INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

DETERMINATION OF COLCHICINE CONTENT IN DRUG BY RP-HPLC

V. Maslarska[1]*, I. Pencheva[2]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University – Sofia, Bulgaria

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy,

Medical University – Sofia, Bulgaria

ABSTRACT

A simple, rapid, precise and accurate RP-HPLC method has been developed for the estimation of colchicine in tablet formulation. A validation and quantity evaluation method has been established by a defined high performance liquid chromatography by using acetonitrile : methanol : water (32:48:20 v/v; pH adjusted to 5.2 with phosphoric acid) as a mobile phase pumped through C18 (250 mm x 4.6mm, 5µm) column. The flow rate was 1.2 ml/min and effluents were monitored at 254 nm. The retention time was 7.56 min and injection volume set at 20µl. The linear regression analysis data for calibration curve showed a good relationship with correlation coefficient of 0.999_8. The concentration range was 17.5– 140 µg/ml. The percentage recovery of colchicine was found to be 99.86%. This selective method is found to be accurate, precise and effectively used for the colchicine in tablet formulation with better chromatographic conditions.

Keywords: Colchicine, RP HPLC method, validation, tablets.

INTRODUCTION

The genus Colchicum (COL) belongs to the colchicaceae family. 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 g/mol. Structure of Colchicine is shown in Fig.1. It is a highly poisonous alkaloid containing various species of colchicum. COL 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 and as drug to treat gout but is also valuable for other diseases such as familial Mediterranean fever, primary biliary cirrhosis and breast cancer[1-8]. Generic colchicine 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[9-11].

Several analytical methods for the determination of colchicine in pharmaceutical preparations, in biological fluids and in plant extracts have been described[12-15]. The aim of the present study is to develop an accurate and reliable method for the quantification of colchicine in tablets using high performance liquid chromatography (HPLC).

EXPERIMENTAL

Materials

All reagents and solvents were of analytical and HPLC grade. Methanol for HPLC was purchased from Merck Ltd. and acetonitrile for HPLC was purchased from Scharlau (Spain). Colchicine standard was purchased from Sigma Aldrich and the tablets were purchased from the local market.

Apparatus

HPLC method development and validation was done on a Shimadzu (Japan) RP-HPLC instrument (LC-20 AD) equipped with a SPD-20A UV/VIS detector,

DGU-20 A_5 vacuum degasser and Lab Solutions software was used.

Chromatographic equipment and conditions
Stationary Phase was Chromosil C18 (250x4.6, 5 µm particle size) and the mobile phase consisted of an acetonitrile: methanol: water (32:48:20 v/v; pH adjusted to 5.2 with phosphoric acid). The mobile phase was filtered through 0.45 µm membrane filter and degassed by using sonicater for about 10 min before use. The sample solutions were also filtered using 0.45 µm membrane filters. The mobile phase was delivered isocratically at a flow rate 1.2 ml/min. All determinations were performed at ambient temperature. The injection volume was 20 µl and the total run time was 9 minutes. The detection was carried out at 254 nm.

Standard and sample solutions
A 20 mg amount of colchicine substance was weighed accurately and dissolved in 20 ml mobile phase in a 20 ml volumetric flask to get 1000 µg/ml. The standard solutions were prepared by further dilution of the stock standard solution with the specified mobile phase to reach the concentration range of 17.5-140 µg/ml.
Twenty tablets were weighed and finely powdered. A portion of the powder equivalent to 2.5 mg of colchicine was accurately weighed and transferred into a 25 ml volumetric flask. Approximately 15 ml mobile phase were added and the solution was sonicated for 25 min. The flask was filled to volume with mobile phase and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration 70 µg/ml.

RESULTS AND DISCUSSION

Specificity
The specificity of the HPLC method was ascertained by analyzing standard drug and sample solutions. The retention time of colchicine was confirmed by comparing the retention time with that of the standard.

Linearity
Linearity of the method was confirmed by preparing colchicine standard curve for the analytical range of 17.5 – 140 µg/ml. The solutions were chromatographed six times, in accordance with the International Conference on Harmonisation. Statistical analysis using the least square regression indicated excellent linearity for colchicine in the mentioned range. A good correlation between colchicine peak areas and drug concentration was observed with $r^2 \geq 0.9998$ (Table 1).

Precision
The percentage label claim, present in tablet formulation, was found to be 99.84 %. A typical chromatogram of colchicine is shown in Figure 2. Precision of the method was confirmed by the analysis of formulation repeated six times (Table 2).

Limit of Detection and Limit of Quantification
The Limit of Detection (LOD) is the smallest amount of analyte in the sample, which can be detected but not necessarily quantified as an exact value. The Limit of Quantification (LOQ) is the lowest amount of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ are calculated as given in Table 3.

Accuracy
To check the accuracy of the developed methods and to study the interference of formulation excipients, analytical recovery experiments were carried out as per ICH guidelines. The results of the recovery studies and their statistical validation data given in Table 4 indicate high accuracy of the proposed method. The percentage recovery was found to be in the range of 98.00-102.0%.

Robustness
As defined by the ICH, the robustness of an analytical procedure describes its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 2% variation in volume of mobile phase composition, ± 0.1 ml/min in flow rate of mobile phase, ±0.1 variation in pH.

CONCLUSION
A simple isocratic RP-HPLC method with UV detection has been developed for determination of colchicine. The method was validated for precision, specificity, accuracy robustness and linearity. The run time is relatively short (9 min), which enables rapid quantification of many samples in routine and quality control analysis of tablets.

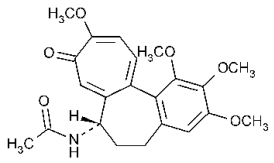

Fig. 1 Chemical Structure of COL

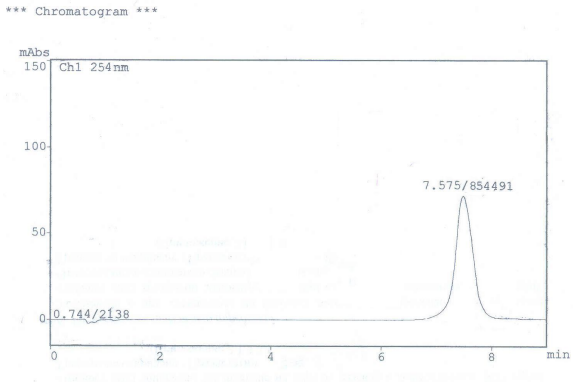

Figure 2: Chromatogram of colchicine

Table 1
Results from study of linearity

Methods	λ, nm	Range (µg/ml)	LinRegrEqu	R^2
RP-HPLC	254	17.5 - 140	y=12156x+659	0.999_8

Table 2
Results from assay of tablet formulation

	Assay %
Sample	**Colchicine**
1	98.97
2	99.85
3	99.57
4	100.1
5	100.6
6	99.93
Average	**99.84**
RSD %	**0.546**

Table 3
Results for LOD and LOQ

Parameters	Results
LOD	2 µg/ml
LOQ	6 µg/ml

Table: 4. Accuracy of Colchicine

Parameters	% Taken	Conc. of sample µg/ml	Recovery	% Recovery
			34.81	99.46
	50.00	35	34.53	98.66
			35.15	100.4
			69.65	99.50
	100.0	70	70.30	100.4
			70.05	100.1
			105.2	100.2
	150.0	105	105.4	100.4
			104.6	99.62
Mean				**99.86**
SD				**0.595**
% RSD				**0.596**

REFERENCES

1. Niel E and Scherramann JM. Colchicine today. Joint Bone Spine. 2006: 73 (6); 672-678.
2. Kaplan MM, Alling DW, Zimmerman HJ, Wolfe HJ, Sepersky RA and Hirsh GS. A prospective trial of colchicines for primary biliary cirrhosis. N Engl J Med. 1986; 315: 1448-1454.
3. Gabrscek L, Lesnicar G, Krivec B, Voga G, Sibance B, Blatnik J and Jagodic B. Accidental poisoning with autumn crocus. J Toxicol Clin Toxicol. 2004; 42 (1): 85-88.
4. Sannohe S, Makino Y, Kita T, Kuroda N and Shinozuka T. Colchicine poisoning resulting from accidental ingestion of meadow saffron (Colchicum autumnale). J Forensic Sci. 2002; 47 (6): 1-6.
5. Inchem, Colchicum autumnaleL. http://www.inchem.org/documents/pims/plant/pim 142.htm; 1-33.
6. Terkeltaub RA. Clinical practice. Gout. N Engl J Med. 2003; 349(17):1647-1655.
7. Drenth JP and Meer JWVD. Hereditary periodic fever. N Engl J Med. 2001; 345(24): 1748-1757.
8. Sakane T and Takeno M. Novel approaches to Behcet's disease. Expert Opin Invest Drugs. 2000; 9(9): 1993-2005.
9. Dollery C, Boobis A, Rawlings M, Thomas S and Wilkings M. Therapeutic drugs. Churchill Livingstone: UK, 2nd ed., 1999; 1: 320-322.
10. Trease GE and Evas WC. Trease and Evans'Pharmacognosy, 13th ed., Bailiere Tindall: London and Philadelphia, 1989; 600-603.
11. Herbert RB and Knagg E. The biosynthesis of the phenethylisoquinoline alkaloid, colchicine, from cinnamaldehyde and dihydrocinnamaldehyde. Tetrahedron Lett. 1986; 27(9): 1099-1102.
12. Sutlupinar N, Husek A, Potesilova H, Dvorackova S, Hanus V, Sedmera P and Simanek V, Alkaloids and Phenolics of Colchicum cilicicum Planta Med. 1988; 54(3): 243-245.
13. Poulev A, Deus-Neumann B, Bombardelli E and Zenk M. Immunoassays for the quantitative determination of colchicine. Planta Med. 1994; 60(1): 77-83.
14. Ondra P, Valka I, Vicar J, Sutlupinar N and Simanek V. Chromatographic determination of constituents of the genus Colchicum. J Chromatogr. 1995; 704(2): 351-356.
15. Al-Fayyad M, Alali F, Alkofahi A and Tell A. Determination of colchicine content in Colchicum hierosolymitanum and Colchicum tinicatum under cultivation. Nat Prod Lett. 2002; 16(6): 395-400.