SPECTROPHOTOMETRIC METHOD FOR THE VALIDATION OF INDINAVIR IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The spectrophotometric methods for the determination of Indinavir in pure and dosage forms have been described in this paper. The present methods involve the determination of Indinavir in pharmaceutical dosage at the given optimum conditions. The stock solution of Indinavir was prepared by dissolving 100 mg of the pure Indinavir drug in 10.0 mL of methanol and made up to 100 mL with distilled water to get a clear solution. Appropriate volumes of this stock solution were diluted step wise to get the working standard solutions of concentrations 200 μg/mL for Method-M_9. The values obtained by the proposed and reference method for formulations were compared statistically with F and t tests and found not to be different significantly. A simple and a cheap UV spectrophotometric method was developed and validated for the quantitative estimation of Indinavir sulphate in capsules as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

Key words: Indianavir, Spectrophotometric methods, Optical studies and accuracy.

INTRODUCTION

Indinavir sulphate 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]. IUPAC name of Indinavir sulphate is [1(1S, 2R), 5(S)]-2,3,5-trideoxy-N-2, 3-dihydro-2-hydroxy-1H-inden-1-yl)-5-[2-[[(1,1-dimethylethyl)amino]carbonyl]-4-(3 pyridinylmethyl)-1-piperazinyl]-2-phenylmethyl)-D-erythropentonamide 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). A detailed literature survey reveals LC methods for the analysis of Indinavir sulphate individually and in various combinations in biological matrices, capillary zone electrophoresis method for the

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analysis of Indinavir sulphate raw material. The specrtophotometric methods reported[2] in the literature for Indinavir revealed that relatively little attention was paid in developing economical methods and therefore, it made a need to develop sensitive and economical visible specrtophotometric methods, which prompted the author in this accord. Rao et al.[3-12] have published their results on different oxide materials, luminescent materials, polymers, glasses and on different drugs in their earlier studies. In the present paper, the authors developed few sensitive UV-visible spectrophotometric for the assay of Indinavir in pure forms that are validated and moreover these developed methods have been extended to pharmaceutical formulations as they are simple, economical and sensitive.

EXPERIMENTAL

Instruments used

Genesys 10 UV-Spectrophotometer 10 mm matched quartz cells procured from Thermo Scientific Company with were used for all spectral measurements. A Systronics digital pH meter [Model-362] was used for pH measurements.

Preparation of reagents

All the chemicals and reagents used were of analytical grade and solutions were prepared with doubled distilled water.

Preparation of stock and working standard solutions

The stock solution (1.0 mg/mL) of Indinavir was prepared by dissolving 100 mg of the pure Indinavir drug in 10.0 mL of methanol and made up to 100 mL with distilled water to get a clear solution. Appropriate volumes of this stock solution were diluted step wise to get the working standard solutions of concentrations 200 μg/mL for Method-M_9.

Procedure for tablets

About ten capsules (INDINAVIN-400 mg) were procured from local pharmacy and contents are removed from the capsules and the powder equivalent to 100 mg of Indinavir was accurately weighed and transferred into a 100 mL calibrated flask, 30 mL of methanol was added and the content shaken thoroughly for 15-20 min to extract the drug into the liquid phase. The volume was finally diluted to the mark with distilled water, mixed well and filtered through Whatman filter paper No 41. The filtrate was made upto mark with distilled water in a 100 mL volumetric flask. A suitable volume of the filtrate was accurately diluted with water and this solution was used for the determination of Indinavir as per the recommended procedures described below.

RESULTS AND DISCUSSION

Method development

It involves the optimization studies for the proposed methods. The optimization studies for the color development for the proposed methods M_8, M_9, M_{11}, M_{13} & M_{14} for the assay of Indinavir were found to be same.

Method-M_9

Aliquots (0.2-1.0 mL; 200 μg/mL) of standard Indinavir were transferred into a series of 10.0 mL calibrated tubes and then solutions of $FeCl_3$ (1.0 mL) and 1, 10-phenanthroline of 1.0 mL were added successively. The total volume in each test tube was brought up to 3.0 mL with distilled water and heated for 10 min in a boiling water bath at 90^oC. After cooling to the room temperature, 2.0 mL of o-phosphoric acid was added in each test tube. The absorbance of the orange red colored complex solution was measured after 5 min at 514 nm against reagent blank prepared similarly. The amount of Indinavir was computed from the Beer-Lambert's plot.

Indinavir possesses secondary nitrogen and functions as electron donor and participates in charge transfer interaction with DDQ. The color species formation in the method appears to be due to the formation of radical anion. Based on analogy the sequence of reaction is given below in the Scheme 1.
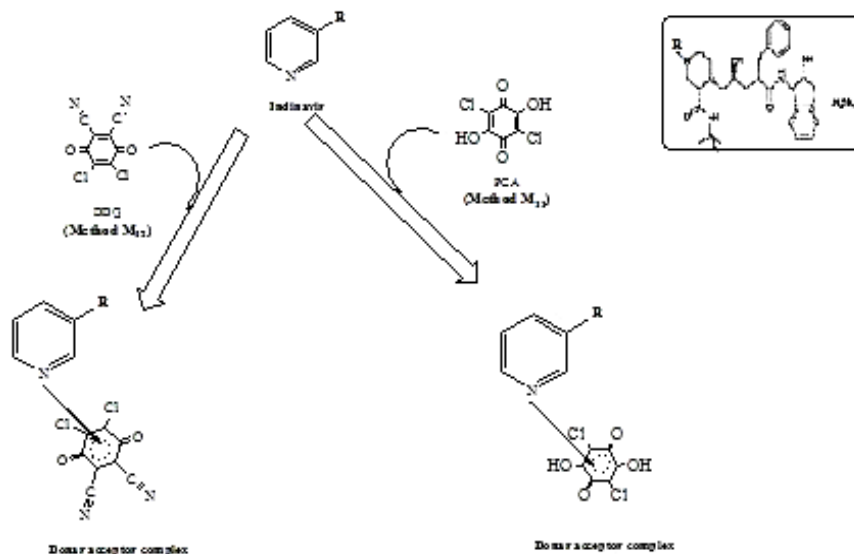
Scheme 1

Indinavir being a base forms an ion association complex with an acidic dye (TPooo, ARS), which is extractable into chloroform from the aqueous phase. The protonated nitrogen (positive charge) of the drug molecule in acid medium is expected to attract the oppositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction. Based on the analogy, the structures of ion association complexes are shown below in Scheme 2.
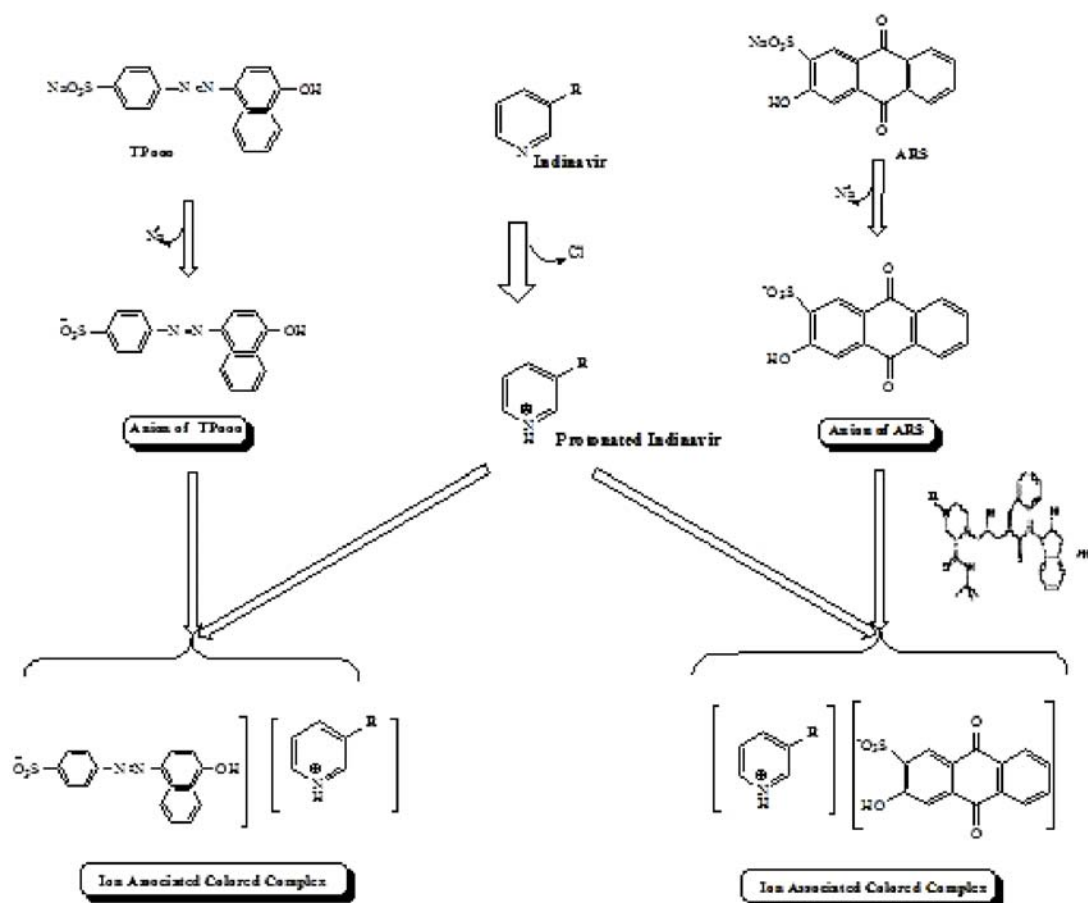

Scheme 2

Spectral characteristics

The absorption spectra were scanned on a spectrophotometer in the wave length region of 340 to 900 nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results

were graphically represented in for M_9 in Fig. 1(a & b). The Beer's law plots of Indinavir in each developed method were recorded graphically.
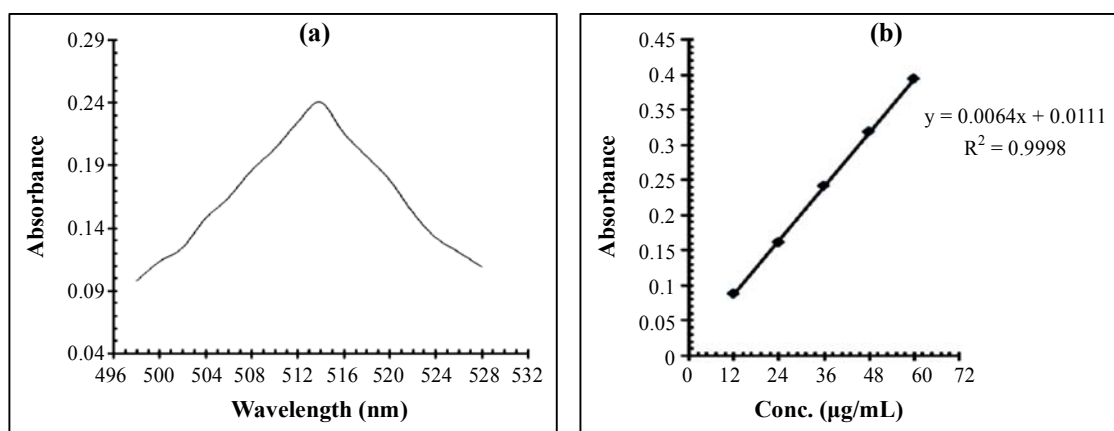
Fig. 1(a & b): Absorption spectra and Beer's law plot of Indinavir for Method-M_9

Recovery studies (Accuracy)

Recovery studies were conducted by analyzing each pharmaceutical formulation in the instance for the active ingredient by the proposed methods. Known amount of pure drug was added to each previously analyzed formulation and the total amount of the drug was once again determined by all proposed methods after bringing the active ingredient concentration within the Beer's law limits (Table 1).

Table 1: The results of accuracy studies) of Indinavir

Proposed methods	IDV in tablet ($\mu g.mL^{-1}$)	Pure IDV added ($\mu g.mL^{-1}$)	Total found ($\mu g.mL^{-1}$)	Pure IDV recovered % ± SD*
Method M_8	20.0	5.0	24.93	99.72
Method M_9	25.0	5.0	29.94	99.80
Method M_{11}	25.0	5.0	30.03	101.00
Method M_{12}	20.0	5.0	25.04	100.16
Method M_{13}	20.0	5.0	24.95	99.80
Method M_{14}	25.0	5.0	24.93	99.72
*Average of six determinations				

CONCLUSION

A simple and a cheap UV spectrophotometric method was developed and validated for the quantitative estimation of Indinavir sulphate in capsules as per ICH guidelines and hence, it can be used for the routine analysis in various pharmaceutical industries. The proposed methods made use of simple reagents, which most ordinary analytical laboratories can afford. The present methods involve the formation of highly stable colored species, which makes it easier for the determination of Indinavir in pharmaceutical dosage at the given optimum conditions. Further, results of statistical parameters and the recovery studies clearly indicated the reproducibility and high accuracy of the proposed methods. Therefore, it is concluded that the proposed visible spectrophotometric methods are suitable and valid for application in assaying of Indinavir related drugs in laboratories lacking liquid chromatographic instruments. Accordingly, it is concluded that the developed UV spectrophotometric method is accurate, precise, linear, rugged and robust and therefore, the method can be used for the routine analysis of Indinavir sulphate in tablets in various pharmaceutical industries.

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