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Research Article

Development and Validation of RP-HPLC Method For Determination of Orlistat in Bulk and Different Branded Capsule Dosage Forms

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Abstract

A simple, specific, precise and accurate reverse phase liquid chromatographic method has been developed and validated for the determination of Orlistat in bulk and multi company capsule dosage forms by using Mobile phase acetonitrile: methanol : distilled water in the ration of (75:15:10) and maintain the pH 3.0 with the addition of ortho phosphoric acid. The method was carried out on a Agilent ZORBAX SB-C18 (150×4.6mm, 3.5µm) column at low rate was 1.0 ml/ min, Detection was carried were monitored at 210 nm. The retention time of Orlistat was found to be 1.542 min. The method was validated according to ICH guidelines. The linearity for Orlistat was in the range of 10-60 µg/ml respectively. The recoveries of Orlistat were found to be in the range of 100.04-101.37%respectively. The method was validated for specificity, precision, linearity, accuracy and robustness. The proposed method can be used for the estimation of orlistat in multiple brand of pharmaceutical capsule dosage form (Zerofat and lipocut) and also calculate system suitability parameter

Keywords: *Orlistat, HPLC, validation, RP HPLC*

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Introduction

Orlistat is an anti-obesity drug 1, chemically it is (S)-((S)-1-((2S, 3S)-3-hexyl-4-oxooxetan-2-yl) tridecan-2-yl) 2-formamido-4-methylpentanoate, it works by inhibiting gastric and pancreatic lipases, the enzymes that break down triglycerides in the intestine. When lipase activity is blocked, triglycerides from the diet are not hydrolyzed into absorbable free fatty acids, and are excreted undigested [1].The chemical structure of ORL was shown in fig.1

Literature review reveals that very few analytical methods were evoked for the estimation of ORL in human plasma. By modern analytical instrument like UV [2], LC- MS/MS [3], stability indicating assays [4], Establishment of impurity profile by HPLC and estimation of drug content in bulk and pharmaceutical dosage forms by HPLC [5,6], was reported. We here in report a simple, rapid and reliable RP-HPLC for the estimation of ORL in bulk and multiple brand capsule pharmaceutical dosage forms (Zerofat and lipocut).

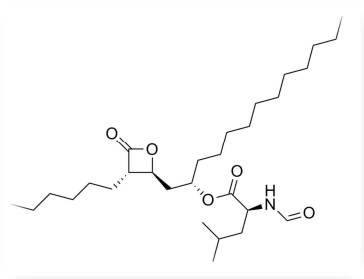

Fig. 1: Chemical structure of orlistat: (S)-((S)-1-((2S, 3S)-3-hexyl-4-oxooxetan-2-yl) tridecan-2-yl) 2-formamido -4-methylpentanoate.

Instrument

JASCO HPLC LC-2000 Plus series, Pump PU-2080, detector UV-2075 plus, Browin software, Agilent ZORBAX SB- C18 (150×4.6mm, 3.5u) column was used.

Selection of Detection wavelength

The detection wavelength was measured by scanning the 10 mg/ml solution of orlistat in methanol, in UV-spectrophotometer, spectra were taken and the wavelength of maximum absorption was selected as 210 nm.

Optimized chromatographic conditions

Chromatographic separation was carried out at room temperature with Agilent ZORBAX SB- C18 (150×4.6mm, 3.5µm) column. Prior to the injection of drug solution, column was equilibrated for 30 min with mobile phase flowing through the system. Mobile phase containing Acetonitrile: Methanol: distilled water in the ratio of the flow rate of mobile phase was maintained at 1.0mL/min and detection was done using UV detector at 210 nm.

Materials and Method

Chemicals and Reagents

Orlistat procured from Shreya pharmaceutical Ltd., Aurangabad. Zero Fat and lipocut tablet procured from local retailer. Orthophosphoric acid is used analytical grade, methanol and acetonitrile used of HPLC grade (Merck Ltd).

Preparation of Sample solution

Twenty Capsules were weighed and finely powdered. Powder equivalent to 60mg Orlistat was accurately weighed into a 100 ml volumetric flask volumetric flask. Add about 60-ml methanol and the mixture was allowed to stand with intermittent sonication for 30 minute to dissolve the drug and then made up to the volume with methanol. Filter the solution through 0.45 nylon membrane filter and working standard solutions of ORL were prepared by suitable dilution of the stock solution with the mobile phase. Ten sets of analyte solution were prepared in the mobile phase containing ORL at a concentration of 10-60 µg/ml for different company capsule dosage form and compare their retention time and Peak area for drug content estimation.

Estimation of ORL in Capsule dosage form

The above prepared solution of orlistat was injected each of the dilutions (20µl) was injected six times in to the column, with flow rate of 1.0 ml/min to obtain chromatogram. From that peak area, the drug content in the capsules was quantified by comparing with calibration curve. Compare the retention time of different brand of capsule and their amount were also compared.

Method Validation: The methods were validated according to International Conference on Harmonization guidelines.

Linearity

The linearity of method is obtained by preparation of calibration curve. The concentrations of analyte were prepared from the stock solution by suitable dilution to obtained desired concentrations for linearity in the range of 10-60 µg/ml.

Figure2. The chromatogram represents the Placebo sample

The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected six times into the column. The calibration curve for ORL was constructed by plotting the mean peak area (Y-axis) versus the concentration (X-axis). It was found to be linear in the concentration range 10-60 µg/ml with good correlation in between concentration and mean peak area, The Regression coefficient was found to be 0.9991 for orlistat.

LOD & LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision. The LOD and LOQ were calculated as

LOD=3.3D/S and LOQ=10D/S

Where D is the standard deviation of the lowest standard concentration and S is the slope of he standard curve. The value of LOD and LOQ are mention in Table 1.

Accuracy by recovery

Accuracy of the method was obtained by performing recovery studies by the standard addition method at different levels of pure standard drug i.e., 80%, 100% and 120% of orlistat to previously analyzed capsule powder sample and mixtures were reanalyzed by the proposed method. From the amount of drug found percentage recovery was calculated. The experiment was conducted in triplicate and results were compared with the expected results and expressed as percentage .The recoveries of orlistat were found to be in the range of 100.04-101.37% , respectively (Table 2).

Table 1: Validation parameters of the HPLC method of Orlistat

Method characteristics	Orlistat
Linearity	10-60 µg/mL
Regression equation	y = 148670x + 94763
Correlation coefficient	0.9991
Theoretical plates	2013
Tailing factor	1.142
LOD (µg/mL)	0.493
LOQ (µg/mL)	1.4929
Precision (RSD, %)	
Intraday (n=3)	0.37
Interday (n=3)	0.498
Repeatability	0.5992
Specificity	No interference of other substance, specific

Precision and repeatability

Precision of the method was performed by intra-day and inter-day studies. For intra-day studies, triplicate of prepared samples of orlistat were analyzed within same day for three different concentration (10, 30, 60 µg/ml). For intra-day studies, triplicate of prepared samples of orlistat were analyzed within same day for three different concentration (10, 30, 60 µg/ml). For inter-day validation, same concentrations of orlistat were determined on three separate days. The % RSD values obtained from peak area for orlistat was observed it should be less than 2% discussing in Table 3.

Table 2: Result of recovery study for Orlistat

Recovery level	Initial amount	Amount added (µg)	Amount recovered (µg)	% Recovery (µg)	SD	%RSD
80	60	48	108.04	100.04	0.431	0.399
100	60	60	121.64	101.37	0.897	0.737
120	60	72	132.95	100.72	0.347	0.261
Average.				100.71	0.465	0.558

n=3

Table 3: Repeatability of orlistat

Concentration (µg/ml)	Area
30	4469864
30	4411854
30	4469598
30	4469688
30	4475102
30	4489254
MEAN	4464227
%RSD	0.5992

Repeatability was determined by applying Orlistat sample six times for each concentration to the HPLC column on the same day. The % RSD values obtained from peak area for Orlistat was observed it should be less than 2% was discussing in Table 3.

Selectivity and specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample matrix for example degradation products, impurities and excipients.

The specificity of the method was established by preparing a placebo solution by following the procedure for the test solution using equivalent weight of the placebo in a portion of the test preparation. Placebo solution was injected into the HPLC system following test conditions, the chromatogram was recorded and measures the responses of the peaks if any. Chromatogram of the placebo has not shown any interference at the retention time of Orlistat. It proves by comparing chromatogram placebo sample (Figure 2) and standard chromatogram (Figure 3).

Robustness

Robustness of the method was determined by analysing standard solutions at normal operating conditions and by changing some operating analytical conditions such as flow rate, pH, and detection wavelength. The conditions

with the variation and the results of variation were mention in Table 5.

System suitability

System suitability was done to verify the repeatability of HPLC method. Theoretical plate, repeatability of retention time and peak area were determined and compared. The result discussed in Table6

Table 6: System suitability parameter

Parameters	Orlistat result	limit
Theoretical plates	2025	N>1500
Repeatability of peak area(n=6)	0.5992	RSD<1%
Repeatability of retention time(Rt) (n=6)	0.4503	RSD<1%

Table 4: Robustness study of Orlistat

Chromatographic changes			
Factor	**Level**	**Assay**	**%Deviation**
Flow rate(ml/min)			
0.8	-0.2	99.03	0.12
1	0	99.15	0
1.2	0.2	100.65	1.51
pH			
2.8	-0.2	100.65	0.7
3.0	0	99.95	0
3.2	0.2	99.05	0.9
Wavelength(nm)			
205	-5	101.86	1.97
210	0	99.89	0
215	5	97.95	1.94

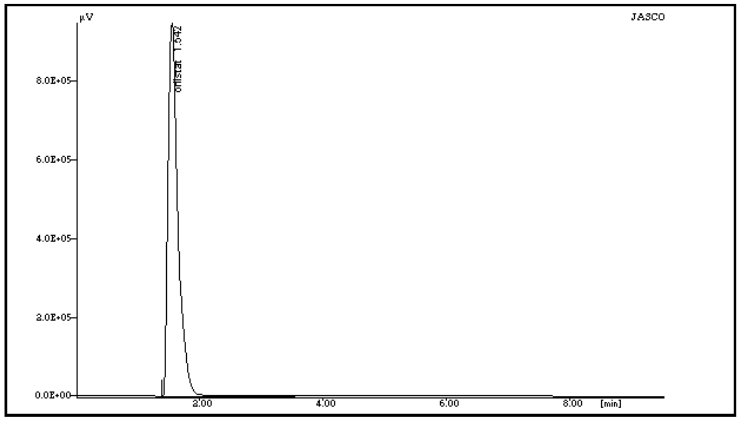

Fig. 3: The chromatogram represents the pure bulk drug of Orlistat

Results and Discussion

The present RP-HPLC method was developed for the quantification orlistat, which can be conveniently employed for routine quality control in bulk and pharmaceutical dosage forms, revealed as simple, rapid, accurate and precise method. The chromatographic conditions were optimized in order to provide a good performance of the assay. A different combination of mobile phases selected on the basis of its polarity different ration of acetonitrile: methanol :distilled water was used finally select mobile phase in the ration (75:15:10) pH 3.0 make with phosphoric acid of mobile phase, These chromatographic conditions gave a retention time of for orlistat was found to be 1.542 min.

A set of six solutions of orlistat at concentrations ranging from 10 to 60µg/ml were prepared. Each sample was analyzed in triplicate; calibration curve was constructed by plotting the peak area against concentration using linear regression analysis. The regression equation was

y= 148670x + 94763 and correlation coefficient was found to be 0.9991 orlistat. Results were shown in Table 1. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive the value of LOD (µg/mL) 0.4932 and value of LOQ (µg/mL) was 1.492 respectively for Orlistat.

The accuracy of the method was determined by recovery studies and the percentage recovery was calculated. The recoveries of Orlistat were found to be in the range of 100.04-101.37 respectively. The methods were specific as none of the excipients interfered with the analyte of interest for this Specificity of the method was checked by injecting the placebo solution, no peaks were found at the retention time of orlistat. Hence, the methods were suitably employed for assaying the commercial Zerofat and lipocut pharmaceutical formulations (Figure 2 for placebo).

For precision study the value of % R.S.D. of at different concentration levels was found to be less than 2%. Orlistat .The obtained %R.S.D. (intra-day precision) values were 0.37 % and % RSD (Inter day) was found to be 0.498 % for orlistat respectively. Repeatability was determined by running minimum of six analyses per sample and evaluating the standard deviation & %RSD for sample by comparing peak area. The value of %RSD

was 0.5992. The robustness of the method was evaluated by deliberately varying the chromatographic conditions of the method such as, flow rate, pH and wavelength. The parameters like tailing factor and retention times showed adherence to the limits. The result of robustness study discuss in (Table 4). The proposed liquid chromatographic method was applied to the determination of orlistat in different brand of capsule dosage form Zerofat and lipocut respectively. The amount of Orlistat in different brand capsule was comparable and result discuss in Table 5.
System suitability was discussed in Table 6 and the result of system suitability indicates that drug was suitable for given developed method.

Conclusion

The proposed RP-HPLC method is simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification.

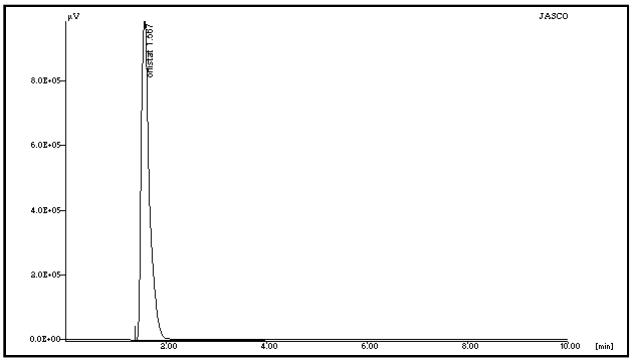

Fig. 4: The chromatogram represents the Orlistat formulation1 (Zerofat).

Moreover the Shorter duration of analysis for Orlistat make these reported methods suitable for routine quantitative analysis in pharmaceutical dosage forms. The recoveries achieved are good by these methods. These method used for comparison of different brand of orlistat capsule dosage form and this method used for comparison of assay and retention time of these formulation.

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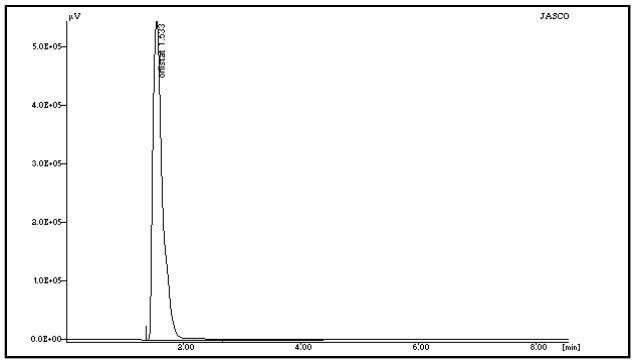

Fig. 5: The chromatogram represent peak of orlistat in formulation 2 (Lipocut capsule).

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