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HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF COLCHICINE IN PHARMACEUTICAL PREPARATIONS AND BIOLOGICAL FLUIDS

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ABSTRACT: A simple and rapid high performance thin layer chromatographic (HPTLC) method was developed for the assay of colchicine in bulk drug, pharmaceutical preparations and biological fluids. The method has a linear range of 4 - 35 µg/ml. The limits of detection and quantification were 1 and 4 µg/ml respectively. The results obtained by the proposed method were compared statistically by means of Student t-test and by the F-test with those of the reported method. The two were shown in good agreement. No interference was observed from common pharmaceutical excipients.

INTRODUCTION: Colchicine is a natural toxic alkaloid of *Colchicum* genera from Liliaceae family and is found in *Colchicum autumnale*. Colchicine is obtained from the corm and seeds of the meadow saffron and other colchicum species. It is mainly used for the treatment of acute gouty and arthritis and for the prophylaxis of the acute attacks. The treatment of acute gouty arthritis is relatively well cured by the use of colchicines as anti-inflammatory agent to urate crystals.

The mechanism is not well known and may be due to several actions (inhibition of leucocytes mobility and activity) [1]. Colchicine also inhibits cellular mitosis by binding to tubulin and is used in the treatment of cancer. It has also been used for the treatment of amyloid deposition in familial Mediterranean fever and primary biliary cirrhosis [2].

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The most frequent adverse effects of oral colchicine are those involving the gastrointestinal tract and may be associated with its antimitotic action [2]. Symptoms of toxicity occur after several hours and are commonly nausea, vomiting and diarrhoea. Multiple organ failure, haemorrhage, bone marrow depression, muscle damage, respiratory distress and myocardial injury are observed after overdose. Death may be due to respiratory depression, cardiovascular collapse or bone marrow depression [2].

Consequently, standard and selective analytical protocol is a must, for the quantification of colchicine in the biological samples and pharmaceutical dosage forms for the purposes of quality control and more significantly for the clinical diagnosis. Literature survey reveals that many analytical methods are reported for determination of colchicine [3-10].

The techniques used for measurement of the selected drugs such as HPLC-MS, LC-ESI-MS and GC-MS, all require highly sophisticated instruments which are relatively expensive and hence are not always available.

On the other hand, spectrophotometric [9, 10] methods are simple and offer appropriate precision and sensitivity, but suffer from strongly interferences of drug excipients. Availability of HPTLC instrument in many laboratories and the simplicity of analytical procedures make the technique very attractive for wide range of applications. A recent literature survey reveals that there are very scanty reports of using HPTLC method for the determination of colchicine. This has encouraged the author to develop simple, rapid, reliable and inexpensive method for the colchicine in pure, biological samples and pharmaceutical dosage forms, to exploit HPTLC technique in pharmaceuticals analyses and given opportunity to analyst to use this technique for the assay of drug and in case of any state of emergency may occur to patients of misusing the drugs.

This paper describes a simple, rapid, inexpensive and sensitive validated HPTLC method for the determination of colchicine in pharmaceutical preparations and biological samples.

MATERIALS AND METHODS:

Instrument: A Camag HPTLC system equipped with Linomate IV sample applicator (Camag, Muttenz, Switzerland), TLC scanner 3 (WINCATS version 1.4.4) and UV cabinet and twin trough glass tank were used for the analysis. The plates were developed in 20x10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). Precoated silica gel aluminium plates $60F_{254}$ (E. Merck, Darmstadt, Germany) with thickness 0.2 mm were used for all determinations.

Reagents and samples: All chemicals and solvents used were of either pharmaceutical or analytical reagent grade. Colchicine was supplies gratis by Dr. Reddy's Laboratories ltd., Hyderabad. Dosage forms of colchicine, manufactured by different firms, were obtained commercially. The body fluids which were received in forensic science laboratory for toxicological analysis were used. These body fluids were said to be collected from a body of person who suspected to be died due to the consumption of plant material of *Gloriosa superba*.

Stock Solution: Colchicine standard (25 mg) was accurately weighed into a 25 ml volumetric flask, dissolved in a minimum quantity of methanol, and diluted to volume with the same solvent to furnish a solution of concentration 1000 µg/ml. This was used as stock solution; further dilutions were prepared using this stock solution.

PROCEDURE:

Assay methodology for pure drug: Aliquots of standard solution of Colchicine (4 -35µg/ml) were applied to aluminium backed silica gel $60F_{254}$ plates as 7-mm bands by means of a Camag Linomat IV applicator with Samples (10 µl) applicator. The plates were developed in a Camag twin trough chamber previously equilibrated with ethyl acetate: methanol (10: 1.4 v/v) as mobile phase for 10 min. The development distance was 8 cm. Plates were then removed from the chamber and dried in a current of air. Densitometric scanning was done at 358 nm by use of Camag TLC Scanner II with CATS3 software. The HPTLC spectrum was shown in **figure 1**.

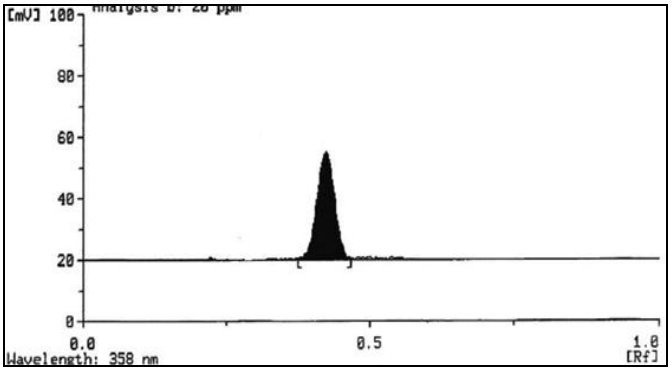

FIGURE 1: HPTLC SPECTRUM OF COLCHICINE

Assay procedure for Pharmaceutical preparations: Twenty tablets were weighed and powdered. An amount of powder equivalent to one tablet was weighed and dissolved in a minimum volume of methanol. This solution was filtered through a Whatman No. 41 paper and the filtrate was collected in a 10 ml volumetric flask and diluted to volume with methanol. This solution (2 ml) was quantitatively transferred to a 10 ml volumetric flask and diluted to volume with methanol to furnish a solution containing 20 µg/ml colchicine. An aliquot was analysed using the procedure described earlier.

Assay procedure for Biological Fluids: Biological fluids (1 ml each) were extracted with 1 ml saturated NH_4Cl and adjusted to pH 9.6 with ammonia and 5 ml of dichloromethane with 5% 2-propanol. After 10 min agitation, the tubes were centrifuged for 5 min at 3500 rpm. The organic layer was transferred into glass tubes and then evaporated to dryness at 45°C.

The residues were reconstituted in 75 µl methanol. An aliquot was analysed using the procedure described earlier.

Validation Parameters: The method was validated for linearity, accuracy, precision, Limit of detection, quantification, specificity and robustness.

Linearity: Linearity was evaluated by analysis of working standard solutions of colchicine at seven different concentrations from 4 to 35 µg/ml, prepared from the stock solution. These solutions (10 µl) were applied to plates, which were then developed and scanned as described above. Peak area was recorded for colchicine and drug concentration was subjected to regression analysis to calculate the regression equations and correlation coefficients. Linearity data and other validation data are given in **table 1**.

Recovery studies (Accuracy): Recovery experiments were conducted to check for the presence of positive or negative interferences from excipients present in the formulation and to study the accuracy of the method. Recovery was determined by the standard addition method. Colchicine standard was added to the formulation at two different concentrations and analysis was performed as described above. Recovery was calculated for each standard at each concentration. The results obtained are listed in **table 2**.

Limit of detection (LOD) and limit of quantification (LOQ): In order to estimate the LOD and LOQ, blank solution (methanol) was spotted 6 times following the same method as explained above. The signal to noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting known concentrations of reference solution until the average responses were approximately 3 or 10 times the standard deviation of the responses for 6 replicate determinations.

Specificity: The specificity of the method was ascertained by analysing standard drug and sample. The spot for colchicine in sample were confirmed by comparing R_f and spectra of spot with that of standard. The peak purity of colchicine was assessed by comparing the spectra at three different levels that is peak start, peak apex and peak end positions of the spot.

Purity of sample spot corresponding to colchicine was determined by taking the spectra and by comparing it with that of standard.

Robustness of the method: By introducing small changes in the mobile phase composition, the effects on the results were examined. The volume of mobile phase was varied in the range of ±5%. The plates were pre-washed by methanol and activated at 60±5ºC for 5, 10 and 12 min prior to chromatography.

Robustness of the method was done at three different concentration levels 0.4, 0.6 and 0.8 µg/ml. Plates were developed in varied volume of mobile phase 8, 10 and 12 ml. Time from spotting to chromatography and chromatography to scanning were also varied and % RSD was determined and found to be less than 2 %.

RESULTS AND DISCUSSION: The proposed HPTLC method for the determination of colchicine using the conditions described above enabled good elution of colchicine (Rf 0.30). Results from calibration showed there was excellent correlation between colchicine peak area and drug concentration in the range 4 to 35 µg/ml.

The regression coefficient was 0.9980 for colchicine. The limits of detection and quantification were 1 and 4 µg/ml, respectively. Average recovery of colchicine from the formulation was 101.27 %, which shows the method is accurate and free from interference from excipients present in the formulation.

Instrument precision and interday and intraday precision were measured to evaluate the precision of the method. The low relative standard deviations obtained (< 2%) indicate the method was highly precise. Robustness was studied during method development by determining the effects of small variation of mobile phase composition (± 2%), chamber saturation period, development distance, and scanning time (10 % variation of each).

No significant change in Rf or response to colchicine was observed, indicating the method was robust. The results are shown in **table 1 and 2.**

TABLE 1 *Rf*, LINEAR REGRESSION, LOD AND LOQ FOR COLCHICINES

Standard	*Rf*	Regression equation	r[a]	% SD	LOD (ng/spot)	LOQ (ng/spot)
Colchicine	0.29	Y = 1938.887 + 13.938*X	0.99801	2.63	10	30

[a] Correlation coefficient

TABLE 2: COLCHICINE RECOVERY INVESTIGATIONS

Compound	Amount of standard added (ng/ml)	Amount of standard detected (ng/ml)	Recovery* %	RSD %
Colchicine	100	103.35 ± 2.92	103.35	2.82
	250	252.61 ± 6.08	101.04	2.40
	500	497.20 ± 4.28	99.44	0.86

* Recovery value by the proposed method is the mean of five determinations.

The applicability of the proposed method to the assay of dosage forms was examined by analysing tablets marketed under different trade names. The results obtained were compared statistically by Student t-test and by the variance ratio F-test with those obtained by the reported method [10]. The Student t-values at 95% confidence level did not exceed the theoretical value indicating that there was no significant difference between the proposed and the reported method. It was also observed that the variance ratio F-values calculated for p=0.05 did not exceed the theoretical value indicating that there was no significant difference between the precision of the proposed and the reported method [10]. The results are tabulated in **table 3**.

TABLE 3: ANALYSIS OF PHARMACEUTICAL PREPARATIONS OF COLCHICINE

Formulation	Label claim mg/tablet	Recovery** ± SD %	
		Reported method [10]	Proposed method
Goutnil (Tablet)	0.5	100.18 ± 0.14	99.1 ± 0.88, F=1.72; t=0.85
Colchicindon (Tablet)	0.5	100.04 ± 0.12	101.1 ± 0.91, F=1.50; t=0.86

**Recovery value by the proposed method is the mean of five determinations. The calculated F- and t- values refer to 95 % confidence limits.

The results of the analysis of peripheral blood, cardiac blood and urine samples are shown in **table 4**. The results obtained are compared with those obtained by the reported method [11]. It was also observed that the colchicine peripheral blood concentration observed in the proposed method (25.4 ng/ml) was much higher than therapeutic concentration range (0.3 to 2.4 ng/ml) [11].

TABLE 4: ANALYSIS OF BIOLOGICAL FLUIDS

Biological fluids	Concentrations ng/ml	
	Reported method [11]	Proposed method
Peripheral Blood	21.9	25.4
Cardiac Blood	22.8	28.9
Urine	148.5	157.5

CONCLUSION: The proposed HPTLC method for the determination of colchicine was simple, rapid and sensitive compared to the reported methods. The utility of the proposed method for the determination of colchicines in dosage form and body fluids has been well demonstrated.

The method did not involve any stringent experimental conditions and was also found to be eco-friendly (low consumption of solvents). Hence, the proposed method could be used for screening and quantitative determination of selected drug.

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