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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF COLCHICINE IN GELATIN NANOPARTICLES FORMULATION

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of colchicine in gelatin nanoparticles formulation. A new validation and quantity evaluation method has been established by a defined high performance liquid chromatography by using buffer (pH 5.2) and methanol (62:38%v/v) as a mobile phase pumped through Zorbax RX C8 (250 mm x 4.6mm, 5µm). The flow rate was 1.0 ml/min and effluents were monitored at 254 nm. The retention time was 8.7 min and injection volume set at 20µl. The column temp was kept at 30°c. The linear regression analysis data for calibration curve showed a good relationship with correlation coefficient of 0.9999. The concentration range was 1.2 – 7.2 µg/ml. The limit of detection and limit of quantification were 0.085 µg/ml and 0.38 µg/ml respectively. The percentage recovery of colchicine was found to be 99.6%. The method is precise for relative standard deviation of 1.23%. The developed method has been validated for accuracy, precision, limit of detection, limit of quantification and linearity as per ICH guidelines. This selective method is found to be accurate, precise, repeatability and effectively used for the colchicine in nanoparticles formulation with better chromatographic conditions.

Keywords: Colchicine, HPLC, Gelatin, Nanoparticles formulation.

INTRODUCTION

The molecular formula of colchicine $C_{22}H_{25}NO_6$ with IUPAC name N-[(7*S*)-1, 2, 3, 10-tetramethoxy-9-oxo 5, 6, 7, 9-tetrahydrobenzo[*a*]heptalen-7-yl] acetamide and molecular weight is 399.44 gm/mol. Structure of colchicine is shown in Fig. 1. Therapeutic indications are acute gouty arthritis, familial mediterranen fever (FMF) and amyloidosis [1]. Colchicine (COLC) is a naturally occurring alkaloid and it is used in human and veterinary medicine. It has been used as an antimitotic agent in cancer research and involving in cell cultures [2]. COLC is used for alleviation of inflammatory process and also for reducing pain and gouts [3-5]. Colchicine has two important biological characteristics:(a) a highly specific association with microtubule proteins [6], and (b) remarkable effect on several basic cell functions including mitosis [7], secretion [8], cell morphology [9], motility [10], micro tubule assembly [11-12], and mitogenic activation [13]. Colchicine can be assayed by HPLC [14-16], or radioimmunoassay [17-18]. The tubulin-colchicine complex can be detected by a fluorometric assay [19]. The aim of the study was to develop a simple, precise and accurate RP-HPLC method for the estimation of colchicine in colchicine loaded gelatin nanoparticles [20-21].

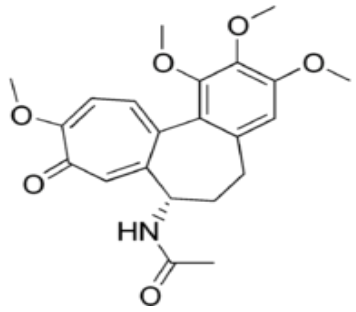


Fig. 1: Structure of colchicine

MATERIALS AND METHODS

Reagents and chemicals

Colchicine with 99.8% purity was obtained as a gift sample from Cadila Healthcare Limited. Colchicine loaded pegylated gelatin nanoparticles were prepared by ethanol precipitation method. Potassium dihydrogen phosphate Methanol, o-phosphoric acid, acetic acid (Merck chemicals) and PVDF filters were used for study.

Apparatus and chromatographic conditions

HPLC method development and validation was done on a Shimadzu (Columbia, MD) RP-HPLC instrument (LC-10AT VP) equipped with a SPD-10 AVP UV detector, injector with 20 µL loop and Class- VP software was used. Stationary Phase was Zorbax RX (Agilend technologies) C8 column (250mm × 4.6 mm ID, 5 µm particle size) and the mobile phase was buffer: methanol (62: 38%v/v; pH adjusted to 5.2 ± 0.05 with phosphoric acid). The buffer solution contains 0.05 M potassium dihydrogen phosphate. The mobile phase was filtered through nylon 0.45 µm membrane filters (Millipore Pvt., Ltd, and Bangalore, India). Flow rate was 1.0 ml/min and injection volume was 20 µL. All weighing were done on analytical balance (Metlor Toledo, United states).

Preparation of mobile phase

The mobile phase was prepared by mixing 620.0 mL buffer and 320 mL methanol; pH was adjusted to 5.2 with phosphoric acid. The mobile phase was degassed for 15 minutes and filtered through 0.45 µm membrane filter before use.

Preparation of diluent

The diluent was prepared by mixing 40 ml water and 60 ml methanol

Preparation of Standard Solutions

Accurately weighed quantity of colchicine standard (30.0 mg) was transferred to a 250 ml volumetric flask and 150 ml methanol was added into it. The resultant solution was sonicated to dissolve. The final volume was made upto the mark with water to obtain a standard stock solution (0.12 mg/ml). An aliquot (5.0 ml) was diluted to 100.0 ml with diluent to obtain a working standard solution of colchicine (6 µg/ml). All the glassware was used of amber colored.

Preparation of Sample Solutions

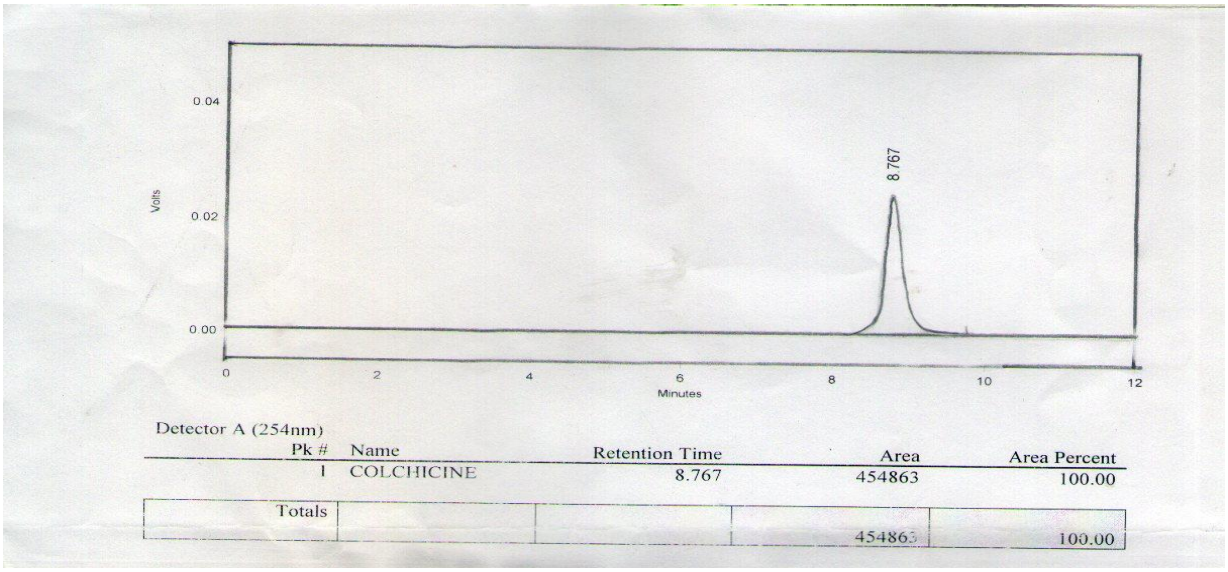
Lyophilized power of PEGylated colchicine nanoparticles was taken (46.3 mg lyophilized powder contains 2 mg colchicine). The accurately weighed powder equivalent to 30 mg colchicine was transferred to 250 ml volumetric flask and 100 ml water was added. The mixture was kept for 30 min to swell the polymer with intermittent shaking. The above solution was treated with 2 ml of acetic acid and kept for 3 hrs to break the gelatin nanoparticles. The solution was sonicated for 10 min. The flask was allowed to stand at

room temperature for 15 min, and the volume was diluted to the mark with methanol. An aliquot (5.0 ml) was diluted to 100 ml with diluent to obtain a sample solution of colchicine (6 µg/ml). The solution was filtered through 0.45µm membrane filter. All the glassware was used of amber colored.

RESULTS AND DISCUSSION

HPLC method

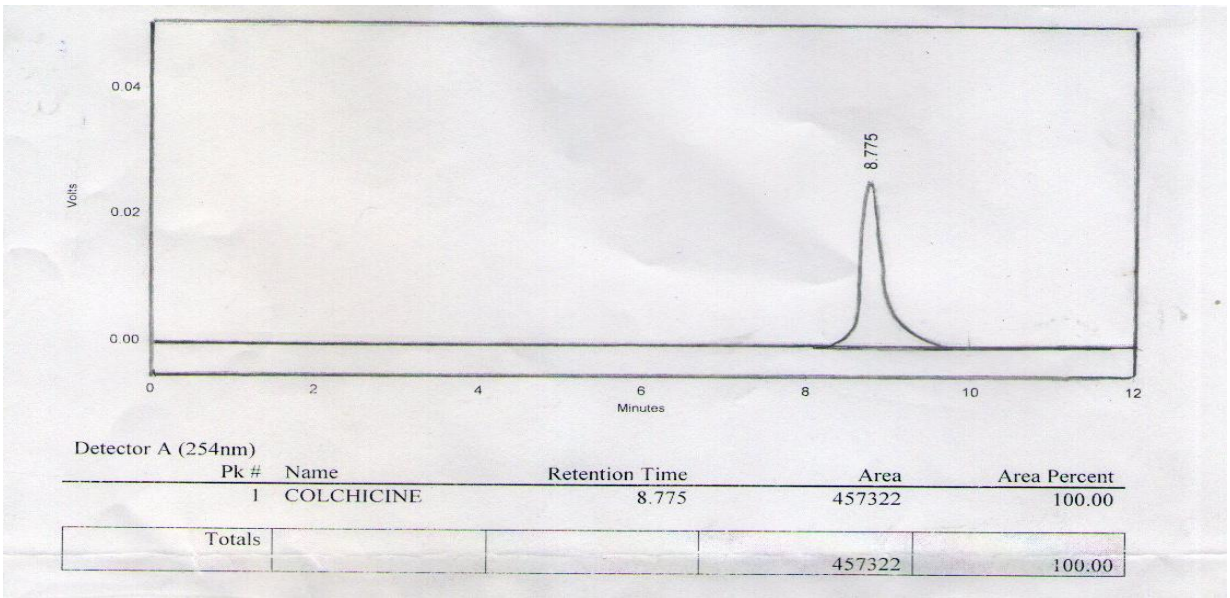
Chromatographic conditions were optimized to get best resolution and peak shape. The mobile phase was selected based on peak parameters; (theoretical plates, symmetry, tailing factor, capacity factor) cost and ease of preparation. A symmetrical peak with good separation (retention time 8.7) was obtained with C8 column and mobile phase containing buffer: methanol (62:38, v/v; pH adjusted to 5.2 ± 0.02 with phosphoric acid). The buffer solution contains 0.05M of potassium dihydrogen phosphate. The flow rate was kept 1.0 ml/min and the column temperature was kept 30°c. Chromatograms of standards and sample are given in Fig. 2 and Fig. 3 respectively. The optimum wavelength for detection and quantification was 254 nm, at which good detector response was obtained with symmetrical peak.



Detector A (254nm)

Pk #	Name	Retention Time	Area	Area Percent
1	COLCHICINE	8.767	454863	100.00
Totals			454863	100.00

Fig. 2: chromatogram of colchicine standard (6 µg/ml)



Detector A (254nm)

Pk #	Name	Retention Time	Area	Area Percent
1	COLCHICINE	8.775	457322	100.00
Totals			457322	100.00

Fig. 3: Chromatogram of colchicine formulation (6 µg/ml)

Method validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [22].

Linearity and Range

The calibration curve was plotted over the concentration range of 20%-120% of sample concentration. Aliquots from (1.0, 2.0.4.0, 5.0, 6.0 ml) standard solution were transferred into a series of 100 ml volumetric flasks and diluted to the mark with diluent. Each of this drug solution (20 µL) was injected three times under the operating chromatographic conditions as described above. Calibration curve was constructed by plotting peak areas versus concentrations of colchicine shown in Fig 4. The plot of the peak area of each sample against concentration of colchicine was found to be linear in the range of 1.2-7.2 µg/ml with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurement are

given in Table 1. The respective linear regression equation being Y= 74927 x + 10100, as shown in Fig 4.

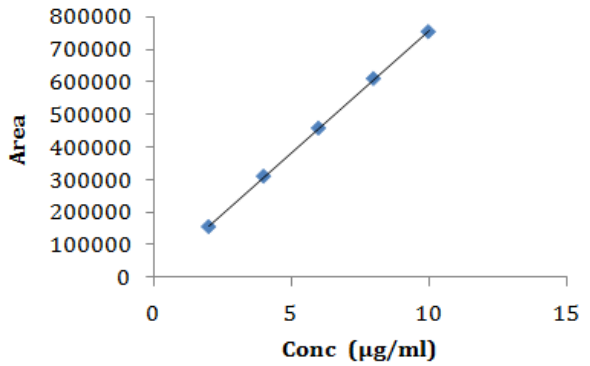


Fig. 4: Calibration curve of colchicine

Table 1: Calibration data of colchicine by RP-HPLC method

Conc (µg/ml)	Area
2	157119
4	312332
6	460126
8	612359
10	756371

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.085 µg/ml and 0.38 µg/ml respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

Accuracy

Accuracy study was performed by recovery study of colchicine. Known amount of standard was added to the placebo sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was performed at triplicate levels.

Table 2: Results of recovery study

Level	Amount taken (Placebo)	Amount taken (Colchicine)	Amount found (Colchicine)	% Recovery	% RSD
50%	138.9	3	3.05	101.67	0.23
100%	138.9	6	6.04	100.67	0.17
150%	138.9	9	8.99	99.89	0.51

Precision

The repeatability was checked by repeatedly injecting solutions of colchicine (6.0 µg/ml). The RSD value for colchicine was obtained 1.1% which indicates method is repeatable. The intraday and interday precisions of the proposed methods were determined by measuring the responses 6 times on the same day and on different days using different make system with different analyst. The % RSD for intra- and inter-day variation is given in Table 3.

Table 3: Results of Intra-day and Inter-day precision

Sample #	Intra-day precision Analyst 1 Shimadzhu make HPLC system		Inter-day precision Analyst 2 Agilend technologies	
	% Assay	% RSD	% Assay	% RSD
Set 1	99.1		101.9	
Set 2	99.6	1.1%	100.8	1.23%
Set 3	98.2		99.1	
Set 4	98.9		100.2	
Set 5	100.6		101.5	
Set 6	101.1		98.9	

System suitability

System-suitability tests are an integral part of method development. System suitability tests are used to ensure satisfactory performance of the chromatographic system. Numbers of theoretical plates (N), retention time (RT) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 6 µg/ml. The results are given in Table 4.

Table 4: System suitability test parameters

S. No.	Retention time (min)	Tailing factor	Theoretical plates
1	8.767	1.35	5877.2
2	8.765	1.34	5870.1
3	8.767	1.35	5869.9
4	8.766	1.35	5876.4
5	8.762	1.34	5874.3
6	8.766	1.35	5865.7
Mean	8.7655	1.35	5872.3
SD	0.0019	0.0052	4.45
% RSD	0.021%	0.38%	0.076%

Robustness

The robustness of proposed method was evaluated by ± 10% change in organic phase ratio, ±10% change in flow rate, ± 0.5 unit change in pH of buffer solution of mobile phase, ±5 °c change in temperature. During this study it was observed that there was no significant change in retention time, asymmetry and theoretical plates of the peak. The interference of excipients was studied by comparing the chromatogram of formulation and standard. The same shape and retention times of peaks showed that there was no interference from excipients.

CONCLUSION

The developed RP-HPLC method was simple, sensitive, precise and accurate hence can be used in routine for the determination of Colchicine in bulk and nanoparticles formulation. The interference of excipients was studied by comparing the chromatography of standards and formulations. The same shape and retention times of peaks showed that there was no interference from excipients.

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