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Determination of Indinavir Sulphate by Visible Spectrophotometry

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Three simple and sensitive visible spectropothometric methods (M_1, M_2, M_3) are developed for the estimation of Indinavir sulphate[1] (INS) in bulk and dosage forms. They are based on the formation of coloured species with sodium nitroprusside-acetaldehyde (SNP-ACD, method-M_1), cobalt thiocyanate (CTC, method-M_2) and citric acid-acetic anhydride (CA-AA, method-M_3) reagents exhibiting maximum absorption at 560, 600, and 560 nm respectively. Methods M_1, M_2, M_3 obeyed Beer's law limits 0.6–6 μg mL^{-1}, 1.4–15 μg mL^{-1} and 1.65–20 μg mL^{-1} repectively and are statistically validated. The proposed methods are selective, simple and economicical for the quantitative determination of Indinavir sulphate.

Key Words: Determination, Indinavir sulphate, Visible spectrophotometry

INTRODUCTION

Indinavir sulphate (INS) is an antiviral drug and is chemically known as [D-erythro-pentonamide - 2,3,5-trideoxy-N-(2,3-dihydro-2-hydroxy-1H-inden- 1-yl) - 5 - (2-[[(1,1-dimethylethyl)amino]carbonyl) - 4 - (3-pyrimidylmethyl) - 1 - piperazinyl]-2-(phenyl methyl)-monohydrate [1(1S, 2R, 5(s)] sulphate 1 : 1 salt. It is an inhibitor of human immunodeficiency virus (HIV) protease. Indinavir binds the protease and inhibits the activity of enzyme. This inhibition prevents the cleavage of viral polyproteins, resulting in the formation of immature non-infection viral particles.

Literature survey revealed that a few methods like HPLC[2] and UV[3] have been reported. There is no analytical report for the estimation of Indinavir sulphate using visible spectrophotometry. The authors have made some attempts in developing visible spectrophotometric methods and succeeded in developing three methods based on the colour formation using the reagents such as sodium nitroprusside-acetaldehyde (SNP-ACD) (method-M_1), cobalt thiocyanate (CTC)

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(method-M_2) and citric acid-acetic anhydride (CA-AA) (method-M_3). These methods are successfully extended to dosage forms containing Indinavir sulphate.

EXPERIMENTAL

A Milton Roy spectronic 1201 and a Systronics 106 digital spectrophotometer with 1 cm matched quartz cells were used for spectral and absorbance measurements.

All the chemicals were of analytical grade and all solutions were prepared in triply distilled water. Freshly prepared solutions were always used. Aqueous solutions of sodium nitroprusside (SNP, E. Merck, 3.35×10^{-2} M), acetaldehyde (10%), phosphate buffer of pH 8.0 (prepared by mixing 30 mL of 0.067 M potassium hydrogen phosphate and 970 mL of 0.067 M disodium hydrogen phosphate and pH adjusted to 8.0) were prepared for method M_1. Cobalt thiocyanate solution (2.50×10^{-1} M, prepared by dissolving 7.25 g of cobalt nitrate and 3.8 g of ammonium thiocyanate in 100 mL of distilled water), citrate buffer solution of pH 2.0 (prepared by mixing 306 mL of 0.1 M trisodium citrate with 694 mL of 0.1 M HCl and pH adjusted to 2.0) were prepared for method M_2. Citric acid-acetic anhydride (BDH, 2.8×10^{-1} M CA in AA) for method M_3 was prepared.

Standard and sample drug solution: An accurately weighed quantity of pure INS (pure or capsule powder) equivalent 100 mg was dissolved in 50 mL of distilled water to get 2 mg/mL stock solution. 5 mL of the stock solution was further diluted with distilled water to get the working standard solution of 100 μg/mL for methods M_1 and M_2.

For method M_3, 25 mL stock solution was mixed with 5 mL of 1 M Na_2CO_3 solution and transferred into 125 mL separating funnel. The free base released was extracted with 3 × 25 mL portions of chloroform. The total chloroform extract was filtered through pledget of cotton containing 2 g anhydrous sodium sulphate and made up to 100 mL with $CHCl_3$ to obtain 500 μg/mL solution.

Analysis of bulk sample

Method M_1: Aliquots of standard INS solution (0.25–1.5 mL 100 μg mL^{-1}) were delivered into a series of 25 mL calibrated tubes containing 15 mL of buffer, pH 8.0. Then 1 mL each of SNP and acetaldehyde were added successively. The volume was brought to 25 mL with distilled water and thoroughly shaken for 2 min. The absorbances were measured at 560 nm against a reagent blank prepared in a similar manner. The coloured product was stable for 1 h. The amount of INS in the sample solution was computed from its calibration curve.

Method M_2: Aliquots of standard INS solution (0.25–1.5 mL, 100 μg mL^{-1}) were delivered into a series of 125 mL separating funnels. Then 2.0 mL of buffer solution (pH 2.0) and 5.0 mL CTC solution were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 mL with distilled water. To each separating funnel 10.0 mL of nitrobenzene was added and contents were shaken for 2 min. The two phases were allowed to separate and absorbance of nitrobenzene layer was measured at 600 nm against a similar reagent blank. The coloured product was stable for 1 h. The amounts of INS in the sample solution was computed from its calibration curve.

Methods M_3: Aliquots of standard INS solution (0.2–1.0 mL 500 μg mL^{-1}) were taken in a series of 25 mL graduated tubes and evaporated to dryness on a water bath. The tubes were cooled to room temperature and then 10.0 mL of CA-AA reagent was added to each tube. The tubes were placed in a boiling water bath for 30 min. The solution in each tube was cooled to room temperature and the volume was made up to the mark with acetic anhydride. The absorbance of coloured solution was measured at 560 nm against reagent blank. The quantity of the drug in the sample was obtained from the Beer's law plot.

RESULTS AND DISCUSSION

The optimum conditions for the development of methods M_1 to M_3 were established by varying the parameters one at a time[4] and keeping others fixed and observing the effect produced on the absorbance of the coloured species.

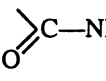
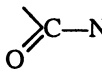
Analytical data

The Beer's law limits, molar absorptivity, Sandell's sensitivity, detection limits[5], regression equation and correlation coefficients obtained by least squares treatments of these results are given in Table-1. The precision of each method was tested by analysing six replicate samples containing 4 μg mL^{-1}, 10 μg mL^{-1}, and 12 μg mL^{-1} of pure drug for methods M_1, M_2 and M_3 respectively. The per cent standard deviation and per cent range of error at 95% confidence level of each method are given in Table-1. Recovery studies were carried out by addition of known standard drug solution to preanalysed sample solution. Results of recovery studies are reported in Table-2.

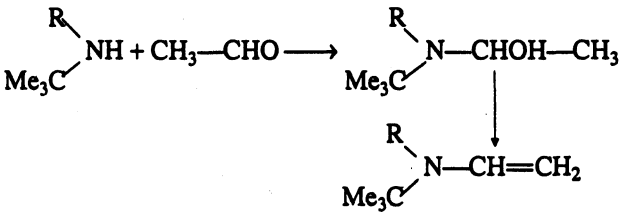
The interference studies in the determation of INS in pharmaceutical formulations revealed that the normally existing excipients and additives like starch, lactose, talc, stearic acid, boric acid, gelatin, magnesium carbonate and sodium lauryl sulphate were found not to interfere even when present in excess of the anticipated amount.

Chemistry of coloured species

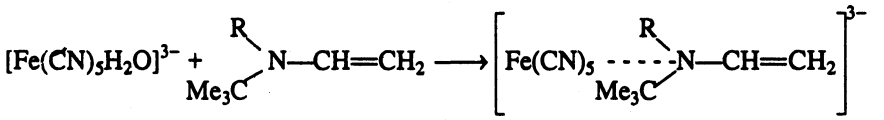
Method M_1: Cullis and Waddington[6] found that many secondary but not primary or tertiary amines react with sodium nitroprusside and acetaldehyde under mild alkaline conditions to give a violet blue coloured compound. Wolfe and Swinehart[7] have reported the formation of $[Fe(CN)_5H_2O]^{3-}$ in aqueous solution of sodium nitroprusside. In proposing the nature of coloured species formation with chloronil-acetaldehyde reagent[8], initial N-alkyl vinyl amine formation of secondary amine with acetaldehyde has been assumed. Even though INS posses-

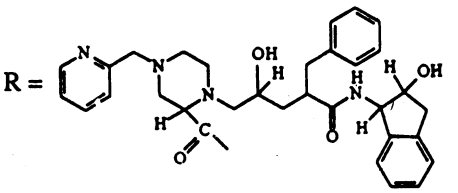
ses two amides ( and ), the former one appears

to behave like secondary amine due to inductive effect of substituent CMe_3 to amide nitrogen. The formation of colour species in INS with this reagent may be assigned through above anology as shown in **Scheme-1.**



$$[Fe(CN)_5NO]^{2-}(Na^+)_2 \xrightarrow{\text{Alkali}} [Fe(CN)_5H_2O]^{3-}$$





Scheme-1

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

Parameters	Methods		
	M_1	M_2	M_3
Beer's law limits (μg mL^{-1}), C	0.6–6	1.35–15	1.65–20
Molar absorptivity (L mol^{-1} cm^{-1})	5.748×10^4	2.634×10^4	2.171×10^4
Sandell's sensitivity (μg cm^{-2} /0.001 absorbance unit)	1.200×10^{-2}	2.700×10^{-2}	3.270×10^{-2}
Detection limit	5.517×10^{-2}	4.106×10^{-2}	1.750×10^{-2}
Regression equation* (Y = a + bc)			
Slope (b)	8.15×10^{-2}	3.7×10^{-2}	3.05×10^{-2}
Intercept (a)	-1.4×10^{-3}	2.0×10^{-4}	1.01×10^{-4}
Correlation coefficient (r)†	0.9999	0.9999	0.9999
Relative standard deviation (%)†	1.2210	0.5850	0.5080
% Range of error (95% confidence limits)	1.2810	0.6140	0.5340

*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

Formulations*	Labelled amount (mg)	Amount found (mg) by proposed methods†			Reference method‡	% Recovery by proposed methods**		
		A	B	C		A	B	C
Capsule-I	400	398.7 ± 1.235 F = 2.85 t = 2.16	397.95 ± 1.265 F = 2.72 t = 1.81	398.17 ± 1.67 F = 1.56 t = 1.9	396.42 ± 2.086	99.69 ± 0.31	99.48 ± 0.417	99.54 ± 0.42
Capsule-II	400	396.43 ± 3.42 F = 1.66 t = 1.59	396.62 ± 2.74 F = 1.09 t = 1.8	395.97 ± 4.13 F = 2.43 t = 1.44	399.0 ± 2.65	99.10 ± 0.86	99.15 ± 0.620	99.03 ± 1.08
Capsule-III	400	397.25 ± 2.07 F = 2.79 t = 0.564	397.82 ± 2.16 F = 3.05 t = 0.79	396.18 ± 0.94 F = 1.76 t = 2.31	398.6 ± 1.24	99.31 ± 0.52	99.46 ± 0.540	99.00 ± 0.24
Capsule-IV	400	398.49 ± 1.257 F = 3.89 t = 1.193	399.4 ± 1.62 F = 2.34 t = 0.64	396.9 ± 3.0 F = 1.46 t = 1.23	400.79 ± 2.48	99.62 ± 0.21	99.85 ± 0.410	99.22 ± 0.75

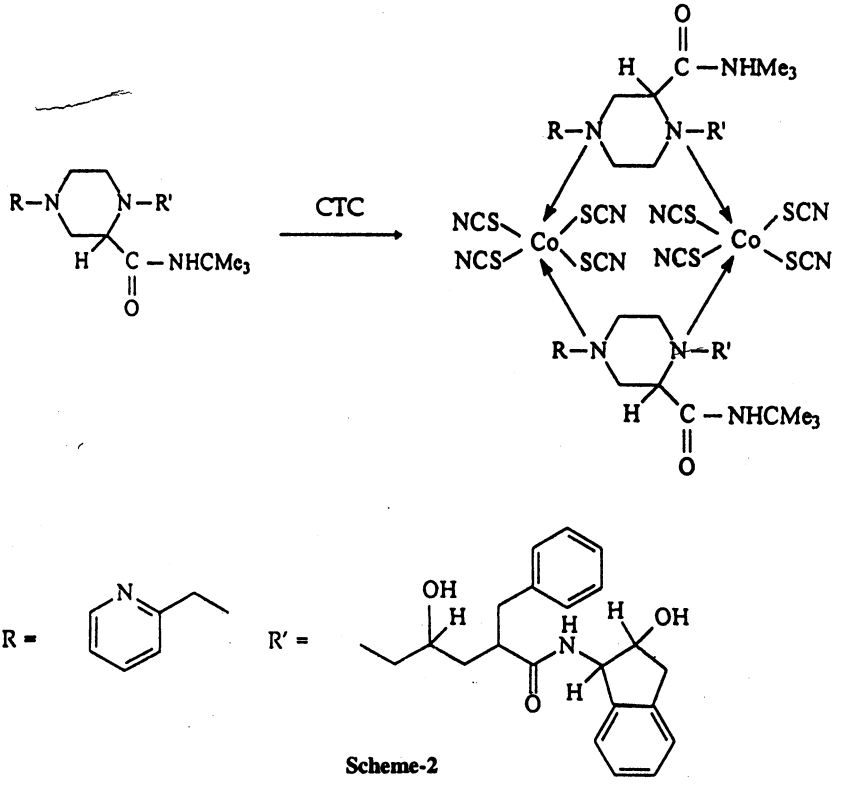
*Different batches from two different pharmaceutical companies.

†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 preanalysed pharmaceutical formulations (average of three determinations).

‡Developed in the laboratory using chloroform solvent (λ_{max} = 236 nm).

Method M_2: Cobalt thiocyanate (CTC) (formed from the combination of ammonium thiocyanate and cobalt nitrate) has been proved to be a valuable chromogenic reagent for the determination of amine compounds[9]. The colour species formed is the coordination complex of the drug (electron donor) and the central metal of cobalt thiocyanate, which is extractable into nitrobenzene from aqueous solution. Formation of the green coloured complex when INS was treated with CTC is due to the presence of the tertiary amino group in it. As INS contains two amino groups in the form of N-substituted piperazine, both the tertiary amino groups may involve in the complex formation based on the analogy of tertiary amine as given in Scheme-2.

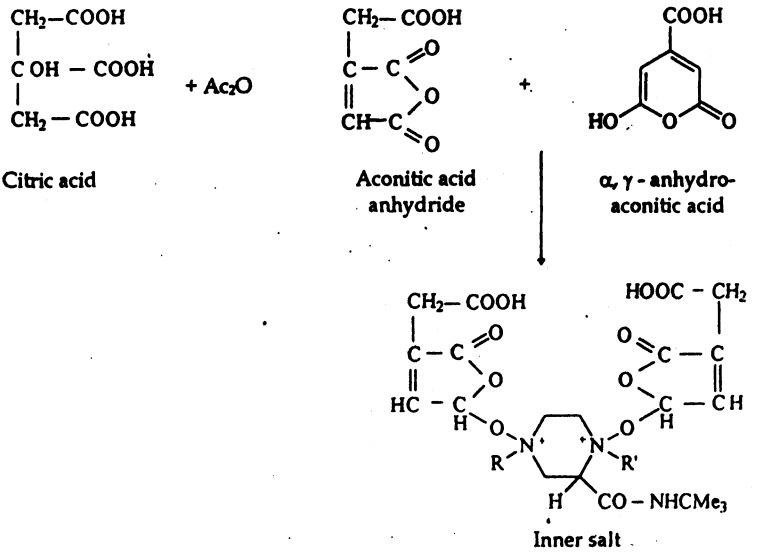


Scheme-2

Method M_3: According to Groth and Dahlen[10], upon suitable action of acetic anhydride-citric acid affords *cis*-aconitic anhydride and α,γ-anhydro- aconitic acid. The latter, when dissolved in acetic anhydride, develops a violet colour due to presence of tertiary amino group. As INS possesses two tertiary amino substitutents it reacts with citric acid-acetic anhydride dehydration product producing a coloured product as shown in scheme-3.

Conclusion

The proposed methods exploit different structural features (method M_1:



Scheme-3

substituted amide which behaves like secondary amine; methods M_2 and M_3: substituted piperazine moiety) of the INS molecule. All the proposed methods have higher λ_{max} values and sensitivity. This is a decisive advantage since the interference from the associate ingredients will be less at higher wavelengths than at lower wavelengths. The sensitivity order of the procedures is $M_1 > M_2 > M_3$. The λ_{max} order of the coloured species is $M_2 > M_1 = M_3$. The proposed methods are simple, rapid and have reasonable precision and accuracy. All the proposed methods are useful for the determination of INS and provide a wide choice, depending on the needs of the specific situation.

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