STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF OLMESARTAN MEDOXOMIL AND AZELNIDIPINE IN COMBINED TABLET DOSAGE FORM

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Abstract:

A stability indicating RP-HPLC method for the simultaneous determination of olmesartan medoxomil (OLM) and azelnidipine from combined tablet dosage form was developed. The separation was accomplished on Inertsil 3V (4.6 mm X 100 mm; particle size 3 µm) column using a mobile phase consisting of potassium dihydrogen phosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) and acetonitrile in gradient elution mode. The analytes were monitored by a photo diode array (PDA) detector set at 255 nm and the flow rate was kept at 2.0 mL min^{-1}. The retention time for olmesartan medoxomil and azelnidipine were 3.148 and 3.704 respectively. Linearity was observed in the concentration range of 10-60 µg/mL for olmesartan medoxomil and 4-24 µg/mL azelnidipine. Both the drugs were subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat and photolytic degradation. The degradants were well resolved from the pure drugs. The method could be used for simultaneous determination of olmesartan medoxomil and azelnidipine in bulk and combined dosage form.

Key words – olmesartan medoxomil, azelnidipine, RP-HPLC, stability-indicating, validation.

1.0 INTRODUCTION

Olmesartan medoxomil (OLM), 2,3-dihydroxy-2-butenyl 4-(1 hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5 carboxylate, cyclic 2,3-carbonate, a prodrug, is hydrolyzed to olmesartan during absorption from the gastrointestinal tract. It is practically insoluble in water and sparingly soluble in methanol. It is available as film coated tablets containing 5 mg, 20 mg, or 40 mg of olmesartan medoxomil in US and Europe. It is indicated for the treatment of hypertension. Olmesartan medoxomil is a white to light yellowish-white powder or crystalline powder with a molecular weight of 558.59.

Azelnidipine (AZL), 3-[1-(Diphenylmethyl)-3-azetidinyl] 5-isopropyl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate, is a dihydropyridine calcium channel antagonist with selectivity for L-type calcium channels. Azelnidipine is offered under the registered trademark CALBLOCK® by Sankyo Co. Ltd. of Japan. CALBLOCK® is offered as an oral tablet administered once daily for the treatment of hypertension and related diseases. Azelnidipine is only slightly soluble in methanol and water and soluble in ethanol, dimethyl sulfoxide, acetic acid and dimethyl fluoride. Olmesartan medoxomil and azelnidipine combination is offered under the registered trademark REZALTAS® by Sankyo Co. Ltd. of Japan. REZALTAS ® is offered as oral tablets containing 10mg/8mg and 20mg/16mg of olmesartan medoxomil and azelnidipine and administered once daily for the treatment of hypertension.

A thorough literature survey has revealed that either olmesartan medoxomil or azelnidipine or combination is not yet official in any pharmacopeia. Few spectrophotmetric[1], potentiometric[2], LC[3-9] methods were reported in the literature for the determination of azelnidipine. Reported analytical methods for the determination of olmesartan medoxomil include spectroscopic[10], HPLC[11-17], UPLC[18], LC-MS[19]. One spectroscopic method was reported for the simultaneous determination of OLM and AZL in combined pharmaceutical dosage forms. To the best of our knowledge, a complete validated stability indicating RP-HPLC method for the simultaneous estimation of OLM and AZL in combined pharmaceutical dosage form was not reported. Therefore, it was

thought worthwhile to develop a simple, precise, accurate reverse phase high performance liquid chromatographic method for the simultaneous determination of OLM and AZL in combined tablet dosage form.

2.0 EXPERIMENTAL

2.1 Chemicals & Reagents

All the reagents were of analytical-reagent grade unless stated otherwise. Milli-Q-water was used throughout the experiment. HPLC-grade acetonitrile, potassium dihydrogen phosphate, orthophosphoric acid were procured from Merck Ltd, Mumbai. All the solvents and solutions were filtered through a membrane filter and degassed before use. Reference standards of OLM, AZL and combined tablets were received from the research development department of Cadila Health care Ltd, Ahmedabad, India.

2.2 Instrumentation

The HPLC system used was of model Waters 2695 equipped with quaternary pump, auto sampler, thermostated column compartment and variable wavelength detector controlled by the empower software. The column used was Inertsil ODS 3 (100 mm X 4.6 mm, 3µm). Column temperature was maintained at 25^0C.

2.3 Chromatographic conditions

Stationary phase	Inertsil ODS 3 (250 mm X 4.6 mm, 3µm) column		
Detection wavelength	255 nm		
Injection volume	25µL		
Flow rate	2.0 mL/min		
Column temperature	25°C		
Sample temperature	5°C		
Buffer	2.0 gm of potassium dihydrogen phosphate is dissolved in water and pH adjusted to 3.0 with orthophosphoric acid		
Diluent	Acetonitrile: water (50:50)		
Mobile phase	Solvent A: Buffer pH 3.0: Acetonitrile(80:20)		
	Solvent B: Buffer pH 3.0: Acetonitrile(20:80)		
Gradient program	**Time (Min.)**	**Solvent A**	**Solvent B**
	0.0	90	10
	4.0	2	98
	4.5	90	10
	6.0	90	10

2.4 Preparation of standard stock solutions

The stock solutions OLM and AZL were prepared separately by dissolving accurately weighed quantity of 60 mg of OLM and 15mg of AZL in 100 mL of diluent. Working solution containing 40 µg/mL of OLM and 16 µg/mL of AZL was prepared from above stock solution.

2.5 Preparation of sample solution

Twenty tablets, each containing 40mg of OLM and 16mg of AZL were accurately weighed and grounded to fine powder. An amount equivalent to 400 mg of OLM was transferred into a 100 mL volumetric flask and about 50 mL of diluent was added, sonicated for not less than 30 min with occasional shaking and made up the volume with diluent. The above solution was filtered through 0.45 µm millipore PVDF filter. The above solution was further diluted to 100 mL with diluent to obtain a concentration of 40 µg/mL of OLM and 16 µg/mL of AZL.

2.6 Stress degradation studies

In order to establish the analytical assay method as stability indicating, the following stress conditions were studied on the combination tablet dosage form containing 40 mg of OLM and 16 mg of AZL as per ICH stability guidelines.

a) Acid hydrolysis: Drug solution in 1N HCl at 70°C for 4 hours.

b) Alkaline hydrolysis: Drug solution in 1N NaOH at 70°C for 4 hours.

c) As such: Sample solution as such for 6 hours

d) Oxidative degradation: Drug solution in 3% hydrogen peroxide at room temperature for 4 hours.

e) Thermal degradation: Tablets were subjected to dry heat at 105°C for 10 hours.

f) Photolytic degradation: The photo degradation was carried out by exposing the tablets samples in solid state to light providing an overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 W h/m^2 for 24 hours.

3.0 Results and Discussion

3.1 Method development and optimization

Stressed samples prepared by a systematic forced degradation study were used for method development trials to optimize the method as a stability-indicating method for simultaneous determination of OLM and AZL. In our preliminary experiments, OLM and AZL were subjected to separation by RP-HPLC, on different commercial columns. Water with acetonitrile and methanol as organic modifiers was used as mobile phase. Broad peaks and tailing were observed for OLM and few degradants. So to improve the peak shapes, phosphate buffer was used in place of water. As the compounds were having varying polarities, a gradient method was tried. The HPLC conditions were optimized by studying the effects of different columns, organic modifiers, and pH of buffer on the separation of OLM, AZL and their degradation products. The separation of OLM, AZL and their degradants was achieved on Inertsil ODS 3 (250 mm X 4.6 mm, 3µm) column and potassium dihydrogen phosphate and acetonitrile combination as mobile phase at a detection wavelength of 255 nm. Fig 1 and Fig 2 shows the separation of OLM and AZL in standard and sample solutions respectively.

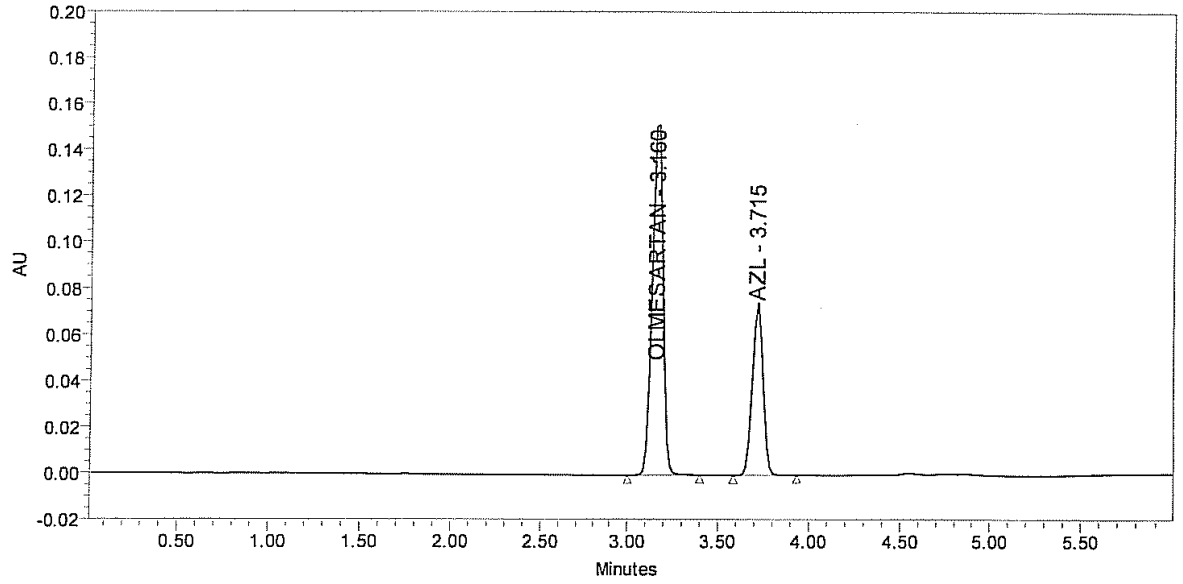


Fig 1: Chromatogram of Standard solution of OLM and AZL

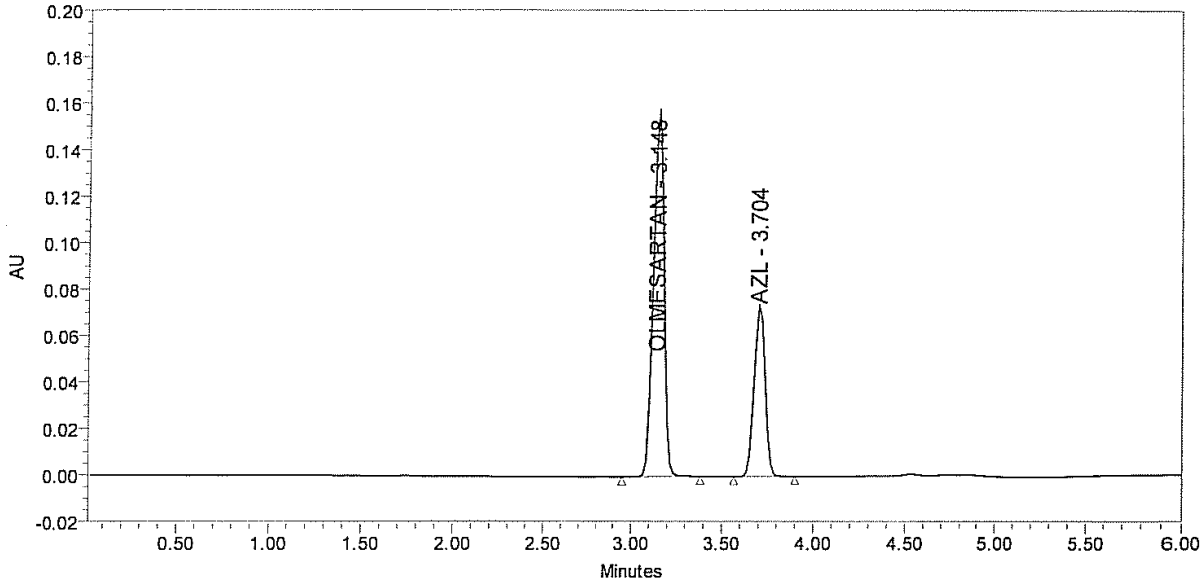


Fig 2: Chromatogram of sample solution of OLM and AZL

3.2 Method Validation

The developed method was validated as per ICH guidelines.

3.2.1 System suitability and precision

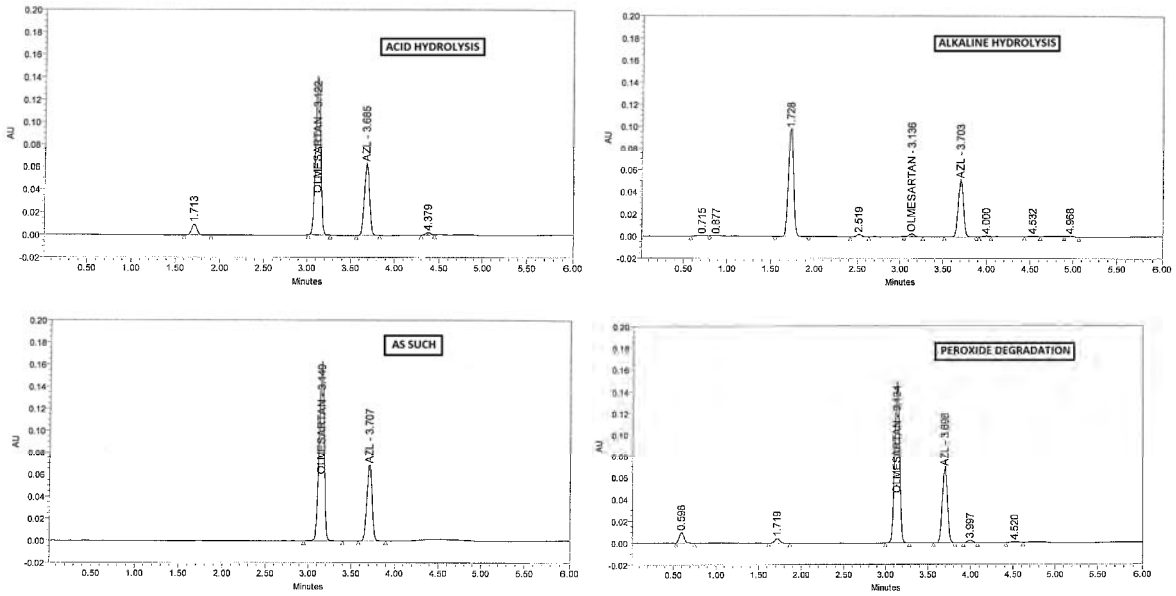
System suitability and precision was demonstrated by making five replicate injections of standard solution. The peak area of OLM and AZL for replicate injection was recorded. The tailing factor and number of theoretical plates was evaluated for the analyte peak. The precision was evaluated by computing the relative standard deviation for the analyte peak area of these replicate injections. The results were within the acceptable limits and were shown in Table 1.

Table 1: System suitability Data

Injection No	Peak area	
	Olmesartan medoxomil	Azelnidipine
1	639206	307026
2	639409	307153
3	639480	306748
4	641346	307172
5	639326	307135
Average	639753.4	307046.8
%RSD	0.140071	0.057466
USP tailing factor	1.01	1.02
No.of Theoretical plates	12842	17394

3.2.2 Specificity

The specificity was evaluated by studying the interference from placebo and degradants with the OLM and AZL peak. Interference from placebo was demonstrated by comparing the chromatograms of placebo, standard solution and sample solution and no interference was observed from placebo. Interference from degradants was demonstrated by performing stress study on formulation under the different stress conditions. The stressed samples of above conditions were prepared as per the test method and chromatographed into HPLC system equipped with a diode array detector. The peak purity of OLM and AZL peak and the assay of formulation were evaluated under each stressed condition. In all the stress conditions, purity angle is less than purity threshold and no purity flag was observed. The study indicates that there is no interference of any degradants with the OLM and AZL peak, which proves that the method is specific and stability-indicating for estimation of OLM and AZL in the formulation.



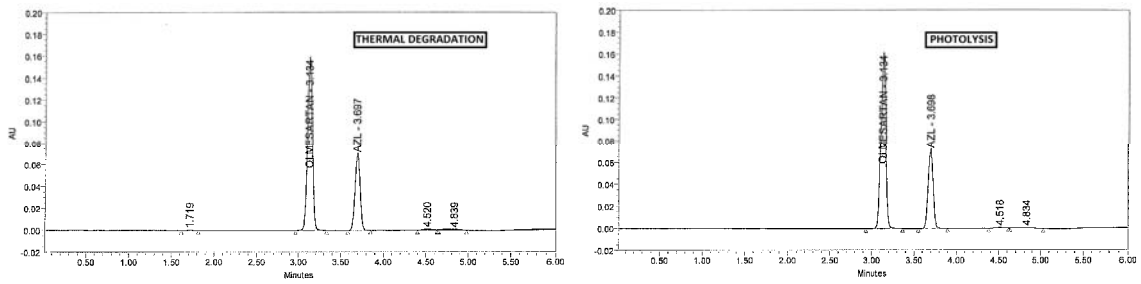


Fig 3: Stress degradation behaviour of OLM+AZL in various stress conditions.

3.2.3 Precision

Method precision was demonstrated by preparing six samples as per the test method. The assay of these samples was determined and the precision of the method was evaluated by computing the percentage-relative standard deviation of the assay results. The % RSD was found to be less than 0.13. The results were shown in Table 2.

The intermediate precision (ruggedness) of the method was demonstrated by carrying out precision study in six replicates of assay on a single batch sample by two different analysts, on two different days and on two different instruments. The % RSD was found to be 0.26 and 0.3 for OLM and AZL respectively.

Table 2: Method precision data

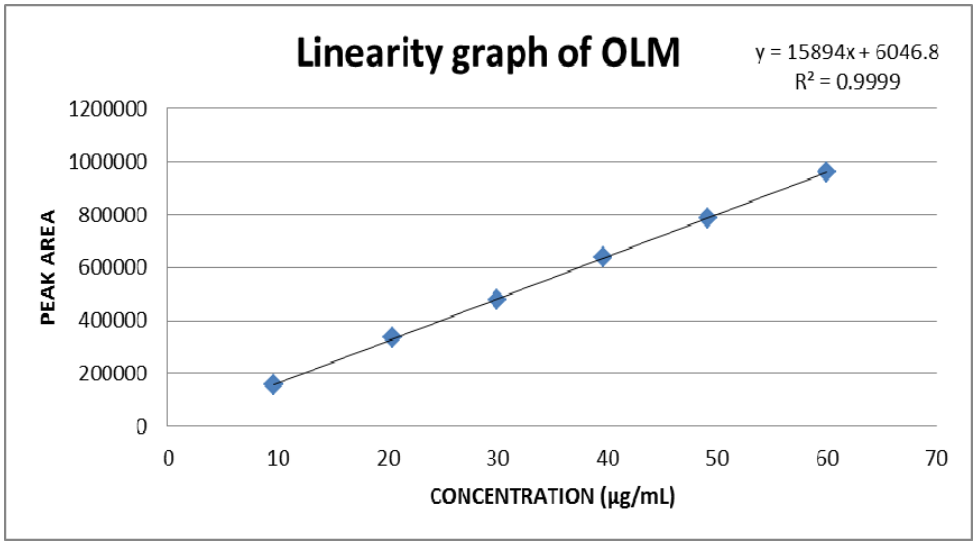
Sample	% Assay	
	OLM	AZL
1	100.2073	100.043
2	99.97606	99.95636
3	100.0066	99.72445
4	100.1164	99.97427
5	99.85623	99.84399
6	100.1006	99.89545
Average	100.0439	99.90625
%RSD	0.123543	0.112247

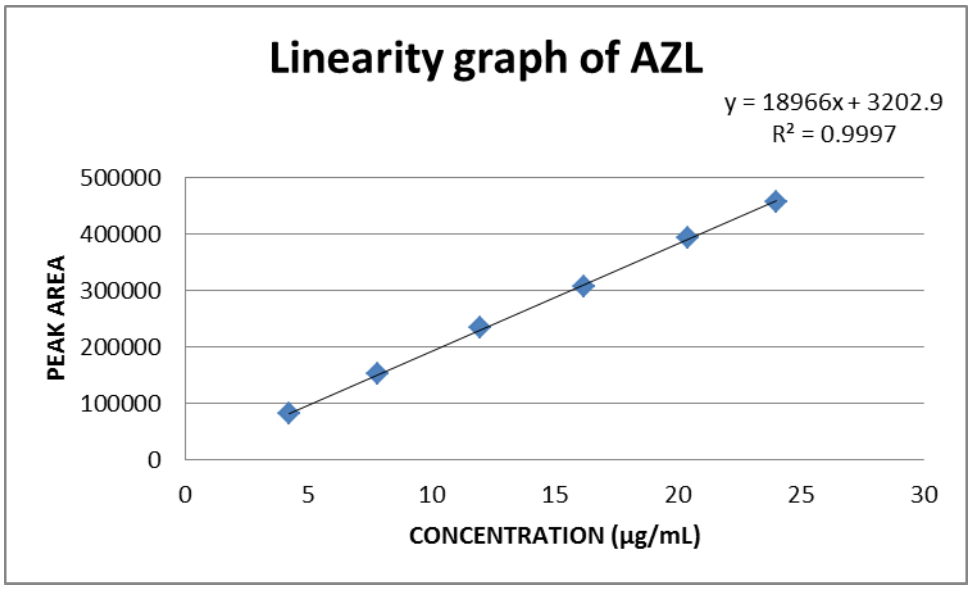
3.2.4 Linearity

The linearity of detector response for OLM and AZL was demonstrated by preparing solutions of OLM and AZL working standard over the range of 25 % to 150 % of target concentration. These solutions were injected into the system and the peak area of OLM and AZL was recorded. A graph of concentration vs peak area of OLM and AZL was plotted separately. The correlation co-efficient between concentration & peak area and y-intercept of the correlation plot were evaluated. The results were shown in Table 3.

Table 3: Linearity Data

Linearity Level	Olmesartan medoxomil		Azelnidipine	
	Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
25%	9.6	156618	4.2	81937
50%	20.4	334098	7.8	151338
75%	30.0	478691	12.0	233780
100%	39.6	639462	16.2	306473
125%	49.2	787410	20.4	392996
150%	60.0	958641	24.0	457239





3.2.5 Accuracy

The accuracy of the test method was demonstrated by preparing recovery samples (i.e. spiking placebo with known quantities of API) at the level of 50 %, 100 % and 150 % of target concentration. The recovery samples were prepared in triplicate in each level. The above samples were chromatographed and the percentage recovery for the amount added was estimated. The precision of the recovery at each level was determined by computing the relative standard deviation of triplicate recovery results. The recovery results are between the range of 98.0 % to 102.0 %, with RSD at each level of less than 0.4 %, proves the method is accurate for the estimation of OLM and AZL in OLM and AZL tablets over the range of 50 % to 150 % of target concentration. The results were shown in **Table 4.**

Table 4: Accuracy Data

Olmesartan medoxomil				Azelnidipine			
Amount Added (mg)	**Amount Recovered (mg)**	**% Recovery**	**% RSD**	**Amount Added (mg)**	**Amount Recovered (mg)**	**% Recovery**	**% RSD**
20.41	20.54	100.64	0.137	7.87	8.01	101.7789	0.126
19.98	20.24	101.3		7.78	7.86	101.0283	
20.51	20.62	100.54		7.92	8.02	101.2626	
39.65	40.06	101.03	0.212	16.25	16.38	100.8	0.133
38.74	39.04	100.77		16.05	16.12	100.4361	
40.05	40.85	102		16.94	17.15	101.2397	
60.01	59.78	99.617	0.318	24.15	24.21	100.2484	0.26
59.88	60.15	100.45		23.85	24.14	101.2159	
60.85	61.78	101.53		24.84	25.29	101.8116	

3.2.6 Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered. The flow rate was altered by 0.2 units, column temperature was altered by 5 units, organic phase ratio of mobile phase was altered by 2 units and pH was altered by 0.2 units. Theoretical plates, tailing factor and % RSD of five replicate standard injections were evaluated. The results prove that the method is robust. The results were shown in the **Table 5.**

Table 5: Robustness Data

Condition	**% RSD**	**Tailing factor**	**Theoretical plates**	**% RSD**	**Tailing factor**	**Theoretical plates**
Normal Condition	0.14	1.01	12842	0.057	1.02	17394
Column Temperature changed by – 5°C (i.e., 20°C)	0.07	1.0	11945	0.056	1.0	18020
Column Temperature changed by + 5°C (i.e., 30°C)	0.04	1.02	13229	0.055	1.04	18622
Organic phase ratio of mobile phase changed by – 2 % absolute [i.e., Buffer solution: Acetonitrile (81: 19) for mobile phase A and Buffer solution : Acetonitrile (21: 79) for mobile phase B].	0.13	1.0	13044	0.056	1.0	18444
Organic phase ratio of mobile phase changed by + 2 % absolute [i.e., Buffer solution: Acetonitrile (79: 21) for mobile phase A and Buffer solution : Acetonitrile (19: 81) for mobile phase B].	0.12	1.0	11910	0.03	1.01	16845
Flow rate changed by – 10 % (i.e. 1.8 mL/min)	0.06	1.0	13202	0.01	1.0	18922
Flow rate changed by + 10 % (i.e. 2.2 mL/min)	0.05	1.0	12564	0.03	1.01	17643

3.2.7 Stability of analytical solution

Stability of standard and sample solution at vial thermostat temperature (i.e., 5°C) was established as mentioned below. Standard and sample solution was prepared as per the test method. The solution thus prepared was chromatographed at regular intervals up to 48 hours. The response of OLM and AZL peak was monitored for both standard and sample solutions. The % deviation of OLM and AZL response from initial for both standard and sample solutions was found to be less than 0.4%. Hence both standard and sample solutions were stable at vial thermostat temperature (i.e.5°C) upto 36 hours.

4.0 CONCLUSION

A new stability-indicating RP-HPLC method was proposed for the simultaneous determination of OLM and AZL in combined dosage form and validated as per the ICH guidelines. The method was found to be simple, selective, precise, accurate and robust. Therefore, this method can be used as routine testing as well as stability analysis of OLM and AZL in synthetic mixtures and combined dosage form.

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