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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR CHRYSOPHANOL IN GEL FORMULATIONS

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ABSTRACT

A simple, accurate, sensitive, precise & reproducible UV spectroscopic method has been developed for the estimation of chrysophanol in pure form and in gel formulations. Chrysophanol was estimated at 225nm in methanol medium. Beer's law was obeyed in the concentration range of 1–10 µg/ml (r2= 0.998) . The method was tested and validated for various parameters according to the ICH(International Conference on Harmonization) guidelines. The detection and quantification limits were found to be 0.57µg/ml and1.72µg/ml, respectively. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 1 %), while being simple, cheap and less time consuming, and hence can be suitably applied for the estimation of chrysophanol in different dosage forms

Keywords: *Spectrophotometric Method, Validation, Accuracy, Chrysophanol, Gel formulation,*

1. Introduction

Infection is a major problem to treat the wound. Antibiotic resistance by the pathogenic microorganism renders drug ineffective and calls for improved designing and development of new drugs. New approach has been developed to isolate active components from botanicals and formulate in to suitable forms. chrysophanol is a 1,8-Dihydroxyanthraquinone derivatives (1,8 DAD) are naturally occurring compounds that have been isolated and separated from various medicinal plants, which belong to various botanical families such as Rhamnaceae(buckthorn,cascara), liliaceae(aloe), polygonaceae(rhubarbs) and Caesalpiniaceae(senna).In our previous study chrysophanol isolated from cassia fistula, posses potent antimicrobial activity against skin infecting pathogenic organisms. In order to exploit chrysophanol, we designed a topical gel formulation of chrysophanol for skin infections.

Various methods have been reported in the literature for the analysis of chrysophanol including thin layer chromatography scanning (TLCS) [1], high performance liquid chromatography (HPLC) with UV–VIS detection [2], PDA detection [3,4] or MS/MS detection [5], capillary electrophoresis with UV–VIS detection [6,7] or electrochemical detection [8,9] However, there were no reports on simple validated UV Spectrophotometric method for estimation of chrysophanol determination. For routine analysis of chrysophanol, a simple and rapid analytical method is preferred. The objective of the present study was to develop simple, precise,

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accurate and validated, economic analytical methods for the estimation of chrysophanol in pure form and in pharmaceutical gel formulations. The developed analytical method was validated as per the ICH (International Conference on Harmonisation) guidelines [10]. Statistical tests were performed on validation data [11,12,13].

2. Experimental

2.1 Materials

Chrysophanol standard compound was from Aldrich Chemical Company,

SYSTRONICS UV-VIS Double Beam Spectrophotometer 2201, The HPLC equipment used was Schimadzu LC- 2010C HT system and Schimadzu HPLC work station (Schimadzu Japan).All chemicals and solvents used were of analytical grade and for HPLC all reagents were HPLC grade.

2.2 Preparation of Standard Stock Solution

1mg Chrysophanol was accurately weighed, transferred to 10 ml volumetric flask and dissolved (if necessary, it may be placed in an ultrasonic bath to dissolve) in 10ml of methanol to give a standard solution of 100µg/ml.

2.3 Determination of λ max.

1ml of standard solution (100µg/ml) was pipetted out into 10ml volumetric flask and made up the volume by adding appropriate quantities of methanol. The absorbance of the resultant solution was scanned in UV range (200-400nm) for maximum absorbance after enabling blank correction for methanol in the above region.

2.4 Procedure for calibration curve

The standard solution were prepared by proper dilutions of the primary stock solution with methanol to obtain working standards in the concentration range of 1-10 µg/ml The absorbance was measured at 225nm against a solvent blank and the calibration curve was plotted. Similarly absorbance of sample solution was measured and the amount of chrysophanol was determined by referring to the calibration curve.

2.5 Estimation of chrysophanol in gel formulation.

For the estimation of the drug in gels, chrysophanol was extracted from 1gm of each gel formulation with 50ml of methanol for 30 min, the resultant mixture was filtered through membrane filter (pore size 0.45µm). From this 2.5ml was pipetted out and made up to 10ml. Then 1ml of the resultant solution was again diluted to 10 ml, the absorbance of the sample was determined spectrophotometrically at 225nm. The concentration of chrysophanol was estimated from the regression equation of calibration curve.

2.6 Analytical Method Validation of the Proposed Method

Linearity

To establish linearity of the proposed method, six separate series of solutions of chrysophanol (1–10 µg/ml in methanol) were prepared from the stock solution and analyzed. Least square regression analysis was performed on the obtained data.

Accuracy

The accuracy of the method is the closeness of the measured value of the true value for the sample. To determine the accuracy of the proposed method, three different levels of drug concentrations were prepared from independent stock solutions and analyzed (n = 6). Accuracy was assessed as the percentage relative error and mean % recovery (Table 2). To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, a known preanalyzed formulation sample and the total concentration was determined using the proposed

methods (n = 6). The % recovery of the added pure drug was calculated as % recovery = [(*Ct*–*Cs*)/*Ca*] x 100, where *Ct* is the total drug concentration measured after standard addition; *Cs*,drug concentration in the formulation sample; *Ca*, drug concentration added to formulation (Table 3).The absorbance of each solution was measured at 225 nm with methanol as blank

Repeatability / Precision

Repeatability expresses the precision under same operating conditions over a short interval of time. It is also termed as intra-assay precision. The precision of an analytical procedure is usually expressed as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The precision of an analytical procedure is usually expressed as the standard deviation, variance, or coefficient of variance of a series of measurements (table:2&3).

Stability Profile

Stability of absorbance is of major importance in Spectrophotometric measurements. The period over which absorbance value at 225nm of chrysophanol in methanol remained stable was investigated using three different concentration 4,5, and 6µg/ml. The absorbance values were measured at 15 min intervals for a period of 1 hour. Data obtained is furnished in table:4

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for chrysophanol by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of of the lowest standard concentration (n = 6) (Table 1).

Range

The range of an analytical procedure is the interval between the upper and lower concentrations of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

3. Results and Discussion

The UV spectra of chrysophanol was measured in the range 200-400 nm against methanol as blank solution (Figure 1). The standard solution showd a broad band of absorption ranging from 200 - 400 nm was observed with λmax at 225 nm, 257,290,347 (figure:1). In previous reports, UV spectral maxima for chrysophanol were 225, 258 or 279 & 432nm [14]. Examining the UV spectra, the maximum UV absorption wavelength of chrysophanol selected for study was 225 nm (Fig:1). And the method was validated by studying the following parameters as ICH guide lines(ICH guide lines 1995) for method validation Table 1.

Calibration curve

For chrysophanol , the linear regression equation obtained with a regression coefficient (r)of 0.999 and standard deviation (SD) of 0.0012 Beer's law was obeyed in the concentration range of 1–10 µg/ml (r2 = 0.998) in methanol medium.

Linearity

The linearity range for chrysophanol was found to be 1–10 µg/ml(r2 =0.998) in methanol medium (Table 1). The low values of the standard error (S.E.) of slope and intercept (Table 1) indicated high precision of the proposed methods. The quality of the fit of the regression equations was supported by the high regression coefficient values (Table 1).

Table1: Validation parameters for UV method of analysis of Chrysophanol

Validation parameters	Chrysophanol
lamda max (λmax).	225nm
Regression equation(y=a+bx)	y=0.134+6.98x
Slope.(S.E.) a	6.98(0.0412)
Y- intercept.(S.E.) a	0.134(0.0012)
Range(µg/ml)	1-10
Correlation coefficient (r)	0.999
Correlation coefficient (*r*2)	0.998
Limit of detection (µg/ml)	0.57
Limit of quantification (µg/ml)	1.7

a=Standard error of mean

Accuracy

The accuracy ranged from 4 to 6 µg/ml (Table 2). The excellent mean %recovery values, close to 100 %, and their low standard deviation values (S.D < 1.0) indicate high accuracy of the analytical methods. The validity and reliability of the proposed methods was assessed by the recovery studies. The mean % recoveries (% R.S.D.) for lower, intermediate and higher concentrations were found to be 100.25 (4µg/ml), 99.6 (5 µg/ml)and 99.83 (6 µg/ml), respectively. The validity and reliability of the proposed method was further assessed via recovery studies by standard addition method (Table:3). These results revealed that any small change in the drug concentration in the solutions can be accurately determined by the proposed analytical method.

Table 2: Accuracy and precision data for the developed method (n=6)

Levels	Estimated concentration by proposed method(µg/ml)a		Mean %Recovery ±S.D	Accuracy(%)b
	Mean±S.D	%R.S.D		
LC(4 µg/ml)	4.01±0.3368	0.842	100.25±0.257	0.025
IC(5 µg/ml)	4.98±0.3696	0.738	99.6±0.498	-0.4
HC(6 µg/ml)	5.99±0.5487	0.915	99.83±0.392	-0.16

a. Estimated concentration of chrysophanol was calculated by linear regression equation.
b. Accuracy is given in %relative error (=100 x (predicted concentration-nominal concentration) / Nominal concentration)

Table 3: Standard addition of chrysophanol for accuracy(n=6).

Drug in formulation (µg/ml)	Pure drug added (µg/ml)	Total drug found by proposed method(µg/ml)	Total drug found by HPLC(µg/ml)	%Recovery	%RSD
5	0	5.02	5.04	100.40	0.915
5	1	5.89	6.03	98.90	1.010
5	2	7.09	7.09	100.67	0.804
5	3	8.04	8.02	100.20	0.674

Precision

Precision was determined by studying the repeatability and intermediate precision.The repeatability results indicated the precision under the same operating conditions over a short interval of time and inter-assay precision. %R.S.D. values for the proposed analytical method were well within the acceptable range, indicating that the method has excellent repeatability and intermediate precision. The % R.S.D. values for the precision studies with real samples of chrysophanol in gel formulation were found to be less than 1 (Table: 2&3).

Detection Limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantified as an exact value. The limit of detection of chrysophanol by the proposed method was found to be 0.57µg/ml (Table 1).

Quantification Limit (LOQ)

The quantification limit of an individual analytical procedure is the lowest amount of analyte in the sample, which can be quantitatively determined with suitable level of precision and accuracy. The limit of quantification of chrysophanol by the proposed method was found to be 1.72µg/ml (Table 1).

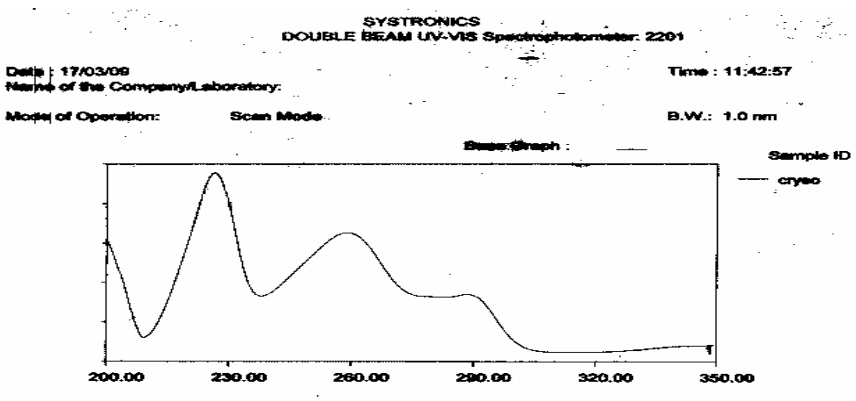
Estimation of formulations

The results obtained for the proposed methods were compared with those obtained using the HPLC method [13]. The calculated student's t-values and F-values did not exceed the theoretical ones at 95% confidence level. Therefore, there is no significant difference between the proposed method and HPLC methods (Table 3).

Table: 4 System precision study/Stability profile(n=6)

Sl No.	Concentration (µg/ml)	Absorbance at 225 nm at time intervals in minutes Mean±SD				
		0	15	30	45	60
1	4	0.554±0.001	0.553±0.003	0.556±0.005	0.554±0.003	0.555±0.001
2	5	0.710±0.001	0.709±0.002	0.710±0.004	0.7108±0.001	0.711±0.002
3	6	0.865±0.001	0.864±0.003	0.865±0.003	0.866±0.002	0.864±0.002

Figure 1



References

1) *Singh NP, Gupta AP, Sinha AK, Ahuja PS High Performance thin layer chromatographic method for quantitative determination of four major anthraquinone derivatives in Rheum Emodi. J Chromatogr A. 2005; 1077: 202–206*
2) *Yan-bin shi, Yan-ping shi, Yan-biao yang, Guang feng Simultaneous determination of tetrahydro palmatine, Magnolol, emodin and chrysophanol in Chinese herbal preparation by RP-HPLC-PDA. 2007; 65:601-606.*
3) *Qu HB, Ma YH, Yu K, Cheng YY Simultaneous Determination of eight active components in Chinese medicine 'YIQING' capsule using high performance liquid Chromatography. J Pharm Biomed Anal.2007; 43:66–72*
4) *Subash CV, Singh NP, Sinha AK Determination of locational variations in the quantity of hydroxyl anthraquinones and their glycosides rhizomes of rheum emodi using High performance liquid chromatography. J Chromatogr A. 2005; 1097:59–65*
5) *Wu YT, Lin LC, Tsai TH Determination of Honokiol and Magnolol in magnolia officinalis by liquid chromatography with tandem mass spectroscopy. Biomed Chromatogr. 2006; 20:1076–1081*
6) *Lv HX, Wang JB, Wang XC, Lin XC, Wu XP, Xie ZH Rapid separation and determination of structurally related anthraquinones in Rhubarb by pressurised capillary electro chromatography. J Pharm Biomed Anal. 2007; 43:352–357*
7) *Tian K, Zhang HG, Chen XG, Hu ZD Determination of five anthraquinones in medical plants by capillary zone electrophoresis with beta-cycledextrin addition. J Chromatogr A. 2006; 1123:134–137*
8) *Wang AF, Zhou Y, Wu F, He PG, Fang YZ Determination of active ingredients in Huangden Yinchen keli by capillary zone electrophoresis with amperometric detection. J Pharm Biomed Anal. 2004; 35:959–964*
9) *Chen G, Xu XJ, Zhu YZ, Zhang LY, Yang PY Determination of Honokiol and Magnolol in cortex Magnoline officinalis by capillary electrophoresis with electrochemical detection. J Pharm Biomed Anal. 2005; 41:1479–1484*
10) *The European Agency for the Evaluation of Medicinal Products, ICH Topic Q2B Note for Guideline on Validation of Analytical Procedures: Methodology GPMP/ICH/281/95, 1996.*
11) *United States Pharmacopoeia, Validation of Compendial Methods, 26th edition, Pharmacopoeial Convention Inc.,Rockville, MD. 2003; pp. 2439–2442.*
12) *Jugoslovenska pharmacopoeia, 5th edition, Savremena administracija, Beograd 2000.*
13) *S. Bolton, Pharmaceutical Statistics: practical and clinical application, 3rd ed., Marcel Dekker, New York, 1997; 216–264.*
14) *J.B.Harborne,Phytochemical methods,3rd ed.,Springer international edition,1988;96-100*

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