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Original Article

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

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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 developed method uses a reverse phase C18 column, INERTSIL ODS 3V column (250X4.6 mm; 5µ), mobile phase consisting of Potassium dihydrogen orthophosphate buffer (adjusted using 30% v/v of ortho phosphoric acid pH 3.5):methanol: acetonitrile in the proportion of 20:40:40 v/v. The mobile phase was set at a flow rate of 1.2 ml/min and the volume injected was 20µl for every injection. The detection wavelength was set at 220 nm.

Results: The developed method resulted in Indinavir sulphate eluting at 3.75 min. Indinavir sulphate exhibited linearity in the range 60-140µg/ml. The precision is exemplified by relative standard deviation of 0.709%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 104ng/ml and 315ng/ml respectively.

Conclusion: An accurate, precise and linear RP-HPLC method was developed and validated for the quantitative estimation of Indinavir sulphate in INDIVIR (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 (Fig.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-pyridinyl methyl)-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.

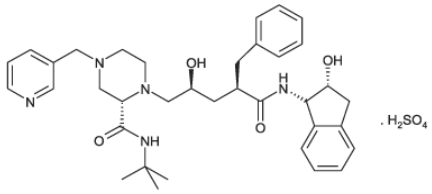

Fig. 1: Structure of Indinavir sulphate

A detailed literature survey reveals liquid chromatographic 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 capsule dosage forms [12-14].

Previous literature reports make use of either dibutylammonium phosphate buffer (pH 6.5) or citrate buffer (pH 5) or triethylammonium phosphate buffer (pH 2.5) as a part of mobile phase. We here report a totally new and a rapid RP-HPLC method for the quantitative estimation of Indinavir Sulphate in INDIVIR capsules using potassium dihydrogen orthophosphate buffer at pH 3.5.

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 [INDIVIR] was procured from Apollo Pharmacy, Hyderabad, India with labelled amount 400mg of Indinavir Sulphate.

Acetonitrile (HPLC grade), Methanol (HPLC Grade), water (HPLC grade), Potassium dihydrogen ortho phosphate (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 LC-20AT VP Liquid Chromatograph comprising a LC-20AT pump, Shimadzu SPD-20A UV-VISIBLE detector and a reverse phase C18 column, INERTSIL ODS 3V column (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 "Spinchrom" software.

A double beam UV-visible spectrophotometer Nicolet evolution 100 having two matched quartz cells with 1 cm light path were used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (1mg sensitivity, Shimadzu BL220H), digital pH meter (Global digital) and sonicator (Citizen) 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 (Fig.2).

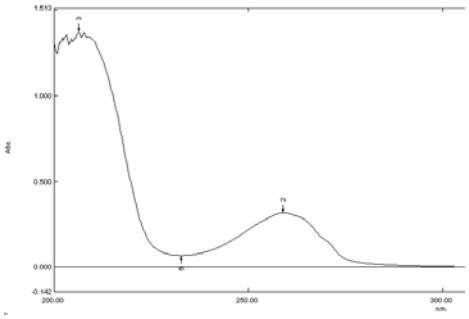

Fig. 2: UV spectrum of Indinavir sulphate

Chromatographic conditions

The developed method uses a reverse phase C18 column, INERTSIL ODS 3V column (250 X4.6 mm; 5µ), mobile phase consisting of Potassium dihydrogen orthophosphate buffer (adjusted using 30% v/v of ortho phosphoric acid pH 3.5):methanol: acetonitrile in the proportion of 20:40:40 v/v. The mobile phase was set at a flow rate of 1.2 ml/min and the volume injected was 20µl for every injection. The detection wavelength was set at 220 nm.

Buffer preparation

The buffer solution is prepared by weighing 2.736g of potassium dihydrogen orthophosphate (KH_2PO_4) and transferring to 1000 ml of HPLC grade water to get 20 mM buffer strength and later pH was adjusted to 3.5 using 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, methanol and buffer in the ratio of 40:40:20 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 diluents (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 equivalent weight to 400mg of Indinavir sulphate was determined. The equivalent 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. Asymmetry factor (A), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Indinavir sulphate at 3.75 min. Fig.3-4 represents chromatograms of blank solution and standard solution (100µg/ml) respectively. The total run time is 5 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 (A) was evaluated for six replicate injections of the standard at the working concentration. The results are given in table 1**.**

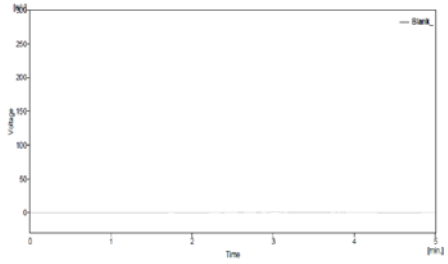

Fig. 3: Typical Chromatogram of Blank solution

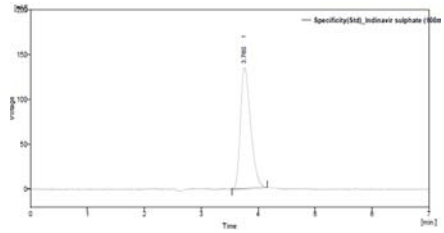

Fig. 4: Typical chromatogram of the standard solution

Table 1: System suitabilitystudies results

Parameters*	Indinavir sulphate
Retention time (min)	3.75
Number Of Theoretical plates (N)	2917
Asymmetry (A)	1.75

* Mean of six injections

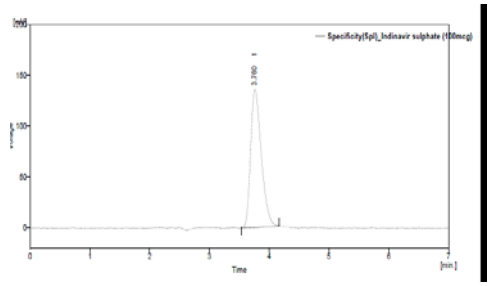

Fig. 5: Typical chromatogram for the tablet formulation

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 [15] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantitiation (LOQ).

Specificity

Fig. 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.

Method precision

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

Repeatability (Intraday 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 2: System precision results of Indinavir sulphate

Injection	Rt (min)	Peak area
1	3.75	1705.17
2	3.76	1710.95
3	3.747	1710.14
4	3.757	1709.11
5	3.75	1701.6
6	3.74	1679.07
Average	3.7512	1702.67
SD	0.0072	12.087
%RSD	0.19	0.71

Table 3: Intraday precision results of Indivair sulphate

Injection	%Assay
1	99.94671
2	100.2854
3	100.238
4	100.1775
5	99.73722
6	98.41654
Average	99.80024
S. D.	0.708
% R. S. D.	0.709

Linearity

Standard solutions of Indinavir sulphate at different concentrations level were prepared. Calibration curve (Fig.6) 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 (60-140µg/ml) for the drug and the results are given in table 4**.** The correlation coefficient of Indinavir sulphate is greater than or equivalent to 0.99, which meet the method validation acceptance criteria and hence the method is said to be linear.

Table 4: Calibration data for Indinavir sulphate

Concentration (µg/ml)	Peak Area 1
60	1012.2
80	1351.2
100	1687.06
120	2034.45
140	2361.75
Regression equation	Y=16.911X-1.8419
Regression coefficient	0.99

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in table 5**.** 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.

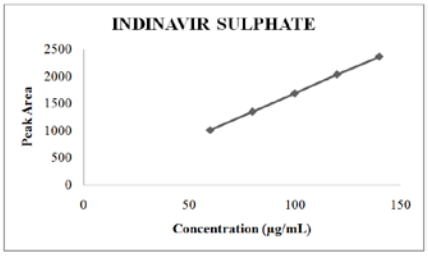

Fig. 6: Linearity graph of Indinavir sulphate

Table 5: Results of Accuracy studies for Indinavir sulphate

Concentration level (%)	*%Mean recovery
80	99.97
100	100.05
120	99.88

*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 limit of detection (LOD). LOQ and LOD were calculated by the use of the equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of response of calibration plot and S is the slope of the corresponding calibration plot. The limit of detection (LOD) and limit of quantitiation (LOQ) for Indinavir sulphate was found to be 0.104µg/ml and 0.315µg/ml respectively.

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 0.709 %. A good linear relationship was observed for the drug between concentration ranges of 60 and 140µg/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.

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CONFLICT OF INTERESTS

Declared None

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