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A new method for spectrophotometric determination of colchicoside

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A facile spectrophotometric method is proposed for determination of Colchicoside (COLSID) in bulk and in dosage forms using ceric ammonium sulphate (CAS). Method involves addition of a known excess of CAS to COLSID and determination of residual CAS with a fixed amount of either Azure B (AB), measuring absorbance at 633nm or Safranin O (SO), measuring absorbance at 519 nm. Calibration graphs are linear over 25.00-50.00 μgml^{-1}, 22.00-38.00 μgml^{-1} and apparent molar absorptivity is calculated to be 2.28 x 10^4 l mol^{-1} cm^{-1} and 2.77 x 10^4 l mol^{-1} cm^{-1} for CAS-AB and CAS-SO respectively. Method is successfully applied to assay of COLSID in pharmaceutical formulations.

Keywords: Azure B (AB), Ceric ammonium sulphate (CAS), Colchicoside (COLSID), Safranin O (SO)

Introduction

Colchicoside (COLSID), an alkaloid, extracted from *Gloriosa superba* Linn, also known as "Meadow Saffron", is used for Gout and also as anticancer drug[1]. COLSID, a glucosylated colchicine, is less harmful to human than other colchicines[2-4]. Due to the action of COLSID on spindle fibre formation during cell division, plant has been identified as a potential anticancerous drug[5]. Immunoassay[6], capillary electrophoresis[7], HPLC[8-11] and spectrophotometry[12] methods have been reported for determination of COLSID. This study presents a simple and sensitive method for spectrophotometric determination of COLSID that is based on oxidation of drug by ceric ammonium sulphate (CAS) and residual oxidant is determined by using either Azure B (AB) or Safranin O (SO).

Experimental

Materials

A UV-2550PC (Shimadzu, Japan) visible spectrophotometer with matched 1cm quartz cells were used for all measurements. All chemicals used were of analytical grade and all solutions were prepared in distilled water. A solution of CAS (0.2%) was prepared by dissolving CAS (0.2 g) in 2M H_2SO_4 and made up to the mark with distilled water in a 100 ml standard flask. Azure B (s d fine chem Ltd., India, 90% dye content, μ= 1.0 X10^5 lmol^{-1} cm^{-1}) (0.01%) and Safranin O (s d fine chem. Ltd., India, 90% dye content, μ= 1.1X10^4 lmol^{-1} cm^{-1}) (0.01%) were prepared with distilled water.

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Standard Solution of COLSID

A stock standard solution (1000 μg ml^{-1}) was prepared by dissolving weighed amount of pure COLSID in distilled water and diluting to a known volume; it was later diluted appropriately with water to get working concentrations for use in spectrophotometric methods A and B, respectively.

Method A

Different aliquots (25.00-50.00 μg ml^{-1}) of COLSID were transferred into a series of 10 ml calibrated flasks using a micro burette. To each flask, CAS (1 ml) was added, shaken well and kept for 10 min. Then added AB (1.5 ml) and diluted to mark with distilled water. Absorbance of each solution was measured at 633nm (Fig. 1a).

Method B

Different aliquots (22.00-38.00 μg ml^{-1}) of COLSID were transferred into a series of 10 ml calibrated flasks using a micro burette. To each flask, CAS (1 ml) was added, shaken well and kept for 10 min. Then added SO (3.0 ml) and diluted to mark with distilled water.

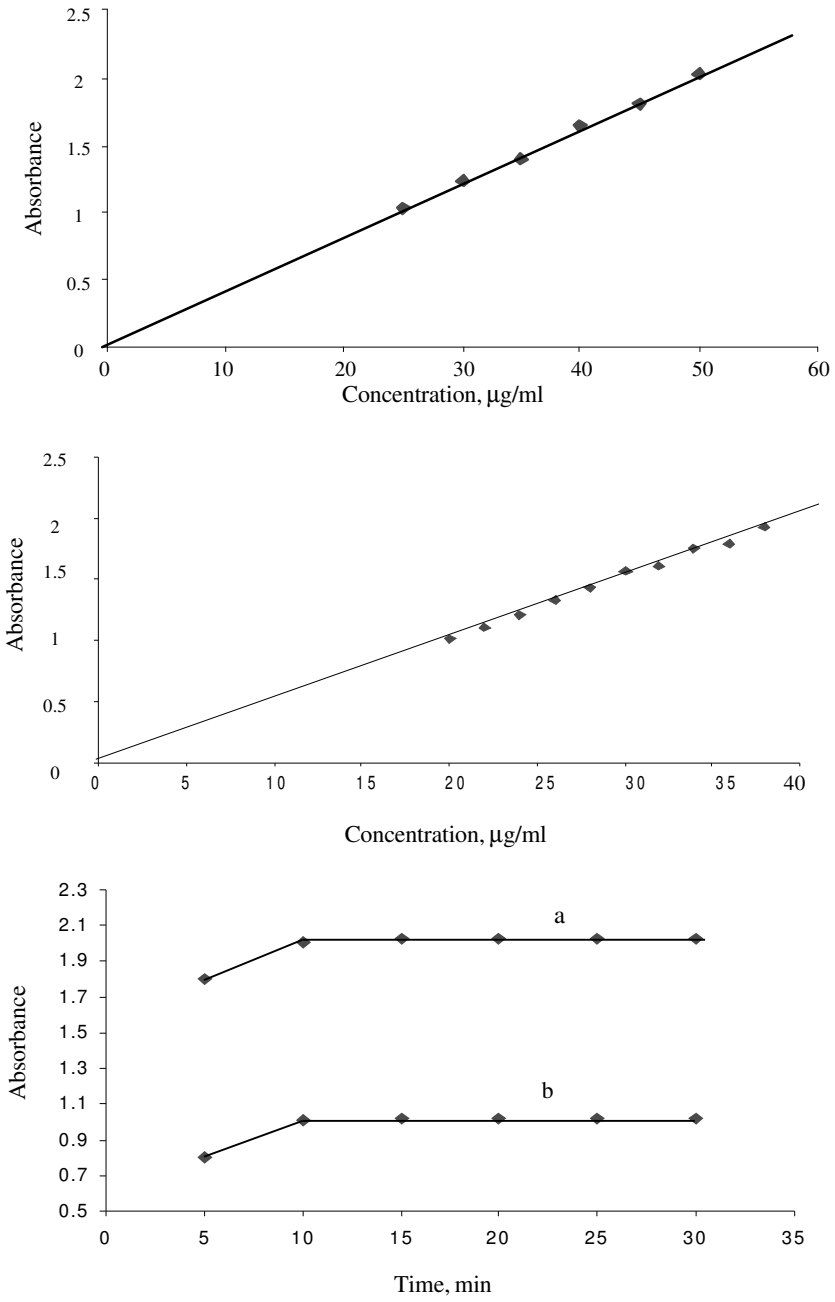


Fig.1—Calibration curve for: a) COSID-Azure B system; b) COLSID- Safranin O system; c) A graph of change in absorbance with time (a, COLSID- Azure B system; b, COLSID- Safranin O system)

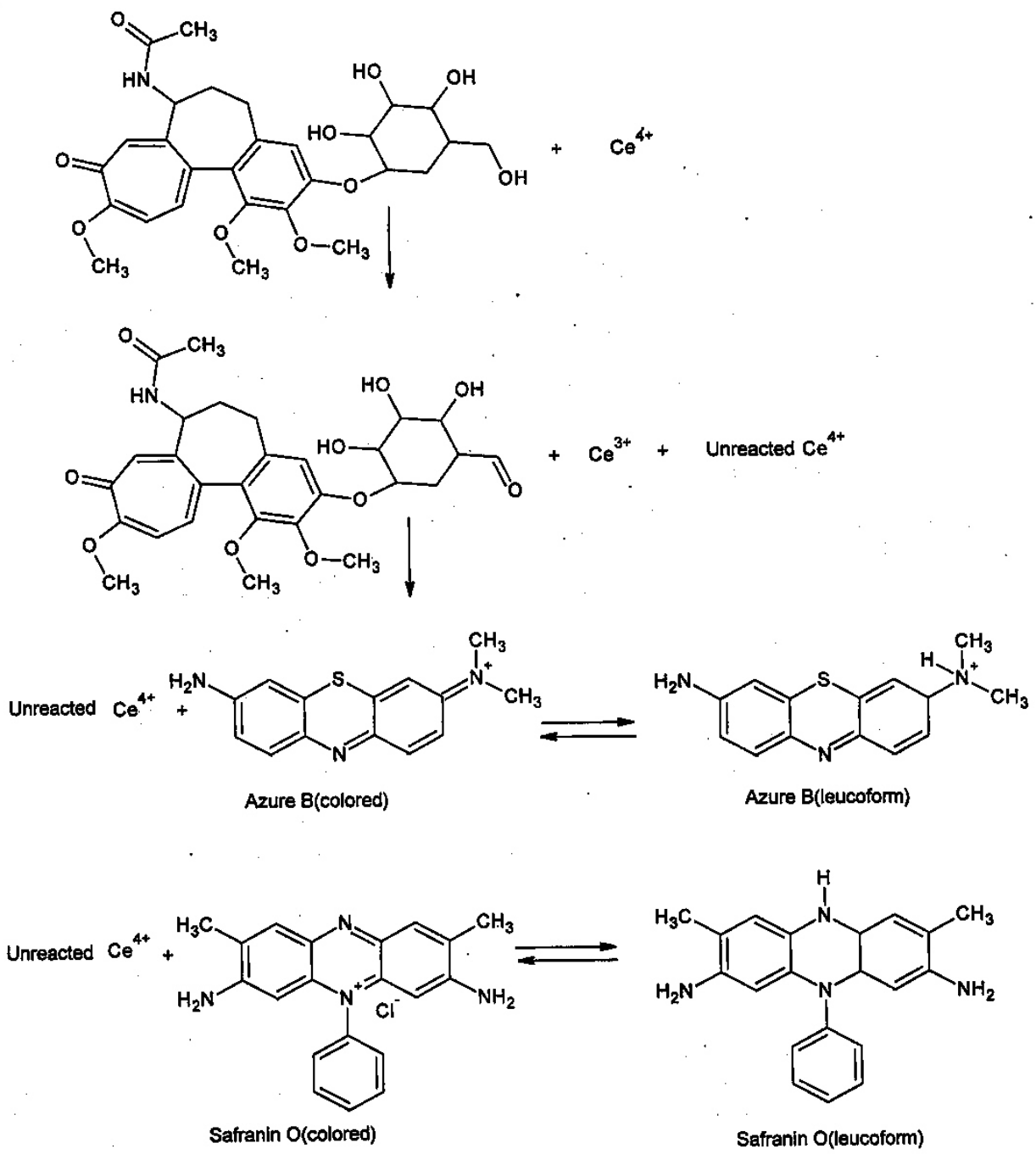
Absorbance of each solution was measured at 519 nm (Fig. 1b).

Analysis of Tablet

A total of 10 tablets of Goutnil (Inga Pharmaceuticals Ltd., India) were ground into fine powder, dissolved in distilled water by stirring for 10 min, transferred into a 100 ml standard flask and made up to volume with distilled water. Above solution was analyzed according the proposed procedures.

Results and Discussion

Method A and B involve oxidation of drug by CAS and residual oxidant is determined by using AB and SO. When known amount of CAS is added to drug solution, drug gets oxidized; excess oxidant bleaches dyes to leuco



Scheme 1

form (Scheme 1). Change in absorbance with time is studied and it is found that after 10 min there is no such variation in absorbance of reaction mixtures (Fig. 1c). Reaction mixtures showed maximum absorbance at 633 nm (Fig. 2) for Method A and at 519 nm (Fig. 3) for Method B.

Analytical Data

A linear correlation was found between absorbance and concentration of drug. Optical parameters (molar absorptivity, Beer's law limit values) were calculated (Table 1). Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to ICH

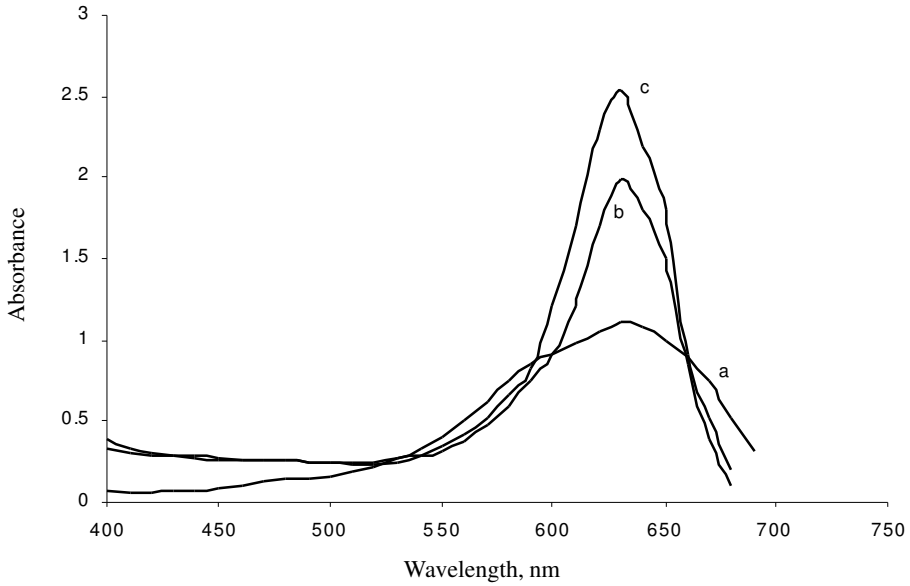


Fig. 2—Absorption spectra for: a) COLSID + CAS + Azure B; b) COLSID + Azure B; c) Azure B

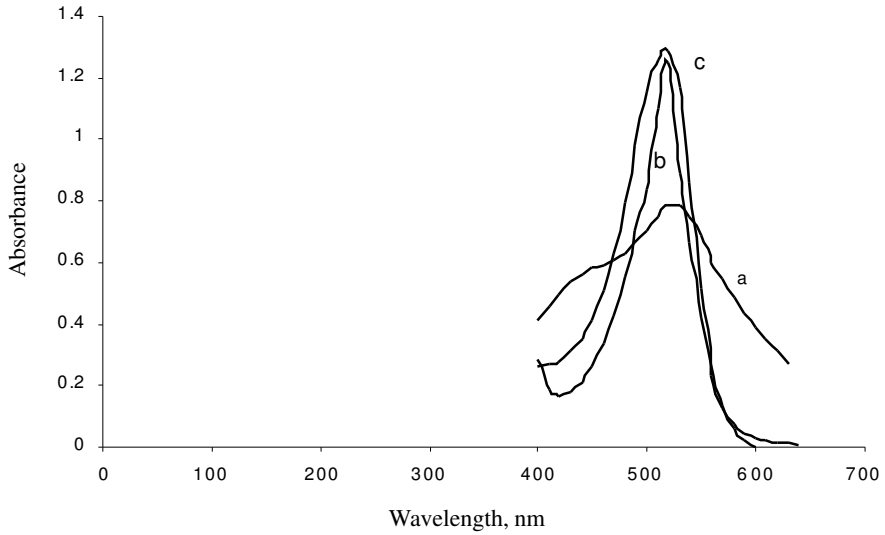


Fig. 3—Absorption spectra for: a) COLSID + CAS + safranin O; b) COLSID + safranin O; c) safranin O

guidelines[13] as LOD =3.3 × σ/S and LOQ = 10 × σ/S, where σ is standard deviation of y-intercept of regression lines (standard deviation of response) and S is slope of calibration curve. Linearity was evaluated by calculation of regression. Sensitivity of proposed methods is determined by calculating sandell's sensitivity (μg/cm^2/ 0.001 Abs unit), which can be defined as smallest weight of substance that can be detected in column of unit cross section.

Accuracy and Precision

For accuracy and precision, solutions containing different concentrations of drug were analyzed. Analytical results are considered to be satisfactory for level of concentrations examined (Table 2).

Interference Study

To investigate effect of excipients and fillers on measurements, standard addition method was carried out. It was observed that talc (70 mg), starch (40 mg), glucose (65 mg) and lactose (55 mg) did not interfere in measurements.

Application

Proposed method had been successfully applied for determination of COLSID in dietary supplements.

Table 1—Analytical parameters

	Method A	Method B
λmax, nm	633	519
Beer's law limit, μg ml^{-1}	25.00-50.00	22.00-38.00
Molar absorptivity, lmol^{-1}cm^{-1}	2.28×10^4	2.77×10^4
Detection limit**	0.0218	0.0074
Quantitation limit**	0.0663	0.0022
Sandell's sensitivity	0.024	0.0197
Regression equation*	Y=a+bX	Y=a+bX
Slope (b)	0.3968	0.5076
Intercept (a)	0.0376	-0.0009
Correlation coefficient	0.9990	0.9971

*Y is absorbance and X is concentration in μgml^{-1}; **Calculated using ICH-guidelines

Table 2—Evaluation of accuracy and precision

Amount taken μgml^{-1}	Amount found μgml^{-1}	RE %	SD μgml^{-1}	RSD %
Azure B*				
25.00	25.22	0.88	0.44	1.75
30.00	30.13	0.43	0.25	0.83
35.00	35.18	0.52	0.48	1.30
40.00	40.16	0.40	0.34	0.85
45.00	45.40	0.88	0.51	1.14
Safranin O**				
20.00	20.36	0.20	0.06	0.32
28.00	27.93	0.25	0.13	0.46
32.00	32.04	0.12	0.11	0.36
36.00	35.86	0.38	0.11	0.32
38.00	37.94	0.16	0.09	0.24

*Mean value of 4 determinations, **Mean value of 3 determinations

Table 3—Assay of tablets

	Labeled amount mg	Amount* found mg	Label claim±SD %
Method A	0.50	0.501	100.18±0.14 t-test=1.039
Method B	0.50	0.500	100.04±0.12 t-test=0.349

*Mean value of 5 determinations, Calculated Student's t-value at 95% confidence level is 2.77

Content of tablet formulation was calculated by applying suitable dilution factor. Calculated student's t-test (Table 3) did not exceed tabulated value, indicating that there was no significant difference between proposed method and tabulated value in respect to accuracy and precision.

Conclusions

Proposed method is simple, sensitive and possesses long dynamic range of determination. It can be applied in quality control laboratories for routine analysis of investigated drugs in raw materials and in pharmaceutical formulations.

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