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A stability-indicating high performance liquid chromatographic assay for the determination of orlistat in capsules

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Abstract

A stability-indicating HPLC method was developed and validated for the quantitative determination of orlistat in capsule dosage forms. An isocratic separation was achieved using a 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 and using a UV detector to monitor the eluate at 210 nm. The mobile phase consisted of methanol:acetonitrile:trifluoroacetic acid (82.5:17.5:0.01, v/v/v). The drug was subjected oxidation, hydrolysis, photolysis and heat to apply stress conditions. Complete separation was achieved for the parent compound and all degradation products in an overall analytical run time of approximately 15 min with the parent compound orlistat eluting at approximately 9 min. 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. The method has the requisite accuracy, selectivity, sensitivity and precision to assay orlistat in capsules. Degradation products resulting from the stress studies did not interfere with the detection of orlistat and the assay is thus stability-indicating.

Keywords: Orlistat; Stability-indicating; HPLC-UV

1. Introduction

Orlistat, *N*-formyl-L-leucine(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-oxo-2-oxetanyl]methyl] dodecyl ester (Fig. 1) [1] is a novel, non-systemically absorbed, antiobesity agent which selectively inhibits the absorption of approximately 30% of fatty components from the diet [2]. Orlistat is a semisynthetic hydrogenated derivative of a naturally occurring lipase inhibitor produced by *Streptomyces toxytricini* [3,4] and is a potent inhibitor of gastric, pancreatic [5] and carboxylester lipases both in vitro and in vivo [6]. This agent reduced weight in obese adults and adolescents with or without co-morbidities such as type 2 diabetes mellitus, hypercholesterolemia, hypertension and metabolic syndrome. These patients had received orlistat for up to 4 years in addition to a hypocaloric diet. Orlistat is generally well tolerated, with gastrointestinal adverse events being the most commonly reported side-effects. Orlistat, in addition to lifestyle and dietary intervention, is thus an attractive option for the treatment of patients who are obese and who have associated co-morbidities or those who are at risk of developing type 2 diabetes [7]. There is a dearth of analytical methods reported in the literature. HPLC [8,9] and gas chromatography [10] methods for the quantitative determination of orlistat in plasma using tandem mass spectrometry have been reported. These methods are complicated, costly and time consuming rather than a simple HPLC-UV method. So it is not feasible to use these highly sensitive methods for the routine quantitative assay of orlistat capsules, where the content of active pharmaceutical ingredient (API) is high in the formulation. To our knowledge, no stability-indicating analytical method for the determination of orlistat in dosage forms has been published. Orlistat is prone to both hydrolysis and thermolysis when stored in conditions above its melting point (42–44 °C), therefore storage over longer periods of time must exclude such conditions [11]. Consequently a simple, precise, accurate, specific stability-indicating HPLC-UV method for the quantitative determination of orlistat in pharmaceutical dosage forms was developed and applied to the assay of orlistat in capsules and bulk form.

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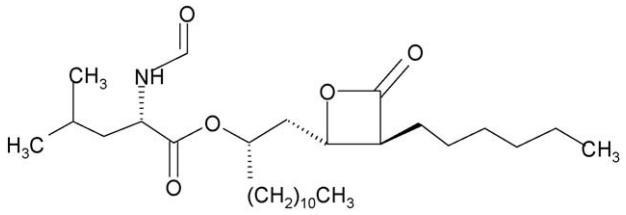

Fig. 1. Structural formulae for orlistat (MW = 495.7).

2. Experimental

2.1. Chemicals and reagents

Orlistat working standard powder was kindly supplied by Hangzhoa Zhongmei Huadong Pharmaceutical Co., China and was used without further purification. Orlistat capsules containing 120 mg orlistat as per label claim were purchased from a local pharmacy. Xenical® (Roche, NJ, USA) capsules were used as the reference formulation. Acetonitrile, methanol, triflouroacetic acid, sodium hydroxide, hydrochloric acid and hydrogen peroxide were obtained from Merck (Darmstadt, Germany). All chemicals were at least of analytical grade 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.

2.2. HPLC instrumentation and conditions

The HPLC system consisted of a Waters® 600 controller solvent delivery module (Waters Chromatography Division, Milford, MA, USA), a Waters® 717 plus autosampler, a solvent degasser, a Waters 2487 Dual λ absorbance detector (Waters Chromatography Division, Milford, MA, USA). A Millenium® (Waters Chromatography Division, Milford, MA, USA) Chromatographic Data System was coupled to the detector via a SAT/IN Module (Waters Chromatography Division) and used to record and evaluate the data collected during chromatographic analysis. The chromatographic separation was performed using a Perfectsil® target ODS-3, 5 μm, 250 mm × 4.6 mm i.d. column. Separation was achieved using a mobile phase consisting of methanol–acetonitrile–trifluoroacetic acid (82.5:17.5:0.01, v/v/v) solution at a flow rate of 0.7 ml/min. The eluent was monitored using UV detection at a wavelength of 210 nm. The column was maintained at ambient temperature and an injection volume of 20 μl was used. The mobile phase was filtered through 0.45 μm nylon filter prior to use.

2.3. Preparation of stock and standard solutions

A stock solution of orlistat (1 mg/ml) was prepared by accurately weighing approximately 20 mg of orlistat into a 20 ml A-grade volumetric flask and making up to volume with HPLC grade methanol. The stock solution was protected from light using aluminium foil and stored for one week at 4 °C and was found to be stable during this period. Aliquots of the standard stock solution of orlistat were transferred using A-grade bulb pipettes into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 0.02, 0.06, 0.1, 0.16, 0.2, 0.26, 0.3, 0.36, 0.5 and 0.75 mg/ml.

2.4. Preparation of capsules for assay

Twenty capsules were opened and the contents weighed and mixed. An aliquot of powder equivalent to the weight of one capsule was accurately weighed into a 100 ml volumetric flask and made up to volume with HPLC-grade methanol. The volumetric flasks were sonicated for 30 min to effect complete dissolution. Suitable aliquots of solution were filtered through a 0.45 μm nylon filter. Suitable aliquot of the filtered solution was added to a volumetric flask and made up to volume with mobile phase to yield starting concentration of 0.2 mg/ml.

2.5. Forced degradation studies of API and capsule contents

In order to determine whether the analytical method and assay were stability-indicating, orlistat capsules and orlistat active pharmaceutical ingredient (API) powder were stressed under various conditions to conduct forced degradation studies [13]. As orlistat is practically insoluble in water and is very soluble in methanol, methanol was used as co-solvent in all studies [14]. All solutions prepared for use in forced degradation studies were prepared to yield starting concentrations of orlistat of 0.2 mg/ml. In all cases, API (1 mg/ml) and capsule contents equivalent to 120 mg orlistat were accurately weighed and prepared for analysis as previously described.

2.5.1. Oxidation

Solutions for oxidation studies were prepared in methanol and 3% H_2O_2 (80:20, v/v) and the resultant solutions refluxed for 30 min in order to facilitate oxidation of the orlistat.

2.5.2. Acid degradation studies

Solutions for acid degradation studies were prepared in methanol and 2 M hydrochloric acid (80:20, v/v) and the resultant solutions refluxed for 30 min.

2.5.3. Alkali degradation studies

Solutions for alkali degradation studies were prepared in methanol and 2 M, sodium hydroxide (80:20, v/v) and the resultant solutions refluxed for 30 min.

2.5.4. Neutral degradation studies

Solutions for neutral degradation studies were prepared in methanol and water (80:20, v/v) and the resultant solutions refluxed for 3 h.

2.5.5. Temperature stress studies

Capsules and API powder were exposed to dry heat (60 °C) in an oven for 3 days. The capsules and API powder were removed from the oven and the contents of 20 capsules were removed and mixed. An aliquot of powder equivalent to the weight of

one capsule and API powder were then prepared for analysis as previously described.

2.5.6. Photostability

Orlistat API, capsule contents and solutions of orlistat were prepared and exposed to light to determine the effects of light irradiation on the stability of orlistat in solution and in the solid state. Approximately 50 mg of orlistat API powder was spread on a glass dish in a layer that was less than 2 mm thick. A solution of API (1 mg/ml) was prepared in Methanol and Water (80:20, v/v). Capsules were prepared in the same way. All samples for photostability testing were placed in a light cabinet (Suntest CPS/CPS$^+$, Atlas Material Testing Technology, Germany) and exposed to light for 40 h resulting in an overall illumination of $\geq$210 W h/m^2 at 25 °C with UV radiation at 320–400 nm. Control samples which were protected with aluminium foil were also placed in the light cabinet and exposed concurrently. Following removal from the light cabinet, all samples were prepared for analysis as previously described.

3. Results and discussion

3.1. HPLC method development and optimization

Herein a novel sorbent material is used and validated for the determination of orlistat. PerfectSil® Target (MZ-Analysentechnik, Mainz, Germany) is an ultra pure silica gel (>99.999%) provided with novel state of the art bonding and end capping technology that is characterized by excellent chemical and mechanical stability and provides excellent peak symmetry for basic analytes. A significant advantage is that it can be used even for 100% aqueous applications. The stressed samples were initially analyzed using a mobile phase consisting of methanol–acetonitrile (82.5:17.5) at a flow rate of 1 ml/min. Under these conditions, as the separation and peak shape were not optimal, the flow rate was changed from 1 to 0.7 ml/min. An improvement was observed in the separation, but the peak shape was still unsatisfactory. An attempt to improve peak shape was made by adding trifluoroacetic acid (TFA) to the mobile phase. The presence of the TFA in the mobile phase resulted in excellent overall chromatography with appropriate peak symmetry and complete baseline resolution. Eventually, a mobile phase of methanol:acetonitrile:trifluoroacetic acid (82.5:17.5:0.01, v/v/v) provided the best chromatographic response and was used for further studies.

3.2. Validation

The method was validated with respect to parameters including linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and recovery.

3.2.1. Linearity

The calibration curves ($n = 3$) constructed for orlistat were linear over the concentration range of 0.02–0.75 mg/ml. Peak areas of orlistat were plotted versus orlistat concentration and linear regression analysis performed on the resultant curve. Three correlation coefficients of $R1 = 0.9998$, $R2 = 0.9997$ and $R3 = 0.9998$ with %RSD values ranging from 0.28 to 3% across the concentration range studied were obtained following linear regression analysis. Typically, the regression equation for the calibration curve was found to be $y = 0.023X + 0.007$.

Table 1
Intra- and inter-assay precision data ($n = 9$)

Actual concentration (mg/ml)	Measured concentration (mg/ml), RSD (%)	
	Intra-day	Inter-day
0.02	0.0205, 2.30	0.020, 3.33
0.15	0.1520, 1.24	0.153, 3.59
0.75	0.7500, 0.42	0.752, 2.70

Data expressed as mean for "measured concentration" values.

3.2.2. LOQ and LOD

The LOQ and LOD were determined based on a signal-to-noise ratios and were based on analytical responses of 10 and 3 times the background noise, respectively [15]. The LOQ was found to be 0.02 mg/ml with a resultant %RSD of 0.4% ($n = 5$). The LOD was found to be 0.006 mg/ml.

3.2.3. Precision

Precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability was investigated by injecting nine replicate samples of each of the 0.02, 0.15 and 0.75 mg/ml standards where the mean concentrations were found to be 0.0205, 0.152 and 0.75 with associated %RSDs of 2.3, 1.24 and 0.42, respectively. Inter-day precision was assessed by injecting the same three concentrations over 3 consecutive days, resulting in mean concentrations of orlistat of 0.02, 0.153 and 0.752 mg/ml and associated %RSD of 3.33, 3.59 and 2.7%, respectively. The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results for orlistat undertaken by two analysts. The %RSD values for intra- and inter-day assays of orlistat in the cited formulations performed in the same laboratory by the two analysts did not exceed 4%, thus indicating the ruggedness of the method (Table 1). The mean retention time of orlistat was 9 min with %RSD of 0.1%.

3.2.4. Accuracy

Accuracy of the assay was determined by interpolation of replicate ($n = 6$) peak areas of three accuracy standards (0.02, 0.15 and 0.75 mg/ml) from a calibration curve prepared as previously described. In each case, the percent relevant error and accuracy was calculated. The resultant concentrations were 0.0206 ± 0.0002 mg/ml (mean $\pm$ SD), 0.152 ± 0.003 mg/ml and 0.754 ± 0.005 mg/ml with percent relevant errors of 3.1, 1.2 and 0.53%, respectively.

3.2.5. Selectivity

The results of stress testing studies indicated a high degree of selectivity of this method for orlistat. The degradation of orlistat was found to be similar for both the capsules and API powder.

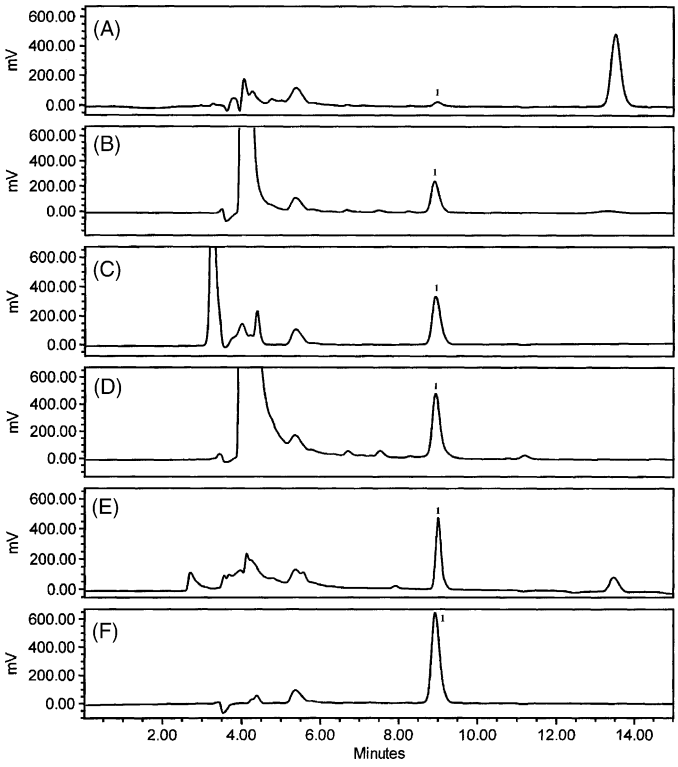

Fig. 2. Typical HPLC chromatograms of: (A) acid hydrolysis-degraded active pharmaceutical ingredient (API); (B) neutral-hydrolysis degraded API; (C) photodegraded API; (D) oxidative degraded API; (E) base hydrolysis-degraded API; and (F) untreated API showing orlistat (1).

Typical chromatograms obtained following the assay of pure bulk sample and stressed samples are shown in Fig. 2.

3.2.6. Recovery

A known amount of orlistat standard powder was added to aliquots ($n = 20$) of capsule contents, mixed and the powder was extracted and diluted to yield a starting concentration of 0.3 mg/ml as previously described in Section 2.4. This solution was analyzed as previously described. The assay was repeated ($n = 9$) over 3 consecutive days to obtain intermediate precision data. The observed concentration of orlistat was found to be 0.299 ± 0.0035 mg/ml (mean $\pm$ SD). The resultant %RSD for this study was found to be 1.18% with a corresponding percentage recovery value of 99.9%.

3.2.7. Stability studies

Orlistat is characterized by an aliphatic hydrocarbon backbone bearing a β-lactone moiety and a *N*-formyl-L-leucyl side chain in δ-position to the lactone C=O C-atom and was suspected to be unstable at high temperature and prone to hydrolysis. Its thermal and hydrolytic degradation have been reported, and all main degradation products isolated, characterized and synthesized [11]. The orlistat degradation products produced by human carboxyl ester lipase have also been described [12]. All stressed samples in both solid and solution state remained colorless. Orlistat was found to be stable under dry heat condition. No decomposition was seen on exposure of solid drug powder to light in a phptostability chamber, whereas photolytic exposure

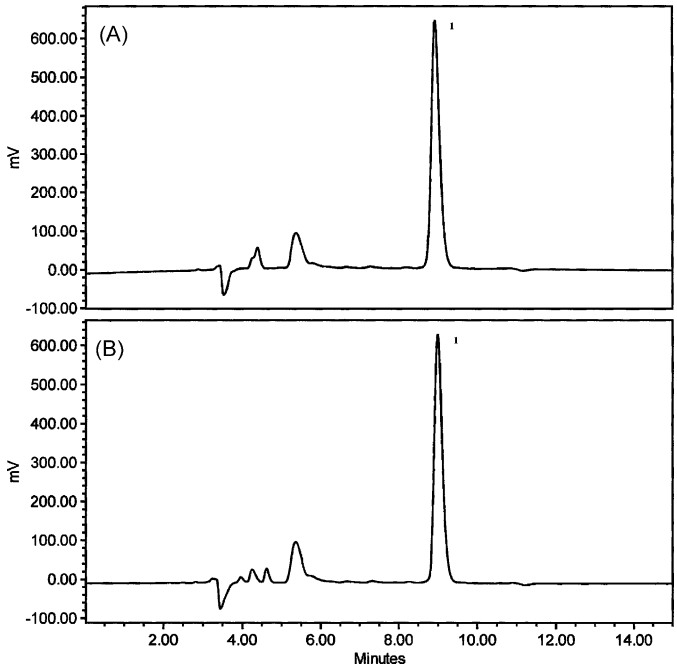

Fig. 3. Resultant HPLC chromatograms following the analysis of a standard solution of orlistat (A) (0.2 mg/ml) and Xenical® capsules (B) showing orlistat (1).

of orlistat API and capsules in methanol and water (80:20, v/v) resulted in 48 and 47.6% decomposition, respectively. The drug was more unstable under acidic stress condition, falling by 95% upon refluxing to 30 min. Orlistat was relatively stable to oxidation. On refluxing the drug in methanol and 3% H_2O_2 (80:20, v/v) for 30 min, around 23% of drug was only degraded. In spite of acidic condition, the drug was found to be more stable in alkaline condition falling by 48% upon refluxing up to 30 min. Three hours after refluxing the drug in methanol and water (80:20, v/v), 60% of drug was degraded. The stability of the stock solution was determined by quantitation of orlistat and comparison to freshly prepared standard. No significant change (<2%) was observed in stock solution response, relative to freshly prepared standard (data not shown).

3.2.8. Assay

The proposed method was applied to the determination of orlistat in a generic brand of orlistat and Xenical® capsules. A typical chromatogram obtained following the assay of Xenical® capsules is depicted in Fig. 3. The result of these assays yielded 100.01% (%RSD = 1.80%) and 99.98% (%RSD = 2.4%) of label claim for the generic brand and Xenical® capsules, respectively. The results of the assay indicate that the method is selective for the assay of orlistat without interference from the excipients used in these capsules.

4. Conclusions

A validated stability-indicating HPLC analytical method has been developed for the determination of orlistat in API and dosage forms. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guide-

lines reveal that the method is selective and stability-indicating. The proposed method is simple, accurate, precise, specific, and has the ability to separate the drug from degradation products and excipients found in the capsule dosage forms. The method is suitable for use for the routine analysis of orlistat in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS [8,9] or GC–MS [10]. These methods are complicated, costly and time consuming rather than a simple HPLC-UV method. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiry dates of pharmaceuticals.

References

[1] M.J. ONeil, A. Smith, P.E. Heckelman, S. Budavari, The Merck Index, An Encyclopedia of Chemicals. Drugs and Biologicals, 13th ed., Merck & Co. Inc., White House Station, New Jersey, 2001, p. 1228.
[2] L.J. Aronne, J. Am. Diet. Assoc. 98 (1998) A13.
[3] E.K. Weibel, P. Hadvary, E. Hochuli, E. Kupfer, H. Lengsfeld, J. Antibiot. 40 (1987) 1081.
[4] E. Hochuli, E. Kupfer, R. Maurer, W. Meister, Y. Mercadel, K. Schmidt, J. Antibiot. 40 (1987) 1086.
[5] S.C. Sweetman, Martindale the Complete Drug Reference, 34th ed., Pharmaceutical Press, London, 2005, p. 1724.
[6] J.B. Harp, J. Nutr. Biochem. 9 (1998) 516.
[7] M.P. Curran, L.J. Scott, Drugs 64 (2004) 2845.
[8] P.K. Bennett, Y.T. Li, R. Edom, J. Henion, J. Mass Spectrom. 32 (1997) 739.
[9] R. Wieboldt, D.A. Campbell, J. Henion, J Chromatogr. B: Biomed. Sci. Appl. 708 (1998) 121.
[10] W.A. Garland, Determination of Tetrahydrolipostatin (Ro 18-0647) in Human plasma by Positive Ion Ammonia Chemical Ionization GC/MS/MS, Report MS-32, Hoffman–La Roche, Nutley, NJ, 1990.
[11] H. Stalder, P.R. Schneider, G. Oesterhelt, Helv. Chim. Acta 73 (1990) 1022.
[12] H. Stalder, G. Oesterhelt, B. Borgstroem, Helv. Chim. Acta 75 (1992) 1593.
[13] ICH Stability Testing of New Drug Substances and Products (Q1AR2), International Conference on Harmonization, IFPMA, Geneva, 2003.
[14] A.C. Moffat, M.D. Osselten, B. Widdop, Clarke's Analysis of Drugs and Poisons in Pharmaceuticals. Body Fluids and Postmortem Material, third ed., Pharmaceutical Press, London, 2004, p. 1370.
[15] ICH Draft Guidelines on Validation of Analytical Procedures: Definitions and Terminology, Federal Register, vol. 60, IFPMA, Switzerland, 1995.