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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC TECHNIQUE FOR CALCULATING BULK AND PHARMACEUTICAL DOSE FORM OF ORLISTAT

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Abstract:
A simple, accurate and rapid RP-HPLC method has been developed for the estimation of Orlistat(ORL) in bulk and pharmaceutical dosage forms using a Zorbax C8 150mm×.4.6mm & 5.0µm particle size in isocratic mode, with mobile phase comprising of 0.1% formic acid :Actonitrile: Methanol in the ratio of 50:30:20 (v/v/v). The flow rate was 1ml/min and detection was carried out by UV detector at 215nm. The retention time for ORL was found to be 3.95 min. The calibration curve was obtained by plotting peak area versus the concentration over the range of 81-189 µg/mL and its percentage recovery was found to be 99.78-100.27%. The %RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column.

***Key words**: Orlistat, HPLC, Isocratic, validation.*

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INTRODUCTION:

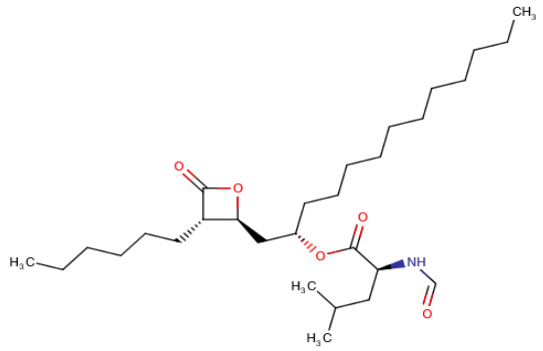


Fig.1.Chemical structure of Orlistat[1]

Orlistat is a lipase inhibitor used in the treatment of obesity that works by inhibiting fat-metabolizing enzymes. It was approved by the FDA for use in combination with a reduced-calorie diet in 1999. This drug is a generally well-tolerated and effective weight-loss aid and is now available in both over-the-counter and prescription preparations, depending on the dosage quantity. Orlistat is a potent and selective inhibitor of various lipase enzymes responsible for the metabolism of fat. It acts in the gastrointestinal (GI) tract via covalent binding to the serine residues located on the active site of both gastric and pancreatic lipase. When orlistat is taken with food containing fat, it partially inhibits the hydrolysis of triglycerides. This decreases absorption of monoaclglycerides and free fatty acids, contributing to weight maintenance and weight loss[2].

EXPERIMENTAL:

Buffer Preparation (0.1% formic acid)[3]:

Accurately transferred 1ml of formic acid in to 1000mL of water and mixed well. Filtered through 0.45µm membrane filter.

Mobile Phase Preparation:

Mixed 500mL of Buffer,300mL of Acetonitrile and 200mL of methanol, degassed by sonication.

Preparation of standard solution

Weighed accurately 135mg Orlistat in 100 ml of volumetric flask and dissolve in 70ml of methanol and make up the volume with methanol From above stock solution 100µg/ml of Orlistat was prepared by diluting 5mlto 50ml with methanol respectively.

Preparation of sample solution:

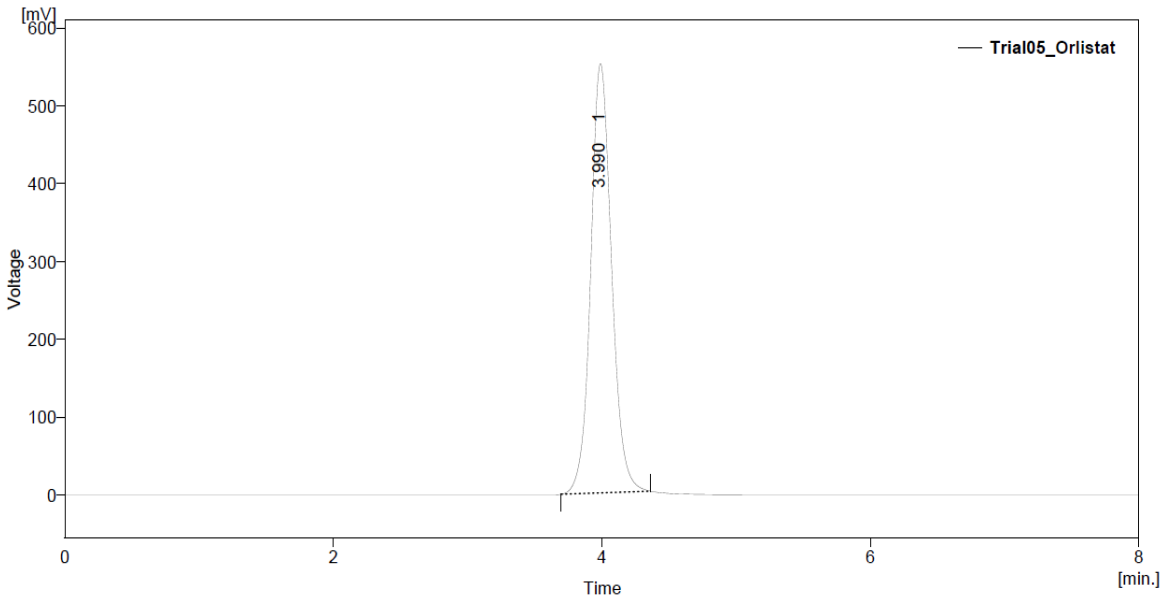
10Capules were weighed as it is, then collected the powder from the 10capsules accurately in poly bag, the weighed empty capsule shells. The calculated average weight of the filled powder by using below formula:

Weight of the 10Capules powder = (10Capsules weight with filled powder- weight of the 10 capsule shells without filled powder) After getting the average weight then weigh the powder equivalent to135 mg of orlistat malate was accurately weighed and transferred into a100ml volumetric flask and dissolved in 70ml of methanol sonicated 15min, then volume was made up to the mark with mobile phase and mixed well[5].

The sample solution was centrifuged at 5000rpm for 10min. Prepared 135µg/mL sample Solution by further diluting 5mL of the above sample stock solution to 100mL with methanol. Then the sample was filtered with PVDF 0.45µm syrnge filter by discarding the 2mL of filtrate.

Preparation of Standard solution

50 mg of Orlistat was weighed and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 100 µg /ml of solution by diluting 5ml to 25ml with mobile phase.



Chromatogram of Trail 4

Obseration:

- Peak shape was good.
- The efficiency was more than 3000 for ORLISTAT
- Tailing obtained below 1.5
- Hence this method was optimized.

METHOD VALIDATION PARAMETERS[5]

Linearity:

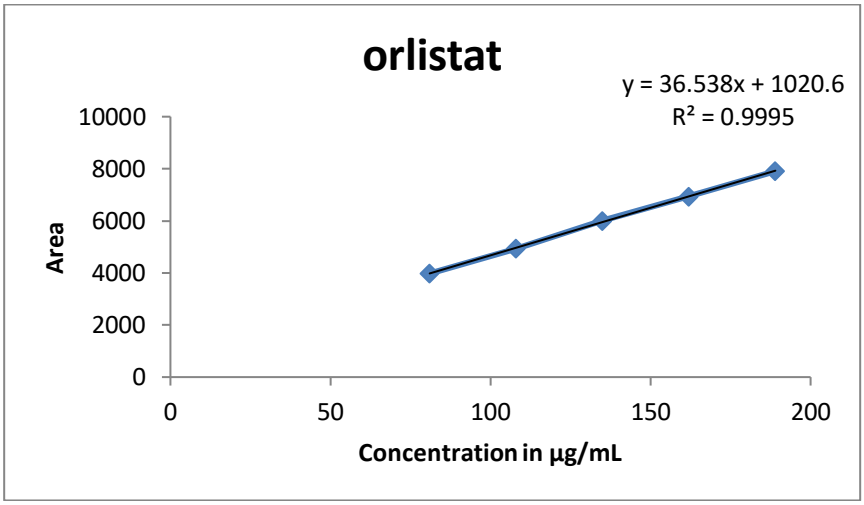
Preparation of the Standard Stock :

Weighed accurately 135mg Orlistat in 100 ml of volumetric flask and dissolve in 70ml of mobile phase and make up the volume with mobile phase and mixed well.

Volume Taken(mL)	Volume diluted to	Concentration(µg/mL)
3	50	81
4	50	108
5	50	135
3	25	162
2.8	20	189

Linearity Results:

S.No	Name of the Solution	Area of Orlistat
1	Linearity solution, Level-1	3976.5
2	Linearity solution, Level-2	4933.711
3	Linearity solution, Level-3 (100%)	6008.974
4	Linearity solution, Level-4 (120%)	6941.284
5	Linearity solution, Level-5 (150%)	7905.307
Slope		1020.6
Intercept		36.58
Correlation coefficient		0.9995



Linearity Graph

System suitability results:.

Name of the Standard	Orlistat	Tailing factor	Plate count
Standard-01	5995.91	1.3	3333
Standard-02	5947.75		
Standard-03	5982.80		
Standard-04	5902.72		
Standard-05	6000.05		
Average	5965.84		
%RSD	**0.7**		

Observation:

System suitability results were met with acceptance criteria, hence system is suitable

System Precision results:

Name of the Standard	Orlistat
Standard-01	5995.91
Standard-02	5947.75
Standard-03	5982.80
Standard-04	5902.72
Standard-05	6000.05
Standard-06	5945.85
Average	5962.51
%RSD	**0.6**

Observation:

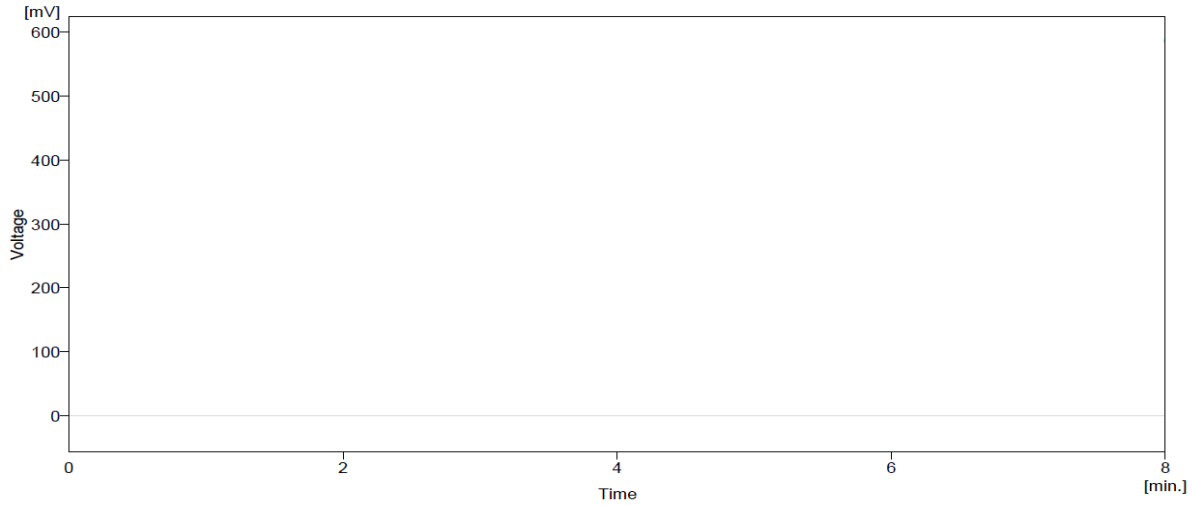
System Precision results were met with acceptance criteria, hence system is precise.

SPECIFICITY

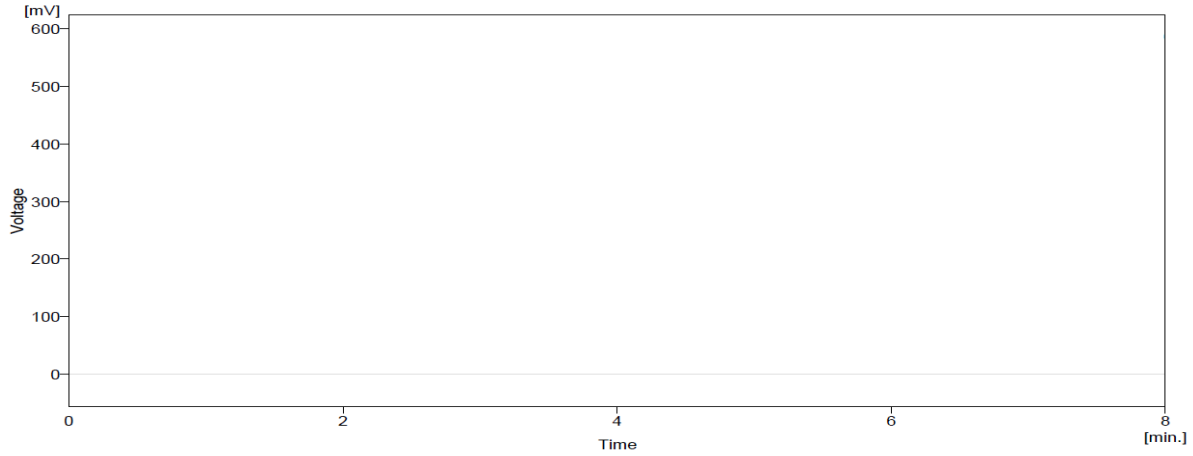
S.No.	Solution details	Area of Orlistat
1	Standard	6085.32
3	Blank	Not Detected
4	Placebo solution	Not Detected
5	Test solution	5947.21

Observation:

There was no interference observed at the retention time of Orlistat due to blank and Placebo Hence System is specific



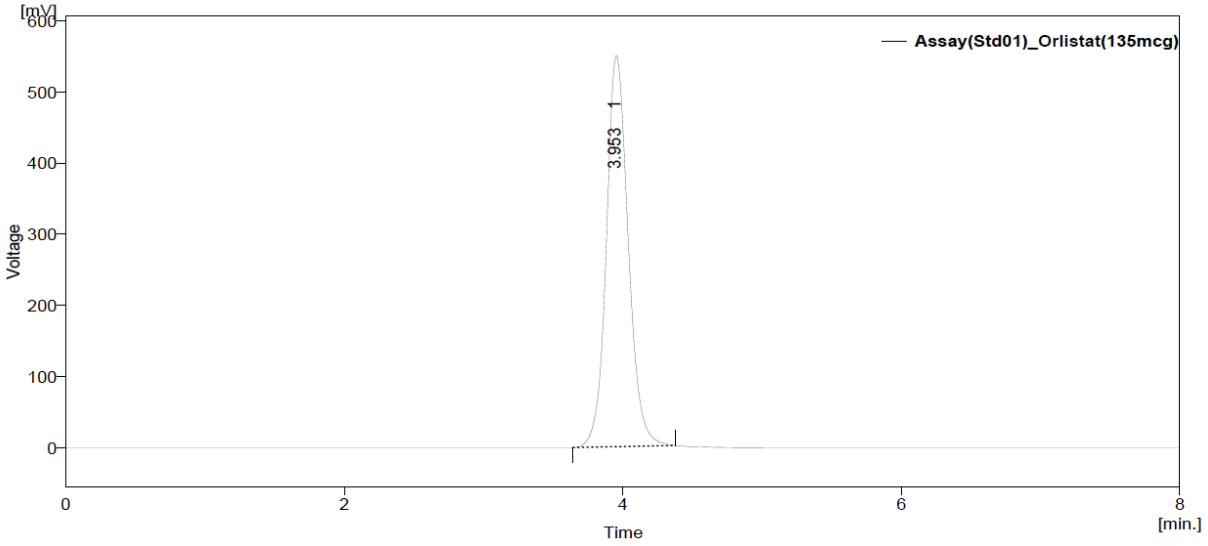
Chromatogram of Blank



Chromatogram of Placebo

Chromatograms of System suitability and System Precision:

Chromatogram of Standard-01



Method Precision Results:

S.No.	Solution details	%Assay of Orlistat
1	Test solution preparation-1	100.7
2	Test solution preparation-2	101.1
3	Test solution preparation-3	101.6
4	Test solution preparation-4	99.9
5	Test solution preparation-5	99.2
6	Test solution preparation-6	99.8
Average		99.2
StdDev		0.91
%RSD		0.9

Observation:

- Mean %Assay Obtained between 90.0 to 110.0%for Orlistat
- The % RSD of % Assay results obtained from Test solution was obtained less than 2.0% for Orlistat
- Hence Method is Precise

Robustness results for Orlistat:

Name of the Parameter	%RSD	Theoretical Plates	Tailing factor
Low flowrate(0.8mL/min)	0.1	3562	1.2
Highflowrate(1.2mL/min)	0.7	3546	1.2
Lower Wavelength(213nm)	0.3	3877	1.3
Higher Wavelength(217nm)	0.4	3641	1.2

Observation: System suitability met the acceptance criteria in Robustness parameters hence method is Robust.

FORCED DEGRADATION RESULTS FOR ORLISTAT[6]:

Name of the Degradation	Condition	Peak Purity	Peak Purity Value	%Assay
Photolytic degradation	1.2mill/LUX Hours	PASS	+	100.1
Thermal Degradation	60°C/7Days	PASS	+	99.4
Acid Degradation	5mL of 3N HCl/4Hrs at 80°C	PASS	+	99.6
Base Degradation	5mL of 3N NaOH Solution/4Hrs at 80°C	PASS	+	99.8
Peroxide Degradation	5mL of 10% H_2O_2/4Hrs at Bench top	PASS	+	94.0
Control Sample	NA	PASS	+	99.9

Observation:

- Peak purity was obtained Pass
- Purity value obtained in Positive
- %degradation 5.8% obtained in Acid degradation
- No interference was observed with treated Blank

DISCUSSION:

The optimum wavelength for the determination of ORLISTAT was selected at 215 nm. Various trials were performed with different mobile phases in different ratios, but 0.1% formic acid: Actonitrile: Methanol (50:30:20) was selected as good peak symmetry and resolution between the peaks was observed. The retention times for the drug were considerably less compared to the retention time obtained for the drugs in the other mobile phase.

The different analytical performance parameters such as linearity, precision, accuracy, and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 81-189 µg/mL for ORLISTAT. From linearity the correlation coefficient R^2 value was found to be 0.999 for ORLISTAT. The proposed HPLC method was also validated for system suitability, system precision and

method precision. The %RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column. The percentage of recovery of ORLISTAT was found to be 99.7% respectively shows that the proposed method is highly accurate.

Hence the proposed method is highly sensitive, precise, and accurate and it successfully applied for the quantification of API content in the commercial formulations of ORLISTAT in Educational institutions and Quality control laboratories.

CONCLUSION:

A new precise, accurate, rapid method has been developed for the simultaneous estimation of ORLISTAT in pharmaceutical dosage form by HPLC.

ACKNOWLEDGEMENT

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