{ml}$
Peak Area*	132603.4533	166423.573	197533.432
%RSD	0.1619	0.9777	0.4213
Mean% recovery	99.82	100.23	99.14

*Mean of three trials.

Limit of Detection (LOD) and Limit of quantification (LOQ)

The limit of detection was found to be 0.088ng/ml and limit of quantification was found to be 0.266ng/ml for AST and similarly for ASP is 1.31ng/ml and 3.98ng/ml respectively. The results are shown in table 2.

Robustness

Robustness was checked by making a slight deliberate change in the experimental procedure by slight change in the temperature, flow rate and pH and no significant change in area and retention time was found in all the parameters and all the values were found to be within 2% relative standard deviation. The results of robustness are given in the table 5, 6, 7.

Table 5. Robustness for flow rate studies (temperature).

Temperature (°C)	Area (AST)	Area (ASP)
25	111802.563	163878.422
30	112293.391	165872.156
35	114609.32	169543.109
Mean	112901.758	166431.229
%RSD	1.327	1.726

Table 6. Robustness for pH studies.

pH	Area (AST)	Area (ASP)
4.4	116011.297	168410.359
4.5	112293.391	165872.156
4.6	113067.297	162874.891
Mean	113790.6617	165719.1353
%RSD	1.723	1.672

Table 7. Robustness for Flow rate Studies.

Flow rate (ml)	Area (AST)	Area (ASP)
0.798	114018.961	165255.6
0.8	112293.391	165872.2
0.802	112194.805	161132.7
Mean	112835.719	164086.8
%RSD	0.909	1.57

Analysis of the marketed products

The proposed method was applied successfully to determine the content of AST and ASP in pharmaceutical product. The results are expressed in terms of percentage in Table 8.

Table 8. Analysis of tablet formulation.

Sl.No.	Parameters	AST	ASP
1	Label claim(mg/tab)	10 mg	75mg
2	Drug content %	99.23±0.3373	100.23±0.9805
3	% RSD	0.3399	0.9783

Conclusion

The proposed HPLC method is simple and the total run time for the two components is less than 9 min. 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.

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