A NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF ORLISTAT IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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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 C_{18} column 150 x 4.6 mm i.d, 3.5µm particle size in isocratic mode, with mobile phase comprising of acetonitrile, water and phosphoric acid in the ratio of 85:15:0.5 (v/v/v). The flow rate was 1ml/min and detection was carried out by UV detector at 205nm. The retention time for ORL was found to be 3.79 min. The proposed method has permitted the quantification of ORL over linearity in the range of 6-60µg/ml and its percentage recovery was found to be 99.78-100.27%. The % RSD of intra day and inter day precision were found 0.49% and 0.57%, respectively.

Key words: Orlistat, HPLC, Isocratic, validation.

Introduction

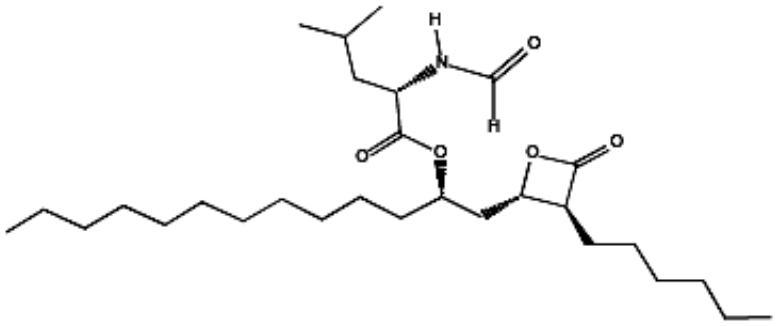

Fig.1.Chemical structure of Orlistat

ORL is a lipase inhibitor for obesity management that acts by inhibiting the absorption of dietary fats. Chemically, ORL is (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[(2S, 3S)-3-hexyl-4-oxo-2-oxetanyl] methyl]-dodecyl ester with empirical formula of $C_{29}H_{53}NO_5$ and molecular weight of 495.7. The chemical structure of ORL was shown in fig.1. Its primary function is preventing the absorption of fats from the human diet, thereby reducing caloric intake [1]. It is intended for use in conjunction with a physician-supervised reduced-calorie diet. ORL is the saturated derivative of lipstatin a potent natural inhibitor of pancreatic lipases isolated from the bacterium *Streptomyces toxytricini*. However, due to simplicity and stability, ORL rather than lipstatin was developed into an anti-obesity drug. Literature review reveals that very few analytical methods were evoked for the estimation of ORL in human plasma

by modern analytical instrument like LC-MS/MS [2, 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 pharmaceutical dosage forms.

EXPERIMENTAL

Reagents& Materials

Pure standard of ORL (99.87%) was obtained as gift sample from Inventis drug delivery systems Pvt. ltd, Hyderabad along with certificate of analysis (COA). HPLC grade Acetonitrile (Qualigens), HPLC grade water, H_3PO_4 (Qualigens), Obelit capsules (Intas Pharmaceuticals), Electronic analytical balance (DONA), Micro pipette (In labs, 10-100µl) were employed in the study. All the glassware employed in the work cleaned with hot water followed acetic anhydride then acetone and dried in hot air oven when ever required. Working environment was maintained in between 18-22^{0}c. How ever, the chemical structure and purity of the sample obtained were confirmed by TLC, IR, melting point, DSC, and XRD studies.

HPLC Apparatus and chromatographic conditions

The HPLC system (Shimadzu co, Tokyo, Japan) consisted of a Shimadzu model LC-10 ATVp, A Shimadzu model SPD-6AV variable wavelength detector (Possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 205nm), A Shimadzu model C-R5A chromatograph integrator module (chart speed at 10mm/min and an attenuation 0), A Shimadzu model SIL-6A auto injector and a Shimadzu module SCL-6A system controller. Isocratic elution of mobile phase comprising of acetonitrile, water and phosphoric acid in the ratio of 85:15:0.5 (v/v/v) with flow rate of 1.0 ml/min was performed on C_{18} ODS analytical column (thermo hypresil, 3.5µm; 150x4.6mm i.d with C_{18} insert (100 A^o, waters limited) as pre column to protect the analytical column from strongly bonded material. Integration of the detector out put was performed using the Shimadzu class Vp soft ware to determine the peak area. They were filtered through 0.45 µm membrane filter and degassed by sonication before use. The flow rate of mobile phase was optimized to 1.0 ml / min which yield a column back pressure of 85-87 kg/cm^2. The run time was set at 10 min and column temperature was maintained at ambient. The volume of injection was 20 µl, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluent was detected at 205 nm.

Procedure recommended

Preparation of mobile phase

Acetonitrile, water and phosphoric acid in the ratio of 85:15:0.5 (v/v/v) were employed as a mobile phase.

Preparation of stock solution of ORL

A stock solution was prepared by dissolving 60mg of standard ORL in a 100 ml volumetric flask containing 70 ml of methanol (HPLC grade) and sonicated for about 15 min and the volume made to the mark with methanol. Daily 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 6-60 µg/ml and each of the these dilutions (20µl) was injected six times in to the column, with flow rate of 1.0 ml/min and peak area of each of the drug concentrations, retention times were recorded.

Construction of linearity

The concentrations of analyte were prepared from the stock solution by taking suitable volume (0.1 - 1 ml) and diluted up to 10 ml to get the desired concentrations for linearity in the range of 6-60 µg/ml. 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) against the concentration (X-axis). It was found to be linear in the concentration range 6-60 µg/ml with good correlation in between concentration and mean peak area.

Estimation of ORL in Capsule dosage form

20 Capsules were weighed and the contents were removed to obtain the average weight powder. A sample of the powder claimed to contain 60 mg of active ingredient, was mixed with 70 ml of methanol. The mixture was allowed to stand with intermittent sonication to ensure complete solubility of drug. Further the resulting solution was passed through 0.45 µm membrane filter followed by adding of methanol to obtain a stock solution of 0.6mg/ml. An aliquot of this solution (1 ml) was transferred to a volumetric

flask and made up to a sufficient volume with mobile phase to get desired concentration of 60 µg/ml. The prepared dilution was injected six times in to the column to obtain chromatogram. From that peak area, the drug content in the capsules was quantified.

RESULTS

The present RP – HPLC method for the quantification of ORL in bulk and pharmaceutical dosage forms, revealed as simple, rapid, accurate and precise method with significant shorter retention time of 3.79 min. The linearity for the detection of ORL was 6-60µg/ml with (R^2=0.996; y = 48.55x-4) the coefficients of variation based on mean peak area for six replicate injections were found to be 0.04-0.43. Results were shown in table-1 and statistical data of calibration curves were shown in table- 2. The intraday and inter-day variations of the method were determined using five replicate injections of three concentrations and analysed on the same day and three different days over a period of two weeks. The result revealed the precision with %RSD of 0.49% and 0.57%, respectively for intra day and inter day. Results were shown in table-3. To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre-analysed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting the solution about five times, at three different concentrations equivalent to 80, 100, and 120% of the active ingredient, by adding a known amount of ORL standard to a sample of known concentration and calculating the recovery of ORL with RSD (%), and % recovery for each concentration. The mean % recoveries were in between 99.78-100.27% and were given in table- 4. The assay for the marketed tablets (OBELIT) was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 99.96 of the labeled claim and no interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results were shown in table-5. To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factor, theoretical plates, limit of detection and limit of quantification and the values were shown in table-6. Ruggedness of the method (intermediate precision) was estimated by preparing six dilutions of the ORL as per the proposed method and each dilution injected in to column. The results were shown in table-7. Robustness of the proposed method was estimated by changing mobile phase composition from acetonitrile: water: phosphoric acid 85:15: 0.5 (v/v/v) to acetonitrile: water: phosphoric acid 90:10:0.5 (v/v/v), changing the flow rate from 1 ml to 1.2 ml/min, changing the temperature ($\pm 5^0$c) and changing the wave length (±5nm) and system suitability parameters were found to be within acceptable limits. Results were shown in table- 8 and indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions. The ruggedness and robustness for the method was performed as per ICH guidelines. Limits of Detection (LOD) and Quantification (LOQ)**,** the limits of detection and quantitation were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$. The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method. The typical chromatograms of ORL standard and tablet dosage form were shown in figure 2, 3.

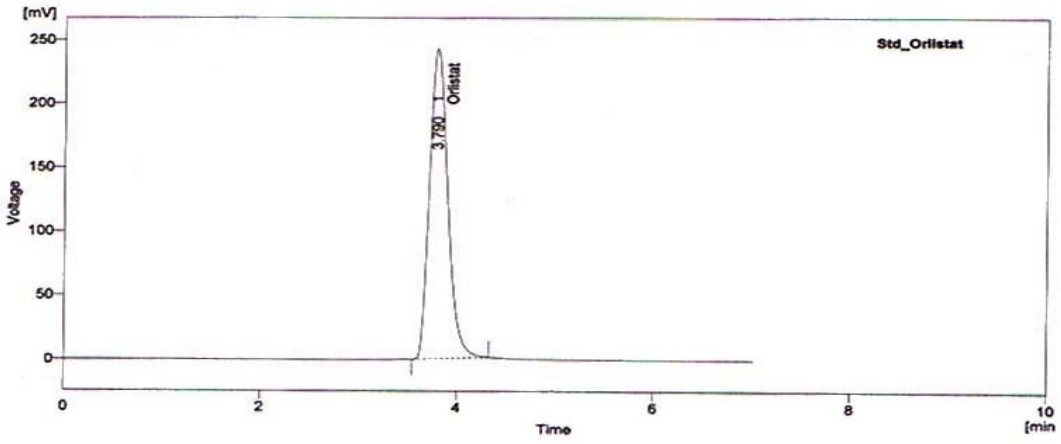

Fig.1. A typical Chromatogram of ORL

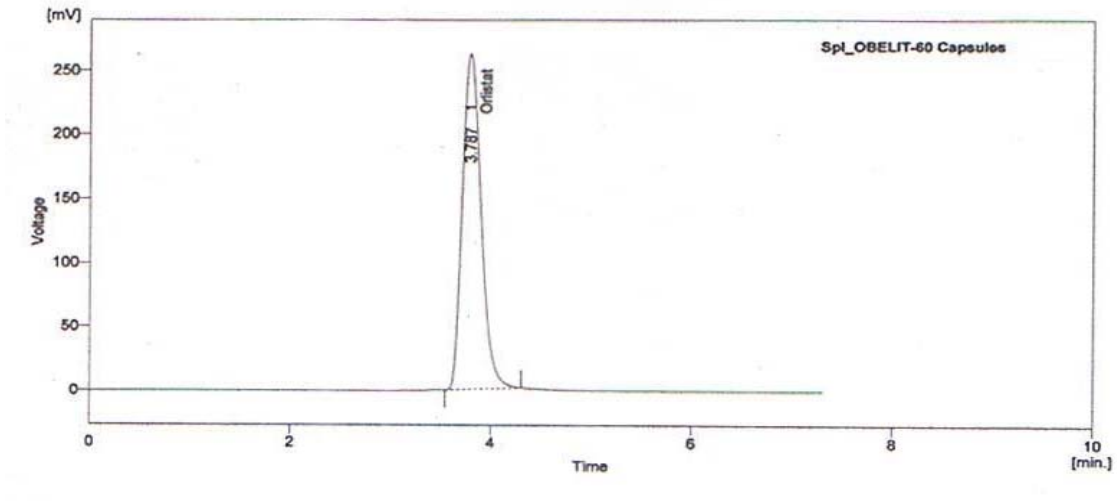

Fig.2. A typical Chromatogram of ORL Capsule

Discussions

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and drug products. The goal of this study was to develop and validate a RP-HPLC method for the estimation of ORL in bulk and pharmaceutical commercial preparations. The main objective of method development was to determine the drug content present in the formulation and its % purity. The chromatographic conditions like mobile phase composition, flow rate was optimized and the method was developed, validated success fully. The selected mobile phase system gave a single sharp peak without interfering peaks. Initial development of the method various mobile phase phases were tried to get sharp peak, finally acetonitrile, water, phosphoric acid in the ratio of 80:15:0.5 (v/v/v) was selected which gave a single sharp peak with retention of 3.79 and tailing factor 1.6. Commercial marketed formulation of ORL was analysed for its contents and % of content was calculated. The proposed method was found to be simple, rapid, economic and accurate and the method was applicable to routine laboratory analysis. The method was validated statistically for various parameters like standard deviation, % relative standard deviation, slope and intercept. The proposed method was following linearity in the concentration range of 6-60µg/ml and obeys the beers lamberts law and above 60 µg/ml the linear plot showing deviation from beers law. Every concentration was injected in to chromatographic system about six times and peak areas were noted. Greater reproducibility was obtained for calibration plots and it was determined by calculating the slope, intercept and %RSD for each standard plot. The method was found to be robust as there was no significant change in the peak area and retention time. The system suitability tests were performed to asses the quality performance of the method. The method was found to be more specific, robust and rugged and most suitable for routine analysis.

Table 1: Concentration Vs Mean Peak area of ORL

Concentration (µg / ml)	Mean peak area*	%RSD
6	294	0.43
12	595	0.21
18	863	0.13
24	1120	0.08
30	1553	0.06
36	1720	0.07
42	1936	0.07
48	2271	0.067
54	2687	0.05
60	2940	0.04

*Mean of six values, $y = 48.55x - 4$, $R^2 = 0.996$.

Table 2: Statistical Data of Calibration Curves of ORL

Parameters	**ORL**
Linearity	6—60µg/ml
Regression equation	48.55x – 4
Standard deviation of slope	0.018
Relative standard deviation of slope (%)	0.037
Standard deviation of intercept	0.158
Correlation coefficient (r^2)	0.996

Table 3: Precision of method

Drug	Concentration (µg/ml)	Observed Concentration (n=5)*			
		Intra day	%RSD	Inter day	% RSD
ORL	6	6.02	0.49	5.98	0.57
	12	11.98	0.25	12.02	0.23
	18	18.02	0.12	17.96	0.15

*Mean of five values

Table 4: Recovery Studies of method

Initial amount of Drug	Amount Added (mg)	Amount Present (mg)	Mean amount found(n=5)*	Mean % recovery
6	8	14.00	13.97±0.21	99.78
6	10	16.00	16.03±0.13	100.18
6	12	18.00	18.05±0.11	100.27

*Mean of five values

Table 5: Estimation of amount of ORL

Brand name of Capsule	Label claim (mg)	Amount estimated(mg)	Mean (±S.d.)	Mean(±S.d)%labeled amount
Obelit	60	59.98	59.98±0.067	99.96±0.04

*Mean of five values

Table 6: system suitability Parameters

Parameter	ORL
Retention time(Min)	3.790
Theoretical Plates	4748
Tailing factor	1.6
Linearity Range (µg/ml)	6-60
Limit of Detection (LOD) (mg /ml)	0.054
Limit of Quantitation (LOQ) (mg /ml)	0.182
Relative standard deviation (RSD)	0.55

Table 7: Ruggedness of method

S.N0	Labeled claim(mg)	Amount estimated*(mg)	Mean ±S.d	%RSD
Set-1	60	60.03	60.03±0.05	0.08
Set-2	60	59.97	59.97±0.04	0.06

*Mean of six values

Table.8: Robustness of method

Parameter	**Variation**	**System Suitability**		
		Theoretical Plates	**Tailing factor**	**% RSD**
Standard	-	4748	0.9	0.23
Flow rate	1-1.2ml	3642	0.85	0.15
Wave Length	-5nm	5565	0.73	0.21
	+5nm	6532	0.72	0.1
Mobile Phase	85:15:0.5 to 90:10:0.5	3123	0.99	0.32
Temperature	-5°C	3346	0.9	0.18
	+5°C	3459	1.1	0.32

Conclusion

The proposed method was simple, accurate and sensitive RP-HPLC method for the estimation of ORL in bulk and Pharmaceutical dosage forms.

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