DEVELOPMENT AND VALIDATION OF SOME NEW UV-VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF INDINAVIR IN PURE AND DOSAGE FORMS

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ABSTRACT

Some new selective accurate and economical spectrophotometric methods for the determination of Indinavir in pure and dosage forms have been described in the present work. These developed methods have been extended to pharmaceutical formulations as they are simple, economical and sensitive. 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. 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-M1, 240 µg/mL for Method-M2, 250 µg/mL for Method-M3, M4, M5 respectively. The effect of wide range of excipients and other inactive ingredients usually present in the formulations for the assay of Indinavir under optimum conditions were investigated. 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. Percent recoveries were determined by adding standard drug to preanalyzed formulations.

Keywords: Indinavir, UV spectrophotometric Methods, Optical Characteristics, Recovery Studies and Precision.

INTRODUCTION

Indinavir sulfate (Fig.-1), chemically known as \[1\{1(S,2R), 5(S)\}-2,3,5-trideoxy-N-2,3-dihydro-2-hydroxy-1H-inden-1-yl\}-3-[2-[[1,1-dimethylethyl]amino]carbonyl]-5-(3-pyridinylmethyl)-1-piperazinyl]-2-phenylmethyl]-D-erythro-pentonamide sulfate (1:1) salt1,2 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 methods3-8 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. The metal elements are able to form a large diversity of oxide compounds. In technological applications, oxides are used in the fabrication of microelectronic circuits, sensors, piezoelectric devices and fuel cells, coatings for the passivation of surfaces against corrosion and as catalysts9-19. Rao et al. have reported their work on different oxide materials in their earlier studies20-54. This paper describes the development and validation of some new UV-Visible spectrophotometric methods 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. The spectrophotometric methods reported3,4 in the literature for Indinavir revealed that relatively little attention was paid in developing economical methods and therefore, it made a needed to develop sensitive and economical visible spectrophotometric methods, which prompted the author in this accord. 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 was 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-M9, 240 µg/mL for Method-M11, M12 and 250 µg/mL for Method-M8, M13, M14 respectively.

**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 What man filter paper No 41. The filtrate was made up to 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 M8, M9, M11, M13 and M14 for the assay of Indinavir were found to be same.

**Method – M9 [DDQ]**

This method involves the reaction between Indinavir and DDQ. The optimum conditions were fixed basing on the study of effects of various parameters, such as volume of DDQ solution, volume of solvents...
used initially and subsequently for dilution and the stability of colored species after final dilution. The absorbance is measured at 542 nm and the results were incorporated in the corresponding procedure.

**Recommended Procedures**

After a systematic and detailed study of the various parameters, as described in optimum condition the following procedures \{M_8\}[FC], proposed for the assay of Indinavir in pure and marketed formulations.

**Method-M_8**

Aliquots (0.5-2.5 mL, 250 µg/mL) of standard Indinavir were transferred into a series of 10.0 mL calibrated tubes and then solutions of NaOH (5.0 mL) and FC (1.5 mL) were added successively. The total volume in each test tube was brought up to 8.5 mL with distilled water. The absorbance of the greenish blue colored complex solution was measured after 5 minutes at 740 nm against reagent blank prepared similarly. The amount of Indinavir was computed from the Beer-Lambert’s plot.

**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 FeCl₃ (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 minutes in a boiling water bath at 90 °C. 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 minutes at 514 nm against reagent blank prepared similarly. The amount of Indinavir was computed from the Beer-Lambert’s plot.

**Method-M_11**

Different aliquots of standard Indinavir solutions (0.5-2.5 mL; 240 µg/mL) were accurately transferred into a series of 10.0 mL volumetric flasks and the total volume was adjusted to 3.0 mL by adding adequate quantity of acetonitrile. To each flask was then added 2.0 mL of 0.1 % p-chloranilic acid, and the content was mixed well and kept aside for 10 min. The mixture was diluted to the volume with acetonitrile and the absorbance was measured at 528 nm against a reagent blank prepared simultaneously. The concentration of the Indinavir was read from the standard graph or computed from the respective regression equation derived using the Beer’s law data.

**Method-M_12**

Aliquots (0.5-2.5 mL) of a standard Indinavir (240 µg/mL) solution were accurately transferred into a series of 10mL volumetric flasks and the total volume was adjusted to 3.0 mL by adding adequate quantity of acetonitrile to each flask. Then 2.0 mL of 0.2 % DDQ solution was added to each flask and the mixture was diluted to the volume with acetonitrile and the absorbance of each solution was measured at 542 nm against a reagent blank.

**Method-M_13**

Into a series of 125 mL separating funnels containing aliquots of standard Indinavir solution (0.5-2.5 mL, 250 µg/mL), 5.0 mL of 0.1 M HCl solution and 2.0 mL of 0.2 % dye solution [TPoo] were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 10 mL with distilled water. To each separating funnel 10 mL of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 485 nm against a similar reagent blank. The amount of Indinavir was deduced from the calibration curve.

**Method-M_14**

Into a series of 125 mL separating funnels containing aliquots of standard Indinavir solution (0.5- 2.5 mL, 250µg/mL) 5.0 mL of 0.1M HCl solution and 2.0 mL of 0.2 % dye solution [ARS] were added
successively. The total volume of aqueous phase in each separating funnel was adjusted to 10 mL with distilled water. To each separating funnel 10 mL of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 450 nm against a similar reagent blank. The amount of Indinavir was deduced from the calibration curve.

**Method Validation**

**Spectral Characteristics**

The absorption spectra were scanned on a spectrophotometer in the wavelength 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$_{11}$ in Fig.- 2a and b and for M$_{14}$ in Fig.- 3a and b respectively. The Beer’s law plots of Indinavir in each developed method were recorded graphically. Figures- 2a and b and Figures-3a and b and their results [i.e, slope, intercept, correlation Beer’s law limits, molar absorptivity, Sandell’s sensitivity and optimum photometric range] were calculated and are reported in Table-1 and Table-2 respectively. The LOD values of Indinavir for each developed method were determined and are reported in Table-1 and Table-2.

![Fig. - 2(a and b)  Absorption spectra and Beer’s law plot of Indinavir for Method-M$_{11}$](image)

**Table-1: Results of Method Validation of the Proposed Methods for the Determination of Indinavir**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>M$_{8}$</th>
<th>M$_{9}$</th>
<th>M$_{11}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{max}$ (nm)</td>
<td>740</td>
<td>514</td>
<td>528</td>
</tr>
<tr>
<td>Beer’s law limits (µg/mL)</td>
<td>12.0-60.0</td>
<td>12.0-60.0</td>
<td>12.5-62.5</td>
</tr>
<tr>
<td>Molar absorptivity (1 mol$^{-1}$. cm$^{-1}$)</td>
<td>$1.72\times10^3$</td>
<td>$1.957\times10^4$</td>
<td>$2.11\times10^4$</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg.cm$^{-2}$/0.001 A.U)</td>
<td>0.0854</td>
<td>0.0753</td>
<td>0.0724</td>
</tr>
<tr>
<td>Regression equation (Y=a+bc);Slope (b)</td>
<td>0.0019</td>
<td>0.0064</td>
<td>0.0054</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0022</td>
<td>0.0011</td>
<td>0.0105</td>
</tr>
<tr>
<td>5 Correlation coefficient (r)</td>
<td>0.9997</td>
<td>0.9998</td>
<td>0.9999</td>
</tr>
<tr>
<td>Relative standard deviation (%)*</td>
<td>0.2428</td>
<td>0.999</td>
<td>1.032</td>
</tr>
<tr>
<td>% Range of error (confidence limits) 0.05 level</td>
<td>0.2791</td>
<td>0.835</td>
<td>0.863</td>
</tr>
<tr>
<td>0.01 level</td>
<td>1.392</td>
<td>1.235</td>
<td>1.377</td>
</tr>
<tr>
<td>LOD</td>
<td>0.0265</td>
<td>0.0170</td>
<td>0.0121</td>
</tr>
</tbody>
</table>

* Average of six determinations considered
**Precision**

The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of Indinavir in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods.

**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-2: Results of Method Validation Obtained by Applying the Proposed Methods for the Determination of Indinavir

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$M_{12}$</th>
<th>$M_{13}$</th>
<th>$M_{14}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{max}$ (nm)</td>
<td>542</td>
<td>485</td>
<td>450</td>
</tr>
<tr>
<td>Beer’s law limits (µg/mL)</td>
<td>10.0-50.0</td>
<td>12.5-62.5</td>
<td>12.5-62.5</td>
</tr>
<tr>
<td>Molar absorptivity (1 mol$^{-1}$, cm$^{-1}$)</td>
<td>2.73x10$^3$</td>
<td>1.160x10$^3$</td>
<td>1.233x10$^3$</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg.cm$^{-2}$/0.001 A.U)</td>
<td>0.0448</td>
<td>0.1262</td>
<td>0.1237</td>
</tr>
<tr>
<td>Regression equation (Y=a+bc);Slope (b)</td>
<td>0.0108</td>
<td>0.0039</td>
<td>0.0039</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0084</td>
<td>0.0050</td>
<td>0.0049</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9994</td>
<td>0.9998</td>
<td>0.9998</td>
</tr>
<tr>
<td>Relative standard deviation (%)*</td>
<td>0.523</td>
<td>1.710</td>
<td>1.540</td>
</tr>
<tr>
<td>% Range of error (confidence limits) 0.05 level</td>
<td>0.437</td>
<td>1.430</td>
<td>1.288</td>
</tr>
<tr>
<td>0.01 level</td>
<td>0.647</td>
<td>2.110</td>
<td>1.905</td>
</tr>
<tr>
<td>LOD</td>
<td>0.0247</td>
<td>0.0289</td>
<td>0.0321</td>
</tr>
</tbody>
</table>

*Average of six determinations considered

**Analysis of Formulations**

Commercial formulations (tablets) containing Indinavir were successfully analyzed by the proposed methods. The values obtained by the proposed and reference method$^4$ for formulations were compared...
statistically with F and t tests and found not to be different significantly. Percent recoveries were determined by adding standard drug to preanalyzed formulations.

**Interference Studies**

The effect of wide range of excipients and other inactive ingredients usually present in the formulations for the assay of Indinavir under optimum conditions were investigated. The commonly used excipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

**Nature of the Colored Species**

Although the structures of the colored species have not been established experimentally, which is beyond the scope of the present investigations, they may be postulated by taking appropriate analogy.

**Method – M$_{11}$**

As Indinavir possesses secondary nitrogen group, it functions as an electron donor and participate in charge transfer interaction with DHQ, which is known as electron acceptor. The color species formation appears to be due to the formation of radical anion.

**Method - M$_{12}$**

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.

**Method - M$_{13}$ and M$_{14}$**

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.

**CONCLUSION**

Some new selective accurate and economical spectrophotometric methods for the determination of Indinavir in pure and dosage forms have been described in the present paper. 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.

**REFERENCES**


[RJC-1449/2016]