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Research Article

Method Development and Validation of Indinavir Sulphate Capsules by RP-HPLC Method

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ABSTRACT

A validated RP-HPLC method has been developed for the determination of Indinavir sulphate capsule dosage form. This method is developed by using a Zodiac ODS hypersil C_{18} column (250mm length, 4.6mm internal diameter and 5µm particle size) and a mixture phosphate buffer pH 5.5, Acetonitrile and Methanol (50:30:20) as a mobile phase. The drug was quantified by a UV detector at 260nm. The method is linear in range of 48µg/ ml to 112µg/ ml. The accuracy of the method was found to be in the range of 98.36% to 101.74%. The proposed method was found to be linear, precise, and accurate for the quantitative estimation of Indinavir sulphate in capsules and can be used for commercial purpose.

Keywords: Indinavir sulphate; RP- HPLC;Method development; Method validation.

1. INTRODUCTION

Indinavir sulphate[1-3] is Chemically (2S)-1-[(2S, 4R)- 4- Benzyl- 2-hydroxyl-5-{[(1S,2R)-2-hyroxy-2,3-dihydro-1H-inden-1-yl]amino} -5-oxopentyl]- N- (2-methy-2-propanyl)4-(3-pyridinyl methyl)-2-piperazine carboxamide sulphate. It is a protease inhibitor used as a component of highly active antiretroviral therapy to treat HIV infection and AIDS. Literature survey reveals, HPLC, RP-HPLC, LC/MS/MS, LC-TMS method for analysis of Indinavir sulphate.

2. MATERIALS AND METHODS

Instrument: The HPLC system used was Schimadzu – SPP equipped with UV detector source of deuterium lamp. The chromatogram was recorded at and peaks quantified by means of PC based software.

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Solvents used: Acetonitrile, methanol and 0.1% ortho phosphoric acid for HPLC grade.

Preparation of phosphate buffer:

Sol 1: Dissolve 13.61gm of potassium dihydrogen phosphate in sufficient water to produce 1000ml.

Sol 2: Dissolve 35.81gm of disodium hydrogen phosphate in sufficient water to produce 1000ml.

Mix 96.4ml of solution 1 with 3.6ml of solution 2

Preparation of mobile phase: Filtered degassed mixture of phosphate buffer, Acetonitrile and methanol in the ratio of 50:30:20

Preparation of standard solution: Dissolve 40mg of drug in 100ml of mobile phase (stock solution). Pipette out 2ml of the solution into 10ml volumetric flask and make up the volume to 10ml.

Preparation of sample solution: Dissolve 60mg of drug in 100ml of mobile phase (stock solution) pipette out 2ml of the solution into 10ml volumetric flask and make up the volume to 10ml.

ASSAY:

Preparation of sample solution: Dissolve 60mg of drug in 100ml of mobile phase (stock solution) pipette out 2ml of the solution into 10ml of volumetric flask and make up the volume to 10ml.

Procedure: 20µ of the blank, standard, and sample was injected into the chromatographic system and area for Indinavir sulphate , the peaks were used for calculating the %assay by using the formula.

$$\text{Assay} = \frac{\text{sample area}}{\text{Standard area}} \times \frac{\text{wt of standard}}{\text{wt of sample}} \times \frac{\text{Dilution of sample}}{\text{Dilution of standard}} \times \frac{p}{100} \times \frac{\text{Avg wt}}{LC} \times 100$$

3. RESULTS AND DISCUSSIONS

Method Development[4-5]: After the number of trials we arrived at the optimized parameter for the estimation of Indinavir sulphate, which are given in Table 1.

Accuracy: The standard solution of accuracy 80%, 100% and 120% was injected into the chromatographic system. Calculate the percentage recovery and mean recovery values. The results are tabulated in Table.2.

Linearity: Each level was injected into chromatographic system and peak area was measured. Plot a graph of peak area Vs concentration (X-axis concentration, Y-axis peak area). The results are tabulated in Table 3.

Precision: The standard solution was injected for 6 times and measured for the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within specific limits. The results are tabulated in Table 4.

Robustness: The flow rate was varied at 0.8ml/min to 1.2ml/min and the wavelength was varied at 258nm to 262nm. The results are tabulated in Table.5.1 and Table 5.2.

Ruggedness: The %RSD for the area of 5 standard injections results in %. The acceptance criteria of the %RSD for the area of 5 standard injections results should not be more than 2%. The results are tabulated in Table 6.

Table 1: Optimized chromatographic conditions

Column	Hypersil ODS
Mobile phase	Phosphate buffer pH 5.5 : Acetonitrile: Methanol (50:30:20)
Flow rate	1ml
Detector Wavelength	260nm
Injection volume	0.02ml
Run time	10min
Retention time	2.960

Table 2: Values showing the results of accuracy

% concentration (at specification level)	Area	Added amount (mg)	Amount found (mg)	% Recovery	Mean recovery
80%	3238.855	64+16	80.13	100.17	
100%	4012.250	80+16	94.43	98.36	100.09%
120%	4577.015	96+16	113.94	101.74	

Table 3: Values showing the results of linearity

S. No	Concentration	Area
1.	48 µg/ml	1881.810
2.	64 µg/ml	2573.972
3.	80 µg/ml	3425.968
4.	96 µg/ml	3940.469
5.	112 µg/ml	4474.837
Correlation coefficient		0.99

Table 4: Values showing the results of precision

Injection (Indinavir)	Area	Retention time
Injection- 1	3425.968	2.967
Injection- 2	3427.591	2.967
Injection- 3	3449.107	2.963
Injection- 4	3425.327	2.957
Injection- 5	3427.957	2.960
Injection- 6	3427.78	2.960
Average	3430.622	2.9623
Standard Deviation	9.119	0.0041
% RSD	0.27	0.138

Table 5.1: Values showing the results of robustness (by varying flow rate)

S. No	Flow Rate (ml/min)	System suitability	
		USP plate count	USP tailing
1.	0.8ml/min	4251	1.82
2.	1.0ml/min	3839	1.68
3.	1.2ml/min	3407	1.76

Table 5. 2: Values showing the results for robustness (by varying wavelength)

S. No	Wavelength	System Suitability	
		USP plate count	USP tailing
1.	258nm	3822	1.68
2.	260nm	3839	1.68
3.	262nm	3796	1.68

Table 6: Values showing the results of Ruggedness

S. No	Area	Retention time
Injection 1	3447.011	2.967
Injection 2	3405.659	2.963
Injection 3	3430.506	2.967
Injection 4	3441.953	2.967
Injection 5	3440.23	2.967
Average	3433.071	2.966
Standard Deviation	16.44	
% RSD	0.4788	

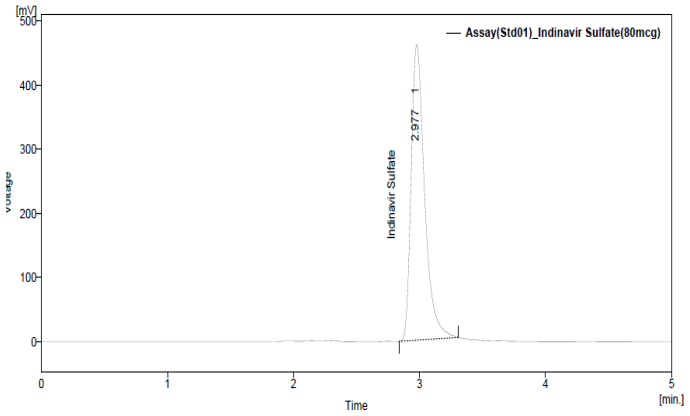

Figure 1: Showing the standard injection chromatogram

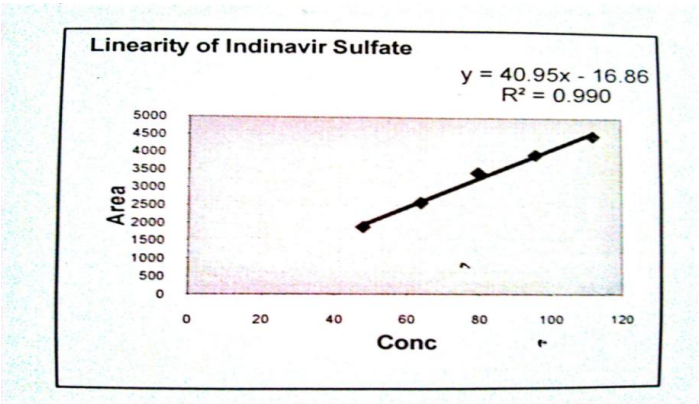

Figure 2: Showing the Linearity graph

4. CONCLUSION

A new method was established for the estimation of Indinavir sulphate by RP-HPLC method. The chromatographic conditions were successfully developed for the estimation of Indinavir sulphate by using Schimadzu- SPD, C_{18} column, flow rate was 1ml/min, mobile phase ratio was (50:30:20) phosphate buffer pH 5.5 : Acetonitrile: Methanol, detection wavelength was 260nm. The retention time was found to be 2.96minutes. The analytical method was validated according to ICH guidelines. The linearity study for Indinavir sulphate was found in concentration range of 48- 112µg/ml and correlation coefficient (r^2) was found to be 0.990 and % recovery was found to be 98.36% to 101.74%. Hence the proposed method was found to be simple, sensitive, accurate, precise, robust, and economical for the determination of Indinavir sulphate in capsule dosage form. The mobile phase is simple to prepare and economical. The sample recoveries were in good agreement with label claims. Hence it can be applied for routine analysis.

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