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Original Article

A RAPID RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF INDINAVIR IN CAPSULES

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ABSTRACT

Objective: To develop an accurate, precise and linear Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH guidelines for the quantitative estimation of Indinavir sulphate (400mg) in capsules.

Methods: The optimized method uses a reverse phase column, Enable Make C18G (250 X 4.6 mm; 5µ), a mobile phase of triethylammonium phosphate buffer (pH 2.5): acetonitrile in the proportion of 50:50 v/v, flow rate of 1.0 ml/min and a detection wavelength of 220 nm using a UV detector.

Results: The developed method resulted in Indinavir sulphate eluting at 2.7 min. Indinavir sulphate exhibited linearity in the range 50-150µg/ml. The precision is exemplified by relative standard deviation of 1.7%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 4.34ng/ml and 13.15ng/ml respectively.

Conclusion: An accurate, precise and linear RP-HPLC method was developed and validated for the quantitative estimation of Indinavir sulphate in INDIVAN (400mg) capsules as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

Keywords: RP-HPLC, Indinavir sulphate, Method development, Validation.

INTRODUCTION

Indinavir sulphate (Figure 1) is a human immunodeficiency virus (HIV) protease inhibitor used for treating acquired immune deficiency syndrome (AIDS). Indinavir sulphate is usually prescribed in combination with other protease inhibitors, nucleoside analogues or reverse transcriptase inhibitors [1-3].

IUPAC name of Indinavir sulphate is [1(1*S*,2*R*),5(*S*)]-2,3,5-trideoxy-N-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl)-5-[2-[[(1,1-dimethylethyl) amino] carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-phenyl methyl)-D-*erythro*-pentonamide sulphate (1:1) salt. The drug has a molar mass of 613.88 g/mol for the free base and 711.88 g/mol for the sulphate salt and is commercially available as capsules (trade name: INDIVAN) containing the equivalent of 400 mg of indinavir free base.

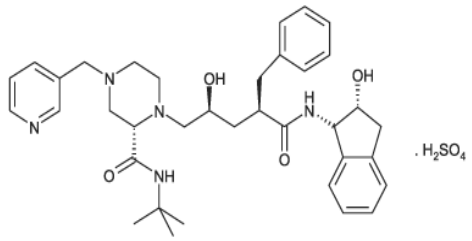

Fig. 1: Structure of Indinavir sulphate

A detailed literature survey reveals LC methods for the analysis of Indinavir sulphate individually and in various combinations in biological matrices [4-10], capillary zone electrophoresis method for the analysis of indinavir sulphate raw material [11], few RP-HPLC methods for the determination of assay of Indinavir in bulk and in capsule dosage forms [12-13]. We here report a totally new and a rapid RP-HPLC method for the quantitative estimation of Indinavir sulphate in INDIVAN capsules.

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure sample of Indinavir sulphate with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [INDIVAN] was procured from APOLLO Pharmacy, Hyderabad, India with labelled amount 400mg of Indinavir sulphate. Acetonitrile (HPLC grade), water (HPLC grade), Triethylamine (AR Grade) and ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India), 0.45µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu Prominence Liquid Chromatograph comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "Lab solutions lite" software. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Shimadzu UV-1800 series, software-UV probe version 2.42) were used in this study.

Method

Selection of wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Indinavir sulphate. Suitable wavelength selected was 220 nm (**Figure 2**).

Chromatographic conditions

The developed method uses a reverse phase C18 column, Enable Make C18G (250 X4.6 mm; 5µ), mobile phase consisting of triethylammonium phosphate buffer (adjusted using 30% v/v of ortho phosphoricacid pH 2.5): acetonitrile in the proportion of 50:50

v/v. The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 20µl for every injection. The detection wavelength was set at 220 nm.

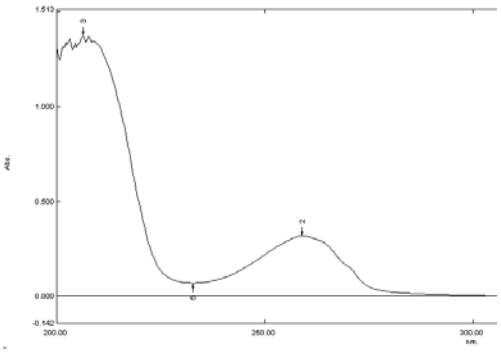

Fig. 2: UV spectrum of Indinavir sulphate

Buffer preparation

The buffer solution was prepared by adding 5 ml of triethylamine to 1000 ml of HPLC grade water and later pH was adjusted to 2.5 using 30% v/v of ortho phosphoric acid in water. The buffer was then filtered through 0.45 µm nylon membrane filter.

Mobile phase preparation

The mobile phase was prepared by mixing acetonitrile and buffer in the ratio of 70:30 v/v and later it was sonicated for 10 minutes for the removal of air bubbles.

Preparation of working standard solution

10mg of Indinavir sulphate was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluent (same as mobile phase) and then sonicated for 2 minutes to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as working standard solution (100µg/ml), 100% target concentration.

Preparation of stock and working sample solution

Ten tablets were weighed separately and the average weight was determined. The average weight was weighed from the ten tablets grinded in a pestle and mortar, transferred to a 100 ml volumetric flask containing 100 ml diluent and then sonicated for 3 minutes, followed by filtration through 0.45µ nylon membrane filter to get sample stock solution of 4mg/ml. 0.25 ml of the above stock solution was pipetted out and made up to 10 ml to get working sample solution equivalent to a concentration of working standard of 100µg/ml.

RESULTS AND DISCUSSION

Method development

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Indinavir sulphate at 2.7 min. Figures 3 and 4 represent chromatograms of blank solution and standard solution (100µg/ml) respectively. The total run time is 4 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*Rt*), number of theoretical plates (*N*) and peak Asymmetric factor were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1.

In order to test the applicability of the developed method to a commercial formulation, 'INDIVAN was chromatographed at working concentration (100µg/ml) and it is shown in Figure 5. The sample peak was identified by comparing the retention time with the standard drug Figure 4. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control.

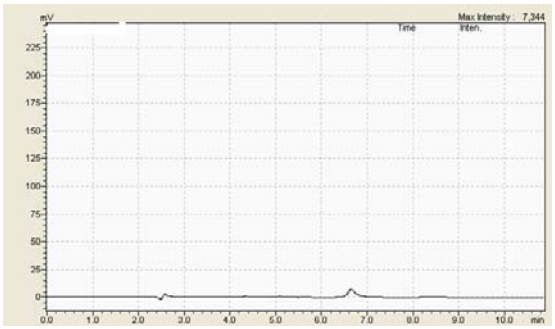

Fig. 3: Typical Chromatogram of Blank solution

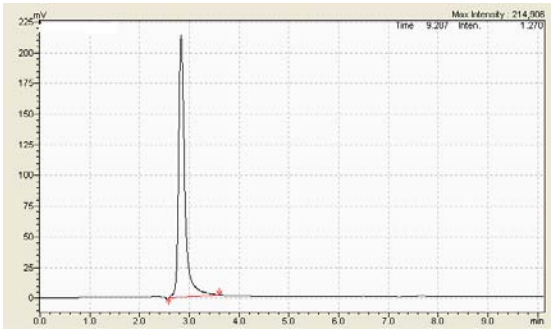

Fig. 4: Typical chromatogram of the standard solution

Table 1: System suitabilitystudies results

Parameters*	Indinavir sulfate
Retention time (min)	2.738
Number Of Theoretical plates (N)	2830
Tailing factor (T)	1.580

* Mean of six injections

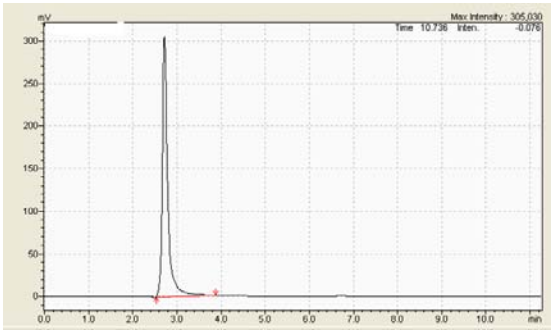

Fig. 5: Typical chromatogram for the tablet formulation

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the

method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines [14] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, limit of detection (LOD) and limit of quantitiation (LOQ).

Specificity

Figures 3-5 for blank, standard drug solution and sample chromatogram reveal that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drugs as blank has no peak at the retention time of Indinavir sulphate. Accordingly it can be concluded that, the method developed is said to be specific.

Precision

System precision

Six replicate injections of the standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in Table 2.

Table 2: System precision results

Injection number (n)	Indinavir sulfate	
	Rt	Peak Area
1	2.799	1802637
2	2.829	1809456
3	2.784	1811117
4	2.797	1800972
5	2.811	1840300
Average		1812896
SD		15917.02
% RSD		0.877

Method precision

Method precision was determined by performing assay of sample under the tests of repeatability (Intra day precision) at working concentration.

Repeatability (Intra day precision)

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (**Table 3**).

Table 3: Intra day precision results

n	Indinavir sulfate % Assay
1	99.02
2	98
3	99.1
4	102
5	101.46
Average	99.9
S.D.	1.722
% R.S.D.	1.723

Linearity

Standard solutions of Indinavir sulphate at different concentrations level (50%, 75%, 100%, 125%, 150% and 175%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding peak area. The results show an excellent correlation between peak area and concentration level of drug within the concentration range (50-150µg/ml) for the drug and the results are given in **Tables 4-5.** The correlation coefficient of Indinavir sulphate is greater than 0.99, which meet the method validation acceptance criteria and hence the method is said to be linear.

Table 4: Linearity of the chromatography system

Drug	Linearity range (µg/ml)	R^2	Slope	Intercept
Indinavir sulfate	50-150	0.9936	18222.44	62118

Table 5: Calibration data for Indinavir sulfate.

% Level	Concentration (µg/ml)	Peak Area 1	Peak Area 2	Peak Area 3
50	50	854043	857555	923599
75	75	1374882	1444948	1521563
100	100	1908429	1912456	1915222
125	125	2380765	2398465	2365851
150	150	2692141	2746449	2798542
Regression equation		Y=18728.3-30779.6	Y=17562.08+149849.1	Y=18376.96+67285.8
Regression coefficient		0.991	0.995	0.995

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in **Table 6.** The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 6: Results of Accuracy studies for Indinavir sulfate

Concentration level (%)	*%Mean recovery
50	100.31
100	99.7
150	101.6

*Mean of three replicates

Sensitivity

The sensitivity of measurement of Indinavir sulphate by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 4.34ng/ml and 13.15 ng/ml.

CONCLUSION

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, limit of detection and limit of quantitation, for the quantitative estimation of Indinavir sulphate in tablets. The precision is exemplified by relative standard deviation of 1.7 %. A good linear relationship was observed for the drug between concentration ranges of 50 and 150µg/ml. Accuracy studies revealed that mean recoveries were between 98 and 102%, an indicative of accurate method. Accordingly it can be concluded that the developed

reverse phase isocratic HPLC method is accurate, precise and linear and therefore the method can be used for the routine analysis of Indinavir sulphate in tablets.

CONFLICT OF INTERESTS

Declared None

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