Chapter VI

Orlistat

6.1. REVIEW OF LITERATURE OF ORLISTAT

1. Patrick K. Bennett et al reported a rapid, sensitive and specific LC/MS/MS/ESI method in SRM mode to quantify tetrahydrolipostatin (Orlistat, Ro 18-0647) in human plasma by a preliminary plasma protein precipitation followed by a simple, one-step liquid-liquid extraction to isolate Ro 18-0647 and its penta deuterated internal standard, Ro 18-0647-d_5, from the biological matrix. Separation achieved using a 2 mm i.d. ×50 mm Deltabond Phenyl column using acetonitrile-2 mM ammonium acetate (90:10) with retention time of the analyte was 1.2 min and the calibration graphs were linear from 0.20 to 10 ng/mL.
2. Ray Wieboldt et al reported the cross-validation of an LC–MS–MS quantitative method previously validated on a triple Quadrupole mass spectrometer. The method has been refined previously on a triple Quadrupole instrument to provide rapid sample throughput with robust reproducibility at sub nano gram detection limits. Optimization of the method on the ion trap required improved chromatographic separation of Orlistat from interfering plasma matrix components co extracted during the initial liquid–liquid extraction of plasma samples. The ion trap produces full-scan collision-induced dissociation mass spectra containing characteristic Orlistat fragment ions that are useful for Quantitation. Data collection on the ion trap required a precursor ion isolation width of 3.0 Da and optimal quantitative results were obtained when three fragment ions were monitored with a 1.8 Da window for each ion. Although a direct cross-validation between the ion trap and the tandem triple Quadrupole mass spectrometer was not possible, quantitative results for Orlistat comparable

to those obtained from the triple quadrupole instrument were achieved by the ion trap with the modified method. The limit of Quantitation for Orlistat in plasma on the ion trap was 0.3 ng mL^{-1} with a linear dynamic range of 0.3 to 10 ng mL^{-1}. Precision and accuracy varied from 4 to 15% over the Quantitation range. The overall results provide an example of the utility of an ion trap in bio analytical work.

3. Mohammadi. A et al reported a stability-indicating HPLC method for the quantitative determination of Orlistat in capsule dosage forms. An isocratic separation was achieved using a mobile phase consisted of methanol: acetonitrile: trifluoroacetic acid (82.5:17.5:0.01, v/v/v) on Perfectsil target ODS-3, 250 mm × 4.6 mm i.d., 5 µm particle size column with a flow rate of 0.7 mL/min by a UV detector to monitor the eluate at 210 nm. The method was linear over the concentration range of 0.02–0.75 mg/mL (r = 0.9998) with a limit of detection and Quantitation 0.006 and 0.02 mg/mL, respectively.

4. Xiao S et al established a method of determining Orlistat in health food by on line HPLC-UV-ESI/MS on an analytical Spherigel C8 column (5 micron, 200 mm x 4.6 mm) with acetonitrile (0.1% formic acid): water (0.1% formic acid) = 80: 20 as mobile phase, the detection wavelength was 2003 mm and exhibiting linearity in the concentration range of 0.3 - 0.6 mg/mL.

5. Effat Souri et al reported a high performance liquid chromatography method with UV detection for determination of Orlistat on a Nova-Pack C_{18} column with an isocratic mobile phase of phosphoric acid 0.1%–acetonitrile (10: 90, v/v) and the UV detection at 205 nm. The method was linear over the range

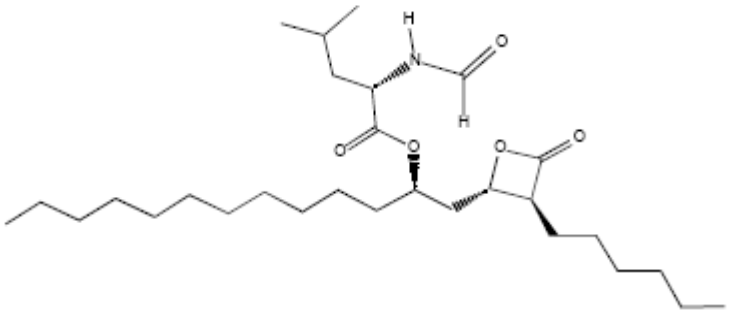
of 10—160 μg/mL Orlistat (r^2>0.9999) and the within-day and between-day precision values were also in the range of 0.10—0.59%.

6.2. DRUG PROFILE OF ORLISTAT

6.2.1. Introduction

Orlistat is a lipase inhibitor for obesity management that acts by inhibiting the absorption of dietary fats and also known as tetrahydrolipstatin. It is a drug designed to treat obesity. Its primary function is preventing the absorption of fats from the human diet, thereby reducing caloric intake. It is intended for use in conjunction with a physician-supervised reduced-calorie diet. Orlistat 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, Orlistat rather than lipstatin was developed into an anti-obesity drug.

6.2.2. Chemistry

Chemical name	Orlistat is (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[(2S, 3S)-3-hexyl-4-oxo-2- oxetanyl] methyl]-dodecyl ester.
Empirical formula	$C_{29}H_{53}NO_5$
Molecular weight	495.7
Chemical structure	 **Fig 6.2.1: Chemical Structure of ORL**
CAS Registry Number	158681-13-1

***6.2.3. Properties*:** It is a single diastereomeric molecule that contains four chiral centers, with a negative optical rotation in ethanol at 529 nm.

6.2.4. Solubility: Orlistat is a white to off-white crystalline powder. Orlistat is practically insoluble in water, freely soluble in chloroform, and very soluble in methanol and ethanol. Orlistat has no pKa within the physiological pH range.

6.2.5. Mechanism of action*:* Orlistat works by inhibiting pancreatic lipase, an enzyme that breaks down triglycerides in the intestine. Without this enzyme, triglycerides from the diet are prevented from being hydrolyzed into absorbable free fatty acids and are excreted undigested. Only trace amounts of Orlistat are absorbed systemically; the primary effect is local lipase inhibition within the GI tract after an oral dose. The primary route of elimination is through the feces.

6.2.6. Contraindications

Orlistat is contraindicated in

- Malabsorption
- Hypersensitivity to Orlistat
- Reduced gallbladder function (e.g. after cholecystectomy)
- Pregnancy and breastfeeding

6.2.7. Use caution with: Obstructed bile duct, Impaired liver function, and Pancreatic disease

6.2.8. Dose : At the standard prescription dose of 120 mg three times daily before meals, Orlistat prevents approximately 30% of dietary fat from being absorbed, and about 25% at the standard over-the-counter dose of 60 mg. higher doses do not produce more potent effects.

6.2.9. Adverse effects

The primary side effects of the drug are gastrointestinal-related like Steatorrhea, it is oily, loose stools, because Orlistat blocks some of the dietary fat from being absorbed, the fat is excreted unchanged in the feces and fecal incontinence, frequent or urgent bowel movements and flatulence.

6.2.10. Precautions

Absorption of fat-soluble vitamins and other fat-soluble nutrients is inhibited by the use of Orlistat. A multivitamin tablet containing vitamins A, D, E, K, and beta-carotene should be taken once a day, at bedtime, when using Orlistat.

6.2.11. Interactions

Orlistat may reduce plasma levels of ciclosporin (also known as "cyclosporin" or "cyclosporine", an immunosuppressive drug frequently used to prevent transplant rejection; the two drugs should therefore not be administered concomitantly, Orlistat can also impair absorption of the antiarrhythmic amiodarone

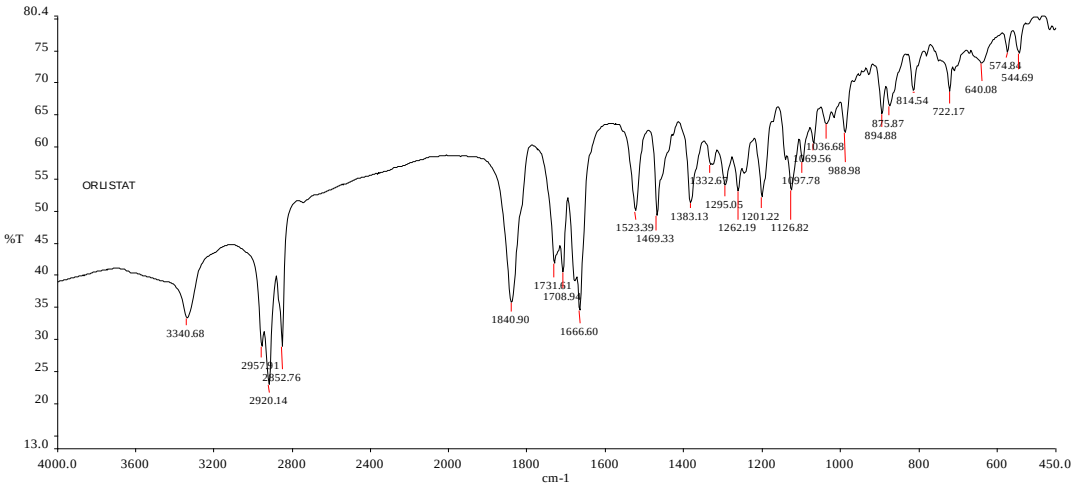
6.2.12. Marketed Available Formulations

Brand Name	Available Dosage Form	Available Strengths	Dosage Frequency
Cobese (Ranbaxy)	Capsule	120mg	
Obelit (intas)	Capsule	120mg	
Orlistatica (torrent)	Capsule	120mg	120mg for adult
Reshape (meyer)	Capsule	60mg,120mg	
Troyslim	Capsule	120mg	
Vyfat	Capsule	120mg	
Zerofat	Capsule	120mg	
Alli	Capsule	60mg	

6.3. AUTHENTICATION OF ORLISTAT

The obtained sample was authenticated by recording the following

- Infra red spectrum
- Thermo gram
- UV Spectrum



Spectrum Name: Orlistat.sp

Description:

Resolution: 4.00 cm-1

Accumulations: 16

Date Created: fri nov 21 13:51:00 2008

Comments:

Analyst: Admin

Fig 6.3.1: Infra red spectrum of ORL

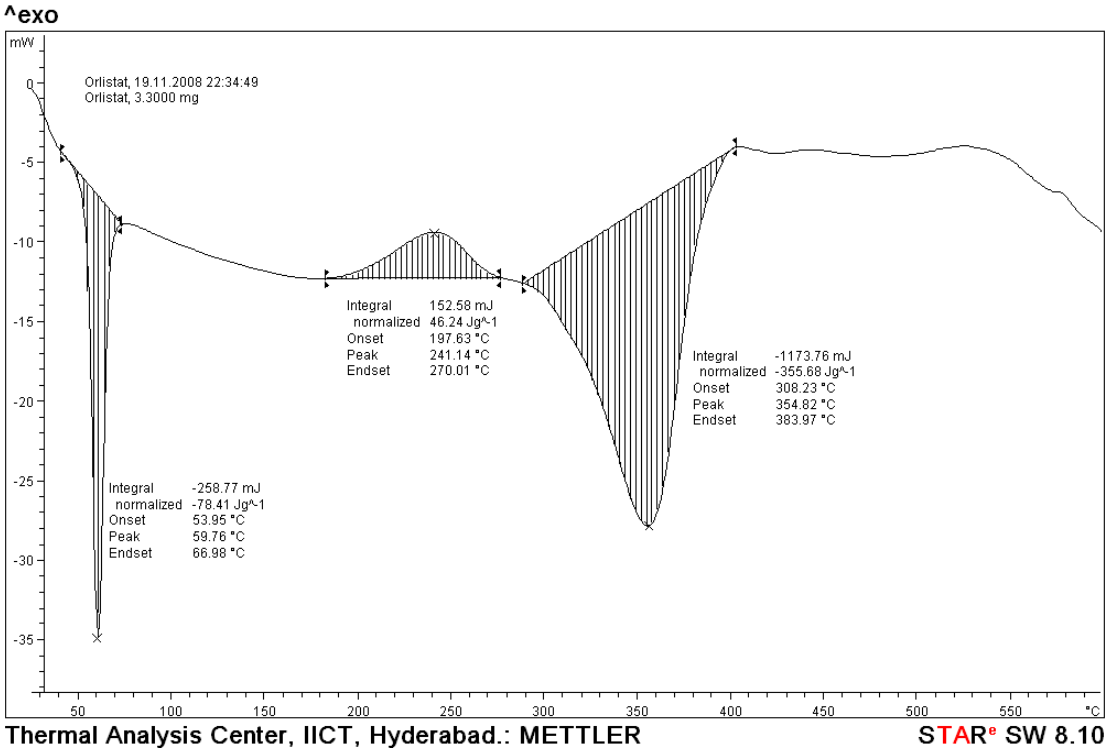


Fig 6.3.2: DSC spectrum of ORL

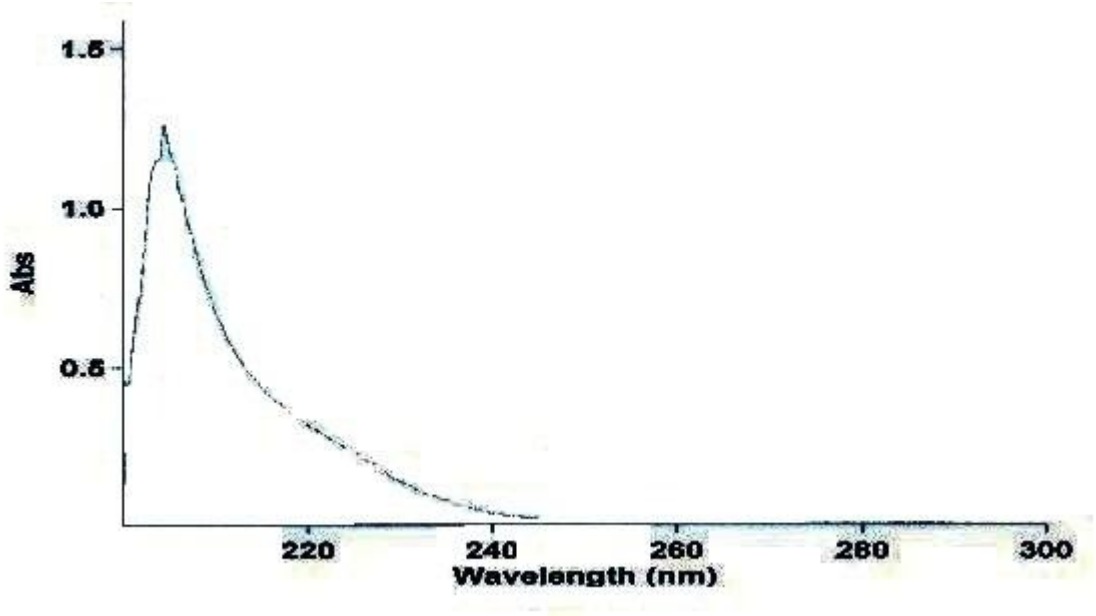


Fig 6.3.3: UV absorption Spectrum of ORL

6.4. A new RP-HPLC method development and validation of Orlistat in bulk and pharmaceutical dosage forms

6.4.1. Introduction

Orlistat (ORL) is a lipase inhibitor for obesity management that acts by inhibiting the absorption of dietary fats. Chemically, it 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. 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 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.

6.4.2. Experimental

6.4.2.1. Materials & Supplies

Pure sample of ORL (Assigned purity 99.87%) was obtained as gift sample from Inventis drug delivery systems Pvt. ltd, Hyderabad along with certificate of analysis (COA). HPLC grade Acetonitrile and water (Qualigens), Phosphoric aicd (Qualigens), Obelit capsules (Intas Pharmaceuticals), Electronic analytical balance (DHONA), 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 when ever required.

However, the chemical structure and purity of the sample obtained were confirmed by IR, DSC studies.

6.4.2.2. HPLC Apparatus and chromatographic conditions

The Shimadzu model HPLC system (Shimadzu co, Tokyo, Japan) consisted of a LC-10 ATVp, SPD-6AV variable wavelength detector (Possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 205nm), C-R5A chromatograph integrator module (chart speed at 10mm/min and attenuation 0), SIL-6A auto injector and 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) pumped from solvent reservoir in to column 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 with flow rate of 1.0 mL/min. Integration of the detector output was performed using the Shimadzu class Vp soft ware to determine the chromatographic parameters. The contents of the mobile phase 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.

6.4.3. Method development

6.4.3.1. Optimization of chromatographic conditions

Initial stages of method development the chromatographic conditions were optimized by performing different trails and the details of chromatographic conditions were shown in table 6.4.1.

Table 6.4.1: Optimized chromatographic conditions

Drug	ORL
Column	C_{18} Column (150 x 4.6 mm i.d, 3.5µm particle size)
Flow rate	1.0 mL/min
UV detection wavelength	205 nm
Mobile phase	Acetonitrile, water and phosphoric acid in the ratio of 850:150:0.05 (v/v).
Column temperature	Ambient
Volume of injection	20 µL
Mode of operation	Isocratic

6.4.3.2. Preparation of mobile phase

HPLC grade solvents of acetonitrile, water and phosphoric acid in the ratio of 85:15:0.5 (v/v/v) were employed as a mobile phase. The contents of the mobile phase were filtered before use through a 0.45 µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1.0 mL/min.

6.4.3.3. Preparation of stock solution of ORL

A stock solution was prepared by dissolving accurately weighed quantity of 60 mg of 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 to obtain the concentration of 0.6 mg/mL. 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, and the peak area, retention times were recorded.

6.4.3.4. Construction of linearity

Ten sets of calibration standards were prepared by suitable dilution of stock solution to get concentrations in the linear 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) and found to be linear in the concentration range 6-60 µg/mL with good correlation in between concentration and mean peak area.

6.4.3.5. 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 and allowed to stand with intermittent sonication to ensure complete solubility of drug. Further the resulting solution was passed through 0.45 µm membrane filter and diluted with methanol to obtain a concentration of 0.6mg/mL. An aliquot of this solution (1 mL) was transferred in to a 10 mL 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 record peak area and retention times. The typical chromatograms of ORL were shown in fig 6.4.1 and fig 6.4.2. From that peak area, the drug content in the capsules was quantified.

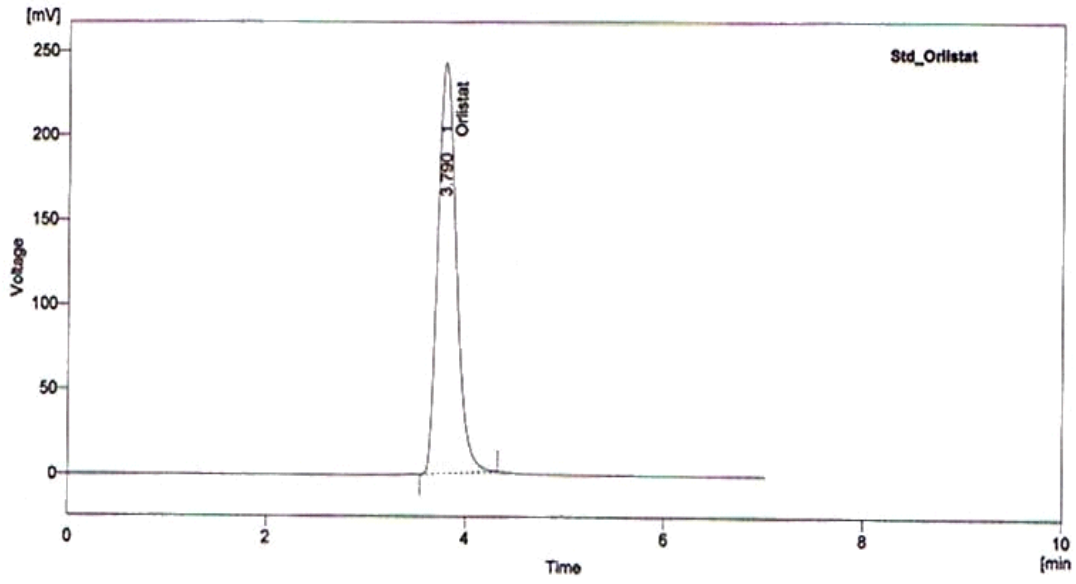


Fig 6.4.1: A typical Chromatogram of ORL

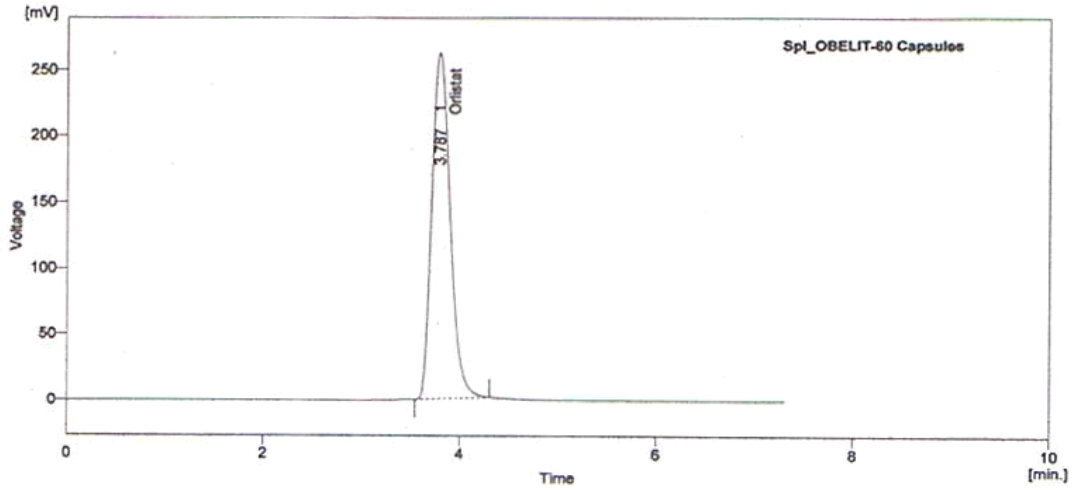


Fig 6.4.2: A typical Chromatogram of ORL Capsule

6.4.4. Method validation

6.4.4.1. Linearity

The linearity for the detection of ORL was 6-60µg/mL with (R^2=0.9996; y = 48.555x-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

6.4.2 and statistical data of calibration curves were shown in table 6.4.3. The calibration curve was shown in fig 6.4.3.

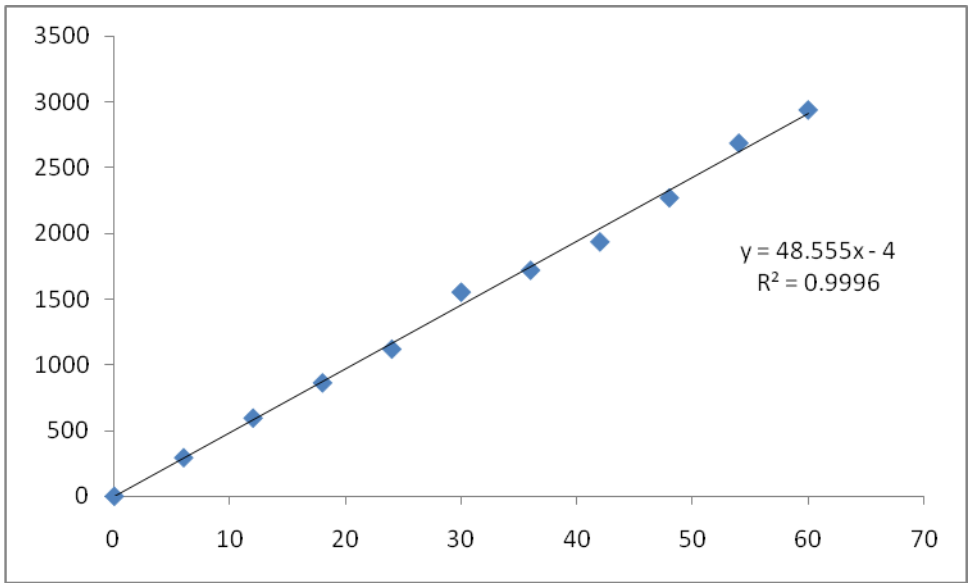


Fig 6.4.3: Calibration curve of ORL

Table 6.4.2: 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, (R^2 = 0.9996, y = 48.55x – 4)

Acceptance Criteria: Correlation Coefficient should not be less than 0.9990

Table 6.4.3: Statistical Data of Calibration Curves of ORL

Parameters	Value
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

6.4.4.2. Precision

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 intraday and inter day less than that of 2.0% indicating the method was more precise. Results were shown in table 6.4.4.

Table 6.4.4: Precision of ORL

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

Acceptance Criteria: RSD should not be more than 1.0%

6.4.4.3. Accuracy

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 those were lying in between the range of 98-102 % indicating that the proposed method was more accurate for the estimation of ORL. The results were given in table 6.4.5.

Table 6.4.5: Recovery of ORL

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

Acceptance Criteria: Recovery should be within 98.0% to 102.0%

6.4.4.4. Assay of ORL

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 6.4.6.

Table 6.4.6: Assay of ORL capsule

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

6.4.4.5. System suitability

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 6.4.7.

Table 6.4.7: system suitability Parameters

Parameter	Value
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

Acceptance Criteria:

1. RSD should not be more than 2.0% for six replicate injections of standard.

2. Tailing for ORL peak is not more than 2.0

3. The column efficiency as determined for ORL peak, Plate Count should be not less than 2000.

6.4.4.6. Ruggedness of ORL

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 in duplicate. The over all RSD less than 2.0% indiacintg the proposed found to be rugged. The results were shown in table 6.4.8.

Table 6.4.8: Ruggedness of ORL

S.NO	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

Acceptance Criteria: Over all RSD should not be more than 2.0%

6.4.4.7. Robustness of ORL

Robustness of the proposed method was estimated by making small chanhes in the exprerimental conditions such as 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 6.4.9 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.

Table 6.4.9: Robustness of ORL

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	-5nm	5565	0.73	0.21
Length	+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

Acceptance Criteria:

1. RSD should not be more than 2.0% for six replicate injections of standard.
2. Tailing for ORL peak is not more than 2.0

3. The column efficiency as determined for ORL peak, Plate Count should be not less than 2000.

6.4.4.8. Detection and quantification limits

Limits of Detection (LOD) and Quantification (LOQ)**,** the limits of detection and quantification 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 = $10\sigma/S$.

6.4.4.9. Specificity

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.

6.4.5: Results and discussion

6.4.5.1. Method development

The development of HPLC methods for the determination of drugs has received greater attention because of their importance in the quality control. The main objective of method development was to determine the drug content present in the formulation and its % purity. The goal of this study was to develop and validate a RP-HPLC method for the estimation of ORL in bulk and pharmaceutical commercial capsule preparations. Initial stages of method development we optimized various chromatographic conditions such as solvent, column, mobile phase composition, flow rate and detection wavelength was optimized and the method was developed, validated success fully. Our experiments and data reported in the literature showed that both the methanol and Acetonitrile could be used an organic modifier in the mobile phase. The use of Acetonitrile as a mobile phase organic modifier resulted in better sensitivity compared to methanol. During method development tests involving the use of mixtures of acetonitrile and different buffer solutions (e.g., Potassium dihydrogen phosphate, potassium phosphate, ammonium acetate) were made to optimize the mobile phase with different pH values and acetonitrile and water, finally acetonitrile, water and phosphoric acid in the

ratio of 85:15:0.5 (v/v/v) has been selected whose mobile phase combination given good peak symmetry, sensitivity, and shorter retention time without interfering peaks. ORL was eluted successfully in the given mobile phase within significant shorter retention time of 3.79 min and gave good single sharp symmetrical peak (tailing factor < 2) without interfering peaks. The typical chromatograms of ORL were shown fig 6.4.1 and fig 6.4.2. The use of hydrophobic stationary phases usually provides adequate retention of organic non polar molecules. The chromatographic separation was achieved using an RP C_{18} column for ORL with symmetrical peak shape. The absorption spectrum of ORL was recorded in methanol and determined detection wavelength as 205 nm. Different flow rates were tested and finally the flow rate of 1 mL/min was optimized. The mode of elution also optimized and isocratic mode was selected than gradient where the elution was achieved using complicated mobile phases. The developed method more advantageous with respect to simplicity, isocratic conditions, shorter run time, low injection volume, less flow rate and inexpensive mobile phases. For the quantification the linearity range was optimized by preparing the solution of ORL in different concentration ranges and finally the range 6-60µg/mL was optimized which follows the beers law. The optimized chromatographic conditions were shown in the table 6.4.1. The proposed method was found to be simple, rapid, economic and accurate and the method was applicable to routine laboratory analysis. The developed was successfully validated as per FDA guidelines.

6.4.5.2. Method validation

The method was validated for various parameters as per FDA guidelines. The linearity of the method was tested by plotting calibration curve using least squares linear regression analysis of concentration and mean peak area. The

method was showing good linearity in the concentration range of 6-60 µg/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 to 0.43. Results were shown in table 6.4.2 and statistical data of calibration curves were shown in table 6.4.3. The calibration curve of ORL was shown in fig 6.4.3.

The intraday and inter day precision studies were performed result revealed the precision with %RSD (0.49% and 0.57%) respectively for intraday and inter day. Results were shown in table 6.4.4. Accuracy studies were performed to know the reliability of the method and the mean % recoveries were in between 99.78-100.27% and were given in table 6.4.5. The method was applied for the estimation of ORL in commercial capsule dosage form having label claim of 60 mg. The average drug content was found to be 99.96% of marketed capsule formulation named OBELIT. The results of assay were shown in table 6.4.6. 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 6.4.7. The method was found be specific, rugged and robust and suitable for routine laboratory analysis.

6.4.6. Summary

A simple, accurate and rapid RP-HPLC method has been developed for the estimation of 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 intraday and inter day precision were found 0.49% and 0.57%, respectively.

6.4.7. Conclusion

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

6.4.8. REFERENCES

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