INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF COLCHICINE IN PHOSPHATE BUFFER SALINE pH 6.4

Arpna patial, Purnima verma*
Department of Pharmaceutical sciences, Lovely Professional University, Phagwara, India

Article Received on: 17/12/11 Revised on: 20/01/12 Approved for publication: 19/02/12

*E-mail: pharmapuruverma@gmail.com

ABSTRACT
The present investigation explores a method to analyze colchicine in phosphate buffer saline pH 6.4. Based on the spectrophotometric characteristics of colchicine, λ_{max} selected was 353.8 nm. Analytical parameters validated were linearity, accuracy, precision, LOD, LOQ and robustness. The developed method demonstrated exemplary linearity in phosphate buffer saline pH6.4 (R^2 = 0.995) for concentration of 6-22 µg/ml with linear equation of y=0.037x+0.005. LOD and LOQ obtained were found to be i.e. 0.145µg/ml and 0.483µg/ml respectively. All validation parameters were observed to be within acceptable range, as recommended by ICH guidelines. The above results confirmed the validity of proposed method for routine estimation of colchicine in phosphate buffer saline pH6.4.

Keywords- UV spectrophotometer, colchicine, Phosphate buffer saline pH 6.4, λ_{max}, ICH guidelines.

INTRODUCTION

Colchicine is an alkaloid, found in the corm and seeds of various species of *colchicum.* Chemically colchicine is, *(S)-N*-(5,6,7,9-tetrahydro-1,2,3,10-tetra-methoxy-9-oxobenzo[a]heptalen-7-yl)acetamide. It is a pale yellow powder with molecular weight of 399.44[1]. Colchicine is a well known ancient remedy for gout[2]. Colchicine is a safe and effective adjuvant to other drugs such as corticosteroids and immunosuppressive drugs for prevention and treatment of recurrent pericarditis[3]. It has also been demonstrated for the treatment of certain dermatological conditions including psoriasis[4], actinic keratoses[5], urticarial vasculitis[6], Behçet's syndrome[7], sweet's syndrome[8], scleroderma[9], amyloidosis[10], cystic acne[11] and erythma nodosum leprosum[12]. Oral administration of colchicine is associated with serious dose dependent gastrointestinal side effects and its accumulation in the body leads to bone marrow suppression[13]. Topical mode of delivery may help avoid typical side effects associated with colchicine[14]. For routine characterization and quality verification of topical dosage form, some specification test such as *in vitro* diffusion study and *in vitro* skin permeation studies are highly recommended to ensure efficacy of topical dosage form[15]. This has necessitated a suitable validated analytical technique for estimation of drug in phosphate buffer saline pH 6.4. UV spectrophotometric analysis is the most common, simple, economical and rapid technique. Moreover, colchicine is an UV active compound. To the best our knowledge no report has yet been published described by the validation of UV method for assay of colchicine in phosphate buffer saline (pH 6.4). Therefore, an attempt was made to develop validation of UV spectrophotometric method for quantification of colchicine taking phosphate buffer saline (pH 6.4) as solvent.

MATERIALS AND METHODS

Materials

All the chemicals were of analytical grade. Pure Colchicine was procured as a gift sample from Alexa biotech Pvt. Ltd., Baddi, India.

Equipment

A Systronic UV- visible spectrophotometer 2202, with 1 cm matched quartz cells were used for the measurement of absorbance. All the glass wares were of analytical grade.

Method validation [16]

Analytical method validation has become an essential part of drug development and characterization. Validation of analytical method involves experimental design to confirm that it is suitable for its intended purpose. The validation tells how good the method is; specifically whether it is good enough for its intended application. The proposed method was validated by determination of following parameters: linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

Selection of λ_{max}

Stock solution of colchicine (1mg/ml) was prepared in phosphate buffer saline (pH 6.4) and sufficiently diluted to get standard solutions. The resultant standard solutions were scanned at wavelength ranging from 200-400 nm. UV scan displayed peaks at two different wavelengths i.e. 245.8 nm and 353.8 nm. Absorbance were noted down and plotted against concentration in concentration range of 2-12 µg/ml and 6-22 µg/ml at 245.8 nm and 353.8 nm respectively and least square regression analysis was carried out. The wavelength exhibited highest R^2 value was selected as λ_{max} for further analysis.

Linearity

Linearity was ascertained by performing five replicated analysis on five independent concentration levels in the range of 6-22 µg/ml. Stock solution of colchicine (1mg/ml) was prepared in phosphate buffer saline (pH 6.4) and dilutions were made to get standard solutions. Absorbance of resultant solutions was observed by UV spectrophotometer at 353.8 nm, plotted against concentration and was treated by linear regression analysis.

Accuracy and precision

Accuracy of the experiment was studied by using recovery studies. To assess precision, intra-day (repeatability) and inter-day (intermediate precision) studies were conducted. Standard samples were prepared in triplicate at three different concentration levels (6, 14 and 22 µg/ml) covering absolute

linear range and results were expressed in terms of % RSD. Intraday precision determined by analyzing standard samples at three different time points of the same day while interday precision was determined by analyzing at three different time points on three different time days.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were estimated by using standard deviation of the response and the slope of the calibration curve. Equations used for calculation are as follows:

$$LOD=3.3\sigma/S,$$
$$LOQ=10\sigma/S$$

Where, σ is the standard deviation of y-intercepts of regression lines and S is the slope of the related calibrations graph.

Robustness

Robustness was established by calculating % RSD for concentration 14µg/ml at three different pH values (pH 6.2, 6.4, 6.6) in triplicate.

RESULTS & DISCUSSION

The proposed method was intended for quantification of colchicine for quality control purposes. Optimum conditions for analysis were investigated. The UV scan of colchicine between 200-400 nm showed the absorption maxima at 245.8 nm and 353.8 nm, as shown in figure 2. Lambert Beer's law was obeyed in the concentration range of 2-12 µg/ml and 6-22 µg/ml for 245.8 nm and 353.8 nm respectively. However the value of regression coefficient was found to be higher at 353.8 nm (R^2 = 0.995), which was selected as best wavelength (λ_{max}) for further measurements. Linearity was established in the range of 6-22 µg/ml with correlation coefficient, R^2 = 0.995. Absorbance range was found to be 0.216-0.819 at 353.8nm. The representative linear equation was y = 0.037x+0.005. Molar absorptivity was estimated as 1.2384×10^4 l/mol/cm. The optical characteristic parameters and overlain spectra of colchicine are shown in table 2 and figure 2 respectively. The accuracy of proposed methods was further accessed via recovery studies. The mean % recovery obtained was 97.66-99.75% which demonstrated the good index of accuracy for the method being validated. The interday and intraday % RSD values obtained were found to be less than 2%, proving that the method was precise and reproducible. The values for LOD and LOQ were found to be in submicrogram level i.e. 0.145µg/ml and 0.483µg/ml respectively, indicating the sensitivity of method. Low % RSD values (less than 2) obtained for robustness test suggested the method was well robust. Being very simple and reliable, the method can be recommended for routine quality control analysis of colchicine in topical dosage form.

CONCLUSION

All of the aforementioned results clearly lead to the conclusion that the described method is simple, sensitive, accurate, precise, robust and cost effective. The proposed method can be applied successfully for quantification of colchicines in PBS pH 6.4 for routine analysis in quality control laboratory.

ACKNOWLEDGMENT

Authors of the present study are thankful to Alexabiotech Pvt. Ltd, Baddi, India for providing gift sample of colchicine.

REFERENCES

1.Indian Pharmacopoeia, Vol. 2, Govt. of India, Ministry of Health and Family Welfare, Ghaziabad, New Delhi; Indian Pharmacopoeia Commission: 2007. p.964.
2.Richette P, Bardin T. Colchicine for the treatment of gout. Expert opin pharmacother 2010; 11(17):2933-8.
3. Markel G, Imazio M, Brucato A & Adler Y. Colchicine for the Prevention of Recurrent Pericarditis. The Israel medical association journal 2008; 10: 69-72.
4. Kaidbey KH, Petrozzi JW & Kligman AM. Topical colchicine therapy for recalcitrant psoriasis. Arch Dermatol 1975; 111:33-6.
5. Grimaître M, Etienne A, Fathi M, Piletta PA, Saurat JH. Topical colchicine therapy for actinic keratoses. Dermatology 2000; 200:346-8.
6. Wiles JC, Hansen RC & Lynch PJ. Urticarial vasculitis treated with colchicine. Arch Dermatol 1985; 121:802-5.
7. Sander HM & Randle HW. Use of colchicine in Behçet's syndrome. Cutis 1986; 37(5): 344-8
8. Suehisa S & Tagami H. Treatment of acute febrile neutrophilic dermatosis (Sweet's syndrome) with colchicines. Br J Dermatol 1981; 105: 483.
9. Alarcon-Segovia D, Ramos-Niembro F, Ibanez de Kasep G, Alcocer J & Tamayo RPJ. Long term evaluation of colchicines in the treatment of scleroderma. J Rheumatol 1979; 6(6): 705-12
10. Chakravarty K, Chanda M. Role of colchicine in primary localised cutaneous amyloidosis. Indian J Dermatol Venereol Leprol 1995; 61:268-9.
11.Jeong S, Lee CW. Acne conglobata: Treatment with isotretinoin, colchicine, and cyclosporin as compared with surgical intervention. Clin Exp Dermatol 1996; 21:462-3.
12. Sarojini PA & Mshana RN. Use of colchicine in the management of erythema nodosum leprosum. Lepr Rev 1983; 54:151-3.
13. Hood RL. Colchicine poisoning. J Emerg Med 1994; 12 (2): 171-77
14 Singh H P, Utreja P, Tiwary A K & Jain S. Elastic Liposomal Formulation for Sustained Delivery of Colchicine: In Vitro Characterization and In Vivo Evaluation of Anti-gout Activity. The AAPS Journal 2009; 11(1):54-64.
15.Paolino D, Cosco D, Muzzalupo R, Trapasso E, Picci N & Fresta M. Innovative bola-surfactant niosomes as topical delivery systems of 5-fluorouracil for the treatment of skin cancer. International Journal of Pharmaceutics 2008; 353:233–242.
16. Anonymous, ICH guidelines. Validation of analytical procedures: text and methodology. Q2 (R1) 2005.

TABLE 1. CALIBRATION DATA OF COLCHICINE IN PHOSPHATE BUFFER SALINE (pH 6.4) AT 353.8

Concentration (µg/ml)	Absorbance*
6	0.216±0.001
10	0.393±0.0005
14	0.545±0.001
18	0.698±0.0005
22	0.819±0.0005

*Each value represents the mean ± SD where, n=5

TABLE 2. OPTICAL CHARACTERISTIC PARAMETERS FOR COLCHICINE IN PHOSPHATE BUFFER SALINE (pH 6.4)

Parameter	Results
Selected λ_{max} (nm)	353.8
Linearity Range (µg/ml)	6-22
Standard Regression Equation	y = 0.037x + 0.005
Correlation Coefficient (R^2)	0.995
Molar absorptivity (l/mol/cm)	1.2384×10^4
Sandell's Sensitivity (µg/ml /cm^2)	0.03
LOD (µg/ml)	0.145
LOQ (µg/ml)	0.483

TABLE 3. RESULTS OF ACCURACY OF COLCHICINE

Concentration of colchicine taken (µg/ml)	Concentration of colchicine observed (µg/ml)*	% Mean Recovery	% RSD
6	5.859 ± 0.040	97.66	0.686
14	13.964 ± 0.040	99.75	0.287
22	21.526 ± 0.026	97.85	0.122

*Each value represents the mean ± SD where, n=3.

TABLE 4. RESULTS OF PRECISION OF COLCHICINE

Concentration of colchicine taken (µg/ml)	Concentration found (µg/ml)*	% Mean Recovery	% RSD
Interday (n=27)			
6	5.964 ± 0.040	99.145	0.671
14	14.026 ± 0.078	100.187	0.556
22	21.561 ± 0.015	98.006	0.070
Intraday (n=9)			
6	5.982 ± 0.080	99.707	1.337
14	14.078 ± 0.026	100.563	0.185
22	21.552 ± 0.054	98.165	0.251

*Each value represents the mean ± SD where, n=3.

TABLE 5. RESULTS OF ROBUSTNESS

Conc. taken (µg/ml)	Solvent (Phosphate buffer saline)	Concentration found (µg/ml)*	% (RSD)
14	pH 6.2	14.076 ± 0.074	0.526
14	pH 6.4	14.054 ± 0.068	0.484
14	pH 6.6	14.42 ± 0.025	0.173

*Each value represents the mean ± SD where, n=3.

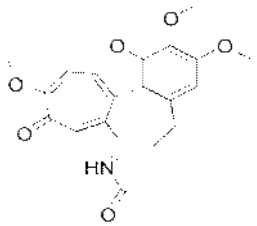

Figure 1. Structure of colchicine

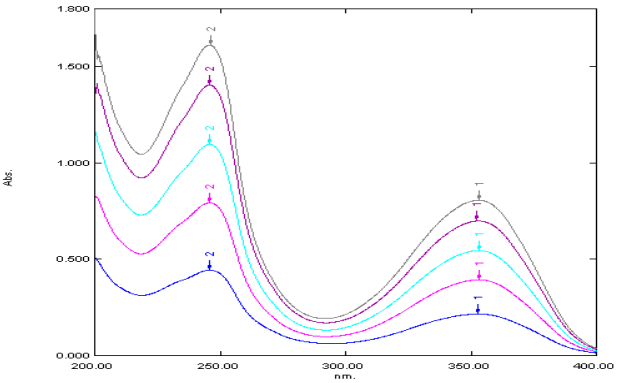

Figure 2. Overlain UV spectra of colchicine in PBS (pH 6.4) in concentration range of 6-22 µg/ml at 353.8 nm

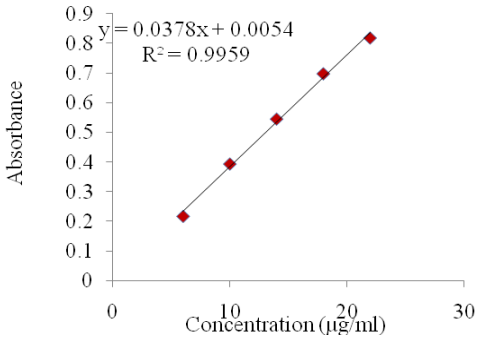

Figure 3. Standard plot of colchicine in PBS (pH 6.4) at 353.8 nm

Source of support: Nil, Conflict of interest: None Declared