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Spectrophotometric Methods for the Determination of Indinavir Sulphate

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Three simple and sensitive visible spectrophotometric methods (**A**, **B** and **C**) have been developed for the estimation of indinavir sulphate in the bulk and dosage forms. Method **A**, involves the addition of excess chloramine-T of known concentration in acidic medium to indinavir sulphate and determination of un-reacted chloramine-T by measure of decrease in the absorbance of dye gallocyanine (GC, λ_{max} 540 nm). Method **B** is based on the reduction of heteropolyacid, phosphomolybdotungstic acid, (FC) reagent by indinavir sulphate producing a characteristic intense blue colour (λ_{max} 760 nm). Method **C** is based on the charge-transfer complex formation between indinavir sulphate and chloranil (λ_{max} 540 nm). Regression analysis, Beer's law plots showed good concentration ranges of 2.5-20, 2-25 and 4.5-40 μg/mL for methods **A**, **B** and **C**, respectively.

Key Words: Determination, Indinavir sulphate, Visible spectrophotometry.

INTRODUCTION

Indinavir sulphate[1], [αR,γS,2S)-α-benzyl-2-(*tert*-butyl carbamoyl)-γ-hydroxy-N-[(1S,2R)-2-hydroxy-1-indanyl]-4-(3-pyridylmethyl)-1-piperazine valeramide sulphate (1:1)], is an antiviral drug which inhibits the activity of human immuno deficiency virus (HIV) protease. It is not official in any pharmacopoeia. HPLC[2] and UV[3] methods were reported earlier for the determination of indinavir sulphate in biological fluids. There is no analytical report for the estimation of indinavir sulphate by using visible spectrophotometry. The authors have made some attempt in this direction and succeeded in developing three methods based on the colour formation using the reagents such as [chloramine-T (CAT)] CAT-GC (Method **A**)[4], Folin-Ciacalteu (FC) (Method **B**)[5] chloranil (Method **C**)[6]. These methods have been successfully extended to dosage form containing indinavir sulphate.

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EXPERIMENTAL

A Systronics 106 Vis spectrophotometer and Milton roy spectronic-1201 UV-Vis spectrophotometers were used for the measurement of absorbance.

All reagents used were of AR grade and prepared by double distilled water. Freshly prepared reagents were always used. Aqueous solutions of CAT (Loba, 1.10×10^{-4} M), GC (Chroma, 2.969×10^{-4} M), HCl (5 M) were prepared for method **A**. Aqueous solution Na_2CO_3 (Loba, 9.43×10^{-1} M), commercially available Folin-Ciacalteu (2 N) were used for method **B**. Chloranil (BDH, 4.09×10^{-3} M) in 1,4-dioxane), DMF (qualigens) were used for the method **C**.

Standard and sample drug solution: Standard indinavir sulphate solution (1 mg/mL) was prepared in distilled water. Working standard solutions of indinavir sulphate (100 and 500 µg/mL for methods **A** and **B**, respectively) were obtained by step wise dilution of stock solution with distilled water. For the preparation of indinavir (freebase from indinavir sulphate), the chloroform solution of indinavir sulphate (20 mL, 2 mg/mL) and aqueous solution of Na_2CO_3 (5 mL, 10 %) were taken in a separating funnel, shaken well for 2 min and chloroformic layer was filtered through a pled get containing 2 g of anhydrous sodium sulphate. The filtrate was made up to 100 mL with chloroform to get working standard solution of 400 µg/mL for method **C**.

Analysis of bulk sample

Method A: Aliquots (1.0-5.0 mL, 100 µg/mL) of standard drug solution were delivered into a series of 25 mL calibrated tubes. Then 1.25 mL of 5 M HCl, 2.0 mL of CAT were added successively to each tube and the solution was diluted to 20 mL with distilled water. After 15 min, 5.0 mL of GC solution was added, mixed thoroughly and the absorbance of the coloured species was measured after 5 min, at 540 nm against distilled water. The blank experiment was carried out in a similar manner. The decrease in absorbance corresponds to consume CAT, which in turn to the drug concentration, was obtained by subtracting the absorbance of blank solution from that of test solution. The calibration graph was drawn by plotting the decrease in absorbance of the dye (GC) against the amounts of drug. The amount of drug in the sample was obtained from its Beer's law plot.

Method B: Aliquots (0.25-1.25 mL, 500 µg/mL) of standard drug solution were transferred into a series of 25 mL graduated tubes. To each tube 1.5 mL of FC reagent followed by 7 mL of 10 % Na_2CO_3 solution were added. The solutions were mixed thoroughly and kept at room temperature for 0.5 h. The solutions were then made up to 25 mL in each tube with distilled water. The absorbance values of the final coloured solutions

were measured at 760 nm with in the stability period (1 min - 5 h) against a reagent blank prepared in a similar manner. The amount of drug was computed from its calibration graph.

Method C: To a series of 25 mL calibrated tubes, aliquots of solution of drug in chloroform (0.5-2.5 mL, 400 μg/mL) in free base form were transferred and chloroform in each tube was removed by heating on water bath. Then 20 mL of 1, 4-dioxane-dimethylformamide (1:9) solution, 1 mL of chloranil solutions were added to each tube. The tubes were heated on a boiling water bath for 0.5 h. They were cooled to room temperature and made up to mark with 1,4-dioxane. The absorbance of coloured species in each tube was measured at 540 nm against reagent blank during the stability period (1 min - 1 h). The amount of drug was computed from the calibration curve.

Analysis of pharmaceutical formulation: An accurately weighed amount of sample (capsule) equivalent to 100 mg of the drug was triturated with 3 × 25 mL of chloroform and filtered. The combined filtrate was diluted to 100 mL with chloroform to get 1 mg/mL stock solution. From this, 50 mL of the solution in chloroform was transferred into a 100 mL volumetric flask and the chloroform in it was completely removed by heating it on a hot water bath. Then the residue was dissolved and diluted to 100 mL with distilled water to get 500 μg/mL aqueous solution for method **B**. 20 mL of aqueous indinavir sulphate (500 μg/mL) solution was further diluted to 100 mL with distilled water to get the working standard solution of 100 μg/mL for method **A**. For the method **C**, 20 mL of 1 mg/mL solution in chloroform was diluted to 50 mL with chloroform to get 400 μg/mL solution and analyzed as described under the procedures for pure sample.

RESULTS AND DISCUSSION

The optimum condition for the development of methods **A**, **B** and **C** were established by varying the parameters one at a time[6], keeping the others fixed and observing the effect produced on the absorbance of coloured species.

Analytical data: The optical characteristics such as Beer's law limits[7], molar absorptivity and Sandell's sensitivity for the methods are given in Table-1. Regression analysis using the method of least squares was made for the slope (b), intercept (a) correlation coefficient obtained from the six different concentrations of drug and the results are summarized in Table-1. The precision and accuracy of these methods were ascertained by estimating the known amount of drug in total solution (12, 15 and 60 μg/mL for methods **A-C**, respectively) and the results are summarized in Table-1.

Commercial formations (capsules) containing indinavir sulphate were successively analyzed by proposed methods. The values obtained by the

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS OF INDINAVIR SULPHATE

Parameters	Method **A**	Method **B**	Method **C**
Beer's law limits (μg/mL)	2.5-20	2-25	4.5-40
Detection limit (μg/mL)	7.01×10^{-2}	1.33×10^{-1}	2.90×10^{-1}
Molar absorptivity (1 mol^{-1} cm^{-1})	1.584×10^{4}	1.779×10^{4}	7.831×10^{3}
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	4.59×10^{-2}	4.0×10^{-2}	9.09×10^{-2}
Regression equation *y = a + bc			
Slope b	2.17×10^{-2}	2.5×10^{-2}	1.09×10^{-2}
Intercept a	1.01×10^{-4}	6.0×10^{-4}	-3.0×10^{-4}
Correlation coefficient (r)	0.9999	0.9999	0.9999
Relative standard deviation (%) **	0.5620	0.4980	0.8330
% Range of error (confidence limit 0.05 level)	0.5890	0.5230	0.8740

*Y = a + bC, where C is concentration.
**Calculated from six determinations.

proposed and reference methods for pharmaceutical preparations were compared statistically by the t-and F-tests and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the preanalyzed formulations. These results are summarized in Table-2.

The interference studies in the determination of indinavir sulphate in pharmaceutical formulation revealed that the normally existing excipients and additives like starch, lactose, talc, stearic acid, boric acid, gelatin, magnesium carbonate and sodium laurel sulphate do not interfere even when present in excess than the anticipated amounts.

Chemistry of coloured species:

Method A: In this method, chloramine-T (CAT) undergoes hydrolysis in aqueous acidic medium to give sodium hypochlorite followed by hypochlorous acid. This reacts with indinavir sulphate (INS) to form the relevant oxidation products, probably a mixture which appears to be reproducible under specified experimental conditions. The remaining hypochlorous acid may be responsible for the bleaching of the colour of GC through destruction of the extended chromophoric system (**Scheme-I**).

Step I

INS + CAT ⟶ Oxidation products of INS + unreacted CAT

Step II

Unreacted CAT + GC ⟶ Oxidation products of GC + GC unreacted
(Colourless) (Coloured)

Scheme-I

TABLE-2
ESTIMATION OF INDINAVIR IN PHARMACEUTICAL FORMULATIONS

Form.*	Labelled amount (mg)	Amount found by proposed methods**			Ref. method†	Recovery (%) by proposed methods***		
		A	**B**	**C**		**A**	**B**	**C**
Capsule	400	399.28 ± 2.09 F = 1.01 t = 2.21	398.92 ± 3.67 F = 3.09 t = 0.91	398.95 ± 2.88 F = 1.9 t = 1.25	396.42 ± 2.086	100.05 ± 0.705	99.75 ± 0.918	99.74 ± 0.720
Capsule	400	398.56 ± 3.22 F = 1.48 t = 1.09	398.75 ± 2.15 F = 1.52 t = 0.30	400.63 ± 3.41 F = 1.66 t = 0.742	399.0 ± 2.650	99.63 ± 0.800	99.68 ± 0.478	100.16 ± 0.850
Capsule	400	400.81 ± 2.43 F = 3.85 t = 0.90	398.59 ± 2.09 F = 2.84 t = 0.90	399.61 ± 1.75 F = 1.98 t = 0.34	398.6 ± 1.240	99.97 ± 0.950	99.64 ± 1.100	99.9 ± 0.438
Capsule	400	398.16 ± 2.43 F = 1.04 t = 1.15	399.11 ± 3.03 F = 1.49 t = 1.02	400.87 ± 1.18 F = 4.44 t = 0.45	400.79 ± 2.480	99.87 ± 0.116	99.77 ± 0.760	100.22 ± 0.190

*Different batches of tablets from four different pharmaceutical companies.
**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 preanalyzed pharmaceutical formulations (average of three determinations).
†Developed in the laboratory (λ_{max} = 236 nm) using chloroform solvent.

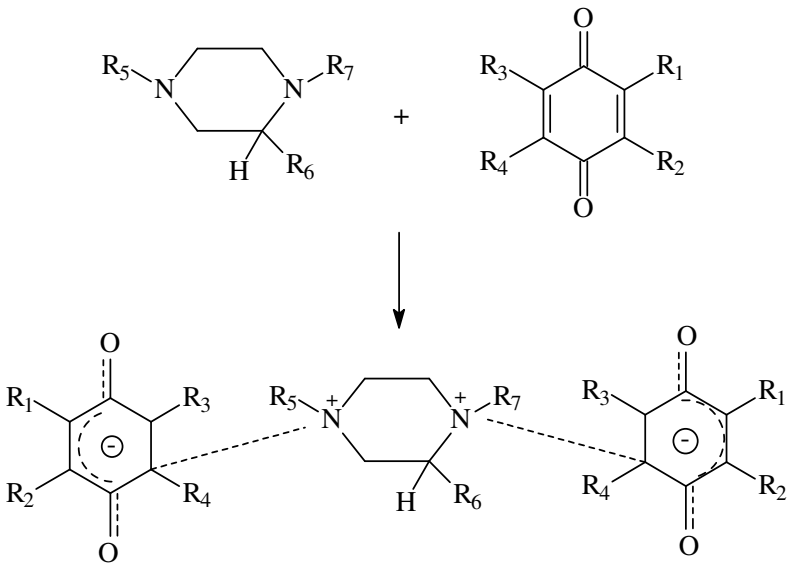
Method B: The colour formation by the FC reagent with indinavir sulphate in this method may be explained in the following manner based on the analogy with reports of the earlier workers[5]. The mixed acids in the FC reagent preparation involve the following chemical species,

$3H_2O, P_2O_5. 13WO_3. 5MoO_3. 10H_2O$ and
$3H_2O, P_2O_5. 14 WO_3. 4MoO_3. 10H_2O$

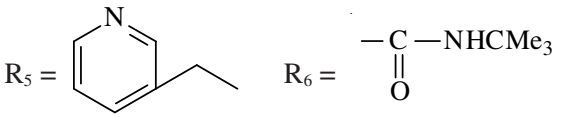
Scheme-II

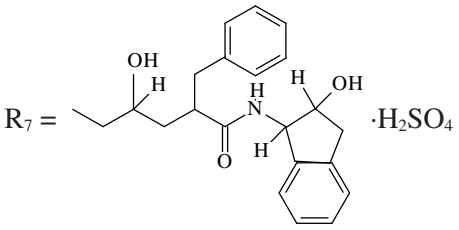
Indinavir sulphate probably affects a reduction of 1, 2 or 3 oxygen atoms from tungstate and/or molybdate in FC reagent (phosphomolybdo tungstate), thereby producing one or more of the possible reduced species which have characteristic intense blue colour.

Method C: The coloured species formation in the methods for the assay of indinavir sulphate appears to be the formation of radical ion through analogy given below (**Scheme-III**). As indinavir sulphate possess two tertiary nitrogens yield EDA complex (a radical anion).



TQ - $R_1 = R_2 = R_3 = R_4 = Cl$





Scheme-III

Conclusion

Even though the estimation of indinavir sulphate has been performed by using three different quinones only chloranil (λ_{max} 540 nm, ε_{max}, 7.831 × 10^3) was preferred over DDQ (λ_{max} 460 nm, ε_{max} 5.809 × 10^3) and chloranilic acid (λ_{max} 510 nm, ε_{max} 6.834 × 10^3) being more sensitive.

All the proposed methods have higher λ_{max} values and sensitivity. This is a decisive advantage since the interference from the associate ingradients will be less at higher wave lengths than at lower wave lengths. The sensitivity order of the procedures is **B > A > C** and the λ_{max} order of the coloured

species is **B > A = C**. The proposed methods are simple, sensitive and have reasonable precision and accuracy. All the proposed methods are useful for the determination of indinavir sulphate and provide a wide choice depending on the needs of situation.

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