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Original Article

A SIMPLE (HPLC–UV) METHOD FOR THE QUANTIFICATION OF COLCHICINE IN BULK AND ETHOSOMAL GEL NANO-FORMULATION AND ITS VALIDATION

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ABSTRACT

Objective: To develop and validate a stability-indicating reversed phase high-performance liquid chromatography (RP-HPLC) method for the determination of colchicine in bulk and ethosomal gel nano-formulation.

Methods: The chromatographic conditions were optimized using stainless steel Hypersil Gold C-18 analytical column with the dimensions of 250 mm x 4.6 mm ID x 5 µm. The mobile phase consisted of acetonitrile and ammonium acetate buffer (20 mmol/l, pH=4.85) in the ratio of 32:68 v/v. The flow rate was set at 1 ml/min and the detection wavelength was 353 nm. The column was maintained at 30 °C and the injection volume was 10 µl. The stability of colchicine in different conditions was investigated by exposing the drug to stress degradation using acid, base, oxidation, heat and light.

Results: There was no interference from excipients, impurities, dissolution media or degradation products at the retention time of colchicine 5.9 min indicating the specificity of the method. The limit of detection (LOD) and the limit of quantification (LOQ) were 8.64 ng/ml and 26.17 ng/ml respectively. The drug showed good stability under heat, acid, oxidation and light, but substantial degradation was observed under alkali condition**.** The procedure was validated for specificity, linearity, accuracy and precision.

Conclusion: A simple, rapid, specific and stability-indicating HPLC–UV method for the determination of colchicine in the pure and ethosomal gel was successfully developed. The developed method was statistically confirmed to be accurate, precise, and reproducible.

Keywords: Colchicine, Ethosomal gel, HPLC-UV, Specificity

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INTRODUCTION

Colchicine (N-[(7S)-1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetra-hydrobenzo[a]heptalen-7-yl]acetamide) has a molecular weight of 399.437, and the molecular formula $C_{22}H_{25}NO_6$ is shown in fig 1 [1]. It is a potent alkaloid obtained from the dried corns and seeds of plants of the genus *Colchicum* which belong to Liliaceae family. Among this family, the commonly used plants are *Colchicum autumnale* "meadow saffron" and *Colchicum autumn crocus* [2-4].

Colchicine has been used for the treatment of acute gout since more than 2000 y ago. It was also used to treat pseudogout and familial Mediterranean fever for several decades. It is highly effective in the treatment of acute gout, especially when given in the first 12–36 h of the gouty attack [3]. However, colchicine for the treatment of acute gout was only approved by the United States food and drug administration (FDA) in 2009, although colchicine tablets have been prescribed in the United States since the 19th century [5].

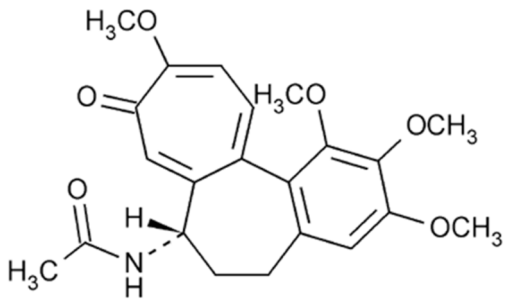

Fig. 1: The chemical structure of colchicine [1]

Ethosomal nanocarriers are lipid based vesicular systems, especially designed for the delivery of therapeutic agents dermally/ transdermally. Numerous ethosomal gels of highly diverse therapeutic agents have been developed and the interest in these formulations is increasing, and they are attracting the attention of many researchers [6-8]. Thus, the need for accurate, simple and sensitive analytical methods to quantify and evaluate the release behaviour of drugs from these formulations is also increasing.

The first few analytical methods developed for the determination of colchicine were involving colorimetric, fluorometric assay, radioimmunoassay, and thin layer chromatography-densitometry methods [9-13]. Based on the literature review, only few methods involving the high-performance liquid chromatography with UV detection are available for determination of colchicine in pharmaceutical formulations [12, 14-17]. Other HPLC-UV methods are reported to detect and quantify the drug in biological fluids and samples [14, 18-21]. Several methods using liquid chromatography coupled with mass spectrometry or tandem mass spectrometry, and gas chromatography/mass spectrometry were also reported to determine colchicine in different biological fluids [22-28], but these methods are time-consuming, complicated, and expensive in comparison to a simple HPLC-UV method. To the best of our knowledge, until now there is no stability indicating HPLC-UV method for the determination of colchicine has been developed. The aim of this study is to develop a simple, rapid, specific and stability-indicating HPLC-UV method for detection and quantification of colchicine in bulk and ethosomal gel formulation. In this work, the linearity range of the calibration curve was extended to make the method suitable for the assay, and also for the *in vitro* release studies of the drug from the prepared ethosomal gel formulation. The method was validated as per the international conference on

harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) guidelines [29].

MATERIALS AND METHODS

Chemicals and reagents

Colchicine (purity>97) was purchased from Acros organics, New Jersey, USA. Acetonitrile (HPLC grade) was purchased from J. T. Baker, Phillipsburg, USA. Ammonium acetate was bought from Bendosen laboratory chemicals, Norway.

Glacial acetic acid was obtained from QRëC, Selangor, Malaysia. Phospholipon 90G® was generously donated by Lipoid LLC, New Jersey, USA. Tween 20® was purchased from R and M chemicals, Essex, UK. Carbopol 940® was a kind gift from Lubrizol Corporation, Ohio, USA. Absolute ethanol (purity ~ 99.7) was bought from Fine Chemicals Inc., USA. Ultrapure water was prepared by Elga Purelab classic UVF water purification system, France. All other organic solvents and chemicals used were either of analytical reagent or of the high-performance liquid chromatography grade.

Instrumentation

The HPLC system was comprised of a Shimadzu (VP series, Kyoto, Japan) pump (LC-20AT VP) with solvent cabinet, a degasser (DGU-20A_3), a column oven (CTO-10S VP), an auto-injector (SIL20A HT VP), UV/VIS detector (SPD-20AD VP) and a computer software (LabSolutions® Version 5.30 SP1).

Chromatographic conditions

The chromatographic separation was performed using a Hypersil Gold C-18 analytical column with the dimensions of 250 mm x 4.6 mm ID x 5 µm, Fisher Scientific, USA. The flow rate was set at 1 ml/min and detection wavelength was 353 nm. The column was maintained at 30 °C and an injection volume of 10 µl was used. The mobile phase consisted of acetonitrile: ammonium acetate buffer (20 mmol/l, pH=4.85) (32:68v/v), then it was filtered through nylon membrane filter 0.45 µm (47 mm) and degassed before use.

Preparation of stock solution, calibration standards and quality control samples

A primary standard stock solution of colchicine (200 000 ng/ml) was prepared by dissolving 4 mg of colchicine powder in 20 ml of the mobile phase, the solution was then vortexed and sonicated for 5 min. The standard stock solution was further diluted with the mobile phase to obtain a working standard solution of 60 000 ng/ml. Solutions for the calibration were prepared by diluting the working standard solution with the mobile phase to give concentrations in the range of 40 to 8000 ng/ml. Three concentrations for the quality control (QC) were prepared at a low, medium and high concentration of 1000, 4000 and 7000 ng/ml, respectively.

Preparation of the ethosomal gel nano-formulation

The ethosomal gel was prepared using the cold method [6]. Briefly, Phospholipon 90G® and Tween 20® were taken in a small round bottom flask and solubilized with ethanol under mixing with a magnetic stirrer. Colchicine containing aqueous phase was added slowly with continuous stirring to obtain the ethosomal system formulation. The ethosomal system was further subjected to extrusion to get the nano-sized vesicles and then incorporated into Carbopol 940® gel base by manual mixing. Blank ethosomal gel formulation is identical to colchicine ethosomal gel but contains no colchicine, was prepared using the same preparation method as the colchicine ethosomal gel nano-formulation.

System suitability studies

The system suitability test was performed to verify reproducibility and the good performance of the chromate-graphic system. The chromatographic parameters, such as, theoretical plates (N), height equivalent to the theoretical plate (HETP) and tailing factor (T) were calculated as per the United States pharmacopoeia (USP) and checked at the three QC concentrations in six injection replicates.

Specificity

The possibility of interferences from mobile phase, ethosomal gel excipients and dissolution media at the retention time of colchicine was assessed by comparing the chromatograms obtained from the standard colchicine solution, mobile phase, blank ethosomal gel formulation, and the *in vitro* release dissolution media (phosphate buffer saline pH=6.4).

Stress degradation studies

Stress degradation study of the drug helps to identify the degradation products, the stability of the molecule and specificity of the analytical procedure. Stress degradation studies under the conditions of acid, base, oxidation, heat and light as per the ICH guidelines [29] were performed on four solutions, namely: colchicine in standard solution and in the ethosomal gel formulation (sample) in addition to the mobile phase and the blank ethosomal gel. Colchicine standard solution of 6000 ng/ml was prepared and used for the study. Colchicine ethosomal gel sample (0.5 g) was dispersed in 20 ml ultrapure water. Then the required volume was taken and diluted with the mobile phase to get the final concentration of 6000 ng/ml. This method was also used to prepare the blank ethosomal gel sample. The mobile phase was used as it is.

Acid and alkali degradation studies

For acid degradation study, two sets of four 10 ml flasks were filled in with 1 ml of colchicine standard, colchicine ethosomal gel, blank ethosomal gel or mobile phase, respectively. Subsequently, 1 ml of 0.5 mol/l HCl was added to all the flasks. The solutions in the first set were neutralized immediately with 1 ml of 0.5 mol/l NaOH, the mobile phase was added up to 10 ml and served as zero hour samples. The solutions in the second set were left on the bench at room temperature (25±2 °C/65 % relative humidity) for 24 h, then neutralized by the same way and the mobile phase was added up to 10 ml and served as 24 h samples. All the samples were injected in triplicate. For the alkali degradation study, a similar procedure was used, but 1 ml of 0.5 mol/l NaOH was added to each flask instead of 1 ml of 0.5 mol/l HCl and the neutralization procedure was done using 0.5 mol/l HCl.

Oxidative hydrogen peroxide (H_2O_2) degradation

Two sets of four 10 ml flasks were filled in with 1 ml of colchicine standard, colchicine ethosomal gel, blank ethosomal gel or mobile phase, respectively. Subsequently, 1 ml of 3 % H_2O_2 was added to all the flasks. The solutions in the first set were immediately added with mobile phase up to 10 ml and served as zero hour samples. The solutions in the second set were left on the bench at room temperature (25±2 °C/65 % relative humidity) for 24 h, then mobile phase was added up to 10 ml and served as 24 h samples. All the samples were injected in triplicate.

Heat degradation study

Two sets of four 10 ml flasks were filled in with 1 ml of colchicine standard, colchicine ethosomal gel, blank ethosomal gel or mobile phase, respectively. The solutions in the first set were immediately added with mobile phase up to 10 ml and served as zero hour samples. The solutions in the second set were heated in a water bath at 80 °C for 2 h, then mobile phase was added up to 10 ml and served as 2 h samples. All the samples were injected in triplicate.

Light degradation

Two sets of four 10 ml flasks were filled in with 1 ml of colchicine standard, colchicine ethosomal gel, blank ethosomal gel or mobile phase, respectively. The solutions in the first set were immediately added with the mobile phase up to 10 ml and served as zero hour samples. The solutions in the second set were stored in a UV cabinet (365 nm) for 24 h, then mobile phase was added up to 10 ml and served as 24 h samples. All samples were injected in triplicate.

Method validation

Linearity

In order for the present HPLC method to be suitable for the assay, content uniformity and *in vitro* release tests, the range of linearity

was extended to be in the range of around 0.5 to 130 % of the target concentration of the drug (6000 ng/ml). Accordingly, the calibration curve was prepared using six solutions of colchicine at concentrations of 40, 100, 500, 2000, 5000 and 8000 ng/ml. Each solution was injected in triplicate. The standard calibration curves for concentration versus peak area were constructed. The homoscedasticity assumption of the linear regression was tested by performing the (F-test) and the linearity was evaluated by weighted least squares regression analysis [30].

Precision and accuracy

Three levels of quality control (QC) samples of colchicine namely low, medium and high concentrations of 1000, 4000 and 7000 ng/ml were prepared from the stock solution and used to determine the method precision and accuracy. For intra-day precision and accuracy, three sets of standard solutions were injected on the same day. For inter-day precision and accuracy, three sets of standard solutions were injected over six consecutive days. Precision is defined as the percentage of the relative standard deviation (%RSD) and calculated by dividing the standard deviation of the calculated concentration by the mean value. While accuracy was presented as the relative percentage of error (%RE) and calculated by dividing the difference between calculated and nominal concentrations of the standard solution with the nominal concentration of the standard solution.

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

The LOD is the lowest amount of analyte that can be detected in a sample, whereas the LOQ is the lowest amount of the analyte that can be quantified in a sample with acceptable accuracy and precision [31]. These were determined by serial dilutions of colchicine stock solutions in order to obtain a signal to noise (S/N) ratio of at least 3.3:1 for LOD and 10:1 for LOQ.

Robustness

The robustness of the developed method was evaluated by deliberately making slight changes in the optimized value of the chromatographic conditions. The evaluated variables were namely: detector wavelength (±2 nm), pH (±0.2), the composition of the mobile phase (±1 v/v), flow rate (±0.1 ml/min) and oven temperature (±2 °C). Colchicine concentration of 4000 ng/ml was used to perform the study. Colchicine peak areas and the %RSD of robustness testing under these conditions were calculated in all cases and the obtained data were analyzed statistically.

Stock solution stability

The stock solution of colchicine (200 000 ng/ml) was kept for 24 h at room temperature (25±2 °C). The stock solution was then diluted to a concentration within the standard calibration curve range of 4000 ng/ml. The responses at 24 h were compared with that of freshly prepared samples at zero hours.

Quantification of colchicine content in the ethosomal gel

Colchicine ethosomal gel (0.5 g) was dispersed in 20 ml of ultrapure water under continuous mixing with a magnetic stirrer for 60 min. The solution was then sonicated in an ultrasonic bath (Branson 5510, USA) for 10 min. A suitable aliquot was removed and diluted with the mobile phase to get the final nominal concentration of 6000 ng/ml and subjected to chromatographic analysis. The drug peak area was referred to the regression equation to get the sample concentration.

Recovery of colchicine

The blank ethosomal gel formulation (0.5 g) was dispersed in 20 ml of ultrapure water, and then 1.2 mg of colchicine (as this amount is equivalent to the amount of colchicine that should be contained in 0.5 g of the colchicine ethosomal gel) was added to the solution under continuous mixing with a magnetic stirrer. The solution was then sonicated in an ultrasonic bath for 10 min.

Then 1 ml of this solution was diluted with the mobile phase in a 10 ml volumetric flask and filtered with 0.45 µm pore size nylon filter to give a final nominal concentration of 6000 ng/ml. The sample of 10 µl was injected into the HPLC system. The drug peak area was referred to the regression equation to calculate the recovery. The analysis was done in triplicate.

Statistics

All the statistical tests of the developed method including the weighted linear regression analysis of the calibration curves, the one-way analysis of variance (ANOVA) and a post hoc Tukey's HSD (honest significant difference) were performed using Minitab® software to analyze the results of the robustness and stock solution stability studies. The difference was statistically significant when $p<0.05$ and all values were expressed as mean±SD.

RESULTS

System suitability studies

The results of retention time, tailing factor (T), theoretical plates (N), and a height equivalent to the theoretical plate (HETP), of the method at three QC concentrations, are shown in table 1. The peak retention time was about 5.9 min, the average theoretical plate was >4500 and the tailing factor was <2, which means that the method met the requirements stated in the United States pharmacopeia [1].

Table 1: Results of system suitability studies of quality control samples of colchicine

Conc. (ng/ml)	Retention time[a]	%RSD	T[a]	%RSD	N[a]	%RSD	HETP[a]	%RSD
1000	5.935±0.0008	0.01	1.057±0.0024	0.23	12591.33±24.22	0.19	19.86±0.04	0.19
4000	5.933±0.0011	0.02	1.060±0.0004	0.04	12736.67±13.94	0.11	19.63±0.02	0.11
7000	5.933±0.0016	0.03	1.063±0.0006	0.05	12776.50±32.69	0.26	19.57±0.05	0.26

[a]mean±SD, n = 6; T: Tailing factor; N: Theoretical plate number; HETP: Height equivalent to the theoretical plate.

Specificity

Fig. 2 shows the representative chromatograms of the mobile phase (A), standard solution of colchicine at a concentration of 6000 ng/ml (B), blank ethosomal gel (C), colchicine ethosomal gel at a concentration of 6000 ng/ml (D) and phosphate buffer saline pH=6.4 for the *in vitro* dissolution media (E). The chromatogram of the colchicine loaded ethosomal gel (fig. 2, D) showed a peak and retention time (5.9 min) similar to colchicine standard solution (fig. 2, B). No interference from mobile phase, blank ethosomal gel formulation (excipients) and the dissolution media were detected at the retention time of colchicine (fig. 2, A, C and E) indicating that the method is highly selective and specific in relation to the medium and excipients used in this study.

Stress degradation studies

The results of the stress degradation studies (acid, alkali, oxidation, heat and light) for the standard solution and the ethosomal gel formulation are shown in table 2. The chromatograms of the stressed standard colchicine solution and colchicine ethosomal gel formulation after 24 h under the different stress conditions are shown in fig. 3 and 4. The drug showed good stability under heat, acid, oxidation and light, but substantial degradation was observed under the alkali condition (fig. 3, B and fig. 4, B). The percentage of drug in the standard solution and ethosomal gel was reduced significantly to 1.63±0.04 and 1.48±0.02 after 24 h, respectively.

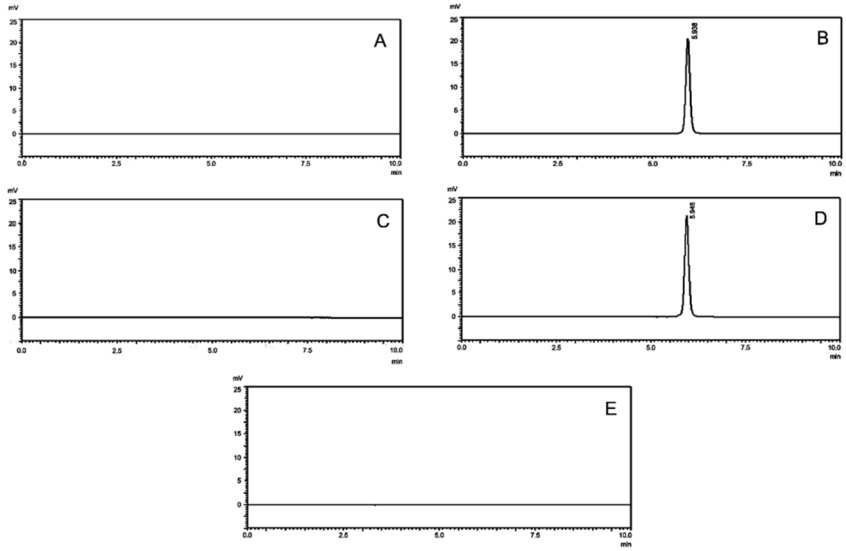

Fig. 2: Chromatograms of A: Mobile phase, B: Standard colchicine solution (6000 ng/ml), C: Blank ethosomal gel, D: Colchicine ethosomal gel (6000 ng/ml), E: Phosphate buffer saline solution

Table 2: Results of the stress degradation studies

Stress degradation studies' results for the standard colchicine solution at 0 and 24h				
Forced degradation condition	**Time (h)**	**Remaining drug (%)[a]**	**Time (h)**	**Remaining drug (%)[a]**
Acid (0.5 mol/l HCl)	0	99.29±0.27	24	97.8±0.38
Base (0.5 mol/l NaOH)	0	99.05±0.36	24	1.63±0.04
Oxidation (3 % H_2O_2)	0	99.82±0.07	24	97.98±0.14
Light (365 nm)	0	99.96±0.08	24	99.50±0.06
Heat (80 °C)	0	99.96±0.08	2	100.01±0.46
Stress degradation studies' results for the colchicine loaded ethosomal gel formulation at 0 and 24h				
Forced degradation condition	**Time (h)**	**Remaining drug (%)[a]**	**Time (h)**	**Remaining drug (%)[a]**
Acid (0.5 mol/l HCl)	0	99.55±0.24	24	96.78±0.09
Base (0.5 mol/l NaOH)	0	97.55±0.02	24	1.48±0.02
Oxidation (3 % H_2O_2)	0	98.05±0.02	24	99.84±0.02
Light (365 nm)	0	99.96±0.04	24	98.96±0.05
Heat (80 °C)	0	99.96±0.04	2	99.77±0.07

[a]mean±SD, n = 3.

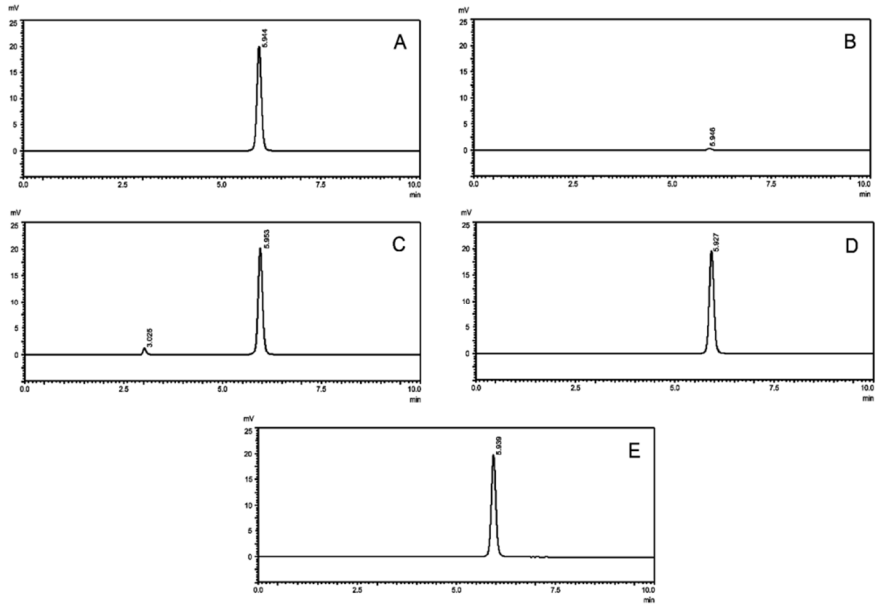

Fig. 3: Chromatograms of stressed standard colchicine solution under A: Acid (0.5 mol/l HCl after 24 h), B: Base (0.5 mol/l NaOH after 24 h), C: Oxidation (3 % H_2O_2 after 24 h), D: Light (365 nm after 24 h), E: Heat (80 °C after 2 h)

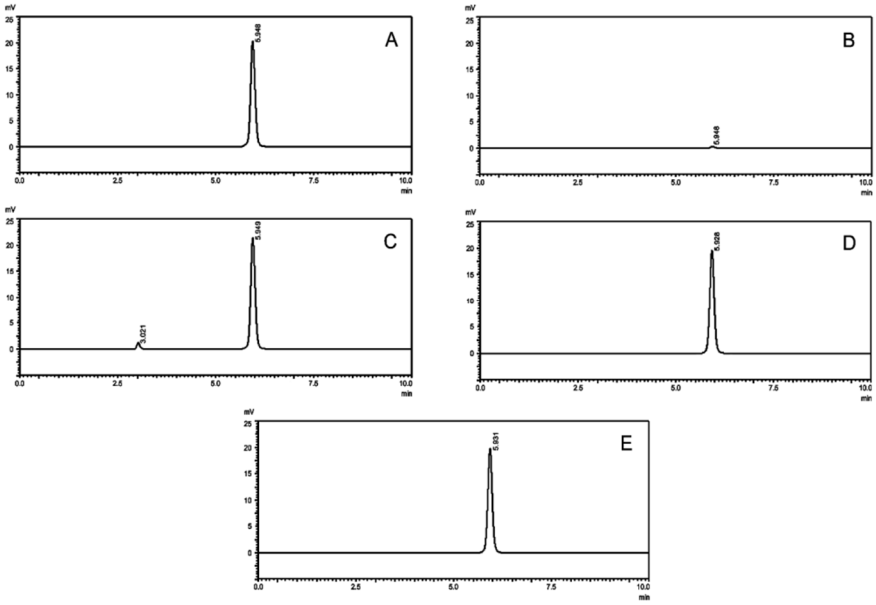

Fig. 4: Chromatograms of the stressed colchicine ethosomal gel samples under A: Acid (0.5 mol/l HCl after 24 h), B: Base (0.5 mol/l NaOH after 24 h), C: Oxidation: (3 % H_2O_2 after 24 h), D: Light (365 nm after 24 h), E: Heat (80 °C after 2 h)

Linearity

The results of the variance test (F-test) confirmed the presence of hetero scedasticity (i.e. F (experimental)>F (tabulated)). So weighted linear regression was applied using the following weighting factors (w_i) 1, 1/x, 1/x^2, 1/$x^{1/2}$, 1/y, 1/y^2 and 1/$y^{1/2}$. The best regression model and weighting factor were selected by comparing the sum of absolute percentage relative error (%RE) values of all models and the results are shown in table 3. A regression model with a weighting factor of 1/x^2 was chosen as it showed the least sum of the %RE across the whole concentration range. The linear regression equation was y= 27.093x+53.1 with a correlation coefficient of 0.9998.

Table 3: Weighted least squares regression analysis

w_i	1	1/x	1/x^2	1/$x^{1/2}$	1/y	1/y^2	1/$y^{1/2}$
a[a]±SD	27.612±0.571	27.656±0.566	27.591±0.653	27.639±0.565	27.655±0.566	27.583±0.657	27.639±0.565
b[a]±SD	138.666±284.682	24.3±11.227	34.713±23.707	41.333±56.922	22.933±10.005	33.063±22.613	40.666±56.887
Mean R^2	0.9999	0.9999	0.9995	0.9999	0.9999	0.9995	0.9999
∑%RE	115.846	22.561	19.814	23.450	22.813	20.302	30.698

[a]mean±SD, n=3; w_i: Weighting factor; a: Slope of regression line; b: Intercept of regression line; R^2: Correlation coefficient; RE: Relative error.

Precision and accuracy

The obtained results of the Intra-day and inter-day precision and accuracy are shown in table 4. The intra-day precision for colchicine ranged between 0.04 % and 0.15 %, while the accuracy was between -0.04 % and 0.39 %. The inter-day precision ranged between 0.14 % and 0.24 %, while the accuracy was between 0.09 % and 0.39 %. The data showed a good degree of precision and accuracy of the method and within the ±2 % range recommended by the United States pharmacopeia [1].

Table 4: Results of intra-day and inter-day precision and accuracy

Theoretical conc. (ng/ml)	Intra-day			Inter-day		
	Found conc. (ng/ml)[a]	Precision (%RSD)	Accuracy (%RE)	Found conc. (ng/ml)[a]	Precision (%RSD)	Accuracy (%RE)
1000	1003.89±0.42	0.04	0.39	1000.94±2.37	0.24	0.09
4000	3998.43±2.96	0.07	-0.04	4004.92±5.77	0.14	0.12
7000	7007.64±10.53	0.15	0.11	7027.68±15.32	0.22	0.39

[a]mean±SD, n = 6.

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

The LOD and LOQ of colchicine in the present method were found to be 8.64 ng/ml and 26.17 ng/ml, respectively.

Robustness

The results of robustness study are presented in table 5. The statistical tests showed that the variations in the pH of the mobile phase and oven temperature did not significantly affect the peak area or the retention time.

On the other hand, the variation in the wavelength of the detection, and flow rate caused significant variations in the peak area and retention time, while the variation in mobile phase composition of acetonitrile did not influence the peak area, but caused significant shifting in the retention time.

Table 5: Analysis of method robustness using colchicine at a concentration of 4000 ng/ml

Parameters	Changes	Mean area[a]±SD	%RSD	Mean retention time (min)[a]±SD	%RSD
Detector wavelength (nm)	355	107895.33±64.34	0.06	5.933±0.0008	0.01
	353	108523±80.05	0.07	5.940±0.0026	0.04
	351	108497±78.26	0.07	5.931±0.0005	0.01
pH of the mobile phase	5.05	108640.33±141.8	0.13	5.934±0.0062	0.11
	4.85	108436.33±83.65	0.08	5.934±0.0012	0.02
	4.65	108639.33±70.87	0.07	5.929±0.0062	0.1
Mobile phase composition (v/v)	(33:67)	108512.67±92.96	0.09	5.599±0.0022	0.04
	(32:68)	108311.33±19.74	0.02	5.935±0.0017	0.03
	(31:69)	108365.33±173.1	0.16	6.332±0.0005	0.01
Flow rate (ml/min)	1.1	99104.33±71.34	0.07	5.427±0.0016	0.03
	1	108412.33±130.9	0.12	5.932±0.0008	0.01
	0.9	120712.33±135.5	0.11	6.555±0.0017	0.03
Temperature (^{0}C)	32	108508.33±72.37	0.07	5.933±0.0009	0.02
	30	108523±83.95	0.08	5.933±0.0026	0.04
	28	108573.67±109.5	0.1	5.929±0.0024	0.04

[a]mean±SD, n=3.

Stock solution stability

The results showed that the percentage of colchicine remained after 24 h kept at room temperature (25±2 °C) was 100.3±0.2 %. There was no statistical difference in peak area between the samples measured at zero time and after 24 h at (25±2 °C), indicating that the stock solution was stable for at least 24 h at room temperature.

Quantification of colchicine content in the ethosomal gel

Colchicine content in the ethosomal gel formulation was found to be 99.86 %.

Recovery studies of colchicine in the ethosomal gel

The experiment was conducted to determine the accuracy of the present method for the quantification of colchicine sample. The recovery of colchicine was calculated from the slope and the intercept of the calibration curve drawn in the concentration range of 40–8000 ng/ml. The percentage recovery of colchicine ranged between 99.54 % and 99.76 % in the ethosomal gel formulation sample.

DISCUSSION

In the development of an HPLC method, the separation of analyte should be optimized at a pH where the retention time of analyte is the least affected by pH changes. Accordingly, the buffer pH should be selected at least±1 pH units from the pK_a of the analyte. This is to ensure that the analyte is 100% ionized or 100% non-ionized at the selected pH for obtaining reproducible retention time of the analyte. Since the pK_a of colchicine is 1.85, thus in the early stages of method development, the pH value of 3.85 was studied. However, this pH is highly acidic and may damage certain HPLC columns that cannot tolerate such a low pH value. Therefore, a higher pH value was tried (4.85), and found that there was no significant difference in peak area of both tested pH values (i.e. 3.85 and 4.85). Accordingly, the pH value of 4.85 was selected as the optimized pH value of the method.

The solubility of the salt used in mobile phase composition should be considered in developing an HPLC method, especially if acetonitrile (the commonly used organic solvent) is present in the mobile phase. In comparison to other studies [16-18, 20], ammonium acetate buffer was selected in this study as it is more soluble in acetonitrile than phosphate buffers. Phosphate buffers may precipitate when mixed with acetonitrile, and this will affect the function of the column and the HPLC device.

Different oven temperatures of 25, 30, 35 and 40 °C were also studied during the optimization of the method. The temperature of 30 °C was found to be the optimum oven temperature as good separation, peak shape and area were obtained.

Several HPLC-UV methods for the quantification of colchicine in pharmaceuticals or biological fluids have been published [12, 14-21]. However, to the best of our knowledge, this is the first developed stability indicating HPLC-UV method for detection and quantification of colchicine. The results of the stress degradation studies confirmed the high specificity of the method for colchicine and revealed that colchicine is highly susceptible to alkali degradation and this might be attributed to the acidic nature of the drug (pK_a = 1.85).

The extended linearity range (0.5 to 130 %) made the method suitable for the assay, content uniformity and *in vitro* release tests. The LOD (8.64 ng/ml) and LOQ (26.17 ng/ml) obtained in the present study were the lowest values among the previously published methods for the quantification of colchicine in pharmaceutical formulations [12, 14-17]. The intra and inter-day accuracy and precision were <1 %, reflecting the high accuracy of the protocol, and that it is meeting the United States pharmacopeia recommendations [1].

CONCLUSION

A sensitive, simple, specific and stability indicating HPLC–UV method for determination of colchicine in bulk and ethosomal gel nano-formulation was successfully developed. The Statistical analysis confirmed that the method was accurate, precise, and reproducible. No peaks interferences with colchicine by the formulation excipients, diluents, dissolution media or degradation products were detected. Colchicine was found to be stable against acid, oxidation, heat and light, but it is highly susceptible to alkali degradation. The method could be used for the routine assay, content uniformity, and the *in vitro* release studies of colchicine from the ethosomal gel nano-formulation.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

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