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DEVELOPMENT AND VALIDATION OF RP-LC-UV METHOD FOR DETERMINATION OF COLCHICINE IN DRUG SUBSTANCE AND DRUG PRODUCT

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ABSTRACT

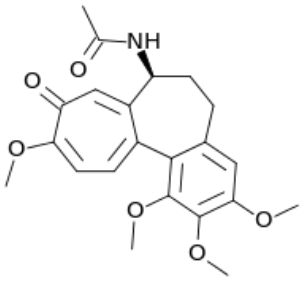
The present work is undertaken with an aim to develop and validate an accurate, precise and rapid method for the estimation of Colchicine in tablets and bulk form. The method is simple, accurate and sensitive. The separation was achieved on high pressure liquid chromatography shimadzu HPLC LC-2010with UV detector, a Reverse Phase C-18 Column (25 cm x 4.6 mm) i.d., particle size 5 µm) using a mobile phase consisting 550mL of phosphate buffer and 450mL of acetonitrile. The HPLC system was equipped with the LC solutions software. Flow Rate: 1.0 ml/min Injection Volume:20µl, Run Time: 12 min, coloum temp: 40°C. The method was validated for specificity, precision, linearity, range, accuracy and robustness. The recovery range for Colchicine is in the range of 99.62–100.03% and the method can be successfully applied for the routine analysis of the drug substance and drug products

INTRODUCTION:

The genus Colchicum belongs to the colchicaceae family. Molecular formula of colchicine is $C_{22}H_{25}NO_6$ (Fig. 1) with IUPAC name N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9- tetrahydrobenzo [a] heptalen- 7-yl] acetamide and molecular weight is 399.44 g/mol. It is a highly poisonous alkaloid containing various species of colchicum. COLC is the main alkaloid obtained from the bulb and seeds of colchicum. It is used in human and veterinary medicine. The medicinal value of colchicum is due, to the presence of (-)-colchicine, the main alkaloid, which was isolated from all species of colchicum. It is widely used in breeding studies, as drug to treat gout but is valuable for other diseases such as familial Mediterranean fever, primary biliary cirrhosis, and breast cancer Generic COLC is available in tablets. (-)- Colchicine, is a phenylethyl isoquinoline derived alkaloid, and it is a poisonous, lipid-soluble alkaloid with a unique 7-memberd aromatic tropolone ring.

Several analytical methods for the determination of COLC in pharmaceutical preparations, in biological fluids and in plant extracts have been described The aim of the present study is to develop an accurate and reliable method for the quantification of COLC in tablets using HPLC.

Fig. 1: Colchicine



2.0 EXPERIMENTAL:

2.1Reagents: O-Phosphoric acid:AR grade
Methanol: HPLC grade, Acetonitrile: HPLC grade, KH_2PO_4ARgrade
Water: HPLC or equivalent grade

2.2Standards and Samples:
Colchicine, ColchicineTablets

2.3Reagent preparation:

2.3.1 Preparation of 0.5% ortho phosphoric acid:
Dilute 1.6 ml of phosphoric acid into 50 volumetric flask and dilute with water

2.3.2 Preparation of phosphate buffer:
Weigh and transfer about 7.2gm of potassium dihydrogen ortho phosphate 1000 mL water sonicate to dissolve the salt and adjust the pH to 5.0 with 0.5% ortho phosphoric acid.

2.3.3Preparation of mobile phase:
Mix 550mL of buffer and 450mL of acetonitrile. Filter and degas.

2.3.4 Preparation of diluent/blank:
Use mobile phase as a diluent.

2.4Chromatographic conditions:
Column: Waters symmetry 5µ C18, 25cm×4.6mm. Detector: 254 nm, Flow rate: 1.0 mL/ minute, Injection volume: 20 µL, Column oven temperature: 40^0C, Sampler temperature: 25^0C. Run time: 12 minutes

2.5Preparation of Standard solution:
Weigh accurately and transfer 25mg of Colchicine working standard into a 250 mL volumetric flask. Add 100mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix.

2.6Preparation of Sample solution:
Weigh accurately and transfer powder equivalent to 50mg of Colchicineinto a 500 mL volumetric flask. Add 250mL of diluent and sonicate for 20 minutes with intermediate shaking then cool to room temperature and dilute to volume with diluent and mix then filter the solution by using Nylon filter.

3.0 METHOD VALIDATION:
The method was validated according to the ICH guidelines for the validation of analytical procedures. The parameters, which were used to validate the method of analysis, were linearity, range, specificity, accuracy, precision and recovery, solution stability.

3.1 System suitability:
To verify that analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set.

3.2 Specificity:
Specificity is the ability of analytical method to assess unequivocally the analyte in the presence of component that may be expected to be present, such as impurities,degradation products and matrix components. Performed the specificity parameter of the method by injecting Blank (Diluent), placebo, standard preparation and sample preparation into the HPLC system. Record the retention times of Blank (Diluent), placebo, standard preparation and sample preparation.

3.2.1 Specificity by degradation studies:
Force Degradation of Colchicinetablets10mg shall be carried out, to confirm that during stability study or throughout the shelf life, any degradation product if found should not interfere with the main peak of Colchicine. In addition, the forced degradation study will help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, water hydrolysis and dry heat) for each of the degradants.

3.2.2 Sample preparation:
Weighed and transferred 50mgequivalent of sample powder into a 500 mL volumetric flask, added about 200mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.3 Placebo preparation:
Weighed and transferred 50mgequivalent of placebopowder into a 500 mL volumetric flask, added about 200mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.4 Acid stressed sample preparation (1.0 N HCL):
Weighed and transferred 50mg equivalent of sample powder into a 500 mL volumetric flask, added 5 mL of 1.0 N HCL kept on water bath at 80^0C for 30 hours. Neutralized with 5mL of 1.0N NaOH. Added about 200 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.5 Alkali stressed sample preparation (1.0N NaOH):

Weighed and transferred 50mg equivalent of sample powder into a 500 mL volumetric flask, added 5 mL of 1.0N NaOH kept on water bath at 80^0C for 30 hours. Neutralized with 5 mL of 1.0 N HCl Added about 200 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.6 Peroxide stressed sample preparation (3.0% v/v H_2O_2):

Weighed and transferred 50mg equivalent of sample powder into a 500 mL volumetric flask, added 5 mL of 3.0% v/v H_2O_2kept on water bath at 80^0C for 30 hours. Added about 200 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.7 Neutral stressed sample preparation:

Weighed and transferred 50mg equivalent of sample powder into a 500 mL volumetric flask, added 5 mL of water kept on water bath at 80^0C for 2 hours. Added about 200 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.8 Thermal stressed (Dry heat) sample preparation:

Weighed and transferred 50mg equivalent of sample powder into a 500 mL volumetric flask heated in hot air oven for 4 hours at 80ºC. Removed and cooled to room temperature. Added about 200 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.3 PRECISION:

The precision of an analytical method is the degree of agreement among individual test result when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurements.

3.3.1 System precision:

The system precision is checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and area of six determination should be measured and calculate relative standard deviation. Injected Blank, Standard preparation six times into the chromatograph. Record the chromatograph. Calculate the relative standard deviation.

3.3.2 Method precision:

In method precision, a homogenous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results of a single batch. Analyzed the sample of Colchicine tablets10mg six times of a same batch as per analytical procedure. Calculated the % Assay of Colchicine with respect to standard preparation.

3.3.3 Intermediate precision:

The intermediate precision was carried out to ensure that the analytical results will remain unaffected with change in instrument, analyst, column and day. Repeated the method precision set by different analyst by using different instrument and different column on different day at different location.

3.4 Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Performed accuracy in different levels, at each level in triplicate Colchicine at 50%, 100%, and 150%. Analysed these samples in triplicate for each level and calculated the % recovery.

3.5 Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Performed the linearity using Colchicine standard in the range of 50% to 150% concentration.

3.6 Range:

The range of analytical method is the interval between the upper and lower levels of analyte that has been demonstrated with a suitability and linearity.

3.7 Robustness:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Figure 2: Linearity graph of Colchicine standard

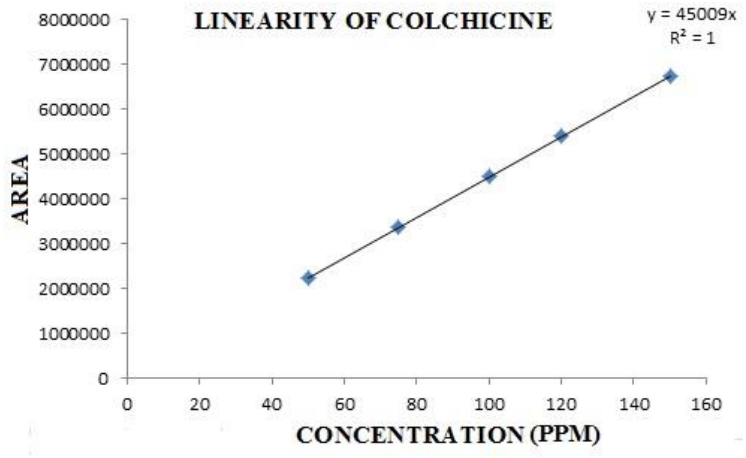


Table 1: Results of Accuracy (%Recovery)

S.no	Levels	Mg added	Mg Recovered	% recovery	Mean %	% RSD
1	50%	50.78	50.45	99.35	99.86	0.168
2		50.49	50.42	99.86		
3		50.52	50.70	100.36		
1	100%	100.25	100.30	100.05	99.62	0.40
2		100.58	100.12	99.54		
3		100.98	100.23	99.26		
1	150%	150.22	150.45	100.15	100.03	0.179
2		150.11	149.85	99.83		
3		150.14	150.40	100.17		

Table 2: Results of Method precision and intermediate precision results:

Parameter	sample	% Assay of Colchicine
Method precision	1	100.05
	2	99.85
	3	101.20
	4	100.02
	5	99.6
	6	100.0
Intermediate precision	1	99.85
	2	99.5
	3	99.8
	4	101.2
	5	99.8
	6	99.6
Mean of 12 determinations		100.0
RSD for 12 determinations		0.628

Table 3: Assay of stressed sample:

ColchicineTablets USP 10 mg	
Stressed condition	%Assay
Sample as such	99.98
Acid (1.0N HCL) Stressed sample	87.12
Alkali (1.0N NaOH) Stressed sample	94.65
3% v/v peroxide Stressed sample	90.12
Neutral Stressed sample	98.95
Thermal Stressed sample	91.25

Table 4: Results of Robustness:

S.no	Parameter	Tailing factor	Theoretical plates	%RSD
1	Original condition	1.02	6800	0.2
2	Increase in flow rate	1.21	6845	0.6
3	Decrease in flow rate	0.99	6798	0.4
4	Increase in column temperature	0.98	6810	0.2
5	Decrease in column temperature	1.01	6851	0.2
6	Increase in organic phase ratio	1.02	6798	0.4
7	Decrease in organic phase ratio	1.09	6801	0.7

Table 5: Results of Filter compatibility:

Filters	%Difference	% Assay
Centrifuged sample	---	99.98
0.45µ Nylon filter	0.03	99.95
0.45µ Nylon filter + prefilter	0.08	99.90
Millipore	-0.07	100.05
PVDF filter (MDI)	1.07	98.91
PVDF filter (PALL)	1.20	98.78

Table 6: Results of Mobile phase stability:

Sl.No	Acceptance criteria	Day-1	Day-2	Day-3
1	The %RSD for 5 replicate injections of standard solution should be not more than 2.0%	0.2%	0.7%	0.8%
2	The tailing factor should not be more than 2.0	1.05	1.02	1.06
3	The theoretical plates should not be less than 2000	6856	6845	6895

Table 7: Results of Stability in analytical solution:

S.no	hours	% Difference in standard solution	% Difference in sample solution
1	1	0.1	0.2
2	2	0.3	0.1
3	4	0.2	0.2
4	8	0.1	0.4
5	16	0.6	1.0
6	20	0.5	1.5
7	24	0.5	2.3
8	26	0.4	3.1
9	28	0.5	3.6
10	48	0.9	8.7

3.7.1Robustness parameters:
Change in column temperature ±5^{0}C. Change in flow rate ±0.2 mL/min. Change in organic phase ±5%

3.8 ADDITIONAL PARAMETERS:

3.8.1 Filter compatibility studies:

3.8.1.1 Sample preparation:

Weighed and transferred 50mg equivalent of sample powder into a 500 mL volumetric flask, added about 200 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well. Centrifuged a portion of this mixture at 4000 rpm for 5 minutes.

3.8.1.2 Placebo preparation:

Weighed and transferred50mg equivalent of placebo into a 500 mLvolumetric flask, added about 200 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well. Centrifuged a portion of this mixture at 4000 rpm for 5 minutes.

3.8.1.3 Centrifuged sample and placebo:

Centrifuged sample and placebo preparation at 4000 rpm for 5 minutes. Collected the supernatant sample and placebo preparation in separate test tubes.

3.8.1.4 Sample and placebo filtered through 0.45µm Nylon filter:

Filtered sample and placebo centrifuged solution through 0.45µm Nylon filter. Collected the sample and placebo preparation in separate vials.

3.8.1.5 Sample and placebo filtered through Nylon + pre filter:

Filtered sample and placebo centrifuged solution through Nylon + pre filter. Collected the sample and placebo preparation in separate vials.

3.8.1.6 Sample and placebo filtered through Millipore filter:

Filtered sample and placebo centrifuged solution through Millipore filter. Collected the sample and placebo preparation in separate vials.

3.8.1.7 Sample and placebo filtered through PVDF filter (MDI):

Filtered sample and placebo-centrifuged solution through PVDF filter (MDI). Collected the sample and placebo preparation in separate vials.

3.8.1.8 Sample and placebo filtered through PVDF filter (PALL):

Filtered sample and placebo-centrifuged solution through PVDF filter (PALL). Collected the sample and placebo preparation in separate vials.

3.9 Stability in analytical solution:

Evaluated the stability in analytical solution by injecting the standard solution and sample solution at regular interval.

4.0 Mobile phase stability:

Evaluated the stability of mobile phase by injecting the standard preparation in conjunctive days and checked the system suitability parameters.

RESULTS AND DISCUSSION:

The chromatographic conditions were optimized and separation was performed on a Waters symmetry 5µ C18, 25cm×4.6mm column using a mobile phase consisting 550mL of phosphate buffer and 450mL of acetonitrile. The proposed mobile phase composition allowed suitable retention time of colchicine and achieved good selectivity towards interference from the excipients of the formulation. Under the chromatographic conditions described, colchicine was eluted about 6.0 min. Good baselines and well-shaped peak can be observed. Calibration curve was constructed using standard colchicine solutions in the range of 50-150 % of test concentration. The linearity of the calibration curve was validated by high value of correlation coefficient (r2 = 1.0).

CONCLUSION

The proposed new HPLC method described in this paper provides a simple, convenient and reproducible approach for the identification and quantification of colchicine in bulk, and pharmaceutical formulations with good presentation. Analytical results are specific, accurate and precise with good recovery values. In short, the developed method is simple, sensitive, easy and efficient having small chromatographic time and can be used for routine analysis.

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