https://doi.org/10.48048/tis.2022.5799

Response Surface Method for the Simultaneous Estimation of Atorvastatin and Olmesartan

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Received: 24 April 2021, Revised: 1 July 2021, Accepted: 8 July 2021

Abstract

The simultaneous estimation of Atorvastatin Calcium (ATS) and Olmesartan Medoxomil (OLM) in bulk and pharmaceutical dosage forms, a new, quick, and cost-effective Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed. The experimental design was used to achieve multivariate optimization of the RP-HPLC experimental conditions. Three independent variables were used to create mathematical models: Acetonitrile content in the mobile phase composition, buffer pH, and flow rate. Here, the applied model was central composite design (CCD) to research the response surface methodology and study the effects of independent factors. The Shimadzu (LC 20 AT VP) HPLC system with Spinchrom software has been used. Zodiac, C18 (250×4.6 ID) 5µm column, phosphate buffers, and acetonitrile were used as mobile phase in the ratio 40:60 v/v with a flow rate 1.15 mL/min. The eluent was monitored at 212 nm using the Prominence UV-Visible detector. The retention time for OLM and ATS was 2.673 and 3.717, respectively. The optimized procedure was validated as per ICH guidelines. The correlation coefficient of OLM and ATS was 0.9869 and 0.9832. The % of recovery was 98.59, 99.68 %. OLM had a LOD of 17.568 µg/mL, while ATS had a LOD of 12.88 µg/mL. OLM had a LOQ of 53.24 µg/mL, while ATS had a LOQ of 39.04 µg/mL. The pH aqueous phase, solvent composition, and flow rate were the most stringent variables affecting the responses, according to the 3D response surface graphs. A new accurate and precise RP-HPLC approach has been developed and validated and used to regularly analyse OLM and ATS.

Keywords: RP-HPLC, Retention time, Validation, Atorvastatin, Olmesartan

Introduction

Olmesartan is an antihypertensive agent that belongs to angiotensin-II receptor blockers. It is recommended for the treatment of high blood pressure and sold as Olmetec® [1,2]. Chemically 5-(2-hydroxypropan-2-yl)-2-propyl-3-({4-(2-(2H-tetrazol 5yl) phenyl) phenyl} methyl) imidazole-4-carboxylic acid and its molecular weight is 446.501 gm/mol [3,4]. Atorvastatin (Lipitor) is a drug class known as statins. It is used to reduce cholesterol. Chemically 7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3, 5-dihydroxyheptanoate with a molecular weight of 557.6319 g/mol [5] **Figure 1**.

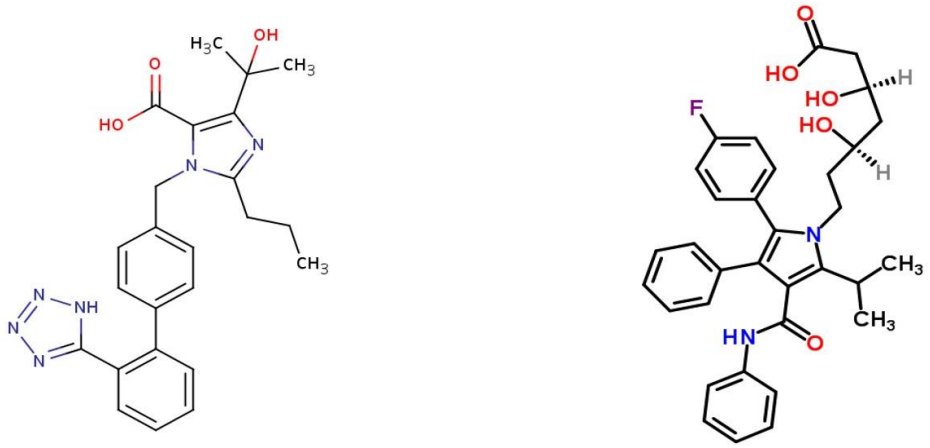

Figure 1 Structures of Olmesartan and Atorvastatin.

The main goal of this study was to develop an improved RP-HPLC system for quality control of OLM and ATS in the pharmaceutical industry, and provide information on the sensitivity of chromatographic factors and their interaction effects on separation characteristics [4,6]. The optimization of chromatographic variables is very complex, such as mobile phase solvent concentration, buffer pH, and flow rate, which significantly impact on chromatographic separation [7]. These independent variables can be conveniently optimized using the Quality by Design (QbD) experiment design method. Quality by Design is a systematic method that uses Design of Experiment (DoE) to achieve the optimal conditions with good quality assurance and involves multi-dimensional combinations and input variables [8]. After approval (ICH Q8 (R2), an experimental design was developed by a Design expert showing a flexible region in which no changes in parameters (e.g., pH, % of organic modifier, etc.) are needed [9]. When more than 1 response (retention time and Asymmetric factor of both drug peaks) must be optimized simultaneously, Derringer's desirability feature is the best choice. For the simultaneous estimation of OLM and ATS from a tablet formulation, we used the same technique for developing and optimizing a new RP-HPLC method [10,11].

Materials and methods

Chandra labs (Pvt), Hyd., provides bulk drugs such as Atorvastatin calcium (ATS) and Olmesartan medoxomil (OLM). The local pharmacy provided ATS (100 mg) and OLM (50 mg) tablets. Potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate, trifluoro acetic acid, and ammonium acetate were used as analytical reagents, and HPLC grade methanol, acetonitrile, and water were used. High-quality HPLC water was provided by the Millipore purification unit.

Instruments used were UV-Visible Spectrophotometer (Nicolet evolution 100), HPLC Shimadzu (LC 20 AT VP) with Zodiac C18 column (250×4.6 mm ID), 5µm Particle size, and UV-Visible Detector and Spinchrom software [12]. The experimental design (Faced central composite) as well as desirability function and data analysis estimates, were created using Design-Expert version 12.1.0.1.

Mobile phases

For the study, phosphate buffer (pH 5.6) and acetonitrile were used in the ratio of 40: 60 v/v.

Buffer preparation

2.95 g of potassium di-hydrogen phosphate (KH_2PO_4) and 0.54 g of potassium di-hydrogen phosphate (K_2HPO_4) were dissolved in 100 mL of water. Using ortho-phosphoric acid, the pH adjusted to 5.6. Both fine particles and gases were separated using the 0.45µ filter [13].

Preparing a mixed standard

Standard stock solutions (µg/mL) of OLM and ATS were prepared by dissolving 100 mg of OLM and 50 mg of ATS in the specified mobile phases. With the mobile phase, the solution was diluted to 100 mL. By adding 1 mL of stock solution to 10 mL of the mobile phase, further dilutions of 100 µg/mL of

OLM and 50 µg/mL of ATS were arranged in 5 replicates of 100 µg/mL of OLM and 50 µg/mL of ATS [13,14].

Preparation of samples for the assay

Standard stocks

Prepare standard stock solutions of OLM and ATS (µg/mL) by dissolving 100 mg of OLM and 50 mg of ATS in the mobile phase. The solution was filtered using a 0.45-µ syringe filter and sonicated for 5 min and diluted to 100 mL with the mobile phase. Additional dilutions are prepared in 5 replicates of 100 µg/mL of OLM and 50 µg/mL of ATS by adding 1 mL of stock solution to 10 mL of the mobile phase [15].

Sample of a tablet

Twenty tablets (100 mg of OLM and 50 mg of ATS found in each tablet) were weighed and crushed into a fine powder and mixed evenly. OLM and ATS (µg/mL) tablet stock solutions were prepared by dissolving a mass equal to 100 mg of OLM and 50 mg of ATS in the mobile phase [16].

Results and discussion

Optimized chromatographic conditions

This method was established using a stationary phase of a zodiac C18 column, 250×4.6 mm ID, 5 m particle size, performed at room temperature, and a mobile phase of phosphate buffer: acetonitrile (40:60 v/v) flow rate at 1.15 mL/min. The injection volume of the sample was 20 µL. At 212 nm, a UV-Visible detector was used. As shown in **Figure 2** and **Table 1**, the retention time for OLM and ATS was 2.673 and 3.717, respectively [17,18].

Table 1 The comparison of experimental and predictive values of different objective functions under optimal conditions.

S.No.	Name	Rt(min)	Peak area	Asymmetry factor	Efficiency	Resolution
1	Olmesartan medoxomil	2.673	813.053	1.259	3708	-
2	Atorvastatin calcium	3.717	284.572	1.143	4770	5.339

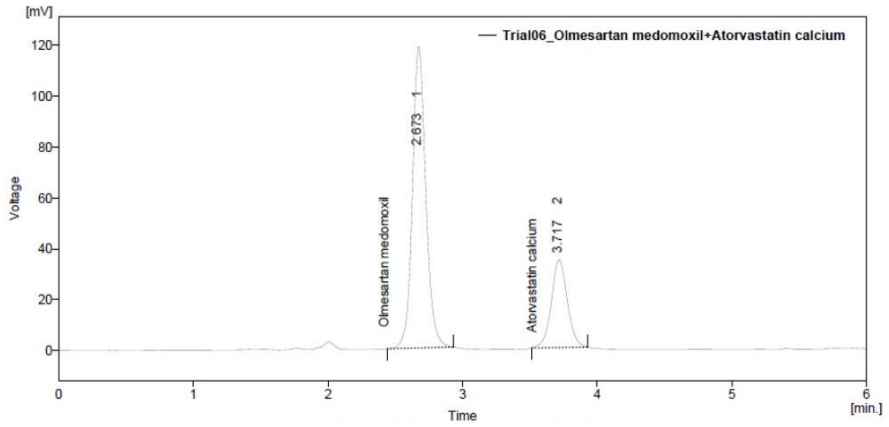

Figure 2 Optimized chromatographic of OLM and ATS.

Experimental design and response surface methodology

The experimental design approach will help to develop a deeper understanding of the interaction of many chromatographic variables on separation efficiency and can be used to optimize separations [19]. The significant chromatographic factors were selected in this study and optimized by a central composite design (CCD) based on preliminary experiments and prior literature knowledge. A CCD design was used

A CCD design was used to monitor the chromatographic response surface and find the best flow rate, mobile phase pH, and % of organic modifier for separation. **Table 2** presents 3 chromatographic variables and levels for which the experimental condition was optimized.

Table 2 Experimental factors and levels used in a central composite design.

Factors	Name	Level (−1)	Level (0)	Level (+1)
A	Flow rate	0.8000	1.15	1.50
B	Solvent %	40.00	60.00	80.00
C	pH of Aqueous Phase	4.50	5.65	6.80

The CCD for 3 independent variables was created using a partial factorial design, 3 replicates of center points, and 5 axial points at an extreme stage. The qualities of the fitted polynomial models were evaluated using the coefficient of determination R^2. The proper optimum condition orientation was identified using Derringer's optimization technique. Several reactions were simultaneously improved [20,21]. The final step is to predict the response and design space using the polynomial equation. Response Surface Methodology (RSM) is a quantitative and statistical approach for problem analysis in which several independent variables, such as solvent, pH, flow rate, and so on, influence dependent variables or responses (e.g., resolution, tailing of a peak, run time). To achieve the best device efficiency, this method was used to optimize the levels of these variables simultaneously, as shown in **Table 3**. RSM allows quadratic models to be described that to explain the response to all chromatographic conditions in the experimental area. Each design variable must be analyzed at a minimum of 3 different levels for the measurement of quadratic regression model coefficients, and accordingly, CCD was used in this optimization study.

Table 3 Central composite design data matrix and responses.

Standard	Run	Flow rate (mL/min)	Solvent (%)	pH of aqueous Phase	Retention time (Rt)	Efficiency	Asymmetric factor
17	1	1.15	60	5.65	4.12	3200	0.52
2	2	1.5	40	5.65	4.13	3850	0.13
8	3	1.5	60	6.8	5.01	4132	0.48
5	4	0.8	60	4.5	4.68	3569	0.37
13	5	1.15	60	5.65	4.37	5132	0.72
3	6	0.8	80	5.65	5.19	4587	0.56
16	7	1.15	60	5.65	2.67	5500	1.2
4	8	1.5	80	5.65	3.47	5300	1.1
14	9	1.15	60	5.65	3.89	5000	1.4
10	10	1.15	80	4.5	3.98	3321	0.81
9	11	1.15	40	4.5	4.05	3468	0.49
6	12	1.5	60	4.5	5.32	4832	0.65
1	13	0.8	40	5.65	4.73	4765	0.73
12	14	1.15	80	6.8	4.89	5024	0.54
7	15	0.8	60	6.8	5.67	5129	0.59
11	16	1.15	40	6.8	5.88	4952	0.94
15	17	1.15	60	5.65	5.75	4987	0.87

OLM and ATS are analyses with a medium polarity. As a result, reverse phase mode is preferable to normal phase mode. For the separation of both analytes, we first tried various reverse phase columns such as C18, cyano, and C8. However, the cyano column separated both analytes poorly, while in C8, OLM eluted early and had a broader peak form. As a result, we concentrated our optimization efforts solely on the C18 column. The combined UV spectra of both drugs show that 212 nm is the best wavelength for detecting OLM and ATS with good response and low baseline noise. Due to variations in the pKa of molecules, the mobile phase pH is a significant factor that drives the selectivity of the process. Based on the literature report, the initial method development was tried on 3 different pH 3.5, 4.5 and 5.5. At pH 4.5 and 5.0, however, high tailing (> 2) was identified with OLM. Acetonitrile, methanol, and, in some cases, tetrahydrofuran are the most common reversed-phase organic modifiers. Because of the high UV cutoff and the existence of peroxide impurities in tetrahydrofuran, which affect the stability of analytes, this organic modifier was not chosen. The peak shape of OLM was improved by using acetonitrile [22].

Design of experiment and design space

Table 3 shows the design for the faced central composite design, which was optimized using all 17 experiments. This design was made up of a 2-level factorial with additional center points in the experimental region's center. The levels of each factor in this study were chosen based on previous scouting experiments. If this method had optimized using the traditional univariate approach, several more experiments would have been needed. At first, it was discovered that at flow rates below 0.8 mL/min, peaks were long, and at flow rates above 1.5 mL/min, the proper separation was not observed.

Similarly, an ideal acetonitrile concentration of 60 % v/v was identified, and the pH of the buffer solution was adjusted between 4.50 and 6.80. The flow rate (0.8 - 1.5 mL/min), buffer pH (4.5 - 6.8), and acetonitrile concentration (40 - 80 % v/v) were all within the ranges. Our main goal was to develop a method with a short run time and symmetric peak shape that allows for accurate drug quantification in a short period of time. As a result, the response was described as the retention time of the last eluting peak (ATS Rt) and the tailing of the OLM (T). **Table 4** lists the statistical parameters obtained from ANOVA for the regression models.

Table 4 Regression model and statistical parameters obtained from ANOVA.

Response	Regression model	Adjusted R^2	Model *p*-value	% CV	Adequate precision
Retention Time	$+4.58 - 0.2925A\ 0.1575B + 0.4275C$	0.89	< 0.001	4.55	17.89
Efficiency	$+4514.59 + 8.00A + 149.63B + 505.87C + 407.00AB - 565.00AC + 54.75BC$	0.82	< 0.001	5.074	20.6
Asymmetric Factor	$0.942 + 0.01375A + 0.09B + 0.02875C + 0.285AB - 0.0975AC - 0.18BC - 0.24225A^2 - 0.06975B^2 - 0.17725C^2$	0.873	< 0.001	0.59	21.06

These models have a probability of $p < 0.05$, indicating that they are meaningful. The modified R^2 was well within reasonable limits ($R^2 > 0.8$), suggesting that the experimental model matches polynomial equations well. The required precision value is a ratio of signal (response) to noise (deviation) that should be greater than 4. The ratio in this study was greater than 25, indicating a sufficient signal, and thus the model is essential for the separation process. The model's reproducibility was determined by a coefficient of variation (C.V.) that is far below the limit of both responses (% C.V. 10). The interaction term with the most significant absolute coefficients among the fitted models is 0.61AC of the Rt model, as shown in **Table 4**. Rt is statistically significant ($p = 0.001$) due to the favorable relationship between A and C. Changing the acetonitrile fraction from low to high causes a rapid shift in Rt at both low and high buffer pH levels, according to the research. Furthermore, raising the buffer pH reduces ATS retention time by a small amount (Rt) at low levels of factor A. When the acetonitrile concentration was set to its lowest level, the buffer pH must be set to its highest level to shorten the study time. This relationship is incredibly synergistic since it resulted in a reduction in study time. The perturbation plots are shown in **Figure 4** to clarify the findings better. This graph illustrates how the response differs as each factor moves away from a chosen reference point, with all other factors kept constant at the reference value in design optimization. Every factor's actual and predicted values were straight lines or curvature, indicating that the response is sensitive to that factor.

Figure 3 displays 2-dimensional color maps with the "green" shade displaying high retention time and efficiency and the "yellow" shade displaying low retention time and efficiency. The working point was chosen from the developed design space through visual inspection, searching for the ATS and OLM retention times that were the shortest. It shows that the retention time of ATS increased as the pH increased to 5.65, the flow rate increased to 1.15 mL/min, and the % of acetonitrile increased to 50 %. Simultaneously, the OLM peak was lowered by decreased acetonitrile content and an acidic pH. At pH 3.2, 60 % v/v acetonitrile, and a flow rate of 1.15 mL/min, we completed faster separation (6.0 min) with good resolution, which is the target of our method.

Figure 4 shows that as the concentration of acetonitrile in the mobile phase decreases, the efficiency of the OLM peak decreases due to a reduction in the interaction with the column's free silanol groups. After processing all of the data with the modeling software (Design Expert@) design structure was developed.

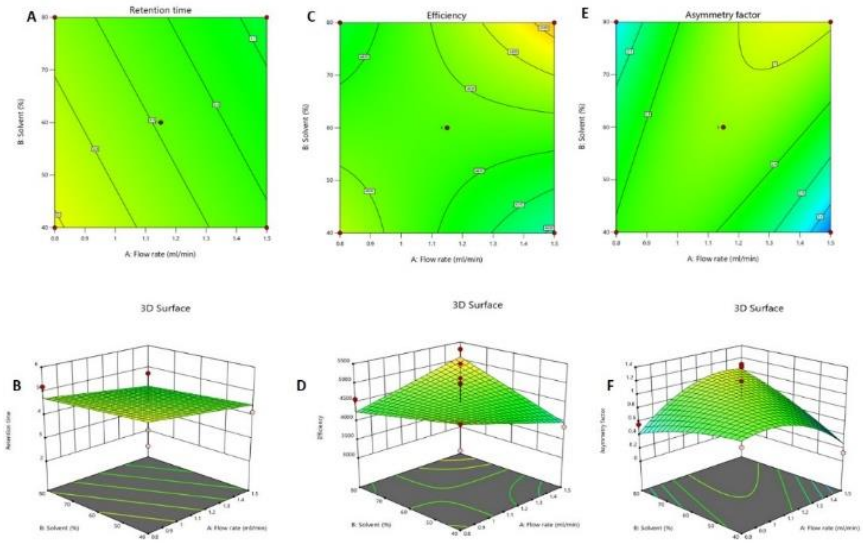

Figure 3 Contour and response surface plots showing the interactive effects, point desirability as suggested by Design-Expert software. Effect of the interaction of flow rate, solvent percentage and pH of aqueous phase on A and B Retention time, C and D Efficiency, E and F Asymmetry factor.

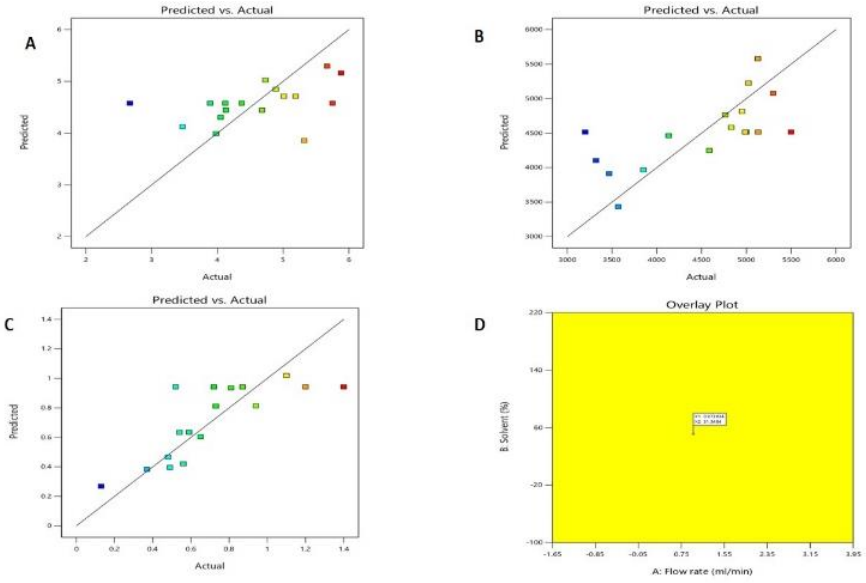

Figure 4 Actual and predicted values of dependant variables, overlay plot of design and point desirability as suggested by Dising Expet software.

Validation

The optimized approach has been tested in compliance with the ICH guidelines [23].

Linearity

By dissolving 100 mg of OLM and 50 mg of ATS in the appropriate mobile phase, standard stock solutions of Olmesartan medoxomil and Atorvastatin calcium have been prepared. The solution was then filtered through a 0.45-micron syringe filter, sonicated for 5 min, and diluted to 100 mL with a mobile phase before being prepared for further dilutions. The relationship between OLM and ATS concentrations (in %) should be linear within the specified range and R^2 value should not be less than 0.9 [24].

Linearity was accomplished using concentrations of 60, 80, 100, 120, 140 µg/mL OLM and 30, 40, 50, 60, 70 µg/mL ATS, as recommended by the ICH are shown in **Table 5**. The correlation coefficients for regular OLM and ATS preparation are 0.9869 and 0.9832, respectively. Since all points are in a straight line and the coefficient of correlation was within limits, the relationship between OLM and ATS concentration and location is linear within the range studied [25].

Table 5 Results for linearity of standards.

	Olmesartan medoxomil		**Atorvastatin calcium**	
S.No.	**Concentration (µg/mL)**	**Peak area**	**Concentration (µg/mL)**	**Peak area**
1	60	520.702	30	189.350
2	80	659.263	40	232.733
3	100	771.231	50	269.831
4	120	879.442	60	306.058
5	140	1,005.606	70	355.197
S.D.	31.6	188	15.811	64
Slope	5.94		4.05	

Accuracy

The accuracy of the procedure was determined by using a recovery analysis. The analysis was carried out by adding the standard drug to the pre-analyzed sample solution 80, 100 and 120 %, adding 5 % of the standard drug solution at each stage. The recovery tests were done 3 times. The method's accuracy was determined by calculating drug recoveries using the standard addition method to determine if the excipients in the formulation developed any positive or negative interference as shown in **Tables 6** and **7** [26].

Table 6 Accuracy of olmesartan medoxomil.

Recovery level	**Amount taken (µg/mL)**	**Area**	**Average area**	**Amount recovered**	**% Recovery**	**Average % recovery**
80 %	100	801.032	812.846	98.64	98.64	98.59 %
	100	816.586				
	100	820.921				
100 %	120	911.538	913.262	118.42	98.68	
	120	911.492				
	120	916.756				
120 %	140	1,000.092	1,010.131	137.83	98.45	
	140	1,014.339				
	140	1,015.961				

Table 7 Accuracy of atorvastatin calcium.

Recovery level	Amount taken (µg/mL)	Area	Average area	Amount recovered	% Recovery	Average % recovery
80 %	50	284.882	286.723	49.28	98.56	99.68 %
	50	287.502				
	50	287.785				
100 %	60	331.682	325.872	60.38	100.64	
	60	311.036				
	60	334.897				
120 %	70	356.491	356.577	69.90	99.86	
	70	357.470				
	70	355.771				

Precision

Prepared samples of OLM and ATS are injected 6 times in the column according to the test procedure. % Relative standard deviation of ATS and OLM assay preparations should be no more than 2.0 % [27]. Precision for ATS and OLM results were shown in **Table 8**.

Table 8 Results of method precision of ATS and OLM.

Atorvastatin calcium			Olmesartan medoxomil		
S.No.	**Rt**	**Area**	**S.No.**	**Rt**	**Area**
1	3.717	286.026	1	2.673	810.419
2	3.717	286.026	2	2.673	810.419
3	3.733	282.016	3	2.687	811.688
4	3.727	288.483	4	2.683	812.647
5	3.740	285.746	5	2.693	831.524
6	3.757	286.026	6	2.707	828.437
Average	3.732	285.721	**Average**	2.6860	817.522
SD	0.015	2.080	**SD**	0.0129	9.735
% RSD	**0.41**	**0.73**	**% RSD**	**0.48**	**1.19**

Limit of detection

$$\text{LOD} = \frac{3.3\,\sigma}{S}$$

where, σ = the standard deviation of the response, S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte [28]. The LODs for OLM and ATS were 17.568 µg/mL and 12.88 µg/mL, respectively.

Limit of quantification

$$\text{LOQ} = \frac{10\,\sigma}{S}$$

where, σ = the standard deviation of the response, S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte. The LOQs for OLM and ATS were revealed to be 53.24 and 39.04 µg/mL, respectively.

Robustness

The solution was prepared according to the test methods injected under various variable conditions, such as flow rate and temperature. The system performance parameters have been contrasted with the precision method parameters [15,19]. The obtained results are mentioned in **Table 9.**

Table 9 Robustness data of atorvastatin calcium and olmesartan medoxomil.

Parameter	Olmesartan		Atorvastatin	
	Rt. (min)	T. factor	Rt. (min)	T. factor
Flow Rate				
0.8 mL/min	3.363	1.333	4.660	1.171
1.0 mL/min	2.673	1.359	3.717	1.143
1.2 mL/min	2.597	1.391	3.490	1.167
Wave length				
210 nm	2.710	1.241	3.770	1.111
212 nm	2.673	1.359	3.717	1.143
214 nm	2.727	1.286	3.787	1.111

Ruggedness

The system's ruggedness was analyzed by measuring the analyst's variance by carrying out the assay by 2 independent analysts as shown in **Table 10** [18].

Table 10 Ruggedness data of atorvastatin calcium and olmesartan medoxomil.

Ruggedness	Olmesartan medoxomil	Atorvastatin Calcium
% RSD	0.73	0.62
Assay Analyst-1	99.39	97.01
Assay Analyst-2	99.70	100.24

Assay

The amount of OLM and ATS present in the formulation was determined by using the formula mentioned below and the results shown in **Table 11**.

$$\%\ \text{Assay} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{\text{LC}} \times 100$$

where, AS: Standard peak area, AT: Sample peak area, WS: Weight of standard, WT: Weight of sample, DT: Dilution factor, AW: Average weight, P: Purity, LC: Label claim.

The quantity of Olmesartan medoxomil and Atorvastatin calcium in the taken dosage form was 99.31 and 99.40 %, respectively, in the given dosage form.

Table 11 Assay results of atorvastatin calcium and olmesartan medoxomil.

	Olmesartan medoxomil		**Atorvastatin calcium**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	825.949	824.612	284.554	287.747
Injection-2	824.058	831.231	288.051	289.831
Injection-3	829.293	827.465	288.444	283.577
Injection-4	823.414	825.068	287.123	287.130
Injection-5	830.957	829.984	285.368	286.687
Average Area	826.734	827.672	286.708	286.9944
Tablet average weight	200.5		200.5	
Standard weight	100		50	
Sample weight	200.5		200.5	
Label amount	100		50	
Standard purity	99.2		99.3	
Amount found (mg)	**99.31**		**49.70**	
Assay (% purity)	**99.31**		**99.40**	

A simple and selective LC method was specified for estimating ATS and OLM in tablet dosage forms. The chromatography was performed with a mobile phase consisting of 40 volumes of KH_2PO_4 buffer, pH 5.8, 60 volumes of acetonitrile, and a detection wavelength of 212 nm. The system has been shown to meet ICH requirements. OLM linearity was found to be 60 - 80 µg/mL, and ATS linearity was found to be 30 – 70 µg/mL. Both statistical and experimental data were suggested that the methods can be used for the dosage forms [29,30].

Conclusions

An RP-HPLC method was developed to assess the dosage formulations of ATS and OLM bulk and combination tablets. For the simultaneous determination of OLM and ATS in the commercial formulation, statistically dependent experimental designs proved to be a practical approach in optimizing selectivity-controlling parameters. The essential factors were designed using central composite design and response surface methodology. The goal of this study was performed by optimizing Rt, efficiency, and asymmetric factor using Derringer's desirability function. This method is linear, reliable, and precise. It has been demonstrated to be simple and effective for OLM and ATS quality control in bulk and tablet formulation.

Furthermore, the previously described method only separates both commonly approached drugs with a longer run time of 10 min using 150 mm columns (5 µm particle sizes). In comparison, our proposed method can quantify OLM and ATS in a run time of 6 min using a novel, univariate statistical techniques. The experimental design and response surface method provides a better understanding of the sensitivity of chromatographic variables and their interaction effects on separation attributes. It also enables the chromatographer to change the objective responses depending on the matrices in which the study must be conducted. This analytical method will be used regularly basis in the future for both bulk and tablet formulations

Acknowledgements

The authors would like to thank Hetero drugs Pvt. Ltd, Hyderabad for their enormous support and providing the API as gift sample. The authors would like to thank Anurag University chairman and management, Gitam University chairman and management for encouraging research and development. Author Vinod Kumar Yata would like to thank Department of Biotechnology, Government of India for providing financial support from "DBT-RA Program in Biotechnology & Life Sciences".

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