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HPLC METHOD DEVELOPMENT AND VALIDATION OF ATORVASTATIN AND ASPIRIN

Piyush Kashyap* and Amit Aggarwal

[*]Maharaja Agrasen University, School of Pharmacy, Village Kallujhanda-174103, Baddi, Himachal Pradesh, India.

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***Corresponding Author**
Piyush Kashyap
Maharaja Agrasen University, School of Pharmacy, Village Kallujhanda-174103, Baddi, Himachal Pradesh, India.

ABSTRACT

In accordance with the guidelines of the International Conference on Harmonization (ICH), a new, straightforward, innovative, accurate, precise, dependable, fast, and linear reverse phase high-performance liquid chromatography (RP-HPLC) method was created and thoroughly validated for the simultaneous qualitative and quantitative estimation of atorvastatin and aspirin in bulk and pharmaceutical dosage form. Validation by HPLC method, the wavelength was selected at the isobestic point at which the two drugs can be detected using UV detectors. The selected wavelength was 255 nm. Using anorthophosphoric acid-adjusted methanol (20:80) mobile phase that contained 0.02 M potassium dihydrogen phosphate, a phenomenex C-18, 5 µm column with 250 x 4.6 mm i.d. in isocratic mode was employed. The effluents were measured at 255 nm, and the flow rate was 1.0 ml/min, Linearity range was determined by external standard calibration method in the concentration range of 20µg/mL to 120µg/mL for atorvastatin and aspirin's acid hydrolysis yielded degradation rates of 2.15% and 2.19%, respectively, after one hour at 60°C. Atorvastatin and aspirin's basal hydrolysis amounts for one hour at 60°C were 2.69% and 1.98%, respectively. At normal temperature, the amounts of oxide degradation of aspirin and atorvastatin were 3.86% and 7.56%, respectively, after three hours. Aspirin and atorvastatin's respective amounts of thermal degradation after a 5-hour period at 110°C were 0.99% and 0.90%.

KEYWORDS: Simultaneous estimation, Atorvastatin, Aspirin, and RP-HPLC.

INTRODUCTION

A dihydroxy monocarboxylic acid, atorvastatin belongs to the statin medication class and is mainly used to decrease blood cholesterol and prevent cardiovascular illnesses. It plays a part as both a xenobiotic and an environmental pollutant. It is a synthetic statin, an aromatic amide, a dihydroxy monocarboxylic acid, a member of pyrroles, and a member of monofluorobenzenes. It has a heptanoic acid functional relationship. It is an atorvastatin's conjugate acid. Several dyslipidemias, such as adult primary hyperlipidemia and mixed dyslipidemia, hypertriglyceridemia, primary dysbetalipoproteinemia, homozygous familial hypercholesterolemia, and heterozygous familial dyslipidemia, can be treated with atorvastatin. For patients with aberrant lipid profiles or cardiac risk factors, atorvastatin is recommended in conjunction with dietary adjustments to prevent cardiovascular events. In people without coronary heart disease but with many risk factors, as well as in patients with type 2 diabetes who do not have coronary heart disease but have several risk factors, atorvastatin can be used as a preventive medication for myocardial infarction, stroke, revascularization, and angina. In individuals with coronary heart disease, atorvastatin may be used as a preventive measure for non-fatal myocardial infarction, fatal and non-fatal stroke, revascularization procedures, hospitalization for congestive heart failure, and angina.

Acetylsalicylic acid belongs to the benzoic acid class, which is salicylic acid with an acetoxy group in place of the hydrogen atom that was formerly linked to the phenolic hydroxy group, an anti-inflammatory non-steroidal medication that inhibits cyclooxygenase. It functions as a prostaglandin antagonist, teratogenic agent, anticoagulant, non-steroidal anti-inflammatory drug, non-narcotic analgesic, inhibitor of platelet aggregation, antipyretic, cyclooxygenase 2 and cyclooxygenase 1 inhibitor, plant activator, drug allergen, prostaglandin antagonist, geroprotector, and EC 1.1.1.188 (prostaglandin-F synthase) inhibitor. It belongs to phenyl acetates, salicylates, and benzoic acid groups. It shares a functional relationship with salicylic acid. It is an acetylsalicylate's conjugate acid. It is prescribed in flu, common cold, neck and back pain, dysmenorrhea, headache, tooth pain, sprains, fractures, myositis, neuralgia, synovitis, arthritis, bursitis, burns, and other traumas to reduce pain, fever, and inflammation associated with these diseases. It is also employed in the symptomatic management of pain following dental and surgical operations. Acetylsalicylic acid in the extra strength formulation is also recommended for the treatment of migraine headache associated with photophobia (sensitivity to light) and phonophobia (sensitivity to sound). Because ASA inhibits platelet aggregation, it is also indaicated for a number of other causes. Among them

are: Lowering the chance of dying from cardiovascular causes when myocardial infarction (MI) is suspected.

MATERIAL AND METHODS

Equipments: The various equipments which were used and their suppliers are given in the following table.

Instruments	Manufacturers/Suppliers
UV Spectrophotometer	Shimadzu UV-1800 UV/Vis. double beam spectrophotometer (Kyoto, Japan).
pH meter	LabIndia, Mumbai
Bath Sonicator	PCI analytics, India
Water bath shaker	Narang scientific works, Pvt. Ltd
Melting point apparatus	Perfit, India
Electronic Balance	Shimadzu, Japan
FT-IR	Nicolet
Centrifuge	REMI, India
Vortex	Perfit, India

Materials: The various chemicals used in the experiment and their suppliers are given in the following table.

Material	Suppliers
Atorvastatin	Centrient Pharmaceuticals, India
Aspirin	Metrochem API Private Limited
Potassium Dihydrogen phosphate	Finar reagents, Ahmedabad
Orthophosphoric acid	Changshu Yanguan Chemical,China
Methanol	Finar reagents, Ahmedabad
HPLC Grade water	Elgareserviour

Preparation of standard stock solution.

i. In the HPLC system, diluent was injected after being filtered by 0.22 µ Millipore membrane filters.
ii. Standard solution setup: A solution containing 2000 µg/mL was obtained by dissolving 20 mg of atorvastatin and 20 mg of aspirin in 10 mL of volumetric flask using diluents. To create a stock solution with a concentration of 200µg/mL, take 5 mL and dilute it up to 50 mL.
iii. Aspirin and atorvastatin calibration curve preparation: To make the necessary dilutions, take 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, and 6 mL from the stock solution. Dilute the solutions up to 10 mL using diluents, filter it through 0.22µ Millipore membrane filters, and then inject it into an HPLC system.

Validation of method

The approach was verified in accordance with the ICH guideline. The parameters that were examined were linearity, accuracy, precision, limit of detection, limit of quantification, robustness and force degradation.

Linearity

The capacity of an analytical method to yield test findings that are exactly proportionate to the analyte concentration in the sample within a specified range is known as its linearity. The interval between the highest and lower levels of analyte that has been shown to be determined within an appropriate degree of precision, accuracy, and linearity is known as the analytical method's range. For aspirin and atorvastatin, the selected linearity ranges were 20–120 µg/mL, respectively. After being filtered using a 0.22 µ filter, each dilution was then injected.

Accuracy

The method's accuracy was calculated as a percentage of recovery. Analysis was done on the three concentration levels: 50%, 100%, and 150%.

Precision

Studies examining variations within and between days proved how accurate the system was. Six consecutive standard injections were made during the intraday studies, and the % RSD and drug peak response factor were computed. In the inter-day variation investigations, the response factor of the drug peaks and the percentage RSD were computed after six consecutive days of repeated standard injections. The established RP-HPLC method was determined to be accurate based on the data collected. In order to determine the percentage RSD, six injections at a concentration of 80µg/mL were used to evaluate the injection's repeatability.

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

In accordance with ICH recommendations, the developed method's LOD and LOQ were examined. There are various methods available for figuring out the LOD and LOQ, depending on whether an instrumental or non-instrumental procedure is used. One of the methods used here was,

LOD= 3.3σ/S and

LOQ= 10σ/S

Where, σ = the intercept's standard deviation

S = calibration curve's mean slope

Robustness

Examining the sample at a lower concentration and purposefully changing the procedure parameters allowed for the study of robustness. Drug reactions were observed to vary, with %RSD being a useful measure. By varying the column temperature, the flow rate, or the mobile phase ratio, the robustness of the procedure was examined.

Force degradation

Acid hydrolysis: Using the stock solution, a 4 mL aliquot of the combined drugs was placed in a 10 mL amber volumetric flask, combined with 1 mL of 2M HCl, and allowed to stand for 1 hour at 60°C ± 2°C after heating on bath samples. After that, they were neutralized with 1 mL of 2M NaOH, diluted to 10 mL using diluents, and the concentration was found to be 80µg/mL. The samples were then sonicated, filtered through 0.22µm membrane filter paper, and introduced into an HPLC system.

Basic hydrolysis: After transferring a 4 mL aliquot of the drug mixtures from the stock solution into a 10 mL amber volumetric flask, it was mixed with 1 mL of 2M NaOH and heated to 60°C ± 2°C. Samples were then neutralized with 1 mL of 2M HCl and diluted to 10 mL using diluents, and the concentration was found to be 80 µg/mL. The mixture was then sonicated, filtered through 0.22µm membrane filter paper, and injected into an HPLC system. Every determination was made using an HPLC.

Oxidative degradation

After transferring a 4 mL aliquot of the combination of both drugs from the stock solution into a 10 mL amber volumetric flask, it was combined with 1 mL of 3% (v/v) hydrogen peroxide and allowed to stand for 3 hours at 35°C ± 2°C. Samples were then diluted with mobile phase up to 10 mL, and the concentration was discovered to be 80µg/mL. In HPLC, all three solutions were injected.

Thermal degradation

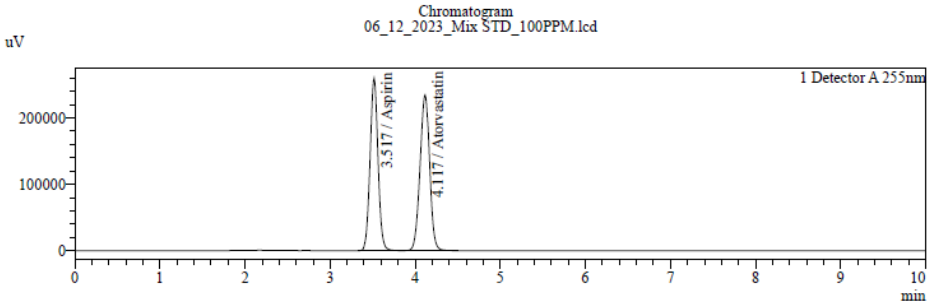
A 10 mL amber volumetric flask was filled with an aliquot of the combination of both medications, made from stock solution containing 4 mL, and heated for 5 hours at 110°C ± 2°C. Following each solution's dilution in mobile phase up to 10 mL, the concentration was determined to be 80 µg/mL, and the mixture was then injected into an HPLC.

RESULTS AND DISCUSSION

HPLC Method

Determination of chromatogram of standard (atorvastatin and aspirin)

On HPLC analysis of standard solution of atorvastatin and aspirin(10µg/mL) chromatogram was optimized and analyzed as per the proposed method. HPLC analysis of blank and standard chromatogram was shown in Figure.



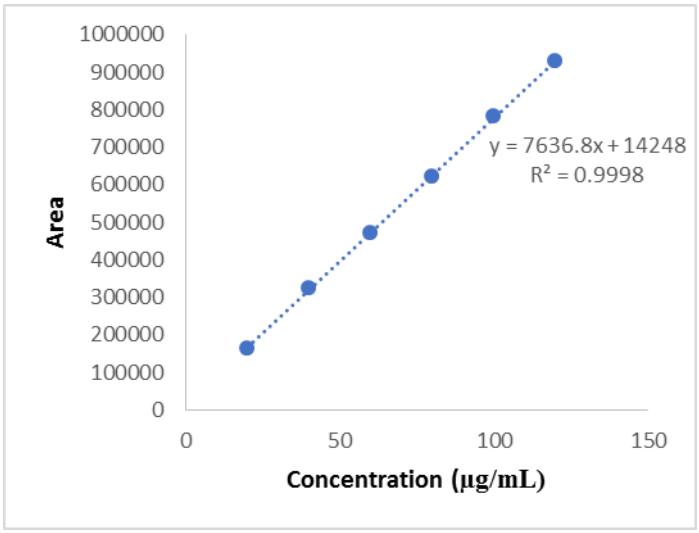
A Representative Chromatogram of Atorvastatin Calcium (AST) and Aspirin (ASP) at 245nm.

Linearity

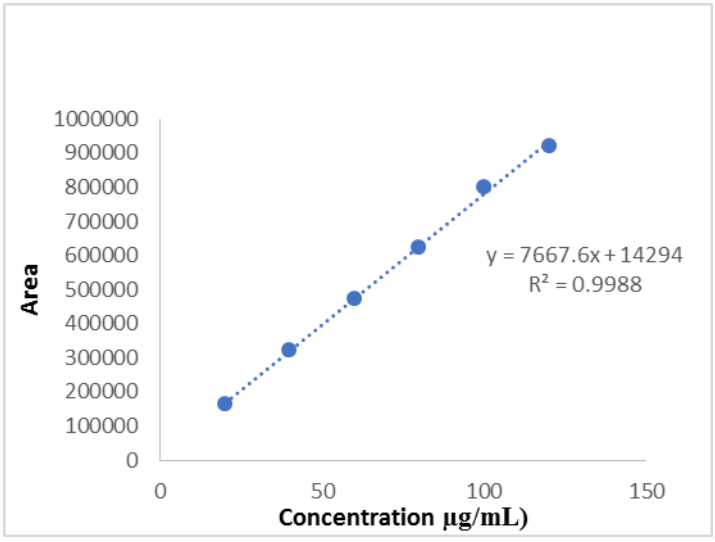
Linearity of Atorvastatin

For atorvastatin, a calibration curve was drawn in a concentration range of 20µg/mL to 120µg/mL. Atorvastatin working stock solutions (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL, and 120 µg/mL) were precisely measured, and each dilution was injected after being filtered through a 0.22 µ filter. Every solution's area was measured at each wavelength. By charting concentration against the location of each reading, the linearity was created.

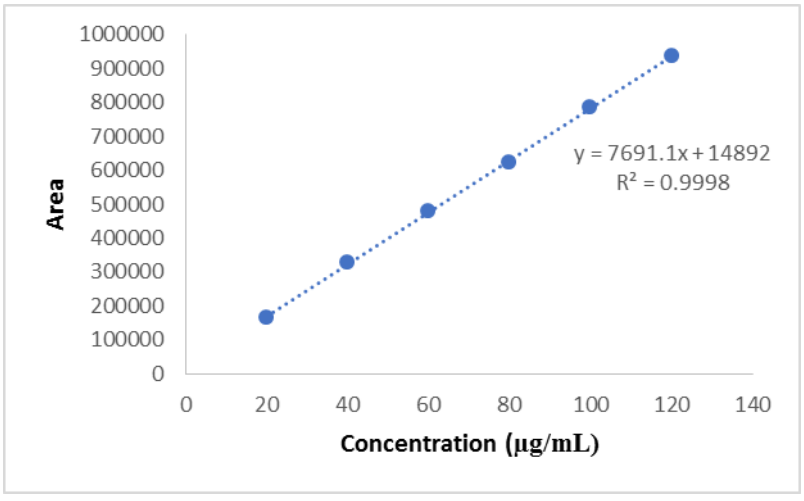
Atorvastatin			
Conc. (µg/mL)	**Linearity 1**	**Linearity2**	**Linearity 3**
20	164745	165574	165044
40	324877	321874	326671
60	471544	472497	480024
80	620084	624874	624789
100	781449	799112	785576
120	930247	922217	937498



Linearity 1 graph of Atorvastatin.



Linearity 2 graph of Atorvastatin.

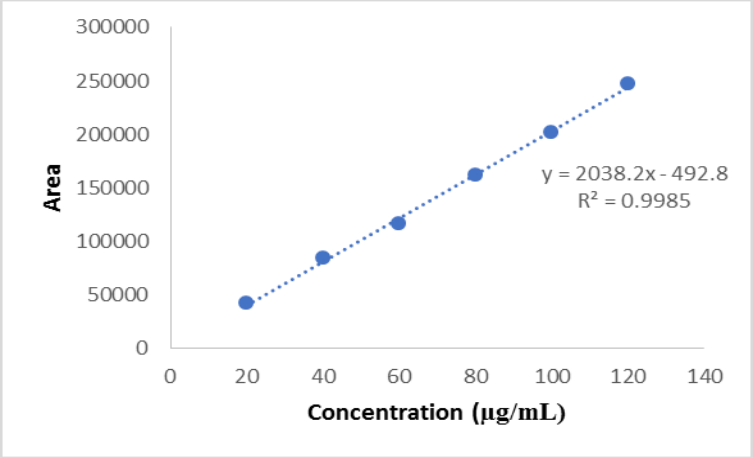


Linearity 3 graph of Atorvastatin.

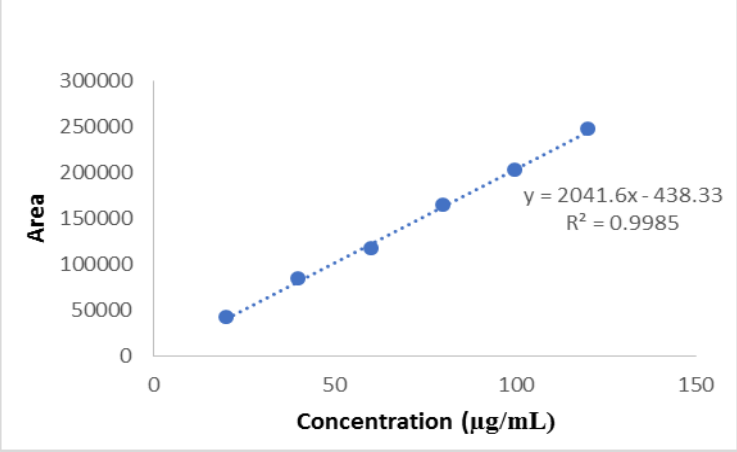
Linearity of Aspirin

For aspirin, a calibration curve was drawn across a concentration range of 20µg/mL to 120µg/mL. Working stock solutions of aspirin (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL, and 120 µg/mL) were precisely measured, and each dilution was injected after being filtered through a 0.22µ filter. Every solution's area was measured at each wavelength. By charting concentration against the location of each reading, the linearity was created.

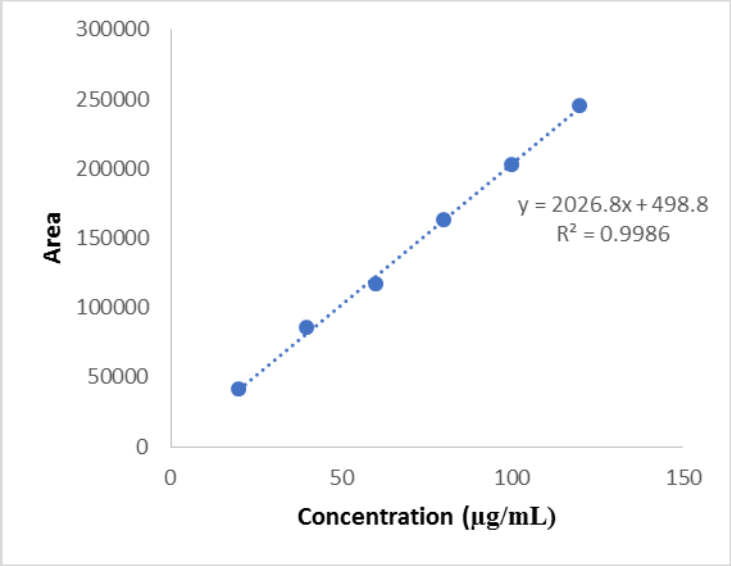
Aspirin			
Conc. (µg/mL)	**Linearity 1**	**Linearity 2**	**Linearity 3**
20	41557	41518	41008
40	83664	83557	85177
60	116795	117057	117240
80	162248	163889	163224
100	202059	201748	202338
120	246784	247055	245269



Linearity 1 Graph of Aspirin.



Linearity 2 Graph of Aspirin.



Linearity 3 Graph of Aspirin.

Accuracy

The method's accuracy was assessed using the standard recovery percentage. Three concentration levels were examined: 50%, 100%, and 150%.

50% Accuracy Study of Aspirin

Accuracy				
	Conc. (µg/mL)	**Area**	**Amount recovered (µg/mL)**	**% recovery**
50%	40	81734	40.23	100.56
	40	81177	39.95	99.88
	40	80104	39.42	98.56
Mean		81005		99.66
SD		828.50		1.02
%RSD		1.02		1.02

100% Accuracy Study of Aspirin

Accuracy				
	Conc. (µg/mL)	**Area**	**Amount recovered (µg/mL)**	**% recovery**
100%	80	163212	80.25	100.32
	80	164509	80.89	101.11
	80	162104	79.71	99.64
Mean		163275		100.35
SD		1203.74		0.74
%RSD		0.74		0.74

150% Accuracy Study of Aspirin

Accuracy				
	Conc. (µg/mL)	**Area**	**Amount recovered (µg/mL)**	**% recovery**
150%	120	245826	120.84	100.70
	120	244950	120.41	100.34
	120	244852	120.36	100.30
Mean		245209.33		100.45
SD		536.29		0.22
%RSD		0.22		0.22

50% Accuracy Study of Atorvastatin

Accuracy				
	Conc. (µg/mL)	**Area**	**Amount recovered (µg/mL)**	**% recovery**
50%	40	320844	39.97	99.92
	40	319574	39.80	99.51
	40	321334	40.03	100.08
Mean		320584		99.84
SD		908.35		0.30
%RSD		0.28		0.30

100% Accuracy Study of Atorvastatin

Accuracy				
	Conc. (µg/mL)	**Area**	**Amount recovered (µg/mL)**	**% recovery**
100%	80	624164	79.54	99.42
	80	626823	79.89	99.86
	80	620246	79.03	98.79
Mean		623744		99.36
SD		3308.52		0.54
%RSD		0.53		0.54

150% Accuracy Study of Atorvastatin

Accuracy				
	Conc. (µg/mL)	**Area**	**Amount recovered (µg/mL)**	**% recovery**
150%	120	923664	118.61	98.84
	120	922885	118.51	98.76
	120	920314	118.18	98.48
Mean		922288		98.69
SD		1753.06		0.19
%RSD		0.19		0.19

The results show that the recoveries are well within the 98%–102% acceptable range, demonstrating a high level of technique sensitivity for analyte detection in the sample. As a result, the approach is precise and useful for drug estimate.

Precision

The suggested procedure was followed in the preparation and analysis of a standard solution containing 80µg/mL of aspirin and atorvastatin.

Repeatability precision study of Atorvastatin

Conc. (µg/mL)	Repeatability		
	Area	Amount recovered(µg/mL)	% recovery
80	624395	79.57	99.46
80	625107	79.66	99.58
80	620548	79.07	98.83
80	623350	79.43	99.29
80	624474	79.58	99.47
80	620104	79.01	98.76
Mean	622996		99.23
SD	1805.61		0.29
%RSD	0.29		0.30

Inter-day precision study of Atorvastatin

Conc. (µg/mL)	Inter-day		
	Area	Amount recovered(µg/mL)	% Recovery
80	619437	78.92	98.65
80	612090	77.96	97.46
80	624894	79.63	99.54
80	618807	78.84	98.55
80	618471	78.80	98.50
80	620557	79.07	98.84
Mean	619043		98.59
SD	4545.79		0.74
% RSD	0.73		0.75

Intra-day precision studyof Atorvastatin

Conc. (µg/mL)	Intra-day		
	Area	Amount recovered (µg/mL)	% recovery
80	629924	80.29	100.36
80	626960	79.90	99.88
80	621647	79.21	99.01
80	626177	79.80	99.75

80	618410	78.79	98.49
80	624716	79.61	99.51
Mean	624639		99.50
SD	4567.14		0.74
% RSD	0.73		0.75

Repeatability precision study of Aspirin

Conc. (µg/mL)	Repeatability		
	Area	Amount recovered (µg/mL)	% recovery
80	164725	81.00	101.25
80	164161	80.72	100.90
80	163069	80.18	100.23
80	163985	80.63	100.79
80	162194	79.75	99.69
80	163822	80.55	100.69
Mean	163659		100.59
SD	997.97		0.61
% RSD	0.61		0.61

Inter-day precision study of Aspirin

Conc. (µg/mL)	Inter-Day		
	Area	Amount recovered (µg/mL)	% recovery
80	162113	79.71	99.64
80	161584	79.45	99.32
80	160054	78.70	98.38
80	161250	79.29	99.11
80	162015	79.67	99.58
80	161817	79.57	99.46
Mean	161472		99.25
SD	829.85		0.51
% RSD	0.51		0.51

Intra-day precision study of Aspirin

Conc. (µg/mL)	Intra-Day		
	Area	Amount recovered (µg/mL)	% recovery
80	162324	79.82	99.77
80	161815	79.57	99.46
80	162430	79.87	99.84
80	162190	79.75	99.69
80	162314	79.81	99.77
80	161847	79.58	99.48
Mean	162153		99.67
SD	239.02		0.15
% RSD	0.15		0.15

Because of the low %RSD readings, the approach was confirmed to be accurate.

LOD and LOQ

Sr. No.	Drug	LOD (µg/mL)	LOQ (µg/mL)
1	Atorvastatin	0.155	0.469
2	Aspirin	0.054	0.164

Based on the response standard deviation and the slope (s) of the calibration curve at approximately the limits of detection and quantification, the method's limits of detection and quantification were computed. The outcomes were within the range.

Robustness

Examining the sample at a lower concentration and purposefully changing the procedure parameters allowed for the study of robustness. Drug reactions were observed to vary, with %RSD being a useful measure. Changes in wavelength and flow rate were used to examine the method's robustness.

Robustness data of Atorvastatinwith deliberate change in flow rate.

Robustness(Flow rate 0.9mL)				Robustness(Flow rate 1.1mL)		
Conc. (µg/mL)	Area	Amount recovered (µg/mL)	% Recovery	Area	Amount recovered (µg/mL)	% Recovery
80	629225	80.20	100.25	625547	79.72	99.65
80	622595	79.33	99.17	623177	79.41	99.26
80	621504	79.19	98.99	632382	80.61	100.76
Mean	624441		99.47	627035		99.89
SD	4178.54		0.68	4779.58		0.78
%RSD	0.669		0.685	0.762		0.780

Robustness data of Aspirinwith deliberate change in flow rate.

Robustness(Flow rate 0.9mL)				Robustness(Flow rate 1.1mL)		
Conc. (µg/mL)	Area	Amount recovered (µg/mL)	% Recovery	Area	Amount recovered (µg/mL)	% Recovery
80	161393	79.36	99.20	162066	79.69	99.61
80	162183	79.75	99.69	160435	78.89	98.61
80	160957	79.15	98.93	161044	79.19	98.99
Mean	161511		99.27	161182		99.07
SD	621.46		0.38	824.17		0.51
%RSD	0.385		0.384	0.511		0.511

Robustness data of Atorvastatin with deliberate change in wavelength

Robustness(Wavelength 250nm)				Robustness(Wavelength 260nm)		
Conc. (µg/mL)	Area	Amount recovered (µg/mL)	% recovery	Area	Amount recovered (µg/mL)	% recovery
80	636287	81.12	101.40	610652	77.78	97.22
80	627338	79.95	99.94	627041	79.91	99.89
80	628296	80.08	100.10	618665	78.82	98.53
Mean	630640		100.48	618786		98.55
SD	4913.56		0.801	8195.17		1.336
%RSD	0.779		0.797	1.324		1.356

Robustness data of Aspirinwith deliberate change in wavelength

Robustness(Wavelength 250nm)				Robustness(Wavelength 260nm)		
Conc. (µg/mL)	Area	Amount recovered (µg/mL)	% recovery	Area	Amount recovered (µg/mL)	% recovery
80	161925	79.62	99.53	160220	78.78	98.48
80	161615	79.47	99.34	159934	78.64	98.30
80	162678	79.99	99.99	159314	78.34	97.92
Mean	162073		99.62	159823		98.24
SD	546.67		0.336	463.15		0.28
%RSD	0.337		0.337	0.290		0.290

There should be no more than 2% RSDs. The percentage RSD for the flow rate change and for change in wavelength was found to be less than 2, falling within the acceptable range. Therefore, the approach is reliable.

Force degradation

Force degradation study of Aspirin

Nature of stress	Storage conditions	Time (h)	Amount of Aspirin remaining (%)	% Degradation
0.1N HCl	60°C	1.00	97.85	2.15
0.1N NaOH	60°C	1.00	97.31	2.69
3% H_2O_2	Room temp.	3.00	92.44	7.56
Thermal	110°C	5.00	99.10	0.90

Force degradation study of Atorvastatin

Nature of stress	Storage conditions	Time (h)	Amount of Atorvastatinremaining (%)	% Degradation
0.1N HCl	60°C	1.00	97.81	2.19
0.1N NaOH	60°C	1.00	98.02	1.98
3% H_2O_2	Room temp.	3.00	96.14	3.86
Thermal	110°C	5.00	99.01	0.99

CONCLUSION

Improving analytical method development and validation in accordance with ICH principles is the goal of the current study. It was determined that the new RP-HPLC method that is suggested is easy to use, fast, sensitive, accurate, exact, and selective in determining the quantitative levels of aspirin and atorvastatin in bulk. The approach is shown to be better than the majority of the documented approaches. Preformulation investigations were conducted to assess the chemical and physical properties of the drug substance prior to validation and method development. The Indian Pharmacopoeia monograph was found to be followed by atorvastatin and aspirin. The capillary method, which complies with the melting point provided in reference, was used to determine the melting points of both medications. The melting point of pure drugs aspirin and atorvastatin were found to be 170-178°C and 137-139°C, respectively. The methanol standard curves for atorvastatin and aspirin were created, and linear regression was used to the resulting absorbance data. A broad band was visible at 245 nm and 361 nm in the ultraviolet spectrum of aspirin and atorvastatin in methanol and water. The overlapped spectraof aspirin and atorvastatin displayed the isobestic point at 255 nm.

The results indicated that the LOD and LOQ for atorvastatin were 0.155µg/mL and 0.469µg/mL and for aspirin were 0.054µg/mL and 0.164µg/mL, respectively. Studies on robustness showed that the developed approaches remained stable even after intentional modifications were made. The wavelength used for the HPLC method of validation was chosen to correspond to the isobestic point at which UV detectors can detect the two medicines. A wavelength of 255 nm was chosen. After numerous attempts, the ideal liquid chromatographic conditions were reached for the separation of aspirin and atorvastatin by choosing the right mobile phase and column. Using an orthophosphoric acid-adjusted methanol (20:80) mobile phase that contained 0.02 M potassium dihydrogen phosphate, a phenomenex C-18, 5 µm column with 250 x 4.6 mm i.d. in isocratic mode was employed. The effluents were measured at 255 nm, and the flow rate was 1.0 mL/min. For parameters that were validated, the technique was shown to be linear, accurate and robust. For aspirin and atorvastatin, the linearity range was established using an external standard calibration method in the concentration range of 20µg/mL to 120µg/mL. The recovery percentage was estimated to be between 98% and 102%, and it was found that every value fell between the ranges. The repeatable analysis of the sample further supported the method's accuracy. As the %RSD values were minimal, the results were judged to be precise. It suggested that the

precision of the procedure is good. The robustness research revealed that the relative standard deviation (RSD) for varying the flow rate, wavelength, and analyst was found to be less than 2, falling within the acceptable range. Thus, RP-HPLC techniques that are straightforward, sensitive, accurate, and precise were created and verified for the simultaneous quantification of aspirin and atorvastatin. The amount of acid hydrolysis degradation for aspirin and atorvastatin after one hour at 60°C was 2.19% and 2.15%, respectively. The amount of base hydrolysis degradation for aspirin and atorvastatin after one hour at 60°C was 1.98% and 2.69%, respectively. The oxide degradation levels of atorvastatin and aspirinafter three hours at room temperature were 7.56% and 3.86%, respectively. The thermal degradation levels of atorvastatin and aspirin afterfive hours were 0.99% and 0.90%, respectively.

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