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Review Article

Review of different approach for determination of Colchicine

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Abstract

Colchicine is primarily used to treat gout, but it can also be used to treat other inflammatory diseases, such as familial Mediterranean fever (FMF). It was initially granted FDA approval in 2009 for the prevention of acute attacks and treatment of them, following a critical trial that demonstrated the medication's effectiveness. Excretion of colchicine and its metabolites occurs via the biliary and urine systems.

Several analytical methods used in the present literature to quantify are gathered and discussed in this systematic study which include UV-visible spectroscopy, spectrofluorimetry, electrochemical methods, and other chromatographic techniques such as HPLC, TLC and capillary electrophoresis. This work investigates the comparative application of several analytical techniques to determine colchicine. The information provided in this review study may be used to effectively carry out additional analytical research for quantitative determination of colchicine in crude drug, pharmaceutical dosage forms and biological fluids.

1-Introduction

One of the most common rheumatic conditions is gouty arthritis. Although the clinical burden of gouty arthritis has long been known, gout is frequently misdiagnosed and mistreated [1]. The Egyptians discovered gout in 2640 B.C., and it was later named “the unwalkable disease” and the “disease of kings” [2]. Nowadays, gout is a well-defined rheumatologic condition that is linked to more than just lifestyle choices. A variety of clinical disorders associated with an excess of serum urate (uric acid) are collectively referred to as “gout.” It can also present a range of clinical manifestations, not just the traditional podagra [2]. Although Colchicine is old drug, it has many new clinical uses, this attributed to the fact that it has many off-label uses like acute and recurrent pericarditis, biliary and hepatic cirrhosis, paget's disease of bone, pseudogout and idiopathic pulmonary fibrosis [3].

Colchicine **(COC) (Fig.1)** is chemically designed as N-(5,6,7,9- tetrahydro-1,2,3,10-tetramethoxy-9 oxobenzol [a]1oltametr-7-yl) acetamide [4]. Colchicine inhibits the migration of leukocytes and other inflammatory cells by disrupting the tubulin polymerization process. Colchicine, corticosteroids, nonsteroidal anti-inflammatory medications (NSAIDs), or a combination of the two drugs should be used to treat acute gouty arthritis [5]. Interstitial nephropathy and uric acid nephrolithiasis are possible in progressive illness, but they usually only happen in patients with more severe and prolonged hyperuricemia [6]. In addition, COC was effectively used in COVID-19 patients, this was attributed to the fact that it is an immunomodulatory drug [3]. It acts by inhibiting the NLRP3 inflammasome which produced by viroporin E, a component of SARS-CoV-2 that is responsible for generation of inflammatory response[7].

COC is official in the British [8] and US [9] pharmacopeias where it is determined by titrimetric and HPLC methods, respectively.

The goal of this review paper was to outline various analytical techniques for figuring out colchicine. These techniques included spectrophotometric, spectrofluorimetric, electrochemical, and chromatographic procedures.

2. chemistry [8]

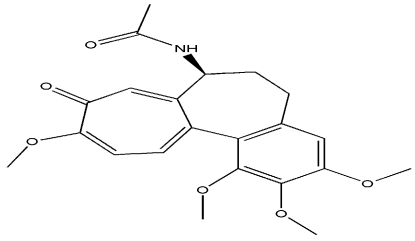

Fig.1: Chemical structures of Colchicine

N-(5,6,7,9- tetrahydro-1,2,3,10-tetramethoxy-9 oxobenzol [a]heptalen-7-yl) acetamide

Molecular formula: $C_{22}H_{25}NO_6$
Molecular weight: 399.4 g/mol
Physical characters: odorless pale-yellow needles or powder, soluble in water, methanol, ethanol and chloroform, pKa 1.85, melting point: 142.0-150.0°C [8].

3. Analytical techniques

3.1. Official and reported analytical methods for the analysis of colchicine.

3.1.1. Official analytical method

Colchicine is determined in different pharmacopoeias (BP [8]and USP [9]) where official titrimetric method in BP [8] using acetic anhydride and toluene as a solvent then titrated against 0.1 M perchloric acid where the endpoint is determined potentiometrically.

COL was assayed in USP [9] via HPLC method, using mobile phase consisting of 0.5 M monobasic potassium phosphate and methanol then pH (5.5 ± 0.05) was adjusted using 0.5 M phosphoric acid. Determination of COL was obtained using 4.6-mm × 25-cm, packing L7 column, UV-detection at 254.0 nm and flow rate at 1.0 mL/ min.

3.1.2. Reported analytical methods.

a. Spectroscopic methods
UV spectrophotometric methods

Sarg et al. [10] described a spectrophotometric method for determination of colchicine under UV light at 366 nm and checked against the colchicine marker.

Spectrofluorimetric methods

A spectrofluorimetric method was developed for determination of colchicine after binding to tubulin to enhance its fluorescence intensity by 300.0 -fold at 430.0 nm. This method did not necessitate the separation of the tubulin-colchicine complex from free colchicine [11].

A spectrofluorimetric method was also described for determination of colchicine in pharmaceutical preparation. The excitation and emission maxima for colchicine in distilled water were 350.0 and 399.0 nm, respectively [12].

b. Electrochemical methods

A voltametric method used to determine colchicine in human urine was developed. Remarkable peak current enhancement showed that acetylene black-dihexadecyl hydrogen phosphate modified coated glassy carbon electrode had great potential in the sensitive determination of colchicine. Good linear relationship was obtained in the range of 1.0×10^{-7}~4.0×10^{-5} mol/L [13].

Bodoki et al. [14] investigated the electrochemical behavior of colchicine by using graphite-based screen-printed electrodes where the anodic differential pulse 2oltametric method in H_3PO_4/$HClO_4$ 0.01 M (pH= 2.05) was selected for quantitative determination of colchicine oxidation peaks at +970 mV versus Ag pseudo-reference over concentration range 85.0–1200.0 ng/ mL.

Electrochemical method was developed for the determination of colchicine in 0.1 M phosphate buffer solution (pH 4.0) upon using differential pulse voltammetry where 1-butyl-3-methylimidazolium tetrachloroferrate was synthesized. This method was developed for quantification of colchicine in commercial pharmaceutical formulations and human plasm [15].

c. Chromatographic methods
Thin Layer chromatography

A validated HPTLC method for determination of colchicine was described. The HPTLC separation was accomplished on an aluminum-backed layer of silica gel GF_{254} using chloroform: methanol (95.0: 5.0, v/v) as a mobile phase. Quantitation was achieved with UV detection at 243.0 nm [10].

Bodoki et al. [16] developed a TLC method for quantitative analysis of colchicine using chloroform: acetone: di-ethylamine (5.0: 4.0 :1.0, by volume) as a mobile phase. The densitometric measurements were carried out at 350.0 nm.

High-performance thin-layer chromatographic method for concurrent analysis of colchicine and khellin was developed. The HPTLC separation was carried out on an aluminum-backed sheets of silica gel GF_{254} using ethylene chloride–methanol (95.0: 5.0, v/v) as a developing system. UV detection was achieved at 245.0 nm [17].

Fahim et al. [18] investigated HP-TLC method for analysis of colchicine using toluene: dichloromethane: methanol, (10.0:10.0:10.0, by volume) as mobile phase where colchicine was detected at 350.0 nm.

High-performance thin-layer chromatography for simultaneous determination of colchicine and gallic acid was performed on pre-coated silica gel 60 GF_{254} upon using ethyl acetate/acetonitrile/water/formic acid/N-dimethylformamide (7.5:1:0.5:0.5:0.5, by volume) as mobile phase. UV analysis was conducted at 360.0 and 287.0 nm for colchicine and gallic acid, respectively [19].

Misra et al. [20] developed high-performance thin-layer chromatography for simultaneous determination of colchicine and gloriosine. Separation was conducted on TLC aluminum plates of silica gel 60 F_{254} using developing system of chloroform: acetone: di-ethylamine (5.0:4.0:1.0, by volume) where densitometric quantification was achieved using 350.0 nm.

High Performance Liquid chromatography

Table 1: **HPLC methods for COC determination and their applications**

Column	Mobile Phase	Flow rate (mL/min)	Detection	Application	Reference
C18 column HPLC	Methanol-acetonitrile (25.0:75.0, v/v)- pH (2.5-5.5)	3.0	350.0 nm	Stability study after hydrolysis and photodecomposition conditions	[21]
C8 column HPLC	Methanol-phosphate buffer (55.0: 45.0 v/v) (pH = 5.5)	1.0	254.0 nm	In pharmaceutical formulations	[22]
Stainless-steel Micropak MCH lo-pm column HPLC	Actonitrile-water (50.0:50.0, v/v)	2.0	245.0 nm	Human plasma and urine	[23]
Zorbax-ODS C18 column HPLC	Methanol: water (60.0: 40.0, v/v)	1.0	243.0 nm	Crude drug and pharmaceutical dosage forms	[10]
Cl8 column HPLC	5.0 mM of l-pentanesulfonic acid-methanol-acetonitrile (60.0: 26.6: 13.4, by volume)- 0.1 M potassium hydroxide (pH= 6.0)	1.5	254.0 nm	Serum or urine	[24]
C18 column HPLC	Acetonitrile: 2.0 mM NH_4COOH, pH= 3.0 buffer (75.0: 25.0, v/v)	0.05	MS/MS	Biological fluids	[25]
C18 column HPLC	Gradient elution (0.5% formic acid-acetonitrile)	1.0	MS/MS	Sheep serum and milk	[26]
C18-Xterra column	Gradient elution (formate buffer (pH= 3.0)-acetonitrile)	0.2	MS/MS	Postmortem body Fluids	[27]
C18 Uptisphere column	Acetonitrile/2.0 mM NH_4COOH, pH= 3.8 buffer (50.0 :50.0, v/v)	0.2	MS/MS	Human plasma	[28]
Inertsil ODS-2	CH_3COONH_4 0.05 M: CH3OH (48.0 :52.0, v/v)	1.0	245.0 nm	Pharmaceuticals and biological fluids	[29]
C18 column HPLC	Acetonitrile – 0.15% phosphoric acid solution (27.0:37.0, V/V)	1.0	350.0 nm	Mouse plasma	[30]
C18 column HPLC	Gradient elution (acetonitrile and 10.0 mmol L^{-1} of ammonium bicarbonate) pH =10.5	0.5	MS/MS	Blood and urine	[31]
Ultimate XB-C18 column HPLC	Gradient elution (0.2% aqueous formic acid- methanol)	0.6	MS	Human blood and gastric juice	[32]
C18 column HPLC	Acetonitrile–10.0 mM NaH_2PO_4, pH= 3.0 (35.0: 65.0, v/v)	1.0	245.0 nm	Pharmaceutical Formulations	[17]
C18 monolithic spin column HPLC	Gradient elution (water with 0.1 % acetic acid - acetonitrile	0.4	MS/MS	Serum, urine, and plants	[33]
RP C18 column HPLC	Gradient elution (acetonitrile- water)	0.3	MS/MS	Herbal cosmetics	[34]
XBridge C18 column HPLC	Water: acetonitrile (75.0 :25.0, v/v)	1.0	MS	Gloriosa superba seeds	[35]
Eclipse XDB-C18 column HPLC	5.0 mM ammonium formate and 0.025% formic acid in acetonitrile (20.0 :80.0, v/v)	0.9	MS/MS	Rat plasma	[36]
An XBridge™ BEH C18 column	Gradient elution 0.01% (*v/v*) ammonia-methanol	0.35	MS/MS	Plasma and urine	[37]

Capillary Electrophoresis (CE)

Separation of colchicine and three toxic alkaloids namely, aconitine, nicotine, and strychnine was accomplished by capillary electrochromatography. Solvent mixture consisting of 2.0% NH_4OH, 20.0 % methanol, and 78.0 % ethyl acetate was utilized to remove the alkaloids from the solid phase extraction column and were detected from 210.0 nm to 400.0 nm [38].

d. Stability indicating methods

Two stability-indicating methods (RP-TLC and HPLC method) have been reported for determination COL only under acidic conditions by the assist of a microwave. The frist method was RP-TLC, where separation was achieved

using mobile phase of water: methanol: diethylamine (70.0: 30.0: 15.0, by volume) flow rate 1.0 mL/min with UV detection at 254.0 nm. The second method was HPLC, where separation was conducted using mobile phase of mixture of water (containing 0.02% diethyl amine): methanol: acetonitrile (50.0: 20.0: 30.0, by volume), flow rate 2.0 mL/min with UV detection at 254.0 nm [39]

Razeq *et al*. have tested COL stability under acidic, alkaline as well as oxidative conditions. COL was separated from its degradation product using RP-TLC, HPLC and spectrophotometric method. HP-TLC method was conducted on pre-coated silica gel 60 GF254 upon using Chloroform-methanol-conc ammonia (8.0: 2.0: 0.1 v/v/v) as a mobile phase and UV detection at 350.0 nm. HPLC method was achieved on kromasil C18 column and a mobile phase of methanol-ammonia (100.0: 1.5 v/v) with UV detection at 246.0 nm. spectrophotometric method was obtained using dual wavelength method at 245.0 nm and 261.0 nm and first derivative of ratio spectra at 302.4 nm [40]

Two stability-indicating methods namely, RP-TLC and HPLC method were developed for simultaneous determination of colchicine and probenecid where COL was tested under acidic, alkaline, oxidative, photolytic, and thermal degradation conditions. HP-TLC method was conducted on pre-coated silica gel 60 GF254 upon using ethyl acetate–methanol–33%ammonia (8.0: 1.0: 1.0, by volume) as mobile phase and UV detection at 254.0 nm. On the other hand, HPLC method was carried out on C18 column using phosphate buffer pH 5.0–acetonitrile (70.0: 30.0, v/v) as a mobile phase, flow rate 1.0 mL/min with UV detection at 254.0 nm [41]

stability indicating spectrophotometric methods have been developed for the concurrent determination of probenecid, colchicine and colchicine degradation product. These methods encompasses ratio difference, derivative ratio and mean centering of ratio spectra with linarity range 0.5–25.0 µg/mL and 1.0–13.0 µg/mL [42]

3. Conclusion

This review summarizes many analytical methods that have been reported in the literature for the identification of COC. The main goal of the review gathering is to collect and analyze as much data as possible on COC analytical methodologies. The results of the investigation indicate that only a small number of analytical procedures based on electrochemical and UV-Vis spectrophotometry are available, and that even fewer publications based on hyphenated methods are available.

Conflict of Interest

The Authors declare no conflict of interest.

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