HPLC Analysis of Orlistat and Its Application to Drug Quality Control Studies

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In this study, a high performance liquid chromatography method with UV detection was developed for determination of orlistat. The chromatographic system consisted of a Nova-Pack C_{18} column, an isocratic mobile phase of phosphoric acid 0.1%–acetonitrile (10 : 90, v/v) and UV detection at 205 nm. Orlistat was eluted at about 6 min with no interfering peak from excipients used for preparation of dosage form. The method was linear over the range of 10—160 μg/ml orlistat (r^2>0.9999). The within-day and between-day precision values were also in the range of 0.10—0.59%. The appropriate dissolution conditions were also determined and applied to evaluate the dissolution profile of orlistat capsules. Optimal conditions were 1000 ml of 3% SLS in water as dissolution medium and paddle at 100 rotation per minute. The proposed method was applied successfully to the determination of orlistat content in capsules and *in vitro* dissolution studies.

Key words orlistat; HPLC; dissolution; drug quality control

Orlistat (Xenical) (Fig. 1), a highly lipophilic tetrahydro derivative of lipstatin, is an inhibitor of gastric and pancreatic lipases required for the hydrolysis of dietary fat (triacylglycerols) in the gastrointestinal tract into free fatty acids and monoacylglycerols.[1,2] A major contributing factor to the development and maintenance of obesity is excessive intake of dietary triglycerides. Inhibition of pancreatic lipase and gastric lipase, leading to prevention of lipid absorption is a treatment of severe obesity.[3,4] Orlistat acts locally in the intestine to block absorption of approximately one-third of all dietary fat.[5] By effectively limiting dietary fat absorption, orlistat promotes weight loss, maintenance of lost weight, and prevention of weight regain in obese patients.[6]

Orlistat yields low, if not undetectable plasma concentrations when administered orally. Quantitative determination of orlistat in human plasma has been reported using LC/MS/MS[7] or GC/MS/MS[8] with a low detection limit. To our best knowledge, there is no pharmacopeial monographs and dissolution tests on orlistat. The dissolution profile has emerged as a valuable quality control tool to assess batch-to-batch release rate properties and to assure the availability of the drug.

This study, describes the development of a fast, accurate and precise HPLC method with isocratic elution for determination of orlistat in pharmaceutical formulations and in dissolution media for drug quality control purposes. The developed method was validated and applied to determination of the content of orlistat in Xenical capsules and to the samples obtained from *in vitro* dissolution studies. The dissolution method was also developed and validated according to USP guidelines.[9]

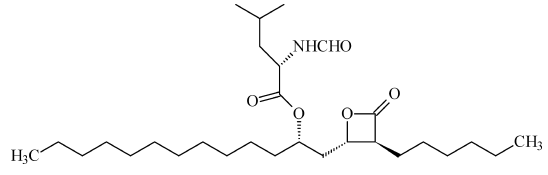

Fig. 1. Chemical Structure of Orlistat

Experimental

Materials Orlistat was from Biocon (Bangalore, India, Lot No: B-0520199) and obtained from Osveh Pharmaceutical Company, Tehran, Iran. Acetonitrile was HPLC grade and purchased from Merck (Darmstadt, Germany). Xenical tablets (Roche, Switzerland, Lot No: M1162C) were purchased from a local pharmacy. All other chemicals and solvents were of analytical grade and prepared from Merck. Deinonized distilled water were used for mobile phase preparation, solubility, assay and dissolution experiments.

Instrumentation The HPLC system consisted of a 600 pump, 710 plus Autosampler and a variable 480 UV detector, all from Waters (Milford, MA, U.S.A.). The data processing system was a multi-channel Chrom and Spec software for chromatography, version 1.5x.

Chromatographic Conditions Separation was achieved using a Nova-Pak C_{18} 4 μm steel column (3.9 mm×150 mm, Waters, Milford, MA, U.S.A.). The isocratic mobile phase pumped at a flow rate of 1 ml/min consisted of orthophosphoric acid 0.1%–acetonitrile (10 : 90, v/v) prepared daily and degassed by passing through a 0.45 μm filter (Millipore, Milford, MA, U.S.A.) and sonication for 10 min. The injection volume was 25 μl and the wavelength for UV detection was 205 nm. All sepatations were performed at room temperature.

Preparation of Standard Solutions Stock standard solution of orlistat was prepared by dissolving appropriate amount of the compound in methanol to give a final concentration of 1000 μg/ml. Standard solutins of orlistat (10, 20, 40, 80, 120, 160 μg/ml) were prepared by subsequent dilution. No decrease in responses was observed after 1 week of storage at 4 °C.

Validation Seven series of standard calibration solutions in the range of 10—160 μg/ml were prepared and analyzed as described above. Calibration curves were constructed by plotting the measured peak area of orlistat *versus* concentrations of standard samples and statistical analysis was performed.

To establish the within-day and between-day accuracy and precision of the method, three replicate of standard solutions at three different concentrations (10, 40, 160 μg/ml) were assayed on one day and three separate days.

Specificity The specificity of the proposed method was performed by analyzing spiked samples with appropriate amount of drug and also samples of the excipients of capsule formulation. The solutions were prepared and analyzed as described before.

Application of the Method The content of 20 capsules were combined and weighed. An amount of powder equivalent to about 120 mg of orlistat was accurately weighed, transferred to a 100 ml volumetric flask, made up to volume with methanol and placed in an ultrasonic bath for 15 min. After filtration through a 0.45 μm membrane filter the solution was diluted with methanol to obtain a concentration of about 120 μg/ml. The drug concentration of six parallels were determined by HPLC using the calibration curve.

To test the content uniformity of orlistat capsules, the content of ten capsules were individually transferred to a 100 ml volumetric flask and methanol was added. The mixture was placed in an ultrasonic bath for 15 min. The solution was diluted ten times and injected to the HPLC system after filtration.

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Solubility Determination The solubility of orlistat was determined in three test media: 1, 2 and 3% of sodium lauryl sulfate in deionized water. An excess of orlistat (100 mg) was placed in 500 ml of dissolution medium using the dissolution apparatus at 37 °C. At different time points (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 h) 1 ml was withdrawn, filtered through a 0.45 μm Millipore filter, diluted 2 times with mobile phase, immediately injected to HPLC system and solubility calculated.

Dissolution Studies Drug release was measured using a dissolution apparatus from Erweka (Heusenstamm, Germany). The apparatus consists of six vessels in a warm bath at 37 °C. The dissolution medium was 1000 ml of 3% sodium lauryl sulfate in deionized water freshly prepared and used within 4 h of preparation. The paddle apparatus was used for capsules and the rotation speed was kept at 100 rpm. To avoid floating, a ring/mesh stainless steel device, which fits under the paddle into the lower portion of the dissolution vessel, was employed. For each dissolution profile, one capsule was added to the medium and samples of 5 ml were drawn at 5, 10, 15, 30, 45 and 60, and 90 min. The dissolution vessels were covered to minimize evaporation.

The solutions were passed through a 0.45 μm Millipore filter and subjected to HPLC system. Sample volumes were replaced by fresh dissolution media reached to 37 °C to maintain a constant total volume.

A standard calibration curve of orlistat in the range of 10—160 μg/ml was used for determination.

Results and Discussion

Chromatographic Condition The chromatographic condition was optimized and separation was performed on a Nova-Pak C_{18} column using a mobile phase consisting of phosphoric acid 0.1% and acetonitrile. The proposed mobile phase composition allows suitable retention time of orlistat and achieve good selectivity towards interference from the excipients of the formulation. Typical chromatograms obtained from standard solution of orlistat and a test solution from dissolution medium are presented in Fig. 2. Under the chromatographic conditions described, orlitstat eluted at about 6 min. Good baseline resolution and peak shape can be observed.

Linearity Calibration curves were constructed using seven series of standard orlistat solutions in the range of 10—160 μg/ml. The equation of linear regression and statistical data are presented in Table 1. The linearity of the calibration curve is validated by the high value of the correlation coefficient.

Accuracy and Precision The accuracy and precision were determined by analyzing three synthetic samples of orlistat at 10, 40 and 160 μg/ml on three separate days. Concentrations were determined using calibration standard curves prepared for each day. Within-day and between-day data are given in Table 2. Good accuracy and repeatability were observed over the entire concentration range. The within-day and between-day variability showed CV values less that 0.59% in all three selected concentrations. The intermediate precision was assessed by comparison of the within-day and between-day data for analysis of orlistat by two analysts using two different HPLC systems. The CV values did not exceed 2%.

Also influences of small changes in the mobile-phase composition (±10%) and percentage of phosphoric acid (±10%) were studied to determine robustness of the method. Peak areas and retention time changes were observed. Peak area values were influenced less than ±0.6%. The retention time of orlistat was influenced <10%. Despite the changes in retention time there is no problem for quantification. The results are summarized in Table 3.

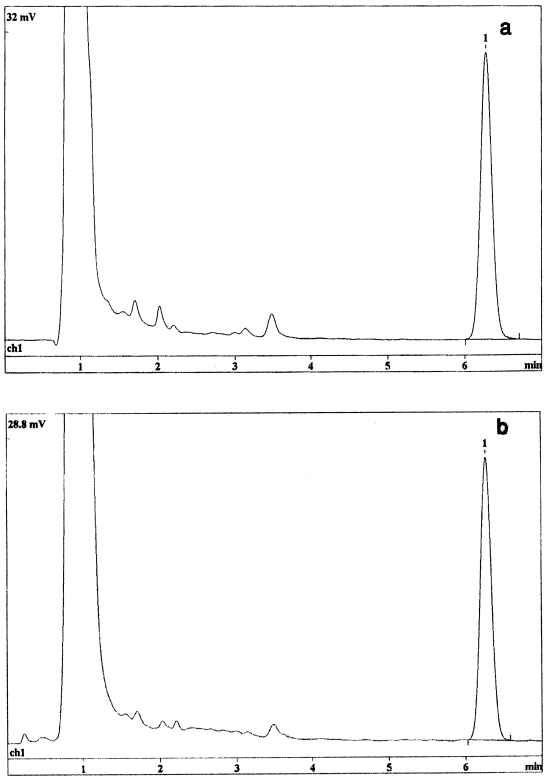

Fig. 2. Chromatograms of Orlistat Standard Solution (100 μg/ml) (a), and Dissolution Medium (3% SLS in Water) Containing Xenical Capsules after 30 min (b)

Table 1. Statistical Data of Calibration Curves of Orlistat

Parameters	Orlistat
Linearity	10—160 μg/ml
Regression equation	$Y=2.628X-0.397$
Standard deviation of slope	0.013
Relative standard deviation of slope (%)	0.495
Standard deviation of intercept	0.215
Correlation coefficient (r^2)	0.9999
Standard deviation of residuals	0.436
Standard deviation of residuals/slope	0.166

Table 2. Precision and Accuracy of Method for Determination of Orlistat ($n=9$; Three Sets for 3 d)

Concentration added (μg/ml)	Concentration found (mean±S.D.) (μg/ml)	C.V. (%)	Error (%)
Within-day ($n=3$)			
10	10.08±0.01	0.10	0.80
40	39.84±0.15	0.38	−0.40
160	159.96±0.21	0.13	−0.03
Between-day ($n=9$)			
10	10.14±0.01	0.59	1.4
40	39.99±0.18	0.45	−0.03
160	160.33±0.64	0.40	0.21

Specificity The specificity test of the proposed method demonstrated that the excipients from capsules do not interfere in the drug peak. Furthermore, well-resolved peaks indicate the specificity of the method (Fig. 2). Thus the proposed HPLC method is useful to quantify orlistat in dissolution

Table 3. The Influence of Small Changes in Mobile-Phase Composition (Method Robustness)

Mobile phase composition	t_R	Peak area
Acetonitrile–phosphoric acid 0.1% (9 : 91)	5.65	291.64
Acetonitrile–phosphoric acid 0.1% (10 : 90)	6.26	291.95
Acetonitrile–phosphoric acid 0.1% (11 : 89)	6.71	292.31
Acetonitrile–phosphoric acid 0.09% (10 : 90)	5.96	294.26
Acetonitrile–phosphoric acid 0.11% (10 : 90)	5.96	293.22

t_R: retention time.

Table 4. System Suitability Parameters

Parameter	Orlistat	Limits
Theoretical plates (n=3)	2066	N>1500
Asymmetry (n=6)	1.03	T<1.5
Repeatability (t_R) (n=6)	0.31	R.S.D<1%
Repeatability (peak area) (n=6)	0.46	R.S.D<1%

t_R: retention time.

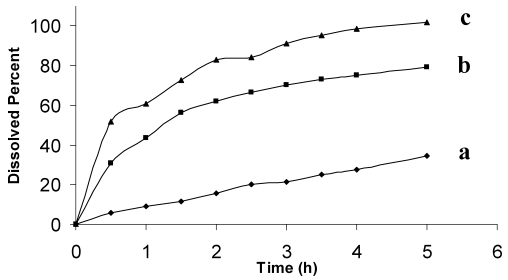

Fig. 3. Solubility of Orlistat in Different Solvents
1% SLS (a), 2% SLS (b), 3% SLS (c).

medium containing pharmaceutical formulation.

Sensitivity The limit of quantification with CV<0.59% was found to be 10 μg/ml for orlistat. The limit of detection with a S/N ratio of 3 was found to be 3 μg/ml.

System Suitability Test System suitability test was done to verify the repeatability of the HPLC method. Theoretical plates, symmetry and repeatability of the retention time and peak area were determined and compared. The results and the limits are summarized in Table 4.

Solution Stability The stability of the stock solution was determined by determination of orlistat and comparison to freshly prepared standard. No significant changes (<1%) was observed in stock solution after 2 d relative to freshly prepared standard.

Solubility Test The concentration of orlistat dissolved in the three dissolution media was measured. The solubility results are presented in Fig. 3. The rate and extent of dissolution of orlistat increased with increasing the amount of sodium lauryl sulfate from 1 to 3%. Taking into account the total volume of the dissolution media (1000 ml) and the dose unit (120 mg) it is concluded that complete dissolution is achieved after 1 h using 3% sodium lauryl sulfate.

Dissolution Study The main problem of this product is its poor solubility in water. Using a surfactant system, such as sodium lauryl sulfate (SLS) is recommended to increase the solubility.[9,10] The concentration of SLS commonly used in dissolution media ranges from 0.1—3% and should be justified. The solubility test showed that orlistat was soluble in

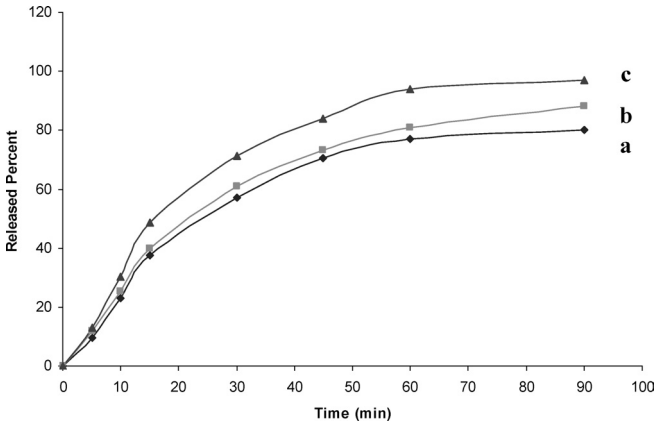

Fig. 4. Dissolution Profile of 120 mg Xenical Capsules (n=6) Using 3% SLS in Water as Dissolution Medium and Paddle at 50 rpm (a), 75 rpm (b) and 100 rpm (c)

Table 5. Content Uniformity Results of Xenical Capsule Commercially Available Using Proposed HPLC Technique

Sample number	Content (mg)
1	120.94
2	118.58
3	119.41
4	120.23
5	121.13
6	118.22
7	119.71
8	121.09
9	120.56
10	119.11
Mean	119.93
S.D.	1.07
R.S.D.	0.89
S.E.	−0.06

3% sodium lauryl sulfate in water. The dissolution test was performed using this dissolution medium. The common stirring speed is 50—100 rpm for paddle method. In this study the stirring speed of 50, 75 and 100 rpm were used to investigate the drug release. It was observed that stirring speed of 100 rpm represents better initial standing up of dissolution, better rate of dissolution and better equilibrium drug release percent. The dissolution profile of orlistat capsules (Xenical) obtained with the described method is presented in Fig. 4. It was observed that more than 70% of drug was dissolved within 30 min.

Assay and Content of Orlistat in Capsules The developed method was applied to assay the weight variation of Xenical capsules. The results of assay were in good agreement with the labeled amount (119.6±0.37) and the error of the determination does not exceed ±0.37. The content of orlistat in capsules was calculated as an average of ten determinations. The experimental results are given in Table 5. The results were very close to each other as well as to the label value of commercial capsules. Recoveries were very close to 100% and prove the suitability and accuracy of the proposed method.

Conclusion

In conclusion, the proposed HPLC and dissolution method

provides simple, accurate and reproducible quantitative method for routine *in vitro* tests of orlistat dosage form. The major advantage of this method is the quick sample analysis without prior separation or purification. Simple sample preparation procedure and a short chromatographic time make this method suitable for processing of multiple samples in a limited amount of time. Finally, as mentioned before no pharmcopeial or published method for determination of orlistat in pharmaceutical dosage forms and dissolution medium has been reported yet. It can be concluded that the proposed method is useful and suitable for quality control tests such as dissolution and content uniformity of commercial capsules of orlistat.

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