Eurasian Journal of Analytical Chemistry
ISSN: 1306-3057 OPEN ACCESS 2019 14 (2): 27-35

Stability Indicating RP-HPLC Method for the Estimation of ORLISTAT in Bulk and Pharmeceutical Dosage Forms

N.V. Venkataramana, Nivedita R Desai, Swamy Sreenivasa, D.B. Aruna Kumar

Received: 28 Feb 2019 ▪ Revised: 08 April 2019 ▪ Accepted: 13 April 2019

Abstract: The current research goal was to develop a novel specific procedure for the quantitation of the Orlistat. The method development was done by using a High-Performance Liquid Chromatography (HPLC). The extensive method development was conducted to identify a right combination of chromatographic conditions and validated as per the regulatory guidelines. The current method overcome the disadvantages of other methods likewise UV and potentiometric titrations. The mixture of mobile phase A and B (50:50) used to elute the Orlistat in isocratic mode. The rapid run time of the method was accomplished by adjusting the flow rate of 2.0mL per minute along with L1-octadecyl chemistry column. The Orlistat peak was detected by using a sensitive Ultra violet detector at 205nm. The method was completely screened with all the key validation parameters likewise suitability, precision, selectivity, linearity, robustness and recovery. The purity threshold of more than 990 proves that the method is free from any possible interferences and the linearity was established at 5 different levels with a correlation coefficient 0.998. The overall %RSD (relative standard deviation) of mean recovery was 0.70%. The marketed commercial formulation of Orlistat capsules was tested on the developed method to confirm the suitability of the method for both bulk active substance and pharmaceutical formulations. Based on the experimental outcome method can be regarded as inventive, and specific to quantitate Orlistat. The developed method can be used directly to test the pharmaceutical formulations and drug substances. Further these methods can be modified to determine the impurities in drug substances and drug products.

Keywords: Orlistat, Reverse Phase-HPLC, Estimation, Specific, Forced Degradation, Regulatory Guidelines, Validated.

INTRODUCTION

Orlistat [1] is chemically,[(2S)-1-[(2S,3S)-3-hexyl-4-oxooxetan-2-yl]tridecan-2-yl](2S)-2-formamido-4-methylpentanoate (Fig. 1) used to treat obesity. Orlistat is the saturated derivative of lipstatin, which is a potential inhibitor of pancreatic lipases isolated from the bacterium *Streptomyces toxytricini.* Because of its relative simplicity and stability, it is chosen over lipstatin for development as an anti-obesity drug. The effectiveness of orlistat in promoting weight loss is definite but modest and also associates with primary side effects such as gastrointestinal-related, and include steatorrhea (oily, loose stools with excessive flatus due to unabsorbed fats reaching the large intestine), fecal incontinence and frequent or

N.V. Venkataramana, Department of Studies and Research in Chemistry, University College of Science, Tumkur University, Tumkuru, India.

Nivedita R Desai, Department of Studies and Research in Chemistry, University College of Science, Tumkur University, Tumkuru, India.

Postgraduate Department of Chemistry and Biochemistry, Ramaiah College of Arts, Science and Commerce, Bengaluru, India.

Swamy Sreenivasa, Department of Studies and Research in Organic Chemistry, Tumkur University, Tumkuru, India.

D.B. Aruna Kumar, Department of Studies and Research in Chemistry, University College of Science, Tumkur University, Tumkuru, India.

E-mail: nirmaldb@rediffmail.com

urgent bowel movements etc., Orlistat produces its effect in small intestine and stomach by forming a covalent bond with lipase enzyme. Orlistat is a crystalline powder having white to off-white color. It is a hydrophobic substance with very limited solubility in polar solvents like water and also within the physiological pH range. Chemically it possesses a strongest acidic PKa of about 12.7 and having low water solubility [2] .

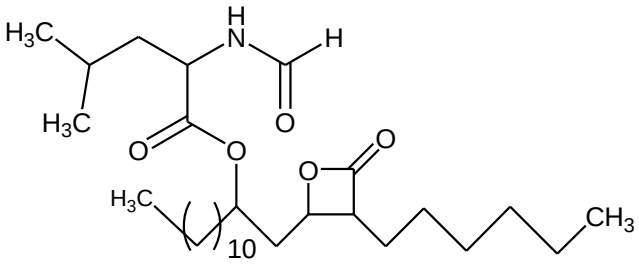

Figure 1: Chemical structure of Orlistat

Stability of any pharmacologically active substance are an important prerequisite, as it affects the safety and therapeutic activity in drug products[3]. It is a basic requirement to perform stability studies and anticipate shelf life of any drug product. The forced degradation is one of the indicators to prove the chemical stability of the molecule. Further, forced degradation studies can be used to understand the possible potential degradants which will be forming upon stability of the molecule. Also, it assists in finding the degradation pathway of the drug substances and drug products [4,5]. An ideal stability indicating method resolves all the inherent impurities from the peak of interest. The forced degradation can be achieved by exposing the samples to many stress conditions. The widely available many regulatory guidelines emphasize to perform the forced degradation as a part of validation and method development. There are vast range of literatures describes about the of the method development, validations and stability analytical procedures. These technical articles also emphasis on stability indicating nature of the assay methodologies. The generally well accepted regulatory bodies/guidelines like ICH (International conference on harmonization), united states food and drug administration, and European medicine agency issued its own documents for validation of analytical methodologies [6-15].

There are few scientific literatures available related to analytical methods for orlistat, this could be due to weak chromophore of molecule. The scientific literature study demonstrated that analytical methods such as Spectrophotometric (Ultra violet) [16,17], RP-HPLC method in bulk, pharmaceutical dosage form [18-22], LCMS methods [23,24]at plasma level as single and combined with other drugs have been developed. There are methods reported for the estimation of Orlistat[25-26]. The UV methods carries an advantage of fast and economical. On the other side it is having many disadvantages like accuracy, variability and reliability. The methods by using LCMS are typically advantaging to determine the sample present in small amount. It brings a disadvantage of owing the instrument i.e it's a highly. In view of above disadvantages, the present research work attempts, to develop and validate a RP-HPLC stability demonstrating novel procedure for Orlistat. The RP-HPLC methods employs a polar mobile phase. As a result, non-polar molecules in the polar mobile phase tend to adsorb to the stationary phase and elute later. The selected molecule, Orlistat is a hydrophobic molecule and suitable for reverse phase chromatographic method of development.

MATERIALS AND METHODS

Reagents and chemicals: Orlistat was obtained as gift samples from Biocon Pvt. Ltd. and all the chemicals and reagents used were AR grade. The organic modifiers used for the study were methanol and acetonitrile, HPLC grade supplied from Merck. The milli Q water was used for the buffer preparation.

Instrumentation: The method development and validation were executed by using Diode array detector/UV Detector. The HPLC supplied from Thermo having a model number of Ultimate CAD 300. The additional instruments used are pH meter, vortex mixer, ultrasonicator, water bath, micro and analytical weighing balance were used for the study.

Chromatographic mode: The reverse phase HPLC mode was selected based on the hydrophobic nature Orlistat for separation. The data analysis software employed was Chromeleon 7.2 version RS4 data analysis software.

Method development: The extensive method development was done to choose a suitable column chemistry. The stationary phase selection was done systematically by selecting a diverse chemistry column like C8 and C18 with array of carbon loading. Also, the development was performed by using a

range of different particle size like 3µm and 5µm. The stationary phase was opted finally due to its efficiency in separating compounds with good peak shapes and less tailing. The column was supplied from Thermofischer. While developing the method the different range of pH like acidic, basic and neutral buffers with different pKa values were tried. The mobile phase with pH having 5.5 found to be suitable for better peaks shape and resolution of all potential degradants from forced degraded samples. The column temperature of 25°C found to retain a peak longer than 12.0 minutes and having a larger system pressure. Hence, the temperature was opted to be 40°C, which assisted in early peak elution, better shape as well low system back pressure. The injection volume between 10µL to 50µL were tried, the higher injection volume resulted in a peak broadening and poor peak shape. The injection volume 25µL found to be optimal and suitable. The final optimized method was considered for the validation as per the ICH regulatory tripartite guideline.

Stationary phase: The stationary phase with C-18, 150 mm x 4.6mm, 3µm (Synchronis) was selected as a suitable column, upon method development.

Buffer Preparation: 0.7 g of sodium phosphate monobasic about 10mM(NaH_2PO_4) was weighed and transferred into a suitable container containing approximately 1000.0 mL of type I water (HPLC water). The solution was sonicated to dissolve and adjusted to pH 5.0with diluted orthophosphoric acid (1mL of 5% Orthophosphoric acid to 10mL of water).

Preparation of Mobile Phase A: Mobile phase solution was prepared by mixing proportionally, buffer, methanol and acetonitrile using a ratio of 31 : 22:47 v/v respectively, the solution was mixed well. The solution was filtered through a 0.45µm membrane filter and degassed the solution for 10 minutes.

Preparation of Mobile Phase B: Used a 100% Acetonitrile as a mobile phase B.

Mode of Analysis: Isocratic mode with 50% of each of mobile phase A and B. Isocratic mode carries an advantage of carrying a constant composition of mobile phase and the polarity of the mobile phase will remain same over the entire chromatographicrun.

Diluent solution: 400 mL of methanol was mixed well with 600 mL acetonitrile in a suitable container.

Standard preparation: 50.45 mg of Orlistat working reference standard was precisely weighed in-to a 100 mL volumetric flask. This was dispersed and dissolved via sonication by adding 60mL of diluent for few minutes with intermittent shaking. The solution was equilibrated to room temperature before making up to volume with diluent and mixed the solution well.

Determination of retention time: The retention time of Orlistat were determined by injecting 25 µL of working standard solution at 2 ml/min flow rate into HPLC chromatograph using UV detector at 205 nm. The 2 ml/min was found to be optimal for better retention time and good peak shape. The chromatography was finalized based on the system suitability and taken forward for validation to confirm the further stability indicating nature.

ANALYTICAL PROCEDURE VALIDATION

To confirm the performance characteristics of the of developed RP-HPLC procedure the validation was performed using parameters as per the ICH guidelines [21,22].

System suitability of developed method was determined by injecting 25 µL of working standard solution at 2 ml/min into HPLC chromatograph using UV detector at 205 nm. The Orlistat is possessed weak chromophore group and shows a better absorbance at the selected wave length. From the chromatograms obtained data such as tailing factor, theoretical plates, and the percentage (%) standard deviation meant for Orlistat peak from replicate injections (5) of standard were calculated.

Specificity was achieved injecting blank solution, working standard solution into the Liquid chromatography system. Verified the retention times of Orlistat peak from working standard preparation. The specificity of developed RP-HPLC method was studied using forced degradation under different stress conditions.

Neat Sample (Sample as such): 100.0 mg of Orlistat representative sample was accurately weighed into a 50.0 mL volumetric flask. This was dissolved via sonication by adding 30mL of diluent for few minutes with intermittent shaking. The solution was the equilibrated to room temperature before making up to volume with diluent and mixing the solution well.5.0 mL of the stock standard was diluted to 20mL in a 20mL volumetric flask with the diluent.

Acid treated sample: (0.1 N HCl) 100.23 mg of Orlistat sample was weighed and transferred to a 50.0mL volumetric flask. Treated the target sample with 2mL of HCL and place it on bench top for 4Hours. The solution was dissolved by sonication by adding diluent. The solution was equilibrated to room temperature before made up to volume with the diluent and mixed well (Stock solution). 5.0 mL of the stock solution was diluted to 20 mL in a 20 mL volumetric flask with the diluent and mixed well.

Alkali Stressed sample:(0.1 N NaOH):100.16 mg of Orlistat sample was weighed and transferred to a 50.0mL volumetric flask. Treated the target sample with 2mL of sodium hydroxide solution and place it on bench top for 2Hours. The solution was dissolved by sonication by adding diluent. The solution was equilibrated to room temperature before made up to volume with the diluent and mixed well (Stock solution). 5.0 mL of the stock solution was diluted to 20 mL in a 20 mL volumetric flask with the diluent and mixed well.

Peroxide Stressed sample: (3.0% w/v):100.12 mg of Orlistat sample was weighed and transferred to a 50.0mL volumetric flask. Treated the target sample with 2mL of H_2O_2 (3.0% w/v)solution and place it on bench top for 4Hours. The solution was dissolved by sonication by adding diluent. The solution was equilibrated to room temperature before made up to volume with the diluent and mixed well (Stock solution). 5.0 mL of the stock solution was diluted to 20 mL in a 20 mL volumetric flask with the diluent and mixed well.

Linearity was done by generating sequence of dilutions in concentration (in ppm) range of 150-600 ppmof Orlistat. 25µl of each solution was injected into HPLC chromatograph. The calibration curve was established (peak area versus the drug concentration in ppm).

Precision Method precision was performed by preparing 6 samples from a single batch. At target working concentration and by injecting 25 µL of working solution.

Accuracy was performed to measure the closeness of the experimental values to the actual amount of samples. The recovery was established at three different levels as per ICH (80%, 100% and 120%) of the target concentrations.

Assay of Orlistat in marketed Sample: The selected marketed formulation ORLICA-120mg supplied from Biocon was taken for the study. Pulverize the content of 10 capsules and weighed the amount equivalent to 100mg dissolved in 200mL of diluent. Dissolved with the aid of sonication and made to final volume with diluent. Filtered through nylon filter and vial for HPLC analysis.

RESULTS

A new stability indicating RP-HPLC procedure to estimate the Orlistat in bulk and pharmaceutical dosage forms was developed and validated. The conditions of developed method were given in **Table 1** and chromatogram obtained were presented in **Figure 2.** The system appropriateness parameters were found to be within the acceptance limit and result were presented in **Table 2.** Developed stability indicating RP-HPLC procedure was found to be selective andout-come were presented in **Table 3** and representative chromatogram of blank and specificity was presented in **Figure 3** and **Figure 4**. The result meets the pre-established acceptance criteria for linearity. Hence the analytical procedure is shown to be linear (regression-coefficient 0.9990) within the concentration series of 150 to 600 pp. The data were presented in **Table 4** and standard calibration graph was presented in **Figure5.** The procedure was found to be precise as the %RSD values were found to be less than 2.0% and the results were presented in **Table 5.**The detailed method procedure was found to be accurate and results were presented in **Table 6.**The assay of Orlistat was found to be **99.3%** and results were presented in **Table 7.** The chromatogram of Orlistat in marketed sample were presented in **Figure 6.**

Table 1: Developed method specification

Column	: Synchronis C18, 150mm x 4.6mm, 3µm.
Flow rate	: 2.0 mL/min
Wavelength	: 205 nm
Column Temperature	: 40°C
Injection Volume	: 25 µL
Runtime	: 15 minutes
Needle wash	: Acetonitrile: water
Elution	: Isocratic mode

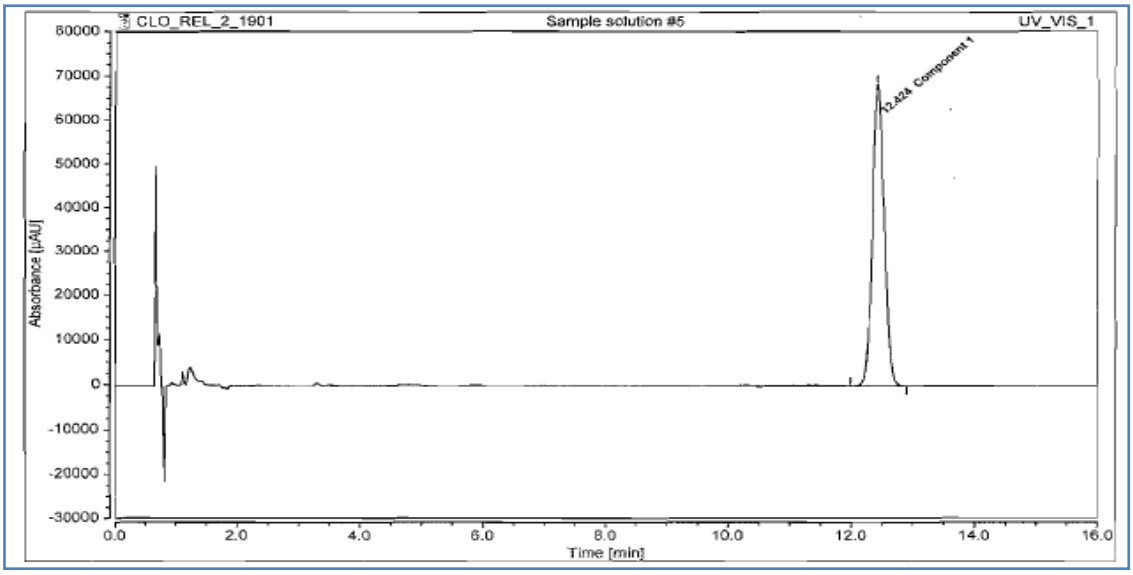

Figure 2: Representative chromatogram of system suitability

Table 2: System suitability parameter data of Orlistat

Set No.	System Precision (Area Count for standard)	Acceptance criteria
1	1366587.016	The system suitability parameter such as % Relative Standard deviation, tailing factor and Theoretical plate count were evaluated for method suitability.
2	1360448.375	
3	1357808.355	
4	1358409.069	
5	1362485.866	
Mean	1361148	
% RSD	0.26	Not more than 2.0
Tailing	1.21	Not more than 2.0
Theoretical Plate	19062	Not Less than 2000

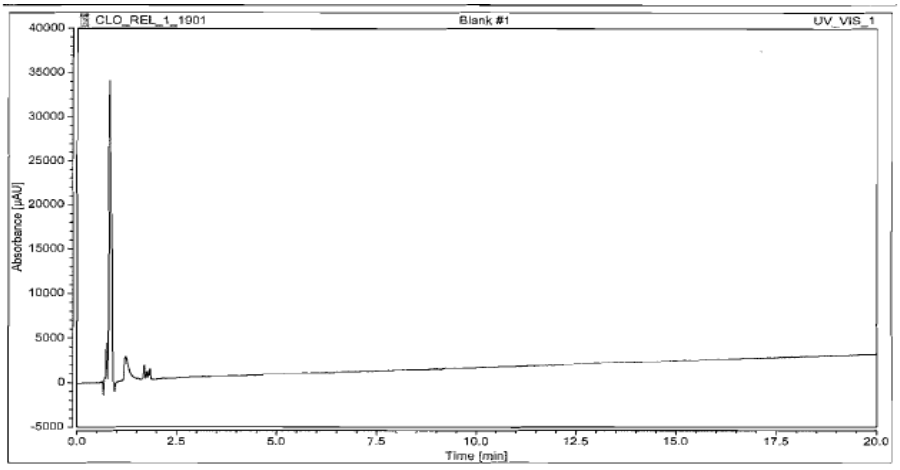

Figure 3: Representative chromatogram of Blank (Early eluting peak from Diluent)

Table 3: Specificity studied data of Orlistat

Stress Condition	Peak Purity**	Purity Match
Sample as Such	P	992
0.1 N HCl Stressed	P	998
0.1 N NaOH Stressed	P	998
3.0%w/v H202 Stressed	P	997

Peak Purity **: 'P' indicates Orlistat Peak is Pure which confirmed by Diode array detector and Dionex Chrome Leon Software.

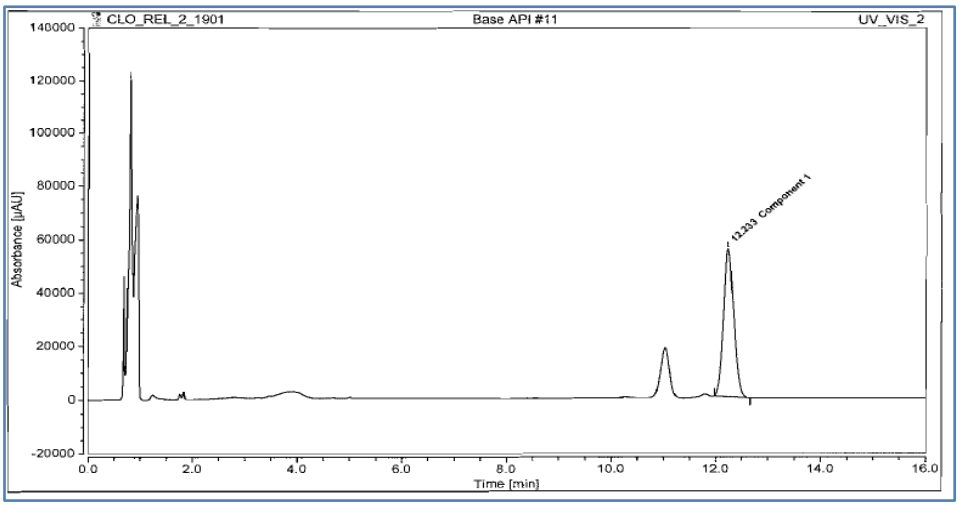

Figure 4: Representative chromatogram of Forced degradation

Table 4: Linearity data of Orlistat

Set. No.	Concentration in ppm	Area count
1	150	463074
2	245	639444
3	370	957856
4	500	1279717
5	600	1527782
Regression Coefficient		0.9991

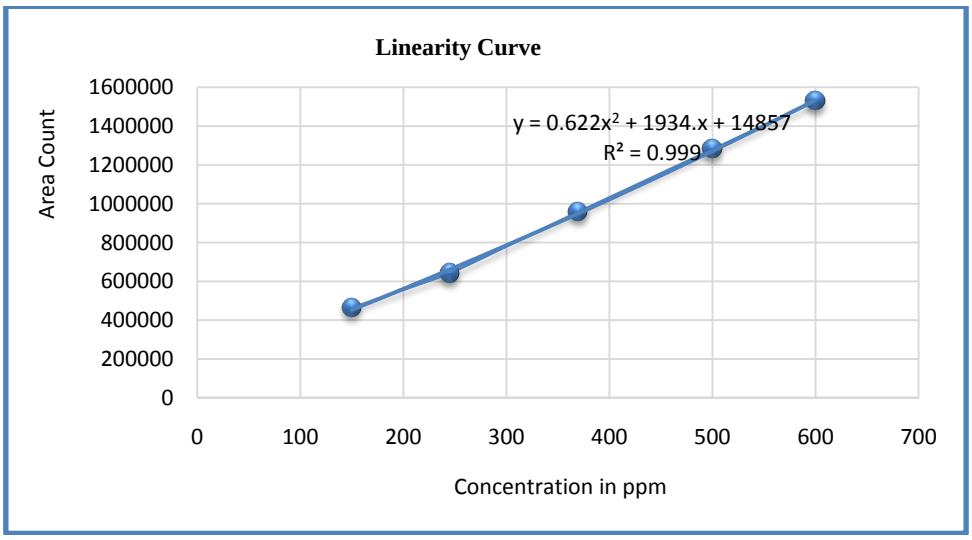

Figure 5: Standard calibration curve of Orlistat

Table 5: Precision study data of Orlistat

Set No.	Method Precision (% Assay)
1	99.0
2	99.2
3	100.1
4	100.8
5	100.6
6	99.9
Mean	99.9
Standard deviation	0.726
% RSD	0.73
Acceptance criteria	NMT 2.0%
% RSD = (Standard deviation *100)/Mean	

Table 6: Accuracy study data of Orlistat

Set. No	Level (%)	Area count	Amount added (mg)	Amount recovered (mg)	Recovered (%)	Average / Mean (%)
1	75%	1005215	37.56	37.26	99.2	99.3
2		994512	36.89	36.86	99.9	
3		988258	37.12	36.63	98.7	
1	100%	1345648	50.12	49.88	99.5	99.2
2		1350121	50.48	50.04	99.1	
3		1335648	49.99	49.51	99	
1	125%	1594878	60.24	59.11	98.1	98.1
2		1582154	60.05	58.64	97.7	
3		1608512	60.52	59.62	98.5	
Over all Mean Recovery						98.9%
Over all % RSD						0.67
Acceptance Criteria						RSD NMT 2.0%

Table 7: Assay data of Orlistat in marketed formulation

Sample No.	% Assay	Average
Sample 1	98.5%	99.3%
Sample 2	100.1%	

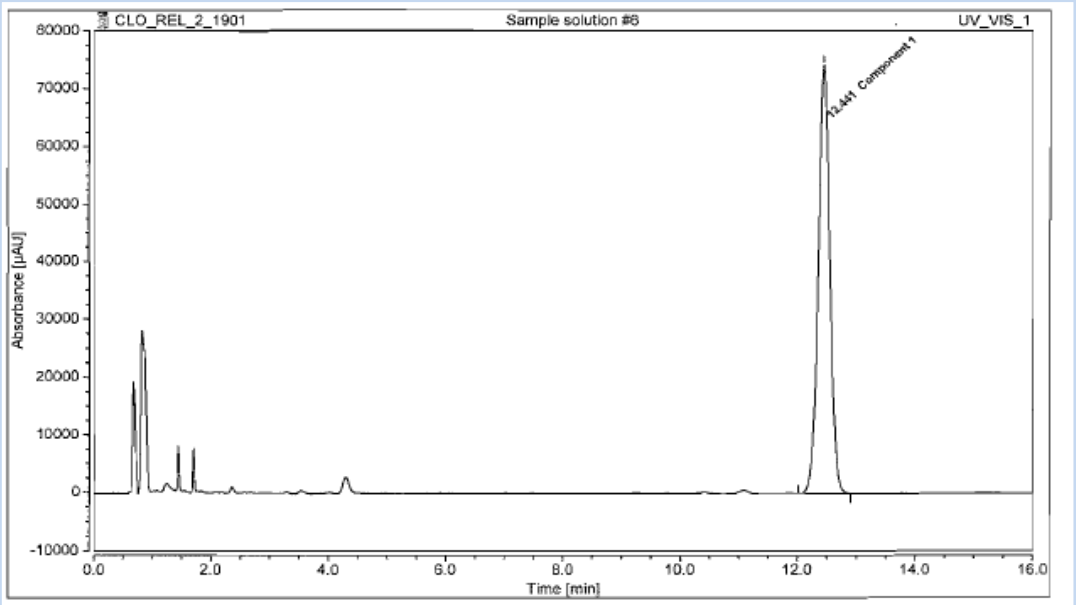

Figure 6: Chromatogram of Orlistat in marketed sample

Table 7: Assay data of Orlistat in marketed formulation

Sample No.	% Assay	Average
Sample 1	98.5%	99.3%
Sample 2	100.1%	

CONCLUSION

The extensive method development was done to identify the suitable conditions for Orlistat by varying different chromatographic parameters. Further, the method suitability was proved by performing the key forced degradation study. The presented method is specific, and peak of interest was very well separated from all potential degradants. The parameters of method validation checked were system suitability, specificity, method precision, method accuracy and linearity. All the parameters considered for method suitability met the predefined acceptance criteria. Statistical analysis data proved that the method developed was accurate, precise and repeatable. The results indicated the suitability of the method to study stability of Orlistat in drug substances as well as drug product. It can be depicted that the developed

procedures may be employed for analysis of routine, development and shelf life stability testing of Orlistat samples. The Shelf life helps in determine the life time usage of the product. The proposed RP-HPLC method is accurate, precise, linear and specific, for the analysis of Orlistat in active and therapeutic dosage forms.

APPLICATIONS

The established method can be used for the routine, quality control, development and stability testing of Orlistat Pharmaceutical formulations and bulk active ingredients. Also, these methods can be further explored as cleaning methods for Pharmaceutical GMP manufacturing facilities.

REFERENCES

[1] https://pubchem.ncbi.nlm.nih.gov./compound/orlistat.

[2] https://www.drugbank.ca/drugs/DB01083.

[3] Blessy M, Ruchi D Patel, Prajesh Prajapati N and Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review, Journal of Pharmaceutical Analysis. 2014;4 (3):159-165.

[4] https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2__Guideline.pdf.

[5] Reynolds DW, Facchine KL and Mullaney JF. Available guidance and best practices for conducting forced degradation studies, Pharm. Technol., 2002;26 (2), 48-56.

[6] Bakshi M and Singh S. Development of validated stability indicating assay methods-Critical review, J. Pharm. Biomed. Anal.2002; 28 (6), 1011-40.

[7] Bakshi M and Singh S. ICH guidance in practice: establishment of inherent stability of secnidazole and development of a validated stability-indicating high-performance liquid chromatographic assay method. Pharm. Biomed. Anal.2004; 36 (4), 769-75.

[8] Bennett P.K, Li Y.T and Edom R, J. Henion. Quantitative determination of Orlistat (tetrahydrolipostatin, Ro 18-0647) in human plasma by high-performance liquid chromatography coupled with ion spray tandem mass spectrometry. MassSpectrum.1997; 32 (7), 739-49.

[9] Jens T. Carstensen and Marcel Decker (2000) Stability-Indicating Assays, Drug Stability: Principles and Practices, 3rd Edition, Marcel Dekker: New York, p 329-84.

[10] http://www.ich.org/products/guidelines/quality/quality-single/article/stability-testing-fnewdrug-substances-and-products.html.

[11] https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5C/Step4/Q5C_Guideline.pdf.

[12] https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3B_R2/Step4/Q3B_R2__Guideline.pdf.

[13] ICH Q1A (R2) (2003) Stability Testing of New Drug Substances and Products. International conference on Harmonisation, IFPMA, Geneva.

[14] ICH Q1B (1996) Stability Testing of New Drug Substances and Products. International Conference on Harmonisation, IFPMA, Geneva.

[15] ICH Q2B (2005) Stability Testing of New Drug Substances and Products. International Conference on Harmonisation, IFPMA, Geneva.

[16] Shweta Maheshwari, Amit P. Khandharb and Anurekha Jain. Quantitative Determination and Validation of Ivabradine HCL by Stability Indicating RP-HPLC Method and Spectrophotometric Method in Solid Dosage Form, Eurasian J. Anal. Chem.2010; 5(1): 53-62.

[17] Kumar TH, Reddy MK, Rishika D and Kumar PR. Estimation of Orlistat by Spectrophotometric method, IJPSR ,2011, 2(9), 2469-2471.

[18] Gaddam P, Dhanalakshmi K and Reddy N. Determination and validation of derivative spectroscopic method for determination of Orlistat in bulk and pharmaceutical dosage form, Int JPharm Sci Rev Res, 2013; 65, 365-368.

[19] Rao MVB, Reddy BCK, Rao TS and Muralidhar P. Reverse phase high performance Liquid chromatographic determination of Orlistat pellets 50.0 %, Rasayan J Chem, 2008;1(3), 636, 638.

[20] Mani Deepika V, Rudraprasad Reddy J, Ch Appa Rao KM and Peer Basha D. A sensitive RP-HPLC method development and validation for the simultaneous estimation of Atorvastatincalcium,

Fenofibrate and Orlistat in tablet dosage form, International Journal Of Pharmaceutical Research & Analysis, 201;, 4, 7-13.

[21] Palakurthi Ashok Kumar, Vinta Bhanu Prakash Reddy and Sivaranjani Thiyaharajan. Quantification of SSRR impurity of Orlistat capsules by normal phase HPLC-UV detector, International Research Journal of Pharmacy, 2012; 3(4),350-355.

[22] Souri E, Jalalizaden H, Kebriace ZA and Zadehvakili B. HPLC analysis of Orlistat and its application to drug quality control studies, Chem Pharm Bull (Tokyo), 2007;55(2),251-4.

[23] Sreekanth Nama, Babu Rao Chandu and Mukkanti Khagga. Validation of Orlistat in bulk and Pharmaceutical dosage form, International Journal of Pharma Sciences and Research (IJPSR), 2010; 1(6), 251-257.

[24] Wieboldt R, Campbell DA and Henion J. Quantitative liquid chromatographic-tandem mass spectrometric determination of Orlistat in plasma with a quadrupole ion trap, J Chromatography Biomed Sci Appl 1998; 708(1-2),121-129.

[25] Alex Schneider, Ludger Wessjohann A, Juliana Severi A and Vilegas Wagner. Comparison of impurity profile of Lipiblock vs Orlistat using HPLC and LCMS/MS,Latin American Journal of Pharmacy, 2012; 31(1),91-96.

[26] Shailendra Bindaiya and AmeetaArgal. Stability indicating assay of Orlistat and its degradation products by HPLC, Bulletin Pharmaceut Res, 2013, 3(2), 44-50.

[27] A. Mahammadi, I. Haririan, N. Rezanour, L. Ghiosi and Walker RB. A stability indicating high-performance liquid chromatographic assay for the determination of Orlistat in capsules Chromatography A, 2006; 1116 (1-2), 153-157.