# Spectrophotometric Determination of Indinavir Sulphate with Precipitation Reagents

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**Indinavir sulphate forms insoluble molecular complex with ammonium molybdate (method A), phosphomolybdic acid (method B) or tannic acid (method C) under acid conditions. In addition, colour reactions have been combined to estimate each precipitant and in turn indinavir sulphate. They are based on the colour formation with released precipitant from the precipitate with chromogenic reagents such as potassium thiocyanate ($\lambda_{max}$ 480 nm for method A) or cobalt nitrate-ethylene diamine tetra acetic acid disodium salt complex ($\lambda_{max}$ 840 nm for method B) or metol-chromium (VI) ($\lambda_{max}$ 590 nm for method C). The methods are statistically validated and found to be precise and accurate.**

Indinavir sulphate (INS) is an antiviral drug and is chemically known as [(αR, γS, 2S)-α-Benzyl-2- (tert. butyl carbamoyl)-γ-hydroxy-N-[(1S,2R)-2-hydroxy-1-indanyl)-4-(3-pyridyl methyl)-1-piperazine valeramide sulphate (1:1)][1]. HPLC[2] and UV[3] methods are reported earlier for the determination of indinavir sulphate in biological fluids. In the present communication three spectrophotmetric methods have been described.

INS undergoes quatitative precipitation in the form of molecular complex with ammonium molybdate (AM, method A) or phosphomolybdic acid (PMA, method B) or tannic acid (TA, method C) when added in excess. In addition to precipitation reactions, colour reactions have also been combined to estimate INS. They are based on the colour formation with released precipitant from molecular complex (AM, PMA or TA) with chromogenic reagents such as potassium thiocyanate PTC, (for AM)[4], Co(II)- EDTA complex (for PMA), metol-Cr(VI) (for TA)[5].

A Systronics model 106 Vis and Milton Roy spectronic 1201 UV/Vis spectrophotometers were used for absorbance measurements. An Elico LI-120 digital pH meter was used for pH determinations.

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Aqueous solutions of AM (E-Merck, Mumbai, $5.1\times10^{-2}$M), PTC (Ranbaxy, Delhi, $5.14\times10^{-4}$M), Concentrated HCl (used as it is) for method A, PMA (Reachem, Chennai, $5.47\times10^{-3}$M), cobalt nitrate (BDH, Mumbai, $1.03\times10^{-1}$M), EDTA disodium salt (SD Fine Chemicals, Mumbai, $1.07\times10^{-1}$M) for method B; TA (Loba Chemie, Mumbai, $1.07\times10^{-3}$M), PMAP (Loba Chemie, Mumbai, $8.71\times10^{-3}$M), Cr(VI) (BDH, Mumbai, $1.01\times10^{-2}$M), buffer pH 3.0 (prepared by diluting 250 ml of 0.2 M potassium acid phthalate and 204 ml of 0.1 M HCl to 1000 ml with distilled water) for method C were prepared in distilled water. Indinavir sulphate was obtained as a gift sample from Cipla, Mumbai.

A standard solution containing 1 mg/ml of INS was prepared in 0.01 M HCl. From this, working standard solutions were prepared by dilution with 0.01 M HCl (200 μg/ml for method A and 100 μg/ml for methods B and C).

An accurately weighed amount of capsule powder equivalent to 100 mg of INS was dissolved in 20 ml of 0.01 M HCl and filtered. The volume of filtrate was made up to 100 ml with 0.01 M HCl to get 1 mg/ml solution. This solution was diluted with 0.01 M HCl to get working sample solution as under procedures described for bulk samples.

In method A, aliquots of standard solution (0.25-1.5 ml 200 μg/ml) were delivered into a series of centrifuge tubes and the volume in each tube was adjusted to 3.0 ml with

0.01 M HCl. One millilitre of AM was added and centrifuged for 5 min. The precipitate was collected through filtration followed by washing with 50% alcohol until it is free from the reagent. The precipitate in each tube was dissolved in 5 ml of acetone and transferred into 25 ml graduated tube. Then 5 ml concentrated HCl and 3 ml potassium thiocyanate solutions were successively added. The tubes were kept aside for 20 min at laboratory temperature. The solution in each tube was made up to the mark with distilled water. The absorbance was measured at 480 nm against a similar reagent blank. The amount of the drug was calculated from Beer's law plot.

In method B, aliquots of standard drug solution (0.25-1.5 ml, 100 μg/ml) were delivered into a series of calibrated tubes and the volume in each tube was adjusted to 3.0 ml with 0.01 M HCl. Then 2 ml PMA was added and centrifuged for 5 min. The precipitate was collected through filtration followed by washing with distilled water until it is free from the reagent. The precipitate in each tube was dissolved in 5 ml acetone and transferred into a 25 ml graduated tube and then 1 ml each of cobalt nitrate and EDTA solutions were added successively. The tubes were heated for 12 min at 60°. The tubes were cooled and the solution in each tube was made up to mark with distilled water. The absorbance was measured at 840 nm against a similar reagent blank. The amount of the drug was calculated from the calibration graph.

In method C, aliquots of drug solution (0.5-2.5 ml, 100 μg/ml) were transferred into a series of centrifuge tubes and the volume in each tube was adjusted to 5 ml with 0.01 M HCl. Then 1.0 ml of tannic acid was added and precipitate obtained was collected by centrifugation of the tubes for 10 min. After draining off the supernatant liquid as completely as possible the precipitate was washed with water (3x1 ml) and the supernatant liquid was drained off as before after each centrifugation. The precipitate was dissolved in 5 ml of acetone and transferred into a series of 25 ml graduated tubes and 10 ml of buffer solution of pH 3.0 was added. Then 1.5 ml of metol (PMAP) and 2 ml of potassium dichromate solutions were added to each tube and the volume was made up to mark with distilled water. The absorbance was measured at 590 nm after 5 min against a reagent blank before 60 min. The amounts of INS were calculated from its calibration graph.

The optimum conditions for maximum colour development of each method (A, B and C) were established by varying the parameters one at a time and keeping the others fixed in the both steps of precipitation and colour formation and observing the effect produced on absorbances of coloured species. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity for the methods are given in Table 1. Regression analysis using linear least squares was made for the slope (b), intercept (a) and correlation coefficient (r) for each system and are presented in Table 1.

Capsules (Indivir-400, Genix Pharma Ltd., Hyderabad)

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS OF INS.

| Parameter | Methods | | |
|---|---|---|---|
| | A | B | C |
| Beer's law limits (μg/ml) | 0.9-12 | 1.95-24 | 1.24-20 |
| Molar absorptivity (l/mol. cm) | $1.992\times10^{4}$ | $7.35\times10^{4}$ | $1.513\times10^{4}$ |
| Sandell's sensitivity (μg/cm²/0.001 absorbance unit) | $3.57\times10^{-2}$ | $9.685\times10^{-3}$ | $4.705\times10^{-2}$ |
| Regression equation (Y=a+bc)* | | | |
| Slope (b) | $5.56\times10^{-2}$ | $1.03\times10^{-1}$ | $2.09\times10^{-2}$ |
| Intercept (a) | $1.39\times10^{-3}$ | $1.33\times10^{-3}$ | $1.1\times10^{-3}$ |
| Correlation coefficient (r) | 0.9999 | 0.9999 | 0.9998 |
| Relative standard deviation (%)** | 0.420 | 0.546 | 0.595 |
| % Range of error (95% confidence limits) | 0.441 | 0.573 | 0.625 |

* Y = a+bc where C is concentration of analyte (μg/ml) and Y is absorbance unit. ** Calculated from six determinations.

TABLE 2: ESTIMATION OF INDINAVIR SULPHATE IN PHARMACEUTICAL FORMULATIONS.

| Sample* | Labeled amount (mg) | Amount found (mg) by proposed methods** | | | **Reference method$^{\Delta}$ | % Recovery by proposed methods*** | | |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | | A | B | C |
| Capsule I | 400 | 398.7±1.53<br>F=1.85<br>t=1.06 | 399.8±3.18<br>F=2.33<br>t=0.072 | 399.8±2.16<br>F=1.07<br>t=1.86 | 396.4±2.09 | 99.75±0.35 | 99.19±1.01 | 99.95±0.54 |
| Capsule II | 400 | 398.6±1.24<br>F=4.55<br>t=0.23 | 396.3±1.95<br>F=1.85<br>t=1.50 | 399.7±2.01<br>F=1.733<br>t=0.95 | 399.0±2.65 | 99.65±0.31 | 99.59±0.49 | 99.95±0.47 |
| Capsule III | 400 | 400.4±0.98<br>F=1.59<br>t=2.1 | 397.4±2.22<br>F=3.20<br>t=1.09 | 398.3±2.54<br>F=4.18<br>t=0.32 | 398.6±1.24 | 100.17±0.30 | 99.35±0.56 | 99.57±0.73 |
| Capsule IV | 400 | 399.4±2.06<br>F=1.45<br>t=0.8 | 399.4±4.1<br>F=2.73<br>t=1.38 | 399.1±4.10<br>F=2.74<br>t=0.26 | 400.8±2.48 | 99.85±0.51 | 99.78±1.03 | 99.97±1.02 |

* Different batches of tablets. ** Average ± standard deviation of six determinations, the t - and F- test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.57. *** Recovery of 10 mg added to the preanalysed pharmaceutical formulations (average of three determinations). Δ Developed in the laboratory using chloroform solvent ($\lambda_{max}$ 236 nm).

containing INS were successfully analyzed by the proposed methods. The accuracy of the methods was ascertained by comparing the results by proposed and reference methods (UV) statistically by the t- and F-tests (Table 2). The results of the recovery experiments by the proposed methods are listed in Table 2.

The interference studies in the determination of INS in the pharmaceutical formulations revealed that the normally existing excipients and additives like starch, lactose, talc, stearic acid, boric acid, gelatin, magnesium carbonate and sodium lauryl sulphate do not to interfere even when present in excess than the anticipated amounts. The results indicate that proposed methods are sensitive, accurate, precise and reproducible. The sensitivity order of procedures is B > A > C and $\lambda_{max}$ of colour species order is B > C > A. The higher $\lambda_{max}$ value of proposed methods have a decisive advantage, since the interference ingredients should be generally far less at higher wave lengths than at lower wave lengths. Thus the proposed methods are useful for the determination of INS in pure samples and pharmaceutical formulations.


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