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Application of TLC-densitometry method for simultaneous determination of lopinavir and ritonavir in capsule dosage form

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ABSTRACT

A rapid and simple high performance thin layer chromatography (HPTLC) method with densitometry at 263 nm was developed and validated for simultaneous determination of lopinavir and ritonavir from capsule dosage forms. Separation was performed on aluminum-backed silica gel $60F_{254}$ HPTLC plates as stationary phase and using a mobile phase comprising of toluene, ethyl acetate, methanol and ammonia, in the volume ratio of 6.5:2.5:0.5:0.5 (v/v) respectively. After development, plates were observed under UV light. The detector response was linear in the range of 6.5 to 20.00 µg/spot and 1.5 to 5.00 µg/spot for lopinavir and ritonavir respectively. The limit of detection was found to be 1.5 ng/spot and 4.6 ng/spot and limit of quantification was found to be 21.00 ng/spot and 5.10 ng/spot for lopinavir and ritonavir respectively. The percentage assay of lopinavir and ritonavir was found between 99.90±1.45 and 101.29±1.95 respectively. The developed method was validated as per ICH guidelines and by recovery studies. The method was found to be simple, rapid, precise and accurate and can be used for routine analysis.

Key words: HPTLC, Lopinavir, Ritonavir, Simultaneous determination.

INTRODUCTION

Lopinavir[1,2], [1S-[1R*, (R*), 3R*, 4R*]]-N-[4 [[(2,6dimethyl-phenoxy) acetyl] amino]-3-hydroxy-5-phenyl 1(phenyl methyl) pentyl] tetra hydro-alpha-(1-methylethyl)-2-oxo-1(2H) pyrimidine acetamide and Ritonavir[1-2], 10-Hydroxy-2-meth yl-5- (1-methylethyl) -1- [2-(1-methylethyl) -4-thiazolyl] -3, 6-dioxo-8, 11-bis (phenyl methyl) -2, 4,7 ,12 –tetra azatridecan-13-oic acid, 5-thiazolylmethyl ester, [5S-(5R*, 8R*, 10R*, 11R*)] are HIV protease inhibitors used in combination therapy. lopinavir and ritonavir (Sustained release capsule with combination of lopinavir 133.3 mg and ritonavir 33.3 mg is available in market by brand name kaletra®) has been shown to be effective against drug-resistant HIV-1[3]. These agents are metabolized by cytochrome P-450 (CYP) 3A in the liver[4-6]. A literature survey reveals analytical methods like HPLC, LC-MS for simultaneous determination of lopinavir and ritonavir in pharmaceutical dosage forms and biological fluids using[8-12] However, no references are reported so far for the simultaneous determination of said drugs by HPTLC method. So it was planned to developed and validate[7] simple, rapid and precise TLC densitometry method for simultaneous estimation of said drugs in combined dosage form of capsules.

MATERIAL AND METHODS

Chemicals and reagents

The lopinavir and ritonavir working standards were obtained as a gift sample from Cipla Ltd. Mumbai. All chemicals and reagents were procured from Qualigens. Capsule dosage forms were procured from local market.

Instrumentation and chromatographic conditions

Chromatography was performed on pre-coated silica gel 60 F_{254} HPTLC plates (Merck). Before use they were pre-washed with methanol and dried in an oven at 105°C for 2 hrs. 10 µL of sample were spotted 8 mm from the edge of the plates by means of a Camag Linomat IV sample applicator. The plates were developed to a distance of 85 mm in a Camag twin-trough chamber previously equilibrated 15 min with mobile phase i.e. toluene:ethylacetate:methanol:ammonia [6.5:2.5:0.5:0.5 (v/v)]. The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. Plates were evaluated by densitometry at 263 nm with a Camag Scanner II, in conjunction with WINCATS software for quantification. The typical chromatogram is shown Fig. 1.

Preparation of standard stock solution

Accurately weigh 25 mg pure standard of lopinavir and 10 mg of pure standard ritonavir transfer to separate 10 mL volumetric flask. The drugs were dissolved in methanol, diluted up to the mark with methanol and mixed well. This gave a standard stock solution of strength 2500µg/mL of lopinavir and 1000 µg/mL for ritonavir.

Preparation of working standard solution

Further the mixture of working standard solution was prepared by diluting 26.6 mL of lopinavir (2500 µg/mL) and 16.65mL of ritonavir (1000 µg/mL) standard stock solution in 50.0mL volumetric flask with methanol to get strength of 1333.00 µg/mL of lopinavir and 333.00 µg/mL of ritonavir.

Preparation of sample solution

Twenty capsules (KALETRA) were weighed and the average weight was calculated. The capsules were crushed tofurnish a homogeneous powder and a quantity equivalent to one capsule (431.01 mg) were weighed in a 100 mL standard volumetric flask. The powder dissolved in methanol and diluted up to the mark with methanol. That solution was then sonicated for 30 min. Then cooled to room temperature and diluted with methanol. The solution was filtered through Whatman No. 41 filter paper and the filtrate was used as sample solution.

Validation Procedures

Linearity

A series of standard curves were prepared from standard stock solutions of both drugs over a concentration range of 6.5-20.00 µg/spot for lopinavir and 1.5-5.00 µg/spot for ritonavir by sample applicator and the plate was developed. The detector response to the different concentrations was measured. The drug peak-area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area. The data were analyzed by linear regression least-squares fitting. The statistical data obtained are given in Table 1.

Limit of detection and limit of quantitation

The limit of detection (LOD) was found to be 1.5 ng/spot for lopinavir and 4.6 ng/spot for ritonavir. Limit of quantitation (LOQ) for lopinavir and ritonavir were determined experimentally by spotting six replicates of each drug at LOQ concentration. The LOQ of lopinavir and ritonavir were found to be 21.00 ng/spot and 5.10 ng/spot respectively.

Assay (from the pharmaceutical preparation)

10 µL working standard solution (13.33 µg/spot of lopinavir and 3.33 µg/spot of ritonavir) and sample solutions were spotted on the plate and the plate was developed and evaluated as described above. The procedure was repeated five times, individually weighing the capsule powder each time. The densitometric responses from the standard and sample were used to calculate the amounts of the drug in the capsule. The results obtained are as shown in Table 2.

Recovery studies

The accuracy of the experiment was established by spiking pre-analyzed sample with known amounts of the corresponding drugs at three different concentration levels i.e. 20, 40 and 60 % of the drug in the capsule (the external standard addition technique). The spiked samples were then analyzed for five times. The results from recovery analysis are given in Table 3. The mean recovery is within acceptable limits, indicating the methods are accurate.

RESULTS AND DISCUSSION

Use of pre-coated silica gel HPTLC plates with toluene:ethyl acetate:methanol:ammonia, in the volume ratio of 6.5:2.5:0 .5:0.5 resulted in good separation of the drug. Figure 1 shows a typical densitogram obtained from lopinavir and ritonavir. Regression analysis of the calibration data for lopinavir and ritonavir showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equations Y=1008.89X+ (-2280.63) for lopinavir and Y= 4310.14X+ (-789.49) for ritonavir. The correlation of coefficient (r^2) obtained was 0.9973 for lopinavir and that for ritonavir is 0.9915. That means a good linear relationship was observed between the concentration ranges 6.5 to 20.00 µg/spot and 1.5 to 5.00 µg/spot for lopinavir and ritonavir respectively. The system suitability experiment was carried out before the determination of lopinavir and ritonavir in unknown samples. The coefficient of variation was less than 2% for replicate measurements of the same sample. This shows that the method and the system both are suitable for the determination of unknown samples. The assay of lopinavir and ritonavir was found to be 99.90% and 101.29%. From the recovery studies it was found that about 100.36% and 100.27 % of lopinavir and ritonavir respectively which indicates high accuracy of the method. The absence of additional peaks in chromatogram indicates non- interference of the common excipients used in capsules.

Table 1: Linear Regression Data for Calibration Curve

Parameters	Lopinavir	Ritonavir
Linearity range(µg/spot)	6.5-20.0	1.5-5.0
r^2 ± SD	0.9973 ± 0.0004	0.9915 ± 0.0002
Slope ± SD	1008.89 ± 10.01	4310.14 ± 6.09
Intercept ± SD	-2280.63 ± 34.97	-789.49 ± 12.27

Table 2: Results of Assay studies from Capsule

Brand	Drug	Labeled amount (mg,n=5)	Amount found (mg, n=5)	%Assay (n=5)	% RSD (n=5)
	Lopinavir	133.3	133.17±2.0	99.90±1.45	1.5
	Ritonavir	33.3	33.73±0.69	101.29±1.95	1.9

Table 3: Results from recovery analysis (n=5)

Excess drug added to the analyte (%)	Theoretical content (mg)	Recovery (%)	% RSD
Lopinavir			
20	(133.3) + 26.66	100.77	0.83
40	(133.3) +53.52	100.36	0.18
60	(133.3) +79.98	100.69	0.44
Ritonavir			
20	(33.3) + 6.66	100.99	0.17
40	(33.3) + 13.32	101.52	0.35
60	(33.3) + 19.98	100.73	0.29

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