SHORT COMMUNICATION

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ORLISTAT PELLETS 50.0%

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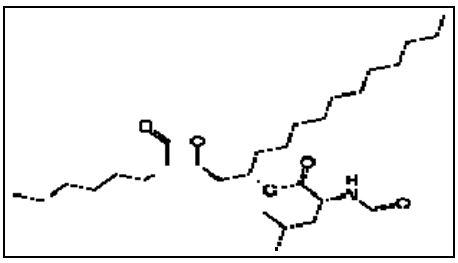
ABSTRACT

A Simple and precise reverse phase high performance liquid chromatographic method has been developed for the determination of Orlistat pellets 50.0%. A Inertsil ODS 3V(5microns,25 x 4.6mm) column, in isocratic mode, with Methanol, Acetonitrile and trifluroaceticacid (82.5:17.5:0.01)as mobile phase. The flow rate is 1ml/minute and effluent is monitored at 210nm.

Keywords: *Orlistat pellets, liquid chromatographic method, Inertsil ODS 3V.*

INTRODUCTION

Orlistat is used for the treatment of obesity. Chemically Orlistat is known as 1-(3-hexyl-4-oxo-oxetan-2-yl)tridecan-2-yl-2-formylamino-4-methyl-pentanoate. Its estimation in commercial dosage form is not reported in any of the pharmacopoeias. A survey of literature reveals that HPLC methods[1,2] are reported for the determination of Orlistat in Quantitative liquid chromatographic–tandem mass spectrometric determination of orlistat in plasma with a quadrupole ion trap. The effect of orlistat on the pharmacokinetics of phenytoin in healthy volunteers also reported. How ever there is no HPLC method reported for its estimation in commercial dosage form. Hence a reverse phase HPLC method for the determination of Orlistat in pharmaceutical solid dosage forms is described.



Orlistat

EXPERIMENTAL

Instrument:

High performance liquid chromatograph, Shimadzu 2010 Rheodyne injector with 100µl loop LC solution computer based data station is used.

Chemicals and reagents:

Reference standard Orlistat is procured from M/S.Biocon ., Acetonitrile HPLC Grade (make E-merck), Methanol HPLC Grade (make E-merck.) and trifluroacetic acid AR grade (make E-merck)

Stationery phase: Inertsil ODS 3V (5microns,25cm x 4.6mm).

Mobile phase preparation:
Prepare filtered and degassed solutions containing a mixture of Methanol, Acetonitrile and trifluroaceticacid (82.5:17.5:0.01)

Standard Preparation:
Transfer about 50mg of Orlistat WS, accurately weighed ,into a 100 ml volumetric flask add 20ml of methanol sonicated for 10 minutes and diluted with Mobile phase to volume of the 100ml flask. Thoroughly mixed and filtered.

Sample Preparation:
Transfer an accurately weighed quantity of the powdered pellets equivalent to about 50.0mg of orlistat into a 100 ml volumetric flask, 20ml of methanol was added, sonicated for 15 minutes and add 50ml of mobile phase, followed by sonication for 30minutes . Cooled up to room temperature. Made up with mobile phase to the volume.

Chromatographic System:
The liquid chromatograph is equipped with a 210-nm detector and a 4.6 mm X 250 mm-Inertsil ODS Column that containing 5-µm packing. The flow rate is about 1.0ml per minute. Chromatograph the standard preparation and record the peak responses as directed by the procedure: the relative standard deviation for replicate injection is not more than 2.0%
Separately inject equal volumes (about 100µl) of the Standard solution and Test solution into the chromatograph, record the chromatograms, and measure the responses for the major peak .Calculate the quantity in mg , of Orlistat $C_{29}H_{53}NO_5$.
The results are tabulated as follows:

Table-1

Semi formulation	S. N0	Label claim %	Amount estimated %	Percentage label claim(%)	Percentage deviation	SD	RSD
PELLETS	1	50.0	50.8	101.6	(+) 1.6	0.326559	0.3216
	2		50.6	101.2	(+) 1.2		
	3		51.0	102.0	(+)2.0		
	4		50.7	101.4	(+)1.4		
	5		50.6	101.2	(+) 1.2		
	6		50.9	101.8	(+) 1.8		

Calibration :
100µl of the above working standard solutions are injected over a time interval of 15 minutes. Evaluation is performed with UV detector at 210nm.The retention time is found to be around 6.618 minutes. Peak areas are recorded and the calibration graph is obtained by plotting peak areas versus concentration.

Assay :
100µl of standard and sample solutions are injected into an injector of liquid chromatograph. The amount of Orlistat calculated by comparing the peak ratio, with that of the standard.(fig.1).

Recovery studies: To study the linearity, accuracy and precision of proposed method, recovery experiments were carried out. Known quantities of standard at two different levels were added to the pre-analyzed sample, the recovery was estimated to be more than 99%.

System suitability test is applied to a representative chromatogram to check various parameters such as efficiency, resolution and peak tailing. The results obtained are shown in Table II that is in concurrence with the USP requirements[4].

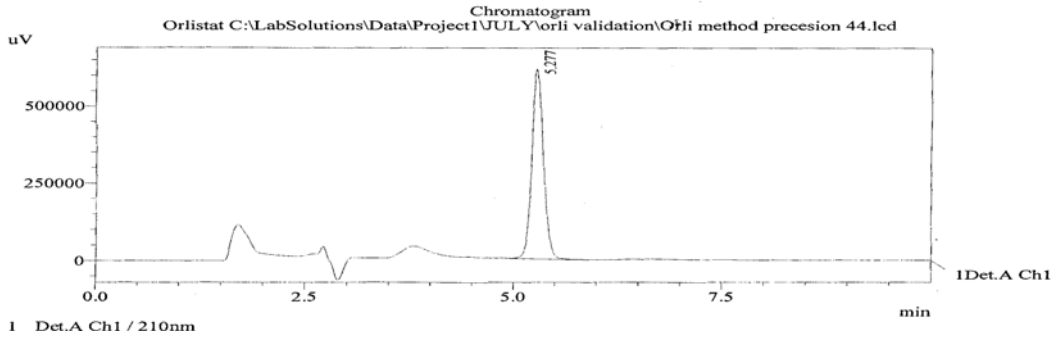


Fig.-1: Chromatogram of sample semi formulation containing Orlistat

RESULTS AND DISCUSSION

Linearity :

The linearity of Orlistat is established by plotting a graph of peak area of standard solutions versus concentration. The linearity is found to be between 100-500µg/ml.

Chromatography :

The mobile phase of a mixture of Methanol, Acetonitrile and trifluroaceticacid (82.5:17.5:0.01)is found to be ideal for analysis of Orlistat .The concentration of Orlistat found to be with in limits and the RSD values are reasonably low.

Table-2

S.NO	Parameter	Orlistat
1	Theoretical plate	6165
2	Tailing factor	1.199
3	RSD Of 6 injection	0.3216

The precision of the method is studied by making 5 injections of standard and very low RSD values indicate good precision. The reproducibility and reliability of the method has been tested by performing recovery studies which showed good results.

CONCLUSION

The proposed method is very simple, rapid and no where involves use of complicated sample preparation. High percentage of recovery shows that the method is free from interferences of the excipients used in the semi formulations. Therefore the method can be useful in routine quality control analysis.

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