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Determination of Diosmin by Visible Spectrophotometry

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Three simple and sensitive spectrophotometric methods (**A-C**) for the determination of diosmin are described. They are based on the oxidative coupling reaction between diosmin and 4-aminophenazone (4-AP)-potassium ferricyanide [$K_3Fe(CN)_6$] (λ_{max} 500 nm, method **A**); or *p*-N,N-dimethylphenylene diamine (DMPD)-chloramines T (CAT) (λ_{max} 680 nm, method **B**); or 2,6-dichloroquinone-N-chlorimide (DCQC) (λ_{max} 540 nm, method **C**). The results obtained by each proposed method is compared statistically by means of student t-test and by the F-test with that of the reported method. They are found to be in good agreement. No interferences are observed from common pharmaceutical excipients. The proposed methods are selective, simple and economical for the quantitative determination of diosmin.

Key Words: Spectrophotometry, Diosmin, 4-Amino phenazone, *p*-N,N-Dimethyl phenylene diamine, Chloramine-T, 2,6-Dichloroquinone N-chlorimide.

INTRODUCTION

Diosmin (DIS), 4*H*-1-benzopyran-4-one-7-[6-*o*-(6-deoxy- a-L-manopyranosyl-β-D-glucopyranosyl)-oxy]-5-hydroxy-2-(3-hydroxy-4-methoxy phenyl], is used as a coagulant and antioxidant. It is also used in the treatment of diseases characterized by capillary bleeding associated with capillary fragility.

A review of literature reveals that HPLC[1-5], MS[6-8], proton and C^{13}NMR[9], IR[10] and UV spectrophotometric[11,12] methods are reported. There is no report on visible spectrophotometric method. The authors made some attempts in this direction by exploiting the characteristic oxidative coupling reactions due to the presence of phenolic-OH groups. In the present paper three visible spectrophotometric methods and their application for routine assay of diosmin in bulk form and tablets are described. These methods are based on the formation of colored oxidative coupling products with the coupling agent (4-amino phenazone, method **A**; *p*-N,N-dimethyl phenylene diamine, method **B**) in the presence of an oxidant ($K_3Fe(CN)_6$), method **A**, chloramines-T method **B**) or 2,6-dichloroquinone N-chlorimide directly (method **C**).

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EXPERIMENTAL

A Systronic model 106 visible and Milton roy spectonic 1201 UV-vis spectrophotometers are used for absorbance measurement. An Elico LI-120 digital pH meter is used for pH measurement.

All the chemicals used are of analytical grade. Aqueous solutions of 4-amino phenazone (Merck, 2.46×10^{-2} M), $K_3Fe(CN)_6$ (BDH, 6.07×10^{-2} M), Na_2CO_3 (Ranbaxy, 9.43×10^{-2} M) for method **A**, *p*-N,N-dimethyl phenylene diamine (Merck, 4.68×10^{-3} M), chloramines-T (Loba, 4.39×10^{-3} M), phosphate buffer of pH 7 for method **B** and 2,6-dichloroquinone-N-chlorimide (Loba, 2.38×10^{-3} M) in isopropanol for method **C** are prepared.

Preparation of standard drug solution: One mg mL^{-1} stock solution of DIS is prepared by dissolving 100 mg of the drug initially in 10 mL of 0.1 M NaOH and making upto 100 mL with distilled water. The stock solution is further diluted to obtained working standard solution of concentration 100 μg mL^{-1} (for method **A** and **B**) and 50 μg mL^{-1} (for method **C**).

Analysis of tablets: Tablets power equivalent to 100 mg of active ingredient is dissolved in 0.1 M NaOH and filtered to remove insoluble portion if any and the working solutions of these methods (**A-C**) are prepared as under standard solution preparation. They are analyzed as under the procedures given for pure samples.

Recommended procedures

Method A: Aliquots of standard drug solution ranging from (0.5-4 mL; 100 μg mL^{-1}) are transferred into a series of 25 mL calibrated tubes. To each tube 0.6 mL of sodium carbonate (9.43×10^{-2} M), 1.5 mL each of 4-amino phenazone (2.46×10^{-2} M) and [$K_3Fe(CN)_6$] (6.07×10^{-2} M solutions are added successively and the total volume in each tube is brought to the mark with distilled water and the absorbance is measured after 10 min at 200 nm against a reagent blank. The colored species is stable for 2 h. The drug concentration is deduced from a calibration curve.

Method B: Aliquots of standard drug solution ranging from (0.5-3.0 mL; 100 μg mL^{-1}) are placed into a series of 25 mL graduated tubes, 9 mL of pH 7 buffer, 1.0 mL each of DMPD (4.68×10^{-3} M) and CAT (4.39×10^{-3} M) solutions are added successively. The volume is made up to mark with distilled water and kept a side for 15 min to allow full colour development. The absorbance is measured at 680 nm against a reagent blank. The coloured species is stable for 2 h. The drug concentration is deduced from a calibration curve.

Method C: Aliquots of standard drug solution ranging from (0.5-4 mL; 50 μg mL^{-1}) are transferred into a series of 10 mL graduated tubes. 0.5 mL of DCQC (2.38×10^{-3} M) is added to each tube and the volume is made up to the mark with isopropanol. The absorbance of the coloured species is measured at 540 nm after 20 min against a reagent blank. The coloured species is stable for 2 h. The amount of drug is computed from the calibration curve.

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedures are established adopting variation of one variable at a time (OVAT) method. The optical characteristics such as Beer's law limits, molar absorptivities, regression equation and correlation coefficients obtained by linear least squares treatment of the results are given in Table-1. The precision and accuracy are found by analysis of 6 separate samples containing known amount of the drug and the results are summarized in Table-1. The relative standard derivation and % range of error at the 95 % confidence level are also given in Table-1. These methods are applied for the determination of diosmin in tablets. These results obtained from the proposed and UV reference methods[12] are compared statistically by means of student t-test and by the variance ratio F-test and no significant difference (Table-2) is observed. It indicates that none of the usual excipients like talc or starch employed in the formation of dosage forms interfere in the analysis of drug by the proposed methods. As an additional check of accuracy, recovery experiments are performed by the standard addition method. These results are also summarized in Table-2.

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

Parameter	Method **A**	Method **B**	Method **C**
λ_{max} (nm)	500	680	540
Beer's law limits (?g/mL)	2-16	2-16	2.5-20
Molar absorptivity (mol/cm)	2.37×10^4	1.9×10^4	1.98×10^4
Sandell's Sensitivity (mg cm^2/0.001 absorbance units)	2.56×10^{-2}	3.2×10^{-2}	3.06×10^{-2}
Regression equation[a] (y)			
Slope (b)	2.92×10^{-2}	3.15×10^{-4}	3.28×10^{-2}
Intercept (a)	-2.5×10^{-4}	-9.28×10^{-4}	-6.12×10^{-4}
Correlation coefficent	0.9997	0.9998	0.9999
Relative standard deviation[b] (%)	0.61	0.58	0.43
% range of error (0.05 level)	0.64	0.61	0.45

a: y = a + bC where C is the concentration in µg/mL and y is the absorbance unit.
b: Six replicate samples.

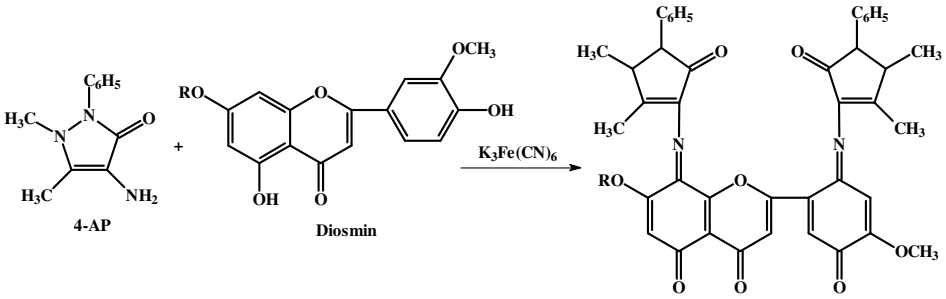
Chemistry of the colored species: Diosmin contains two phenolic-OH groups which are free in *p*-position. In method **A**, diosmin condenses with 4-amino phenazone in the presence of oxidizing agent [$K_3Fe(CN)_6$], to give colored N-substituted quinoneimine. In method **B**, it condenses with the *in situ* intermediate (*p*-N,N-dimethyl benzoquinonediimine (DMPD), PDBQI formed DMPD by the action of an oxidant (CAT) to give colored indodye. In method **C**, it condenses with 2,6-dichloroquinone N-chlorimide directly at pH 7.0 to give coloured indophenol. The coloured species are represented in **Scheme-I**.

TABLE-2
ASSAY AND RECOVERY OF DIOSMIN IN PHARMACEUTICAL FORMULATIONS

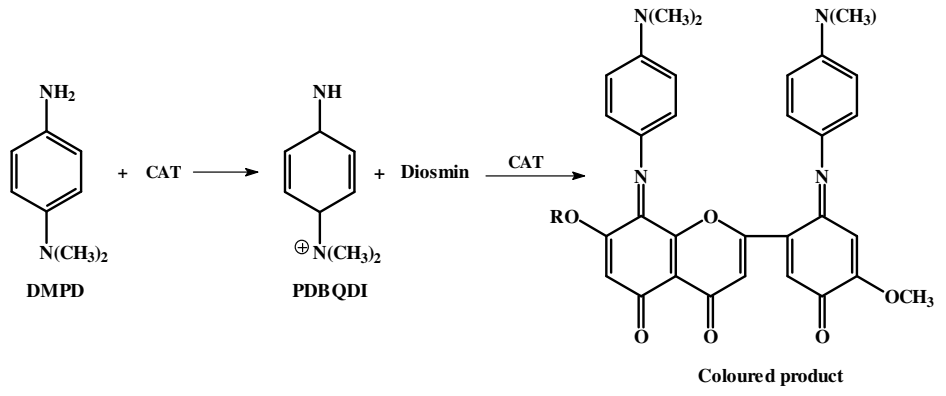
F.[a]	L.A. (mg)	Amount found by proposed methods[b] (mg)			Reference methods	% Recovery by proposed methods[c]		
						A	B	C
Tab. I	150	149.9±0.53 F = 2.74 t = 0.17	149.9±0.61 F = 3.63 t = 0.37	149.4±0.23 F = 1.93 t = 1.16	150.5±0.32	100.2±0.2	99.91±0.4	99.82±0.1
Tab. II	150	150.0±0.53 F = 2.58 t = 0.3	150.1±0.60 F = 3.31 t = 1.85	149.9±0.38 F = 1.13 t = 0.77	149.9±0.33	99.76±0.2	100.08±0.4	99.96±0.2
Tab. III	450	448.9±0.97 F = 1.02 t = 0.53	448.8±1.2 F = 1.5 t = 1.85	449.2±0.7 F = 1.96 t = 1.8	448.8±0.98	99.94±0.3	99.94±0.3	99.83±0.1
Tab. IV	300	300.7±0.64 F = 3.38 t = 1.73	300.8±0.79 F = 4.4 t = 0.92	300.0±0.37 F = 1.35 t = 0.4	300.4±0.43	100.05±0.3	100.26±0.2	100.03±0.2

a: Different batches of tablets from four different pharmaceutical companies; b: Average ± standard deviation of six determinations, the t and F-test value refer to comparison of the proposed method with the reference method. Theoretical values at 95 % confidence limit F = 5.05, t = 2.57; c: Recovery of 10 mg added to the pre analyzed pharmaceutical formulations (average of three determination); LA = Labelled amount.

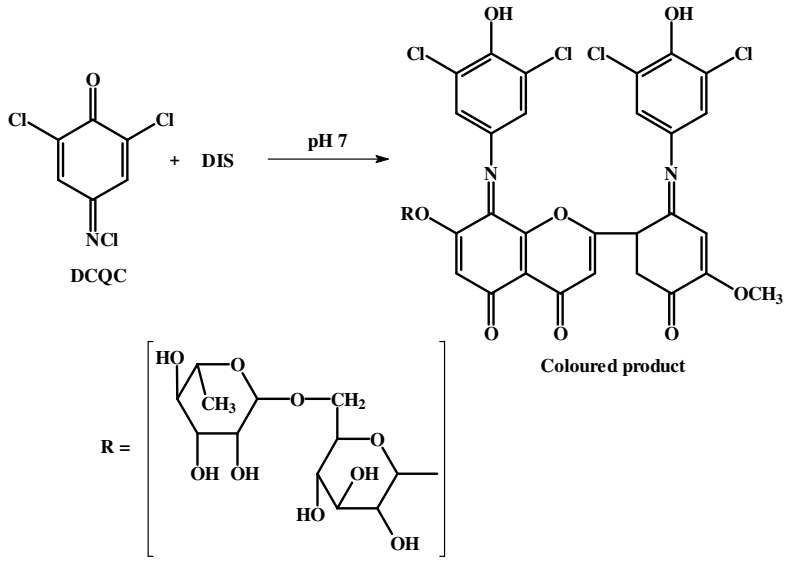
Method-**A**



Method-**B**



Method-**C**



Scheme-I

All the methods are simple and sensitive and with good precision and accuracy and can be used for routine quality control analysis of diosmin in pure form as well as pharmaceutical formulations. The order of sensitivity among the proposed methods in the determination of diosmin is A > C > B > R.

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