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A Rapid RP-HPLC Method Development for Quantitative Estimation of Indinavirin Pure and Dosage Forms

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ABSTRACT

A simple, accurate and sensitive RP-HPLC method stability indicating RP-HPLC method has been developed and validated for the analysis of Indinavir. The studies on selection of mobile phase and flow rate basing on peak parameters were extensively carried out. Acetonitrile, water and phosphoric acid in the ratio of 80:15:5 (v/v/v) were employed as a mobile phase in the present assay. The accuracy of the developed method was demonstrated at three concentration levels in the range of 50–150 %.The recoveries of the Indinavir from a series of spiked concentrations and the percentage recoveries were found in the range of 99.73 to 99.96 %. The % drug content of tablets obtained by the proposed method for Indinavir was found to be 99.99 %, respectively. The developed method was said to be simple, selective and accurate and is useful for the assay of Indinavir in dosage forms and can be further employed in the quality control analysis of bulk manufacturing and formulations units.

Keywords: Indinavir, RP-HPLC, Validation, Recovery Studies, Precision and Ruggedness.

INTRODUCTION

Indinavir sulfate (Figure-1), chemically known as [1(1*S*,2*R*), 5(*S*)]-2,3,5-trideoxy-N-2,3-dihydro-2-hydroxy-1H-inden-1-yl)-5-[2-[[(1,1-dimethylethyl)amino]carbonyl]-4-(3pyridinylmethyl)-1-piperazinyl]-2-phenylmethyl)-D-erythro-pentonamide sulfate (1:1) salt [1, 2], is a potent protease inhibitor of Human Immunodeficiency Virus (HIV) widely used in the treatment against the acquired immune deficiency syndrome (AIDS) and is prescribed in combination with other protease inhibitors, nucleoside analogues or reverse transcriptase inhibitors. Literature survey revealed that few analytical methods [3-8] have been reported for the estimation of Indinavir in dosage forms. In this accord the author attempted, to develop and validate simpler, economic, rapid, precise and accurate analytical methods with good sensitivity for quantitative analysis of Indinavir in pure and marketed formulations in accordance with International Conference on Harmonization (ICH) guidelines.

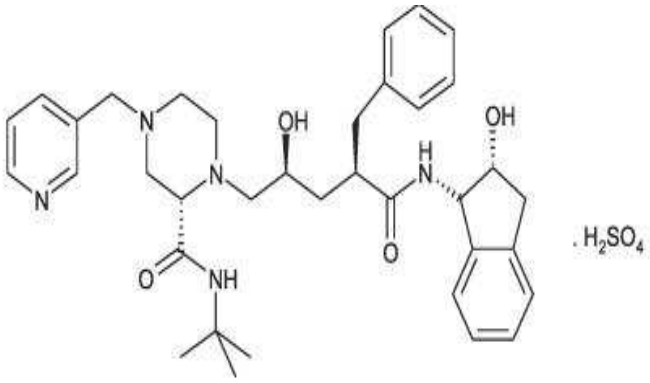

Figure-1: Molecular Structure of Indinavirsulphate

This paper describes the development and validation of some new RP-HPLC for the assay of Indinavir in pure and dosage forms. It briefs the chemical name, structure, therapeutic importance, analytically useful functional groups, and commercially available formulations and is divided into two sections. The specrtophotometric methods reported [3, 4] in the literature for Indinavir revealed that relatively little attention was paid in developing economical methods. Rao et al. have reported their work on different oxide materials in their earlier studies [9-29]. A detailed literature survey revealed few RP-HPLC methods for the determination of assay of Indinavir in bulk and in dosage forms [7, 8]. In the view of the above importance of stability testing, the authors therefore, developed and validated a stability-indicating RP-HPLC assay method for Indinavir in pure and in pharmaceutical dosage form as per ICH guidelines. The author's investigations and the experimental work carried out were incorporated in this present manuscript and moreover these developed methods have been extended to pharmaceutical formulations as they are simple, economical and sensitive.

MATERIALS AND METHODS

Chemicals and Solvents: Indinavir standard (99.9%pure) was obtained asgifted sample from Hetero drugs Ltd, Hyderabad. Tablets of Indinavir [INDINAVIN] were purchased from local pharmacy. HPLC grade Acetonitrile (Qualigens), orthophosphoric acid of AR grade were obtained from Sd. Fine Chemicals Ltd. and HPLC grade water was obtained from a Milli-QRO water purification system.

Instumental Appratus: The HPLC analysis of Indinavir was carried out on HPLC system with Waters 2695 alliance equipped with binary HPLC pump, ThermoHypersil BDS C-18 column (250mmx4.6mm, particle size) and Waters 2998 PDA detector. The output signal was monitored and processed using the built-in Empower 2 software. Electronic analytical balance (DONA) and Micro pipette (In labs, 10-100µl) were employed in the present analysis. All the glassware employed in the present analysis were cleaned with hot water and dried in hot air oven whenever required.

Mobile Phase Preparation: Acetonitrile, water and phosphoric acid in the ratio of 80:15:5 (v/v/v) were employed as a mobile phase in the present assay. Before use, this mobile phase was degassed and filtered through 0.45µ membrane filter.

Diluent Preparation: Methanol is used initially as diluents for extractingthe drug and consequent dilutions were made with mobile phase.

Preparation of Standard Solution: Accurately weighed about 50.0mg of Indinavir and transferred into a 100mL volumetric flask then, add 60mL of methanol and sonicated to dissolve. Cool the solution to room temperature and diluted to mark with methanol [stock solution]. Daily working standard solutions of Indinavir were prepared with mobile phase containing Indinavir at a concentration of 5.0-15.0µg/mL and each of the these dilutions (20µl) was injected six times in to the column, with flow rate of1.0 mL/min and peak area of each of the drug concentrations, retention times were recorded.

Analysis of Marketed Sample (Dosage Forms): Ten capsules of market formulations purchased from local pharmacy were weighed and the contents were removed to obtain the average weight powder.. An accurately weighed portion of the powder, equivalent to about 50 mg of Indinavir was transferred to a 100mL volumetric flask followed by the addition of 70mL of methanol. The solution was sonicated at controlled temperature for 30min and diluted to volume with methanol and mixed thoroughly. Filter the solution through 0.45µm membrane filter. Further, different concentrations that obey within the linearity limits was prepared by transferring of different aliquots of this solution into a series of 10mL volumetric flask and diluting the mark with the same mobile phase. These prepared dilutions were injected six times into the column to obtain the respective chromatograms. From that peak area of the chromatograms, the content of Indinavir in the capsules was quantified.

RESULTS AND DISCUSSION

Method Development: In the present study the development of a new stability indicating RP-HPLC method for Indinavir involved the optimization studies of various chromatographic conditions (i.e, using different column, different buffer and different mode of HPLC run).Initially the method development was started with the use of two different columns C_8 and C_{18}. Of the two columns [C_8 and C_{18}], ThermoHypersil BDS C-18 column (250mmx4.6mm, particle size) gave satisfactory resolution at 3.550mins run time. Secondly a study on selection of mobile phase and flow rate basing on peak parameters (height, area, tailing, theoretical plates, capacity factor and resolution) was extensively carried in the development of the proposed method. The mobile phase composition of Acetonitrile and water in the ratio of 80:15(v/v) at void volume eluted Indinavir with long retention time. The best results were obtained when the mobile phase composition of Acetonitrile, water and phosphoric acid in the ratio of 80:15:5 (v/v/v) at a flow rate 1.0 mL/min. With this mobile phase Indinavir eluted at a retention time of ~ 3.550 minutes (Figure-2)**.** Finally, a flow rate of 1.0 mL/min with an injection volume of 20µL and UV detection at 232nm was found to be best for analysis of Indinavir. The chromatogram of Indinavir standard using the proposed method is shown in(Figure-2).System suitability results of the proposed method are presented inTable-1.

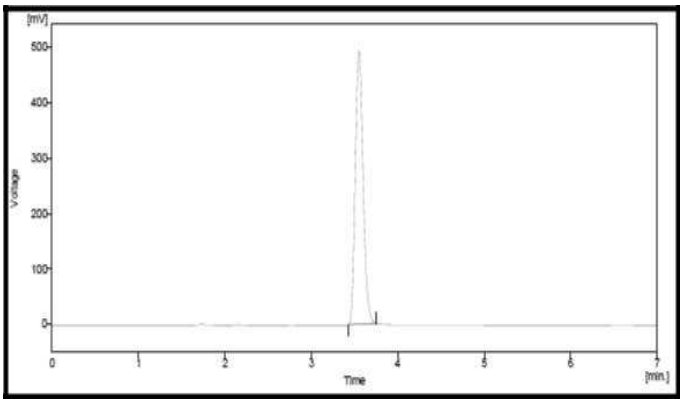

Figure-2: Validative Chromatogram of Indinavir

Table-1: System Suitability Parameters

PARAMETERS	INDINAVIR
Retention time	3.550
USP Plate count	6539
USP Tailing	1.292
Linearity Range (µg/mL)	5.0-15.0
Limit Of Detection (LOD) (µg/mL)	0.0698
Limit Of Quantitation (LOQ) (µg/mL)	0.2339

HPLC Conditions**:** The injection volume of sample was 20µL. An isocraticmobile phase containing Acetonitrile, water and phosphoric acid in the ratio of 80:15:5 (v/v/v) was carried out with the flow rate of 1.0 mL/min at ambient column temperature. Before the analysis, the mobile phase was degassed and filtered through a 0.45 µm membrane filter. The photodiode array UV-detector was set to a wavelength of 232 nm for the detection and chromatographic

runtime was 10 minutes. The entire HPLC system was equilibrated before making each injection. The work was carried out in an air-conditioned room maintained at temperature 25 ± 2 ^{o}C.

Procedure:Prior to the assay the column was equilibrated with the mobile phase for about 30 min at a flow rate of 1.0 mL/min. With the above optimized chromatographic conditions a steady base line was recorded. 20µL of daily working standard and formulation sample solutions of Indinavir were prepared with mobile phase containing Indinavir at a concentration of 5.0-15.0µg/mL were injected separately(six times) into the column the HPLC system at a flow rate of 1.0 mL/min and peak area of each of the drug concentrations, retention times were recorded.

***Method Validation*:** The developed RPHPLC method extensively validated for assay of Indinavir using the following parameters.

Specificity

Blank and Placebo Interference: A study to establish the interference ofblank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution showed no peaks at the retention time of Indinavir peak revealing that the diluent solution used in sample preparation do not interfere in the assay of Indinavir in dosage forms. Similarly chromatogram of placebo solution showed no peaks at the retention time of Indinavir peakindicating that the placebo used in sample preparation do not interfere in the assay of Indinavir in dosage forms.

Forced Degradation

Control Sample: Weighed and finely powdered not fewer than 20 tablets. Accurately weigh and transfer powder equivalent to 50mg of Indinavir into a 100mL volumetric flask, containing 70mL of methanol, sonicated for 30minutes with intermittent shaking at controlled temperature and finally diluted to the mark with methanol and mixed. Filter the solution through 0.45µm membrane filter. Transfer 5.0mL of the above solution into a 100mL volumetric flask and diluted to volume with the same diluent.

Acid Degradation Sample: Accurately weigh and transfer powder equivalent to50 mg of Indinavir into a 100mL volumetric flask, containing 70 mL of methanol and sonicated for 30minutes with intermittent shaking at controlled temperature. Then add 10mL of 5N acids to the same flask and refluxed for 30min at 60°C, then cooled to room temperature and neutralized with 5N NaOH and finally diluted to volume with methanol and mixed. Filter the solution through 0.45µm membrane filter. Transferred 5.0mL of the above solution into a 100mL volumetric flask and diluted to volume with diluent (Figure-3).

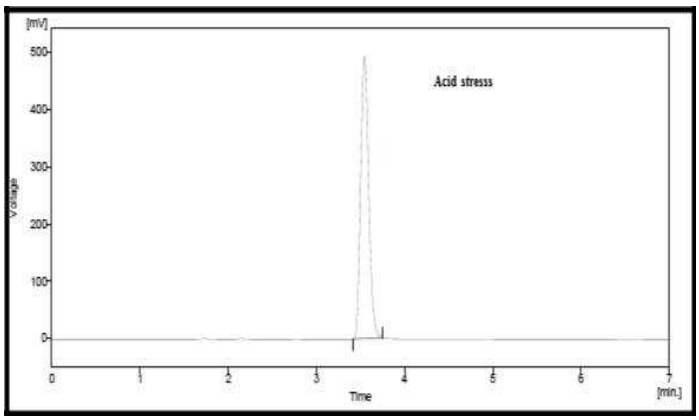

Figure-3: Validative Chromatogram of Indinavir in Acid Stress

Base Degradation Sample: Accurately weigh and transfer powder equivalent to50 mg of Indinavir into a 100 mL volumetric flask, containing 70 mL of methanol, and sonicated for 30 minutes with intermittent shaking at controlled temperature. Then added 10 mL of 5 N Base (NaOH), refluxed for 30 min at 60 °C, then cooled to room temperature, neutralized with 5 N Acid (HCl) and diluted to volume with methanol and mixed. Filter the solution

through 0.45 µm membrane filter. Transferred 5.0 mL of the above solution into a 100 mL volumetric flask and diluted to volume with diluent (Figure-4).

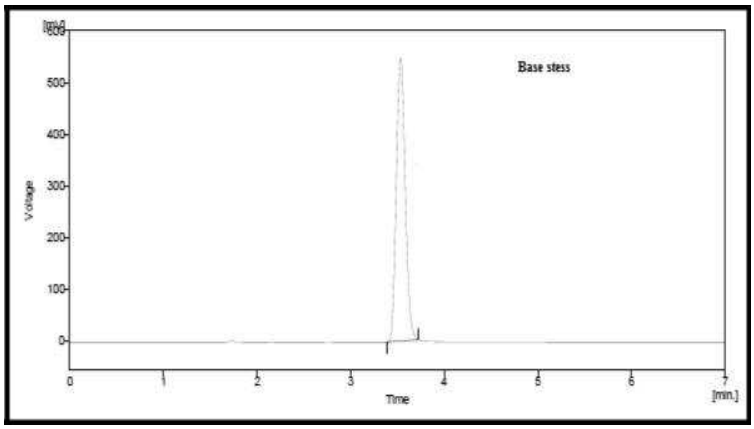

Figure-4: Validative Chromatogram of Indinavir in Base Stress

Peroxide Degradation Sample: Accurately weighed and transferred equivalent to 50 mg of Indinavir into a 100mL volumetric flask added about 70mL of methanol and sonicated for 30minutes with intermittent shaking at controlled temperature. Then added 2.0mL of 30% peroxide, refluxed for 30min at 60°C, then cooled to room temperature and diluted to volume with methanol and mixed. Filter the solution through 0.45µm membrane filter. Transferred 5.0mL of the above solution into a 100mL volumetric flask and diluted to volume with diluents. It was observed from these studies that there were no marked degradation in the chromatograms obtained revealing the good stability indicating of the proposed method.

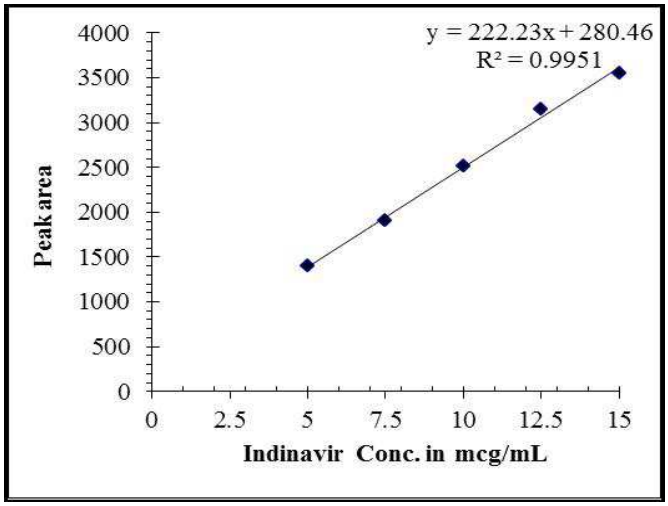

Figure-5: Linearity Curve of Indinavir

Linearity & Detector Response (Lod&Loq):Linearity was evaluated by analysis of working standard solution of Indinavir at seven different concentrations. The range of linearity was from 5.0 – 15 µg.mL^{-1}. The peak areas obtained with respect to their concentration of Indinavir was subjected to regression analysis to calculate the calibration equations and correlation coefficient. The calibration plot and the regression data results (Slope, intercept and correlation coefficient [r^2]) so obtained for Indinavir are represented in Figure-5 & Table-2. These results showed that within the concentration range mentioned above, there was an excellent correlation between peak areas and concentration of Indinavir respectively. The LOD and LOQ of Indinavir were experimentally determined by six replicate injections. The LOD and LOQ values of Indinavir were found to be 0.0698 µg/mL and 0.2339 µg/mL respectively.

Precision: The precision of the method was demonstrated by inter day and intraday variation studies. In present study the intraday studies were made, six repeated injections of standard and sample solutions and the response factor of drug peaks (Figure-6**)** and percentage RSD were calculated. The results of the above precision studies of Indinavir are summarized in Table-3 indicted that developed RP-HPLC method is precise.

Table-2: Calibration of the RP HPLC for the Estimation of Indinavir

Concentration (µg.mL)	Area (mAU)
5.0	1396.995
7.5	1902.193
10.0	2512.491
12.5	3152.335
15.0	3549.803
Regression equation; Intercept (a)	280.46
Slope (b)	222.23
Correlation coefficient	0.9951
Standard deviation on intercept (S_a)	5.175928
Standard deviation on slope (S_b)	8.972152
LOD	0.0698
LOQ	0.2339

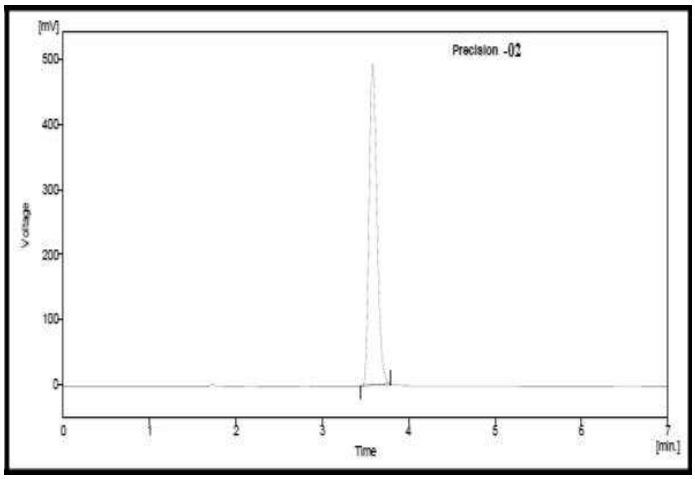

Figure-6: Precision Chromatogram of Indinavir

Table-3: Results of Method Precision

S No	Name	Area
1	Injection-1	3118.275
2	Injection-2	3104.769
3	Injection-3	3112.789
4	Injection-4	3108.741
5	Injection-5	3110.113
6	Injection-6	3113.555
Avg		3111.374
Std Dev		4.615877
% RSD		0.148

Accuracy: The accuracy of the method was determined by analyzing a known quantity of drug substance corresponding to 50%, 100% and 150% of the working concentration of Indinavir was added. Each set of addition were repeated three times .The accuracy was expressed as the percentage of analyte recovered by the assay. The validative chromatograms obtained at each accuracy level were represented. The recoveries of the Indinavir from a series of spiked concentrations and the percentage recoveries were found in the range of 99.73 to 99.96%. These results indicated that the proposed RP-HPLC method is highly accurate for the assay of Indinavir in dosage forms.

Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions (change in flow rate and column temperature). No marked changes in the chromatograms demonstrated that the HPLC method developed is robust.

Ruggedness: The ruggedness of the proposed RP-HPLC method was evaluated by a different analyst and different analyst and different instrument in the same laboratory. The %RSD for peak areas of Indinavir was calculated. These results revealed that the %RSD was within the limits indicating that the developed RP-HPLC method was found to be rugged.

***Analysis of Marketed Formulation*:** Analysis of marketed formulations (INDINAVIN) of Indinavir was carried out by using the proposed method under the above described optimized HPLC conditions. The % drug content of tablets obtained by the proposed method for Indinavir was found to be 99.99%, respectively. The results are given inTable-4.

Table-4: Results of Analysis of Tablet Containing Indinavir

PHARMACEUTICAL FORMULATION	AMOUNT OF INDINAVIR*		% RECOVERY
	LABELLED	FOUND	
INDIVAN	400 mg	399.96	99. 99 %

**Average of three determinations*

CONCLUSION

Reversed phase liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the determination of Indinavir in pure and formulations. Based on peak purity results, obtained from the analysis of force degradation studies, it can be concluded that the absence of coeluting peak along with the main peak of Indinavir indicated that the developed method is specific for the estimation of Indinavir in presence of degradation products As no attempt was made to identify the degraded products, the proposed method can be used as a novel stability indicating method for assay of Indinavir in bulk drug and also for in its dosage forms. The developed method gave a linear calibration curve ranging from 5.0 -15.0 µg/mL. The % RSD for precision and accuracy was found to be less than two, which revealed that the results obtained are within acceptance criteria. Finally it is concluded that the proposed RP-HPLC method is sensitivity, precise, economical, reproducible and stability indicating and can be applicable for the analysis of Indinavir in pure and in Pharmaceutical formulations.

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