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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF COLCHICINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The present study describes a method to analyze colchicine using UV spectrophotometry. Based on the spectrophotometric characteristics of colchicine, λ_{max} selected was 246nm. The method was validated as per ICH guidelines and the parameters validated were linearity, range, accuracy, precision, LOD and LOQ. The method demonstrated linearity (r^2 = 0.995) in the range of 2-20 µg/mL with linear equation y=0.006x+0.011. LOD and LOQ were found to be 1.81 and 5.47 µg/ml, respectively. The proposed method can be used for routine analysis of colchicine using water as solvent in bulk and tablet dosage forms.

KEYWORDS: UV spectrophotometer, colchicine, λ_{max}, ICH guidelines, Method development, Validation.

INTRODUCTION

Colchicine is an alkaloid, found in the corm and seeds of various species of colchicum. Chemically it is (S)-N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl) acetamide (Fig.1). It is well known remedy for Gout[1] and also an effective adjuvant to other drugs such as corticosteroids and immunosuppressive drugs for prevention and treatment of recurrent pericarditis.[2] It is also used for certain dermatological conditions including psoriasis[3], actinic keratosis[4], urticarial vasculitis[5], scleroderma[6], cystic acne[7], erythma nodosum leprosum[8], behcet's syndrome[9], sweet's syndrome[10], amyloidosis.[11]

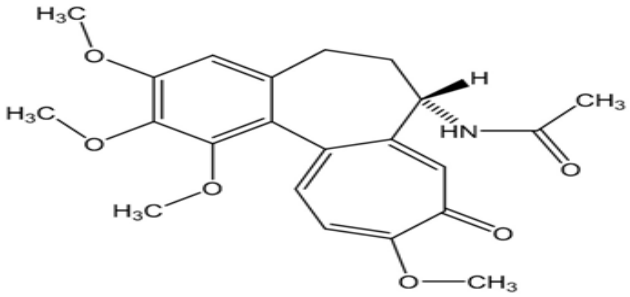

Fig 1: Chemical structure of Colchicine.

The literature survey reveals that there is only one UV spectrophotometric method reported for estimation of colchicine using phosphate buffer saline pH 6.4.[12] The solvent used for the analysis was phosphate buffer pH 6.4. Hence, the present work is performed to develop UV spectrophotometric analysis of colchicine using more economic solvent i.e. water.

MATERIALS AND EQUIPMENT

Working standard of colchicine was procured from KAN Phytochemicals Pvt Ltd (Haryana, India) as gift sample. Shimadzu UV-1800 (Kyoto, Japan) with 1 cm cuvettes was used for the measurement of absorbance.

EXPERIMENTAL

Analytical procedure

The stock solution of colchicine was prepared at concentration of 1 mg/mL in water. Working solutions of appropriate concentration were prepared by diluting the stock solution using water. The absorbance of the solutions was measured using UV-Vis spectrophotometer at λ_{max} of 246 nm.

Method validation parameters

The developed method was assessed for linearity, range, accuracy, precision, limit of detection and limit of quantitation.

RESULTS AND DISCUSSION

Method development

Colchicine was tested for dissolution in water, methanol, ethanol, 0.1M hydrochloric acid solution and 0.1M sodium hydroxide solution. The drug was showing good solubility in water, and hence was selected as the solvent. When the working standard solution of colchicine was scanned in UV range between 200 and 400 nm, the drug was showing maximum aborbance at 246 nm, which was selectrd as $\lambda_{max.}$

Linearity and Range

Linearity was performed by taking eight non-zero concentrations of colchicine in the range of 2-20 µg/mL (Fig.2). The absorbance was measured at 246 nm and the graph was plotted taking concentration on X-axis and absorbance on Y-axis (Table 1). Correlation coefficient (r^2) obtained was 0.995, proving the method to be linear in the range of 2-20 µg/mL.

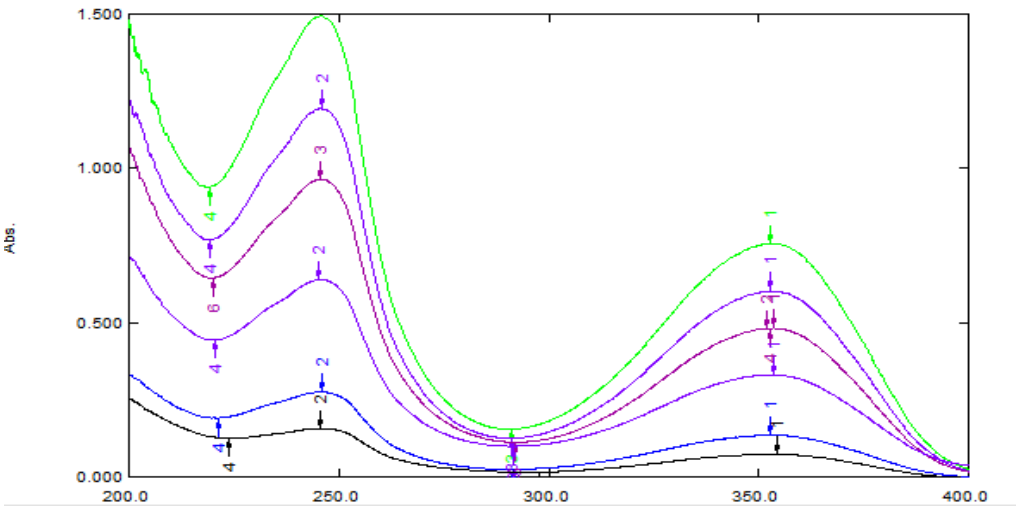

Fig.2 Overlain spectra of Colchicine.

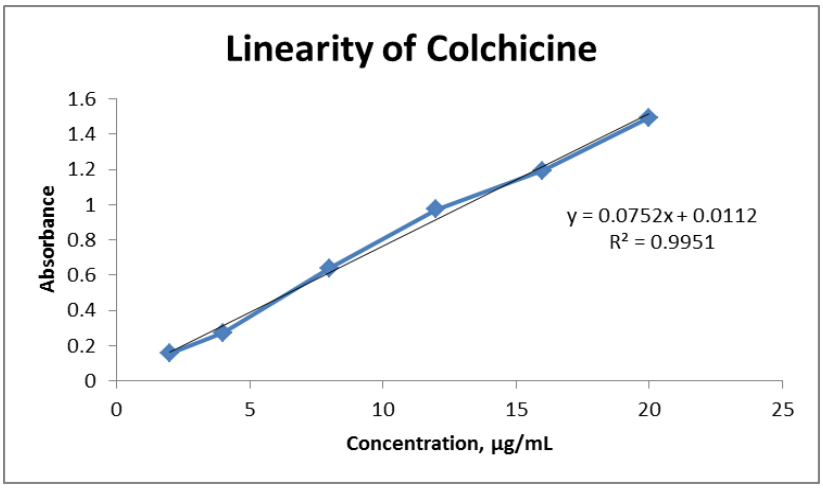

Fig. 3: Calibration curve of colchicine at 246 nm.

Table 1: Linearity data of colchicine at 246 nm.

S.No.	Concentration (µg/mL)	Absorbance
1.	2	0.157
2.	4	0.273
3.	8	0.639
4.	12	0.974
5.	16	1.194
6.	20	1.494

Accuracy

The accuracy of the developed method was determined at three levels. The sample solutions were spiked at 50%, 100% and 150% concentration levels and the % recovery was calculated (Table 2). It was found to be in the range of 98.06 – 100.77%, proving the method to be accurate.

Table 2: Accuracy data.

S.No.	Amount of Marketed formulation (µg/ml)	Amount of API added (µg/ml)	Absorbance at 246 nm	Amount found (µg/ml)	% Recovered
1.	8	4	0.918	12	100.77
2.	8	8	1.192	16	98.41
3.	8	12	1.482	20	98.06

Precision

To assess the precision, intra-day study was conducted by measuring the absorbance of the working standard solution at concentration of 10 µg/mL at six different time points within same day. Inter-day study was conducted by measurement for six different days. The %RSD calculated was found to be 0.20% for intra-day precision and 0.91% for inter-day precision (Table-3).

Table 3: Intra-day and inter-day precision data.

S.No.	Absorbance of Intra-day precision	% RSD	Absorbance of Inter-day precision	% RSD
1.	0.848	0.197	0.828	0.909
2.	0.843		0.835	
3.	0.846		0.820	
4.	0.846		0.836	
5.	0.846		0.842	
6.	0.847		0.831	

LOD and LOQ

Limit of detection and limit of quantitation were determined from the slope and standard deviation of calibration graph. The LOD and LOQ were found to be 1.81 and 5.47 µg/mL, respectively.

Analysis of formulation

The developed UV method was used for analysis of marketed tablet dosage form (Brand name -Zycolchin). The sample solution was prepared at concentration of 10 µg/mL using

water as solvent and its absorbance was measured at 246 nm. The concentration of sample solution was back calculated from the linear equation. It was found to be 97.64%.

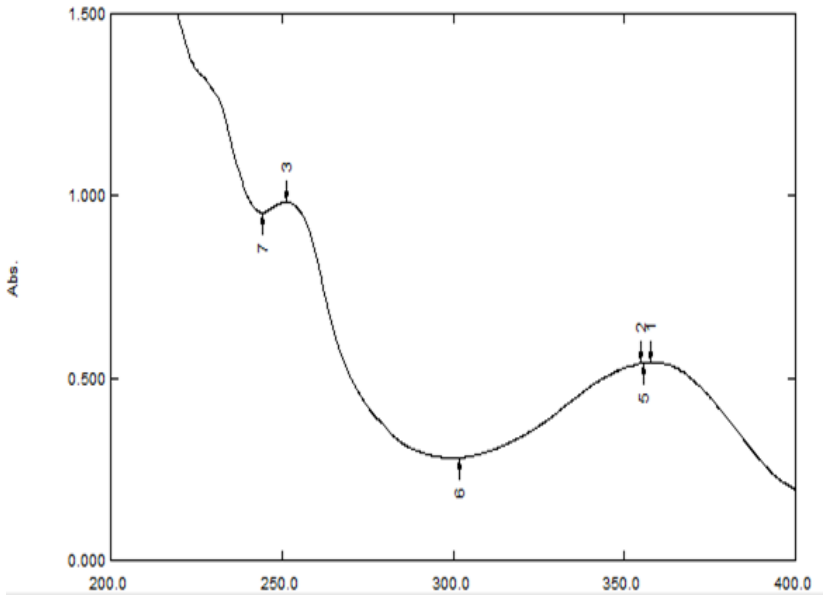

Fig. 4 UV spectra of marketed formulation.

Table 4: Analysis of marketed formulation.

Brand name	Label claim (mg)	Amount found (mg)	% assay
Zycolchin	0.5	0.493	98.60

CONCLUSION

The UV method developed for estimation of colchicine in bulk and tablet dosage forms is simple, accurate, precise and sensitive. The usage of water as solvent let it to be more economic method. The proposed method was also successfully applied for quantitative estimation of marketed tablet dosage form and hence can be used for routine analysis.

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