INTRODUCTION

Orlistat is used to treat obesity. Chemically Orlistat is known as 1-(3-hexyl-4-oxo-oxetan-2-yl)tridecan-2-yl 2-formylamino-4-methylpentanoate. A survey of literature reveals that HPLC methods\textsuperscript{1,2,3} are reported for the determination of HPLC Analysis of Orlistat and Its Application to Drug Quality Control Studies. The effect of orlistat on the pharmacokinetics of phenytoin in healthy volunteers, Orlistat in Quantitative liquid chromatographic–tandem mass spectrometric determination of orlistat in plasma with a quadrupole ion trap. However, there is no HPLC method reported so far 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.

ABSTRACT

In this study, a high performance liquid chromatography method was developed for the determination of orlistat with UV detection. The chromatographic system consisted of an Inertsil ODS 3V (5 microns, 25x4.6mm) column, an isocratic mobile phase of Methanol, Acetonitrile and trifluoroaceticacid (82.4:17.5:0.01). The flow rate is 1ml/minute and effluent is monitored at 210nm. Orlistat was eluted at about 6.0min with no interfering peak from excipients used for preparation of dosage form. The method was linear over the range of 10-150µg/ml orlistat ($r^2>0.9999$). The within-day and between-day precision values were also in the range of 0.182-0.265%. The appropriate dissolution conditions were also determined and applied to evaluate the dissolution profile of orlistat pellets. Based on the stability and basic nature of the drug, dissolution experiments were conducted in 1000ml of 0.1N Hydrochloric acid with 2% sodium lauryl sulphate with paddle stirring at 100 rotations per minute (rpm). Dissolution was found to be not less than 75% in 45. Dissolution medium and paddle at 100 rotation per minute. The proposed method was applied successfully to the determination of orlistat content in pellets and for in vitro dissolution studies.

Key words: Drug release, HPLC, orlistat pellets 50%.

METHOD

Instruments

High performance liquid chromatograph, Shimadzu 2010 Rheodyne injector with 100µl loop LC solution computer based data station.
**Chemicals and reagents**

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

**Stationery Phase**

Inertsil ODS 3V (5microns, 25cm × 4.6mm).

**Mobile phase preparation**

Prepare filtered and degassed solutions containing a mixture of Methanol, Acetonitrile and trifluoroacetic acid (82.5:17.5:0.01)

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**Drug release**

**Apparatus 2; 100 rpm**

**Medium**

0.1N hydrochloric acid containing 2% sodium lauryl sulphate

**Time: 45 minutes**

Determine the amount of C_{29}H_{53}NO_{5} dissolved, using the following method. Medium-0.1N Hydrochloric acid containing 2% sodium lauryl sulphate-Transfer an accurately 8.3mL of hydrochloric acid in 1000mL water, add 20g of sodium lauryl sulphate and mix.

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

**Mobile phase preparation**

Transfer about 50mg of Orlistat WS, accurately weighed, to a 100 ml volumetric flask add 20ml of methanol, sonication for 10 minutes and dilute with Medium to volume. Mix and filter. Transfer 2.0ml of this solution to a 10 ml volumetric flask, dilute with medium to the volume and mix.

**Chromatographic System**

The liquid chromatograph is equipped with a 210-nm detector and a 4.6 mm X 250 mm-Inertsil ODS Column that contains 5-µm packing. The flow rate is about 1.0 mL per minute. Chromatograph the standard preparation and the record the peak responses as directed for procedure: the relative standard deviation for replicate injection is not more than 2.0%

**Procedure**

Separately inject equal volumes (about 100µl) of the Standard solution and Test solution into the chromatograph, record the chromatograms, and measure the peak responses. Determine the amount of Orlistat C_{29}H_{53}NO_{5} dissolved.

**Calibration**

100µl of the above working standard
solutions are injected at a time interval of 15 minutes. Evaluation is performed with UV detector at 210nm. The retention time is found to be around 5.305 minutes. Orlistat. Peak areas are recorded and the calibration graph is obtained by plotting peak areas versus concentration.

**Dissolution**

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%.

**RESULTS AND DISCUSSION**

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 requirements4.

**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 10-150µg/ml.

<table>
<thead>
<tr>
<th>Semi formulation</th>
<th>Media</th>
<th>BOWL NOS</th>
<th>% Release</th>
<th>Average</th>
<th>Limits</th>
<th>SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td></td>
<td>1</td>
<td>84.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>2</td>
<td>84.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>1000ml of 2% SLS</td>
<td>3</td>
<td>83.88</td>
<td>84.28</td>
<td>75.0% release</td>
<td>0.297</td>
<td>0.349</td>
</tr>
<tr>
<td>E</td>
<td>in 0.1 N</td>
<td>4</td>
<td>84.53</td>
<td>83.86</td>
<td>in 45 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Hydrochloric acid</td>
<td>5</td>
<td>84.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Table 1: The results are tabulated as follows**

**Table 2**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Tamsulosin HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Theoretical plate</td>
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<tr>
<td>2</td>
<td>Tailing factor</td>
<td>1.054</td>
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<tr>
<td>3</td>
<td>RSD of 6 injection</td>
<td>0.349</td>
</tr>
</tbody>
</table>

**Chromatography**

The mobile phase of mobile phase of Methanol, Acetonitrile and trifluoroacetic acid (82.4:17.5:0.01) is found to be ideal for analysis of Orlistat. The concentration of Orlistat found to be within limits and the RSD values are reasonably low.

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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