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Research Article

QUANTIFICATION OF SSRR IMPURITY OF ORLISTAT IN ORLISTAT CAPSULES BY NORMAL PHASE HPLC UV DETECTOR

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ABSTRACT
A simple and efficient NP-HPLC UVD method was developed and validated for the quantitative determination of Orlistat SSRR Impurity in Orlistat dosage forms. An isocratic separation was achieved by using a Chiral Pack IA, 250 mm × 4.6 mm, 5 µm particle size column with a flow rate of 1.0 ml/min and using a UV detector at 205 nm. The mobile phase consists of n-Hexane and Isopropyl alcohol (70:30, *v/v*). The drug was subjected oxidation, hydrolysis, photolysis and heat to apply stress conditions. Complete separation was achieved for the parent compound and SSRR Isomer of Orlistat in an overall analytical run time of approximately 45 min with the parent compound Orlistat eluting at approximately at 5 min. The method was linear over the concentration range of 3.1 - 40 µg/ml with a limit of detection and limit of quantification 1.0 µg/ml and 3.1µg/ml respectively. The method has been the requisite accuracy, selectivity, sensitivity and precision. Degradation products resulting from the stress studies did not interfere with the detection of Orlistat and SSRR Impurity and the test method is thus stability-indicating.

KEYWORDS: Normal phase, Column temperature, SSRR of Orlistat, Development & validation.

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.
SSRR Isomer impurity (Fig. 2) is one of the process related chiral isomer of Orlistat and it was formed from SRR isomer of Oxetanone during manufacturing process of Orlistat.
There is a dearth of analytical methods reported in the literature[7-12] but no method has been reported for the determination of Orlistat SSRR Impurity by using HPLC with UV detection. The vendor was developed HPLC with ELSD detector method for the estimation of SSRR Impurity of Orlistat.
The objective of this work is to develop and validate a simple, precise and accurate HPLC method with UV detection and it should be stability indicating. The developed normal phase high performance liquid chromatographic technique equipped with UV detector method[13-14] was validated according to ICH guidelines[15] and found to be a simple, precise, specific, linear, accurate rugged and robust and stability Indicating method.

EXPERIMENTAL

Materials and Chemicals

SSRR isomer of Orlistat is a process related impurity which is obtained from Ranbaxy Laboratories. Isopropyl alcohol and n-Hexane (Merck India Limited) of HPLC grade were used for mobile phase preparation and for sample preparation.

Equipments

The analysis was carried out in Agilent HPLC equipped with UV detector and Agilent HPLC with PDA Detector. Data acquisition was carried out by using Empower Software.

Chromatographic conditions

The separation of Orlistat and SSRR isomer impurity were achieved by using a mobile phase of a mixture of n-Hexane and Isopropyl alcohol in the ratio of 70:30, *v/v* respectively. The chromatographic separation was achieved in the normal phase mode. The high performance liquid chromatography equipped with UV detector at 205 nm and by using a Chiral Pack IA, 250 mm × 4.6 mm, 5 µm particle size column with a 1.0 ml/min flow rate, 10 µl of injection volume and 10 °C of column heater temperature. The separation was achieved within the 45 min for the Orlistat and SSRR Isomer of Orlistat, the retention time of the both peaks were 5.4 and 13.0 respectively. The detection and peak purity establishment of active and SSRR impurity were achieved using photo diode array (PDA) detector.

Diluted standard preparation

A standard stock solution of Orlistat (1000 µg/ml) was prepared by using the diluent (mobile phase). The final concentration of diluted standard was 20 µg/ml.

Sample preparation

An aliquot of powder equivalent to the weight of 100 mg Orlistat was accurately weighed into a 10 ml volumetric flask and made up to volume with diluent to yield concentration of 10 mg/ml.

RESULTS AND DISCUSSION

The analytical test method of related substances plays a major role in pharmaceutical solid dosage forms for quantification of impurities which are present in the dosage forms. The main aim of this study is to get the separation of SSRR Impurity of Orlistat from the main analyte without interference. Different mobile phases in different ratios of Isopropyl alcohol and n-Hexane were tried for separation of SSRR impurity from main analyte. The chromatographic separation has been achieved in a mode isocratic program by

using single mobile phase consist of a mixture of n-Hexane and Isopropyl alcohol in the ratio 70:30, *v/v* respectively. The resolution in between SSRR impurity and Orlistat peaks found more in developed isocratic method. The typical retention times of Orlistat and SSRR Impurity are 5.4 and 13.1 minutes respectively. The SSRR impurity pertaining to active moiety is estimated at specific wavelength of 205 nm.

The developed HPLC method was validated and found to be specific, precise and accurate and also stability indicating for the estimation of SSRR Impurity in Orlistat capsules formulation. A typical chromatogram of diluent, diluted standard and SSRR impurity spiked sample was shown in the Fig.3-5.

Optimization of test method

The test method chromatographic conditions were optimized by using a Chiral Pack IA, 250 × 4.6 mm, 5 µm with a mobile phase containing a mixture of n-Hexane and Isopropyl alcohol in the ratio 70:30, *v/v* respectively. The column temperature was maintained at 10 °C with a flow rate of 1.0 ml/min. The Retention time of Orlistat and SSRR isomer of Orlistat were found to be 5.4 and 13.1 minutes respectively. The Relative retention time of Orlistat SSRR isomer was found to be 2.42 with respect to the main analyte peak. The developed test method is capable to separate SSRR impurity from main analyte with a good resolution. The tailing factor for both peaks and resolution were found to be 1.0 and not less than 3.0 respectively. The peak purity of main analyte and SSRR impurity were found within the limits and no purity flag was observed in purity results table of Empower software.

Extraction procedure for Orlistat and SSRR impurity

The extraction of Orlistat and SSRR impurity from pharmaceutical formulations in capsule form as achieved through the selective extraction of the active ingredient with n-Hexane and Isopropyl alcohol in the ratio of 70:30, *v/v* respectively. The finished product containing volumetric flasks were sonicated in Ultra Sonic power sonicator for 5 min to extract the drug moiety from the pharmaceutical formulations. Suitable aliquots of solution were filtered through a 0.45 µm Nylon filter and used as a test.

Establishment of Relative Response Factor for Orlistat SSRR impurity

Prepared and injected a series of solutions consisting of SSRR Impurity and Orlistat in the range of 0.10% to 0.30% (0.1%, 0.15%, 0.2%.0.25%, 0.3%) levels. The load of 10 µl injection volume of sample was injected into the liquid chromatograph and calculated the Relative Response Factor (RRF) of SSRR Impurity with respect to Orlistat from the calibration curve data. The RRF value of SSRR was found to be 0.96. The Relative Retention Time (RRT) of SSRR Impurity was found to be 2.42.

VALIDATION

System suitability

The system suitability parameters were evaluated by making the injection of test sample spiked with SSRR impurity. The system is deemed to be suitable as the tailing factor ≤ 1.5 and the resolution between Orlistat and SSRR impurity is >3.0.

Specificity

In order to determine whether the developed analytical method was stability-indicating, Orlistat capsules and Orlistat active pharmaceutical ingredient (API) were stressed under various conditions to conduct forced degradation studies. As Orlistat is practically insoluble in water and is very easily soluble in diluent.

The specificity was examined by analyzing a solution of a placebo, which consisted of all the excipients and capsules shell without the drug substance as per ICH guidelines (ICH, Q2B). The degradation was observed for Orlistat during stress conditions like UV light, Sunlight, Heat, Water and also acidic, basic, Oxidation, Hydrolysis and Humidity conditions. The acidic, basic, and oxidation stress conditions studies were carried out by refluxing API for 6hours with 1N HCl, 1N NaOH and 3% Hydrogen peroxide respectively. The thermal degradation was carried out by heating the drug product at 40°C for 24hours and the photo degradation was performed exposing the drug product to 1.2 million lux hours and 200 watt hours/m^2 in photo stability chambers. All the stress conditions and purity angle and purity threshold values are reported in Table.1 and Fig.6.

Precision

The precision of test method was evaluated by analyzing six samples of Orlistat by spiking SSRR impurity at target concentration level (i.e. 0.2%) with respect to test concentration. The %R.S.D of SSRR impurity from six sample preparations were found to be 1.0 which indicates that precision of the method was precise for quantification of SSRR impurity (Tab.2).

Linearity

Linearity of 'SSRR impurity of Orlistat' was conducted from LOQ level to 200% of the target concentration (i.e. 0.2%).The linearity graph was plotted for "SSRR impurity of Orlistat' in between concentration versus detector response (area). The correlation coefficient was found to be 0.998. Linearity plot is shown in Fig.7.

Accuracy

The % recovery of SSRR impurity of Orlistat and Orlistat were determined by spiking the SSRR impurity of Orlistat and main analyte at five different levels starting from 50% to 150% of the target concentration level (i.e. 0.2%) of the impurity. The % recovery of impurity and main analyte were found within the limits as per ICH guidelines (Tab.3).

LIMIT OF DETECTION & LIMIT OF QUANTIFICATION

LOD and LOQ Establishment

A series of different concentrations of SSRR of Orlistat solutions were prepared in diluent. The limit of detection and limit of quantification were established by based on signal to noise ratio. The signal to noise ratio 3 for LOD and 10 for LOQ were found. The LOQ concentration was found to be 0.028% for Orlistat SSRR impurity (Tab.4).

Precision at LOQ Level

The precision at limit of quantification level was determined by preparing six spiked solutions of Orlistat SSRR impurity at LOQ level. The % RSD for the impurity from six preparations was found within the limits as per ICH guidelines.

Accuracy at LOQ Level

Samples were prepared in triplicate by spiking with Orlistat isomer impurity (SSRR) at a concentration level of limit of quantification and injected into HPLC. The mean %recovery of impurity was found within the limits as per ICH guidelines.

Solution stability

The solution stability of the standard and impurity were studied for about 2 days on bench top and in refrigerator. The

aged solutions were injected in to liquid chromatograph against with freshly prepared standard solution; the samples were stable for a period of 2 days on bench top.

Robustness

The robustness was investigated by varying the conditions with respect to change in the flow rate, column temperature and IPA composition in mobile phase. The study was conducted at different flow rates of 0.8ml/min and 1.2ml/min instead of 1.0ml/min to study the effect of the change in flow rate. The column temperature was adjusted to 5°C and 15°C instead of 10°C column oven temperature, to study the effect of the change in column temperature. Organic phase composition (IPA) variation was studied 90% and 110% in mobile phase, to study the effect of organic phase composition variation in mobile phase. The method was found to be robust with respect to flow rate, column temperature and organic phase composition without any changes in system suitability parameters such as tailing factor and RRT of SSRR impurity (Tab.5).

CONCLUSION

The simple, selective, isocratic mode normal phase high performance liquid chromatographic method provides selective quantification of SSRR impurity without interference of blank, placebo, thereby affirming stability-indicating nature of the method. The proposed method is highly selective, reproducible, specific and rapid. The developed method was robust in the separation and quantification of Orlistat and SSRR Impurity. This developed method can be applied successfully for the quality control of commercial and routine analysis. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies.

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Table 1: The results table of specificity of the method

Sample No.	Stress Conditions	Purity angle	Purity threshold	Purity flag
1	Acid stress	1.003	1.026	NO
2	Base stress	0.228	1.036	NO
3	Oxidation stress	0.509	1.017	NO
4	Sunlight stress	0.612	1.028	NO
5	UV light stress	0.675	1.026	NO
6	Thermal stress	0.572	1.024	NO
7	Humidity stress	0.574	1.028	NO
8	Water stress	0.587	1.027	NO

Table 2: The results table of precision of the method

Sample No.	SSRR impurity	
	RRT	% Imp (n)
1	2.4	97.5
2	2.4	99.0
3	2.4	98.0
4	2.4	97.5
5	2.4	100.0
6	2.4	98.0
Average	2.4	98.5
%RSD	0	1.0

n=6

Table 3: The results table of Accuracy of the method

Sample No.	Spike level (n)	'µg/ml' added	'µg/ml' found (recovered)	Mean % recovery
1	50%	10.222	9.533	93.3
2	75%	15.333	14.867	97.0
3	100%	20.444	18.500	90.5
4	125%	25.555	23.200	90.8
5	150%	30.666	28.967	94.5

n=3

Table 4: LOD and LOQ of SSRR Orlistat impurity

Name of the Impurity	Test Name		Signal to Noise ratio		% Impurity at LOQ level
	Conc. at Limit of Detection (µg/ml)	Conc.at Limit of Quantification (µg/ml)	LOD	LOQ	
SSRR impurity	1.0	3.1	3.0	10.4	0.028

Table 5: System suitability results from robustness

Parameter	Flow rate (ml/min)			Column temperature (°C)			Organic phase (IPA) variation (±10%)		
	0.8	1.0	1.2	5	10	15	90	100	110
Tailing factor for Orlistat peak	1.0	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0
RRT of SSRR impurity	2.50	2.40	2.48	2.78	2.40	2.24	2.61	2.40	2.48

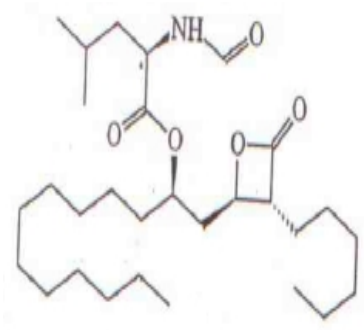

Figure 1: Chemical Structure of Orlistat

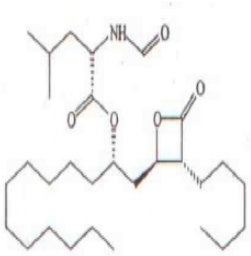

Figure 2: Chemical Structure of Orlistat SSRR Impurity

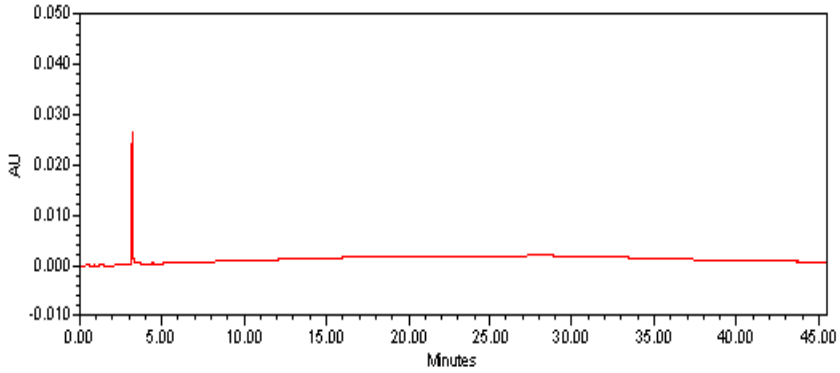

Figure 3: A typical chromatogram of related substances blank

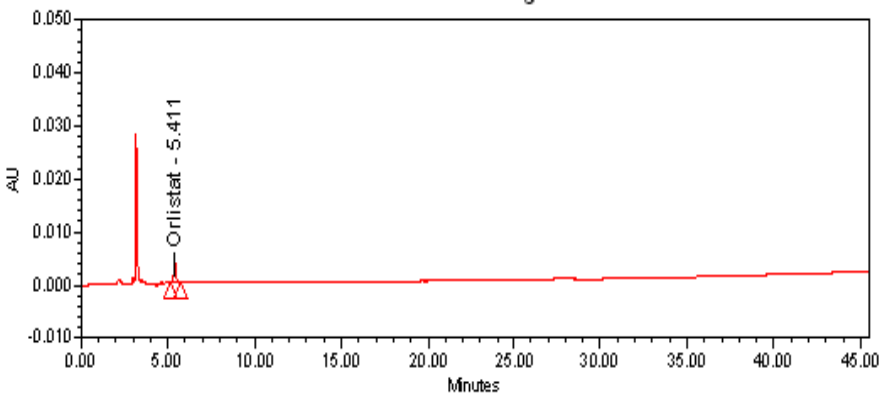

Figure 4: A typical chromatogram of related substances diluted standard

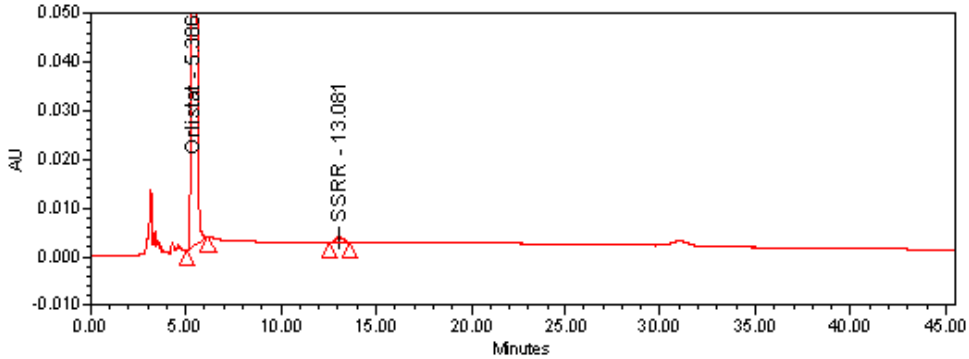

Figure 5: A typical chromatogram of related substances SSRR-impurity spiked sample

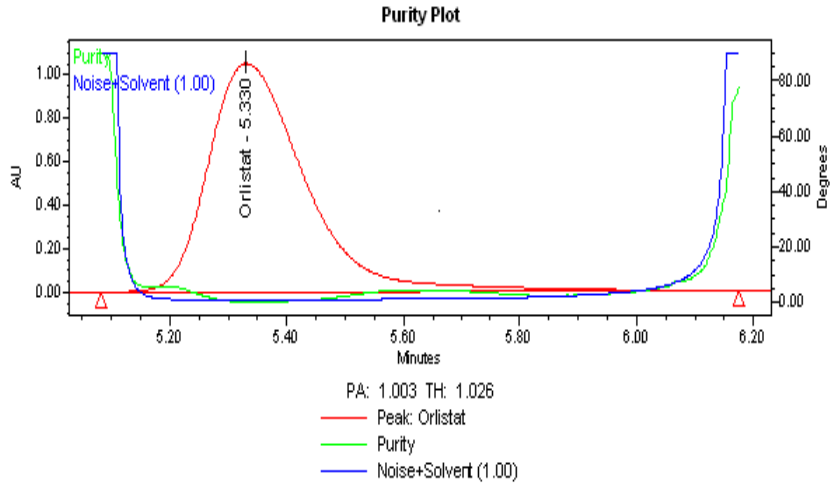

Figure 6: Purity results chromatogram

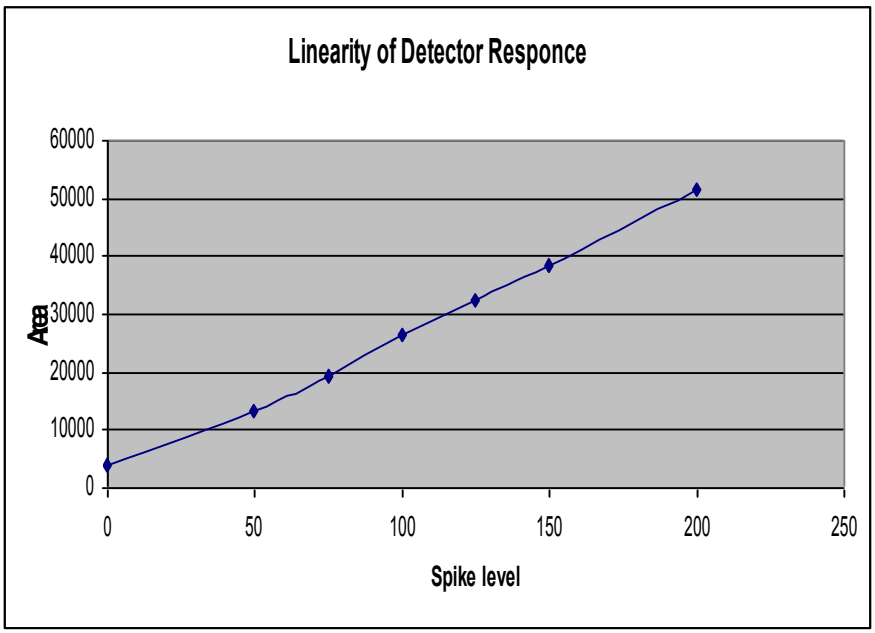

Figure 7: Linearity plot for Orlistat SSRR Impurity

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