DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF ORLISTAT AND ITS FORMULATIONS

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Abstract:

A simple, accurate, precise, robust and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of orlistat in the capsules formulation. Orlistat was chromatographed on silica gel 60 F254 TLC plate, as a stationary phase. The mobile phase was toluene: ethyl acetate: phosphopolymbdic acid (6:4:0.3 v/v/v) which gave a dense and compact spot of orlistat with a R_f value of 0.39±0.03. The quantification was carried out at 581.00 nm. The method was validated in terms of linearity, accuracy, precision, robustness and specificity. To justify the suitability, accuracy and precision of the proposed method, recovery studies were performed at three different concentration levels. Statistical analysis proved that the proposed method is accurate and reproducible with linearity in the range of 1000 to 6000 ng/spot. The limit of detection and limit of quantification for orlistat were 25 and 78 ng/spot, respectively. The proposed method can be employed for the routine analysis of orlistat as well as in pharmaceutical formulations.

Keywords: HPTLC densitometry, orlistat, capsules, validation

I. INTRODUCTION

Orlistat, N-formyl-L-leucine(1S)-1-[[(2S,3S)-3-hexyl-4-oxo-2-oxetanyl]methyl]dodecyl ester[1] (fig.1), is a gastric and pancreatic lipase inhibitor that limits the absorption of dietary fat. It is used together with dietary modification in the management of obesity [2]. Orlistat has been determined by a variety of methods and liquid chromatography is the most commonly used method of analysis for determination of orlistat[3-5], Spectrophotometric[6-8], HPTLC[9]and stability studies[10-12] methods are available. However; they are highly sophisticated, costly and time consuming.

Nowadays, high performance thin layer chromatography (HPTLC) has become a routine analytical technique due to its advantages of reliability in quantitation, analysis at microgram and even in nanogram levels and

cost effectiveness. This method is advantageous since large number of samples can be simultaneously subjected to analysis. The amount of solvent required in comparison to HPLC is very less. This reduces the time and cost of analysis and possibilities of pollution of the environment. HPTLC also facilitates repeated detection (scanning) of the chromatogram with same or different parameters. Hence, the present investigation was undertaken to develop and validate a simple, rapid, accurate, precise and specific HPTLC method for determination of Orlistat.

II. MATERIALS AND METHODS

Orlistat was procured as a gift sample from Murli Krishna Pharma Private Ltd. Pune (M.H.), India. Silica gel 60 F254 TLC plates (20×20 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were purchased from Merck Ltd, Mumbai and used as stationary phase. Analytical grade methanol, toluene, ethyl acetate and phosphomolybdic acid (96%) were all obtained from Merck life science private limited, Mumbai, India.

HPTLC Instrumentation:

Thin layer chromatography was performed on 10×10 cm aluminium backed TLC plates coated with 250 mm layer of silica gel 60F254 (E. Merck, Darmstadt, Germany). The plates were prewashed by methanol and activated at 105-110° for 15 min prior to use for chromatography. The samples in methanol were spotted as 6 mm wide bands at a distance of 10 mm from the bottom and 20 mm from the sides of the plate, under continuous flow of nitrogen by means of a Camag Linomat 5 sample applicator (Camag, Muttenz, Switzerland) fitted with a 100 µl syringe. A constant application rate of 150 nl/s was employed and the distance between adjacent bands was 8 mm. The plates were then conditioned for 20 min in a pre-saturated twin-trough chamber (Camag, Muttenz, Switzerland, 10×10 cm^2) with the mobile phase, toluene:ethyl acetate:phosphomolybdic acid (6:4:0.3 v/v/v), in one trough and the plate in the other trough. The plate was then placed in the mobile phase and ascending development was performed upto a distance of 70 mm from application position at ambient temperature. After development, plates were air dried and densitometric scanning was performed at a wavelength of 581 nm with Camag TLC scanner III operated in the reflectance-absorbance mode and controlled by WinCATS software

(V1.2.1). The slit dimension was kept at 6×0.45 mm and scanning speed employed was 20 mm/s. Evaluation was done using linear regression versus peak areas.

Preparation of standard orlistat solution and samples:

A fresh stock solution of orlistat was prepared in Methanol (1000 µg/ml). Standard solutions were prepared by dilution of the stock solution with methanol to give solutions containing of 100, 200, 300, 400, 500 and 600 µg/ml. One microlitre from each standard solution was spotted on the TLC plate to obtain a final concentration range of 1000-6000 ng per spot. The standard curve was replicated six times on different plates.

Analysis of formulation:

The developed method can be applied in determination of orlistat in capsules. The drug from the capsules was extracted by dissolved in methanol in a 50 ml volumetric flask. The resulting solution was suitably diluted at desired concentration (2000 ng). Two microlitres of the solution was applied on TLC plate followed by development, and scanned as described above. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

Precision:

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (2000 ng per spot of orlistat). The intra-day and inter-day precision for the determination of orlistst was carried out at three different concentration levels of 1000, 4000 and 6000 ng per spot.

Recovery study:

In order to determine the recovery, known quantities of a previously analysed reference standard corresponding to 80, 100 and 120 percent of the label claim were spiked during the procedure, i.e. to 120.6 mg of capsules (label claim 60 mg), 0.8, 1 and 1.2 mg/ml of orlistat was added. Then drug was extracted by employing methanol as a solvent for extraction and analyzed as described above. The recovery was calculated by comparing the resultant peak areas with those obtained from pure standards in methanol at the same concentrations.

Sensitivity:

In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted six times following the same method as explained above. Solutions containing 500–1000 ng of orlistat were spotted on TLC plate. The signal to noise ratio was determined. An LOD was considered as 3:1 and LOQ as 10:1.

Robustness

The robustness study was evaluated with respect to the minute but a deliberate alteration in the chromatographic conditions, the result of the study delivers the reliability of the analysis. The change in Chromatographic parameters, e.g. composition of mobile phase, volume of mobile phase, chamber saturation time, detection wavelength. The effects of such deliberate changes on peak area and % assay were calculated, which found 98.56 % to 101.32 %. as well as % RSD for retardation factor found 2.2101%.

III. RESULTS AND DISCUSSION

There are several spectroscopic and chromatographic methods reported for assay of orlistat, but HPTLC method of analysis in capsules has not been reported so far and so the aim was to develop and validate HPTLC method of analysis for orlistat bulk and capsules.

A suitable solvent system for the composition of the mobile phase for development of chromatogram was optimized by testing different solvent mixtures of varying polarity. Various mobile phases were evaluated. Use of chloroform, methanol, aetonitrile as single component and a short saturation time of 15 min gave a necklace effect. So toulene:acetone (5:5, v/v), toulene:methanol (6:4, 4:6 v/v), toluene: acetic acid (10:3, 9:3 v/v), toluene:acetonitrile (4:6 v/v), toluene:ethyl acetate (8:2 v/v) were tried. The best results were obtained using toluene:ethyl acetate:phosphomolybdic acid (6:4:0.3 v/v/v). This mobile phase showed a good resolution and a compact spot of orlistat. Densitometric scanning of all the tracks at λmax 581 nm showed compound with Rf value 0.392±0.03 (single spot), identified as orlistat as shown in Fig.2. The method was successfully used in the analysis of orlistat from the capsules without interference of the formulation excipients.

Under the experimental conditions employed, the lowest amount of drug which could be detected was found to be 25.38 ng/spot and the lowest amount of drug which could be quantified was found to be 76.93 ng/spot, with a

relative standard deviation <5%. The calibration curve was found to be linear in the concentration range of 1000-6000 ng/spot (n=3). Fig. 3 shows 3D overlay of HPTLC densitograms of standard plot of orlistat, using 60F254 TLC plates and toluene:ethyl acetate: phosphomolybdic acid (6:4:0.3, v/v/v). Peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficients. The one way ANOVA ($p>0.05$) shows no significant difference in the values of each calibration curve. The regression data as shown in Table 1 and fig. 4 showed a good linear relationship over the concentration range studied. Fig. 5 shows Repeatability of orlistat standard and capsules formulations spot obtained using the developed method. There was no interference from the formulation component and Rf values of orlistat standard and orlistat in the capsules was found to be same thereby confirming the selectivity of the method.

The recovery of orlistat ranged from 99.52% to 100.60%, average of 100.06 %, as shown in Table 2. These results showed high efficiency of orlistat from formulation components and confirms that the proposed method can be used for determination of orlistat in capsule formulations.

For precision determination, two and five microliter aliquots of sample containing 3000 and 5000 ng orlistat were analyzed according to the proposed method. In order to control the scanner parameters, one spot was analyzed several times. By spotting and analyzing the same amount (3000 ng) several times (n=5) the precision of the automatic spotting device was evaluated. The developed assay method was applied for the estimation of orlistat from two different capsule formulations. The drug content of the formulations was found to be 60.63 mg and 59.35 mg, which corresponds to 101.05±1.8611 and 98.92±0.8666 percentage label claim respectively as shown in table 3. This confirms that the method was successfully applied to the formulation analysis. The relative standard deviation (% RSD) for the analysis of 5 replicate applications indicated good instrumental precision and assay for the proposed HTLC method (% RSD consistently less than 2). Accuracy and repeatability and intermediate precision studies of orlistat at different levels were given in Table 4. The percentage RSD was found to range from 1.79 to 0.99%, averaging to 1.39 %. This confirms the good precision on the developed assay method.

The proposed HPTLC method combined with densitometric analysis was found suitable for determination of orlistat. Statistical data analysis proves that the method is precise and reproducible for the analysis of orlistat. The system being economical can be employed for the routine estimation of the drug in pharmaceutical formulations as well as in bulk drug analysis.

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Table1: Linear Regression Data for the Calibration Curves

Linearity Range (ng/spot)	R^2±S.D.	Slope	Intercept
1000-6000	0.9960±0.0029	$2x10^{-6}$	0.002

Table 2: Recovery Studies of Orlistat From Capsules

Amount of drug analysed (mg)	Capsules formulation concentration (mg/ml)	Amount of drug added (mg/ml)	Total Amount drug recovered	% recovery ±S.D.
60	1.0	0.8	59.71	99.52±0.0081
60	1.0	1.0	60.26	100.40±0.2232
60	1.0	1.2	60.57	100.60±0.0881

Table 3: Results for Instrumental Precision And Assay of Capsules Formulation By HPTLC

Capsules	Weight of capsule powder taken (mg)*	Amount of drug estimated (mg/capsule)*	% Label claim*
Cap 1	120.4	60.63	101.05±1.8611
Cap 2	118.2	59.35	98.92±0.8666

*Average of Five replicates

Cap 1: Lipocut 60, Lupin pharmaceutical, Pvt LTD. Kalina, Mumbai (Label Claim 60mg)

Cap 2: O Stat 60, Arlisto pharma, pvt Ltd. Mumbai (Label Claim 60mg)

Table 4: Accuracy and Precision Data of HPTLC Assay for Orlistat

Amount of Orlistat spotted (ng)	Amount detected (ng) (mean±SD)	RSD (%)
Inter-day (n= 3)		
2000	2030±1.0138	0.9988
4000	4091±1.5031	1.4696
6000	6097±0.6939	0.6829
Intra-day (n=3)		
2000	2096±1.8782	1.7916
4000	4157±1.5031	1.4460
6000	6263±1.2619	1.2089

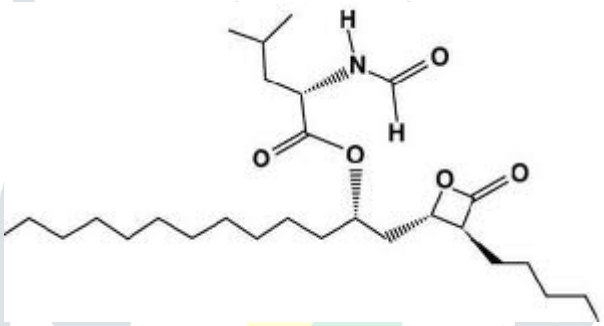

Fig. 1: Chemical structure of Orlistat

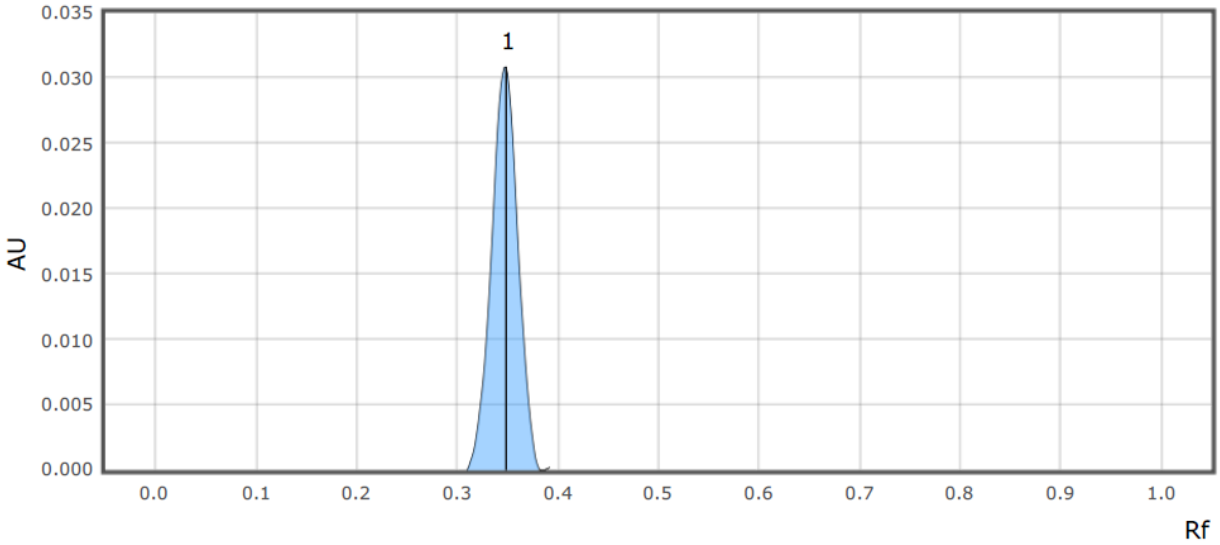

Fig. 2: HPTLC densitogram of orlistat 2 µl per spot with R_F 0.382 (Analysis was done using silica gel 60 F254 TLC plate as a stationary phase and toluene: ethyl acetate:phosphomolybdic acid (6:4:0.3 v/v/v) as mobile phase)

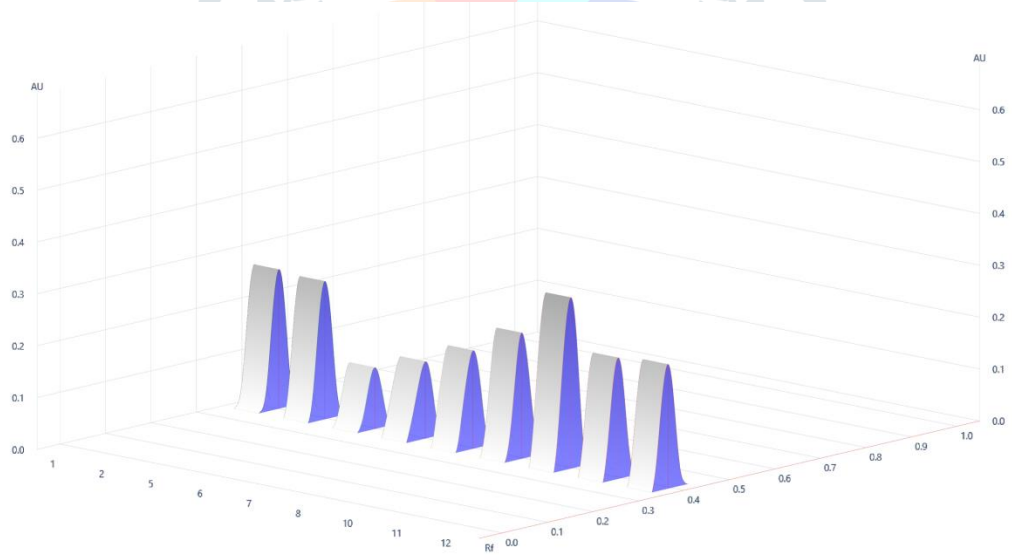

Fig. 3: Three dimentional overlay of HPTLC densitograms of standard plot of orlistst. Analysis was done using 60F-254 TLC plates as stationery phase and toluene-ethyl acetate:phosphomolybdic acid (6:4:0.3, v/v/v) as mobile phase.

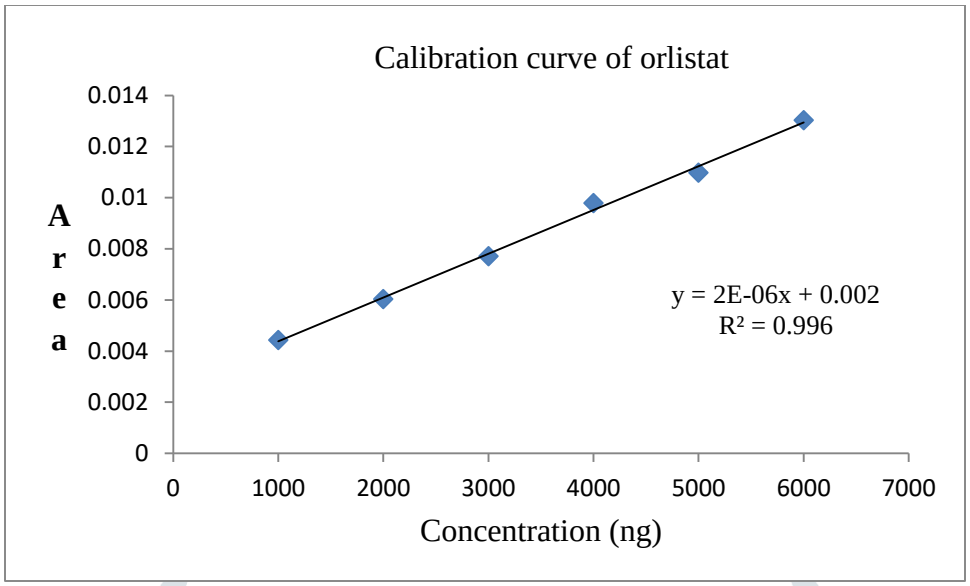

Fig. 4: Linear regression curve of the orlistat standard plot. The regression coefficient was found to be R^2= 0.9960 for the equation y=2E-6x-0.002

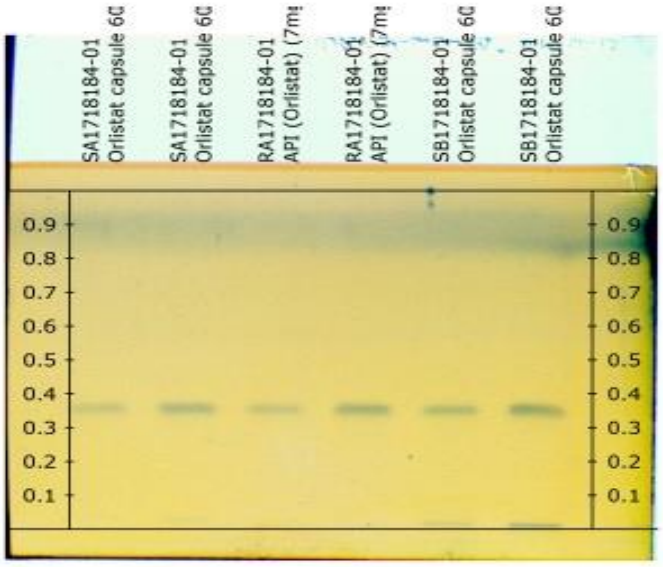

Fig. 5: Repeatability of orlistat standard and capsules spot in tungsten lamp at 581 nm (Analysis was done using silica gel 60 F254 TLC plate as a stationary phase and toluene:ethyl acetate:phosphomolybdic acid (6:4:0.3 v/v/v) as mobile phase for both standard and capsules)