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Stability indicating reverse phase HPLC analytical method development and validation for quantitative determination of orlistat in canola oil

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ABSTRACT

A rapid, linear, sensitive and specific reverse phase HPLC method was developed and validated to quantify Orlistat in canola oil. Method was developed by using different solvents of different ratios with temperature to enhance the miscibility of oil and solubility of drug. Complete extraction of drug from oil is achieved and can be detected with the analytical run time of approximately 12min. The method has been found to be linear and shows stability of orlistat. A method with isocratic separation was achieved using a C-18, 150mm×4.6mm i.d.,5µm particle size column with a flow rate of 2.0mL/min with a UV detector to monitor the elute at 210nm. The mobile phase consisted of buffer (0.1% ortho phosphoric acid): acetonitrile.

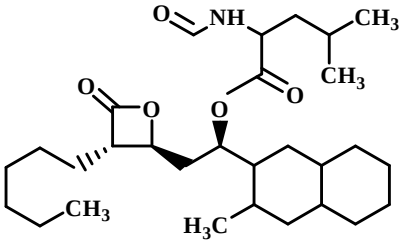
KEYWORDS

Orlistat;
Canola oil;
HPLC;
UV detector;
API (Active pharmaceutical ingredient)

INTRODUCTION

Orlistat is a lipase inhibitor for obesity management that acts by inhibiting the absorption of dietary fats of approximately 30% of fatty components from the diet[1] Orlistat is (S)-2- formylamino-4-methyl-pentanoic acid (S)-1-[[(2S, 3S)-3-hexyl-4-oxo-2-oxetanyl] methyl]-dodecyl ester (Structure 1) having empirical formula of $C_{29}H_{53}NO_5$, and its molecular weight is 495.7[2]. It is a single diastereomeric molecule having four chiral centers. 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.

The major challenge involved in the method development was developing reverse phase chromatographic condition and the drug extraction from the vehicle used for the formulation that is insoluble in all aqoues media which is canola oil, an aliphatic acid and hydrophobic in nature.



Structure 1: Structure of orlistat (MW = 495.735g/mol)

A new approach for method development was made by using the methanol in combination with temperature. The drug extraction was found 100% with

appropriate peak symmetry and complete baseline.

EXPERIMENTAL

Chemicals and reagents

Orlistat standard was provided by Ranbaxy Research Laboratories (Gurgaon, Haryana). Orlistat solution in canola oil was prepared by in house in various mg/ml concentrations.

Ortho-phosphoric acid (85 % w/w, Sd-fine-chem)

Acetonitrile (Merck)

Methanol (S d-fine-chem/Qualigence)

Solvents and chemicals were of HPLC and analytical grade respectively and used as received. Purified HPLC grade water was obtained by reverse osmosis and filtration through a Milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all solutions.

HPLC instrumentation and conditions

The HPLC system consisted of a Waters® 2965 controller solvent delivery module (Waters Chromatography Division, Milford, MA, USA), a Waters autosampler, a solvent degasser, a Waters 2996 PDA detector (Waters Chromatography Division, Milford, MA, USA). A Empower® version 3.0, Chromatography Division, Milford, MA, USA) Chromatographic software used to record and evaluates the data collected during chromatographic analysis. The chromatographic separation was performed using a kromosil C-18, 150mm×4.6mm i.d., 5µm particle size column with a flow rate of 2.0 ml/min and using a UV detector to monitor the elute at 210nm. The mobile phase consisted of buffer (0.1% ortho phosphoric acid): acetonitrile in the ratio of 10:90(v/v). The diluent used for sample preparation was methanol. The elute was monitored using UV detection at a wavelength of 210nm. The column was maintained at 30°C temperature and an injection volume of 50µL was used. The mobile phase was filtered through 0.45µ membrane and degas prior to use.

HPLC method development and optimization

Method development has been started by selecting the suitable diluent for miscibility of the canola oil and solubility of the orlistat as well, for the complete extraction of drug from canola oil.

The miscibility of canola oil was initially analyzed by using methanol, acetonitrile, buffer methanol and in there different combination the oil was found to be immiscible in all. Orlistat is found to be soluble in methanol and acetonitrile. Solution mixture of orlistat and canola oil was also checked for miscibility by using THF, acetonitrile, methanol and chloroform. Both of them are found soluble in chloroform. (Above Results are summarized in the TABLE 1)

It was concluded that reverse phase method development was not possible under all the above conditions.

Extraction with hexane was carried out by taking drug in canola oil and mixing with 50ml of hexane and 100ml of Acetonitrile in separating funnel. Two layers were formed. Acetonitrile layer has been injected after filtering with an APFB1.0µm + PTFE 0.45µm glass fiber filter .The extraction of drug was found to be 90%.

Addition of 2ml of alkali (0.01N NaOH) and 2ml of acid (0.01N HCl) was done separately during extraction to see the effect of acidic and basic environment on solubility of oil and extraction of drug but it has been found that there is no role of pH in extraction of drug.

A new approach for method development was

TABLE 1 : Solubilityof orlistat and canola oil mixture in different solvents

S.no	Experiment	Canola oil	Orlistat
1	Canola oil + Methanol	Immiscible	-
2	Canola oil + Acetonitrile	Immiscible	-
3	Canola oil + Methanol :Buffer (50: 50)	Immiscible	-
4	Canola oil + Acetonitrile :Buffer (50: 50)	Immiscible	-
5	Orlistat + Hexane	-	Insoluble
6	Orlistat + Methanol	-	Soluble
7	Orlistat + Acetonitrile	-	Soluble*
8	Canola oil + Orlistat + THF	Miscible	Insoluble
9	Canola oil + Acetonitrile +Orlistat + THF	Immiscible	Poor extraction
10	Canola oil +methanol +Orlistat + THF	Immiscible	-
11	Canola oil + Orlistat + Chloroform	Miscible	Soluble
12	Canola oil + Acetonitrile +Orlistat	Immiscible	Poor extraction

***Solubility in methanol is found to be better than in acetonitrile**

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made by using methanol in combination with temperature. The sample is prepared by adding orlistat and canola oil with methanol, sonicated at 40-50ºC for 5 minute, allowed to cool, filtered with an APFB1.0µm + PTFE 0.45µm glass fiber filter after attaining the room temperature and injected.

A novel sorbent material is used for the determination of orlistat is kromosil with porous, spherical, silica-based HPLC packing material for analytical chromatography. (Kromasil packings provided with novel state of art bonding and end capping technology that is characterized by excellent chemical and mechanical stability and provides excellent peak symmetry for analytes while also presenting excellent properties of chemical purity, stability, surface properties, pore size and pore uniformity. Kromasil silica has a smooth surface, combining perfectly spherical silica with a very smooth surface is a great combination when some one looking for silica with a long lasting lifetime

The drug extraction was found 100% with appropriate peak symmetry and complete baseline resolution.

The stability of the stock solution was determined by quantification of orlistat (heated) in comparison to freshly prepared orlistat (without heating). No significant change was observed in response (Figure 1)

Preparation of standard and sample solutions

1. Preparation of standard solutions

A standard solution of orlistat (0.5mg/ ml) was prepared by accurately weighing approximately 25mg of orlistat working standard in 50ml volumetric flask, added 30ml of diluent, sonicated for 2 minutes and diluted to the volume to get the concentration of 0.5mg/mL solution and filtered through APFB1.0µm + PTFE 0.45µm glass fiber filter.

2. Preparation of sample

Pipette out desired volume of sample solution into a required volumetric flask containing approximately 3/4 volume of methanol, rinse the pipette (3-4 times) with methanol sonicate at 40-50ºC for 5min cool it at room temperature dilute up to the volume with methanol keep at room temperature for 10min, prepare the sample solution to get a concentration of 500µg/mL approximately with diluent and filter through APFB1.0µm + PTFE 0.45µm glass fiber filter.

Validation

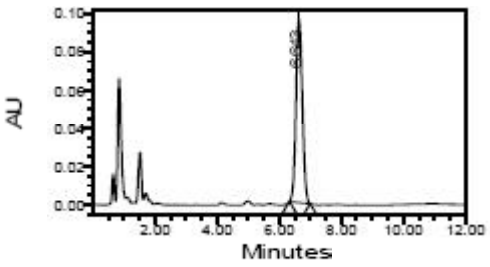
The method was validated with respect to parameters including Linearity, Precision, Stability, Robustness, Ruggedness and Selectivity

1. Linearity

The calibration curves (n = 5) constructed for orlistat were linear over the concentration range of 0.350-0.600mg/ml. Peak areas of orlistat were plotted versus orlistat concentration and linear regression analysis performed on the resultant curve. The correlation coefficients of R = 0.9998, across the concentration range studied were obtained following linear regression analysis.

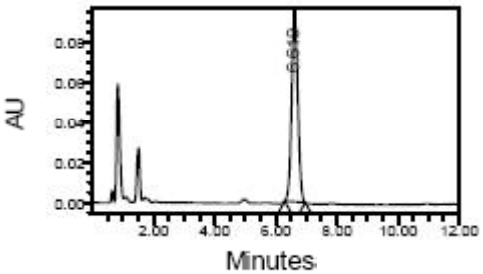
2. Precision

Precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability was investigated by injecting six replicate injection of orlistat standard of same concentration as sample and



	Injection	Name	Retention time (min)	Area (µV*sec)	Purity 1 angle	Purity 1 threshold
1	1		6.64	1369125	2.702	9.575

Orlistat without heating in methanol



	Injection	Name	Retention time (min)	Area (µV*sec)	Purity 1 angle	Purity 1 threshold
1	1		6.62	1313911	1.166	4.682

Orlistat after heating in methanol

Figure 1: Heating effect on orlistat

the %RSD of 0.21 was found . Intra-assay precision data are obtained by repeatedly analyzing, in one laboratory on one day, aliquots of homogeneous sample, each of which independently prepared according to method procedure Sample solution (500µg/ml) was injected in six replicate where the mean concentrations were found to be 99.42%w/w with %RSDs of 0.27.

3. Stability in analytical solution

Stability in analytical solution was assessed by injecting the 0.5mg/ml concentration for 15 hrs with approximately 1hrs delay resulting in associated cumulative RSD of 1.22%.

4. Selectivity

The results of stress testing studies indicated a high degree of selectivity of this method for orlistat and its possible degradation products.

5. Robustness

By changing temperature of column (±5 degree centigrade)

By decreasing the temperature RSD was coming 0.76% and on increasing Temp RSD is found to be 0.97%

Variation in mobile phase composition (±2)

By decreasing the Organic percentage in mobile phase the RSD has been found 1.77% and on increasing RSD has been found to be 1.98%

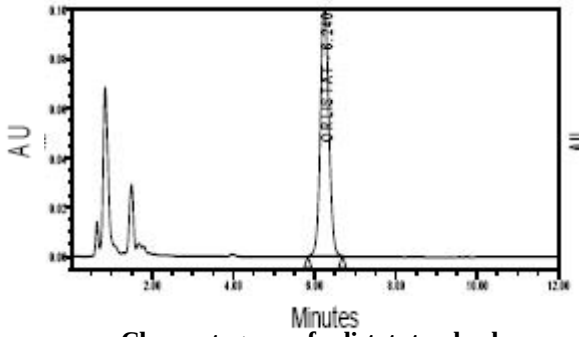
Variation in wavelength of detection (±5)

By decreasing the wavelength the RSD has been found 1.71% and on increasing RSD has been found to be 0.50%.

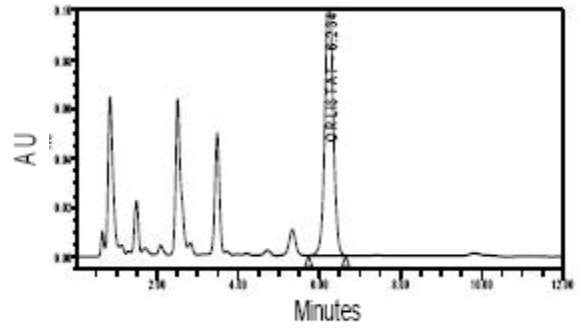
7. Forced degradation studies of sample

In order to determine whether the analytical method and assay were stability-indicating, orlistat in canola oil was prepared and stressed in under various conditions to conduct forced degradation studies. All solutions prepared for use in forced degradation studies were prepared in methanol to yield starting concentrations of orlistat of 0.5 mg/ml and evaluated for peak purity and in all stress condition orlistat peak purity were passes and results are summarized in TABLE 2.

7.1. Oxidation



Chromatogram of orlistat standard



Chromatogram of orlistat in canola oil

Figure 2 : Typical chromatogram of orlistat standard and formulation in canola oil

TABLE 2: Summary of force degradation result

S.no	Stress condition	Time	% of degradation orlistat
1	Acid hydrolysis (1.0N Hydrochloric acid)	Initial	8
2	Alkaline hydrolysis (0.005M Sodium hydroxide)	Initial	45
3	Oxidation (6.0% Hydrogen peroxide)	Initial	12

Solutions for oxidation studies were prepared as described above and add 1mL of 6% H_2O_2 (30% v/v).

7.2. Acid degradation studies

Solutions for acid degradation studies were prepared in methanol and add 1mL of 1M Hydrochloric acid (80:20, v/v).

7.3. Alkali degradation studies

Solutions for alkali degradation studies were prepared in methanol and add 0.5mL of 0.005M, sodium hydroxide (80:20, v/v).

RESULTS AND DISCUSSION

The proposed method was applied to the determination of orlistat in canola oil. Eventually, a mobile phase

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TABLE 3: Results of stability studies

Conc.	Time points	Condition	Assay (%w/w)
1	Initial		105.25
	7 Day	25ºC	107.50
2	Initial		103.08
	7 Day	25ºC	105.04

of buffer (0.1% ortho phosphoric acid : acetonitrile) in the ratio of 10:90 (v/v) with a run time of 12 minutes with a flow rate of 2.0ml/min, Orlistat eluted at about 6 min, provided the best chromatographic response and was used for further studies.

The method was found to be linear over the concentration range of 0.350-0.600mg/mL

A typical chromatogram obtained following the standard assay and formulation depicted in figure 2. The results of the assay indicate that the method is selective for the assay of orlistat in this formulation.

Supporting experiment

The stability studies carried out in-house for one week. The study has been carried out for different mg/mL solution of orlistat in canola oil with four stability time points of 0Hr, 6Hr, 24Hrs, 72Hrs and one week. The Results indicate that the drug is stable. Stability data are shown in TABLE 3.

CONCLUSIONS

To our knowledge no stability-indicating reverse phase analytical method for the determination of orlistat in canola oil has been published. There are few analytical methods of HPLC[3,4] and gas chromatography[5] reported in the literature for the quantitative determination of orlistat. It is not feasible to use these highly sensitive methods for the routine quantitative assay of orlistat in canola oil.

Consequently a HPLC analytical method has been developed for the determination of orlistat in canola oil. The proposed reverse phase HPLC-UV method is simple and linear and has the ability to separate the drug from excipients and can be applied for the quantification of orlistat in canola oil where the content of active pharmaceutical ingredient (API) is high in the formulation. The simplicity of the method allows for application in laboratories that lack of sophisticated analytical instruments such as LC–MS or GC–MS.

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