

# Analytical Methods for Determination of Muscle Relaxant Thiocolchicoside in Pharmaceutical Preparations- A Review

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**Abstract:** Thiocolchicoside is a centrally acting muscle relaxant and used in combination with many NSAIDs for the treatment of various musculoskeletal disorders. As it is less sedative than other centrally acting muscle relaxants hence frequently prescribed for low back pain (LBP), orthopedic, traumatic and rheumatologic disorders. It is available in market in single component and as multicomponent formulations. Various analytical methods are available for determination of thiocolchicoside in drug substances and formulated products. The present article summarizes more than 100 analytical methods including all types of chromatographic, UV-Visible spectrophotometry and radio immune assays with their percentage of utility for determination of thiocolchicoside in biological matrices, bulk material and different pharmaceutical formulations.

**Keywords:** Analytical methods, thiocolchicoside.

## INTRODUCTION

Thiocolchicoside (THC) is a semisynthetic derivative of colchicoside (natural compound) which is obtained from the seeds of *Gloriosa superba* and *Colchicum autumnale*. It is an analogue of colchicines, as they share the same benzo (alpha) heptalenic moiety [1]. It is used as muscle relaxant for the treatment of painful muscle contractions in acute and chronic rheumatic conditions, in traumatology and in patients with acute low back pain [2]. Moreover, Anti-inflammatory and analgesic effects of this drug have also been reported in animal models [3]. It is used since several years in the European countries, but in India the first formulation containing thiocolchicoside was approved in the year 2008 [4]. THC is available in combination therapy with many NSAIDs for treatment of acute low back pain and painful muscle spasms.

## CHEMISTRY

Thiocolchicoside (THC) (Fig. 1) is a 2-Glucoside analog of Thiocolchicine and known as 2-demethoxy-2- glucosidoxythiocolchicine. The Molecular formula is  $C_{27}H_{33}NO_{10}S$ ; and molecular weight is 563.62 g it occurs as yellow crystalline powder and is soluble in water, methanol, 0.1N HCl, 0.1N NaOH. It contains not less than 98.0 per cent and not more than 102.0 per cent of  $C_{27}H_{33}NO_{10}S$ . Thiocolchicine is chemically, *N*-[(7*S*)-5,6,7,9-Tetrahydro-1,2,3-trimethoxy-10-(methylthio)-9-oxabenz[*a*]heptalen-7-yl]acetamide and its semi-synthetic derivative THC is, *N*-[(7*S*)-2-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-5,6,7,9-Tetrahydro-1,3-dimethoxy-10-(methylthio)-9-oxabenz[*a*]heptalen-7-yl]acetamide [5].

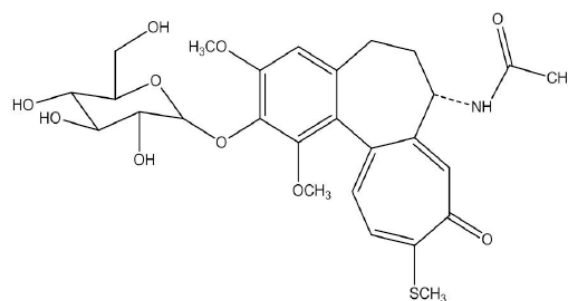


Fig. (1). Chemical structure of thiocolchicoside.

## PHARMACOLOGY

### Mechanism of Action

THC is a centrally acting Non-benzodiazepine muscle relaxant. It acts as a weak postsynaptic GABA agonist [6]. TCC has been used clinically for more than 35 years as a muscle-relaxant, anti-inflammatory, and analgesic drug, but its molecular targets and mechanisms of action are still under investigation. This compound has been shown to inhibit the binding of [<sup>3</sup>H] GABA (g-aminobutyric acid) or [<sup>3</sup>H] strychnine to rat cerebro-cortical or spinal cord membranes, respectively, *in vitro* as well as to corresponding autoradiographic sections *in vivo*. Moreover, TCC was shown to interact preferentially with a subpopulation of GABA-ARs with low-affinity binding sites for GABA. Although its precise mechanisms of action remain unknown, TCC has been thought to act as a GABA-AR agonist that induces depression of the central nervous system and, in turn, myorelaxation [7].

### Pharmacokinetics

On IM administration THC shows *C*<sub>max</sub> values of 113 ng/mL (4 mg dose) and 175 ng/mL (8 mg dose) in 30 min. After oral administration, no THC is detected in plasma. Only two metabolites were observed with maximum plasma concentrations one hour after THC administration [8].

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## Dosage Forms and Recommended Dose

Oral, parenteral and topical formulations of THC are available in India. The maximum recommended oral dose is 8 mg every 12 hours for not more than 7 consecutive days. The maximum intramuscular dose should be 4 mg every 12 hours, for up to 5 days [8].

## Contraindications

THC is contraindicated in patients hypersensitive to the active substance, during the entire pregnancy period, during lactation and in women of childbearing potential not using contraception. The drug is also contraindicated in children less than 16 years of age [8, 9].

## Adverse Effects

THC has shown some side effects like anaphylactic reactions, such as pruritus, urticaria, angioneurotic oedema; anaphylactic shock following intramuscular injection, somnolence, vasovagal syncope, usually occurring in minutes following the intramuscular injection; diarrhea, gastralgia, nausea, vomiting and allergic skin reaction and phototoxicity.

On 21<sup>st</sup> November 2013, the European Medicines Agency's Committee on Human Medicinal Product recommended that the authorized uses for THC containing medicines for use by mouth or injection should be restricted across the European Union. In addition, the dose of thiocolchicoside by mouth or injection should be restricted [8].

Thiocolchicoside given at doses of 6–12 mg/kg is unable to induce, in intact rats, any electrical or behavioural paroxysmal activity. However, when this compound is applied topically to the pia, given by microinjection to cerebral cortex, or administered parenterally at doses of 6 mg/kg to rats with minimal lesions of the dura and arachnoid membranes, it displays within a few minutes, a powerful convulsant activity. It is also reported that thiocolchicoside presented seizures in a patient when treated with a much lower dose (0.25 mg/kg for the cumulative dose or 0.05 mg/kg for a single dose) [10, 11].

## Recent Safety Alerts for Thiocolchicoside

The review of thiocolchicoside was triggered by the Italian medicines regulatory agency, AIFA, following new experimental evidence which suggested that THC was broken down in the body into a metabolite called M<sub>2</sub> or SL59.0955 that could damage dividing cells, resulting in aneuploidy (an abnormal number or arrangement of chromosomes) [9].

## ANALYTICAL METHODS FOR DETERMINATION OF THICOLCHICOSIDE

The analysis of pharmaceuticals is an integral part of drug development process. From many years; analytical techniques like UV/Vis-Spectrophotometry, Spectrofluorimetry, Atomic Absorption Spectrometry, Capillary-Electrophoresis, Liquid-chromatography, Gas-chromatography Mass-spectrometry, Luminescence, Voltammetry and Polarography have been studied for the analysis of pharmaceutical compounds. Amongst all these methods, chromatographic methods have been widely exploited and preferred over other methods [12]. THC is a commonly used muscle relaxant in the treatment of

acute painful muscle spasms. It is available in combined treatment with many non-steroidal anti-inflammatory drugs (NSAIDs) like Aceclofenac, Diclofenac, Paracetamol, Ketoprofen, Etodolac, Etoricoxib, Lornoxicam and Floctafenine and the literature reports vast number of analytical methods such as UV-spectrophotometry, HPLC, TLC-densitometry and LC-MS/MS for the determination of THC in biological matrices, bulk material and the corresponding pharmaceutical formulations.

Consequently, the objective of the present review is to compile the analytical methods published so far for estimation of THC in biological samples including metabolites and degradants, bulk materials and pharmaceutical formulations. The percentage utility of these techniques is calculated and shown in Fig. (2), from which it can be noticed that High-Performance Liquid Chromatography (HPLC) and Spectrophotometric methods have been most extensively used. Apart from this, the present review will be useful for selecting the diluents Fig. (3) and the analytical procedure for the assay of THC with different concentrations ranges, selective accuracy and precision in various biological material or pharmaceuticals.

## Spectrophotometry [13-29] and Spectrofluorimetry [30]

In the literature, nearly twenty seven UV- Spectrophotometric methods have been reported so far for estimation of THC alone and in combined dosage forms as well as a single Spectrofluorimetry method has been also established for THC from injection. Table 1 illustrates the summary of the reported UV-Spectrophotometric and Spectrofluorimetry methods indicating the basic principle, matrix used,  $\lambda_{max}$ , solvent and linearity range.

### Methods for Analysis as a Single Component

Eight methods for estimation of THC in a capsule dosage form as a single component have been developed. The first, second and third method employ first order, second order and third order derivative spectroscopy to reduce spectral interference. The fourth method employs selection of area under curve in wavelength region of 254 – 264 nm using 0.1 N Sodium hydroxide solution prepared in double distilled water [13]. Two simple and sensitive spectrophotometric methods have been developed for the quantitative estimation of THC from its capsule formulation. The first method is a zero order UV spectrophotometric method using distilled water as solvent; the drug showed absorption maximum at 259.8 nm and linearity was observed in the concentration range of 5.0 – 50  $\mu\text{g/mL}$ . The second method is area under curve selecting the wavelength range of 269.8 - 249.8 nm [14]. In other method, utilizing UV-Spectrophotometric procedure, based on the linear relationship between the THC concentration and the  $\lambda_{max}$  amplitude at 279 nm [15].

### Methods for Analysis in Combined Dosage form with Other Drugs

As THC is available with many NSAIDs, analgesics and antipyretics in combined pharmaceutical formulations; literature revealed range of UV-Spectrophotometric methods for simultaneous determination of THC in combined dosage forms.

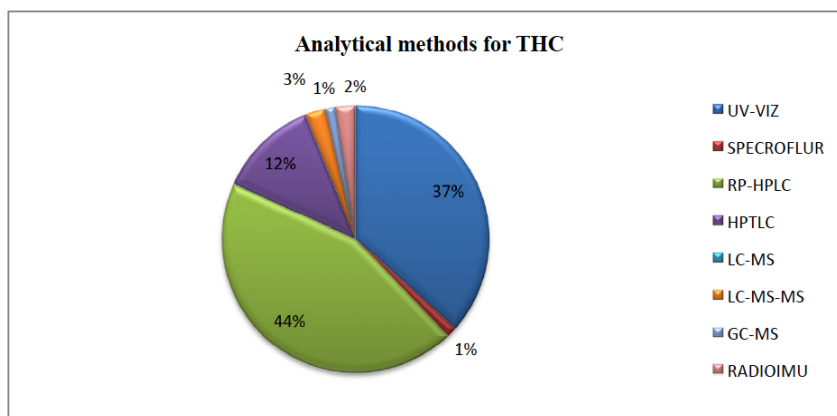


Fig. (2). Different analytical methods employed for determination of thicolchicoside.

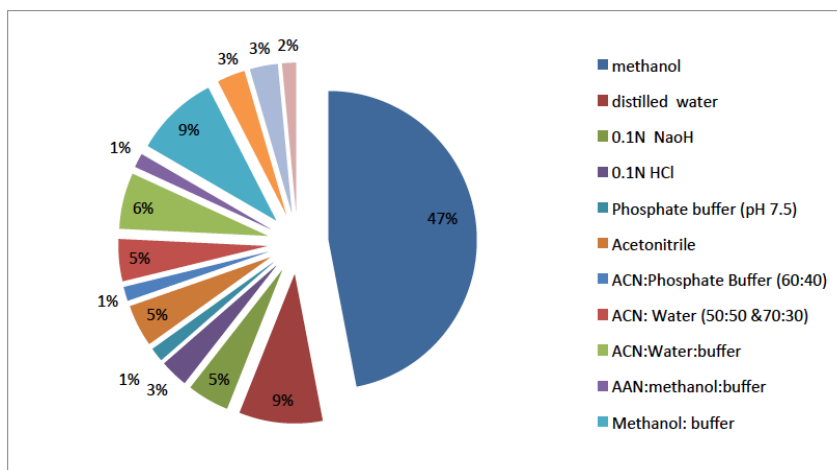


Fig. (3). Diluents used for the analysis of THC in different analytical methods.

Two simple, rapid, accurate and economical methods have been developed for the estimation of THC and DCF-P in capsules. The first method; involves formation of Q-absorbance equation at 264 (isoabsorptive point) and at 259 nm, while the second method; involves formation of simultaneous equation at 259 and 271 nm, using water as solvent [16].

Two methods; absorption correction and area under curve have been established for the quantitative estimation of THC and DCF-P from tablet formulation using methanol as a solvent. In absorption correction method, quantitative estimation of THC was carried out by subtracting interference of diclofenac using experimentally calculated absorption factor, also Beer's plot was constructed for DCFP and THC in solution mixture at different concentration (25:4, 37.5:6, 50:8, 62.5:10, 75:12  $\mu\text{g}/\text{mL}$ ) levels. For the simultaneous equations using area under curve; 252.56 - 260.59 nm ( $\lambda_1 - \lambda_2$ ) and 278.51 - 285.53 nm ( $\lambda_3 - \lambda_4$ ) have been selected as a sampling wavelength ranges for estimation of DCFP and THC [17].

Two methods for simultaneous determination of DCFP with THC in combined dosage form have been developed. The first method is based on first derivative of the ratio spectra ( $^1\text{DD}$ ) measuring the amplitudes at 268.78 nm and 355.62 nm. For the second method, two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug [18]. A simple method employed the

formation and solving of simultaneous equation using 259 nm and 277 nm as the wavelength for forming equation has been also developed for DCFP with THC using 0.1 N NaOH as a solvent [19]. A single method, using multicomponent mode of analysis of the instrument is used for the simultaneous estimation of the two drugs THC and DCF-P respectively [20]. Three spectrophotometric methods (A–C) were described for simultaneous determination of THC with DCF-S in capsule formulation. The first 'method A' is absorbance correction method and determines concentration of DCFS directly from calibration plot by measuring absorbance at 276.6 nm and THC was determined after correction for absorbance of DCF-S at 372.8 nm. The second method is based on first order derivative spectroscopy to overcome spectral interference from other drug and wavelengths 278.6 nm and 243.2 nm were selected for the determination of the DCFS and THC, respectively. In the third method, diclofenac sodium was determined by plotting the difference in absorbance at 244 and 269 nm (difference is zero for THC) against the concentration of DCFS. Similarly for the determination of THC, the difference in absorbance at 266.8nm and 290 nm (difference is zero for DCFS) was plotted against the concentration of DCFS [21].

Two methods for simultaneous estimation of THC and DCFP in combined dosage form have been described. In the first method; the wavelengths 276.5 nm ( $\lambda_{\text{max}}$  of DCFP) and

**Table 1. Spectrophotometric methods used for determination of thiocolchicoside alone and in combined dosage form.**

Sr. No.	Name of drug	Sample matrix	Method		Detection ( $\lambda$ max) nm	Linearity range	Comments	Ref.
1	THC	Capsule	A	Zero order	259.0 nm	THC- 2.5 – 50 $\mu$ g/mL	Simple and accurate	[13]
			B	First order	252.0 nm			
			C	Second order	260.0 nm			
			D	AUC	254.0 -264.0 nm			
2	THC	Capsule	A	Zero order	259.8 nm		Simple and economical	[14]
			B	AUC	269.8-259.8 nm			
3	THC	Capsule	Zero order		257.0 nm	THC-2-38 mg/mL	Simple	[15]
4	THC+DCF	Capsule	A	Q-value/Analysis	264 nm and 259 nm	THC- 2-40 $\mu$ g/mL DCF- 1-50 $\mu$ g/mL	Economic	[16]
			B	Simultaneous equation				
5	THC+DCF	Tablets	A	Absorption correction	264.99 nm and 373.84 nm	DCF-25-75 $\mu$ g/mL THC-4-12 $\mu$ g/mL	Higher linearity range	[17]
			B	Area Under Curve (AUC)	DCF 252.56-260.59 nm and THC 278.51-285.53 nm			
6	THC+DCF	Tablets	A	Ratio Derivative	268.78 & 355.62 nm	12.5-75 $\mu$ g/ml	Different linearity and complex	[18]
			B	Dual Wavelength	252.47nm and 260.74 nm	25-75 $\mu$ g/mL		
7	THC+DCF	Capsule	Simultaneous equation		259 nm and 277nm	THC 4 - 24 $\mu$ g/mL & DCF 10 - 60 $\mu$ g/mL	Simple and economical	[19]
8	THC+DCF	Capsule	Multicomponent mode		254,259,265,271,286	THC-20-100 $\mu$ g/mL and DCF-20-100 $\mu$ g/mL	Economical	[20]
9	THC+DCF	Capsule	A	Absorbance correction method	276.6 nm and 372.8 nm	DCF-5-30 $\mu$ g/mL and THC-10-60 $\mu$ g/mL	Sensitive	[21]
			B	First order derivative	278.6 nm and 243.2 nm			
			C	Dual wavelength	244nm and 269 nm			
10	THC+DCF	Capsule	A	Simultaneous Equation method	260.0 nm THC and 276.5 nm DCF	THC-1-10 $\mu$ g/mL and DCF- 6.25-62.5 $\mu$ g/mL	Easy and economical	[22]
			B	Absorbance Correction method	276.5 nm DCF, 373.0 nm is isoabsorptive point of THC			
11	THC+ DCF	Capsule	A	absorption correction method	THC-397.80 nm and DCF- 271.0 nm	THC-5-35 $\mu$ g/mL and 10-70 $\mu$ g/mL	Accurate	[23]
12	THC+ ACE	Tablet	A	AUC	264.5-254.5 nm for THC and 279.0-269.0 nm for ACE	THC and DCF- 4-36 $\mu$ g/mL	Precise and selective	[24]
13	THC+ ETD	Tablet	Absorbance Ratio Method		260 nm and 232nm	ETD-15-100 $\mu$ g/mL and THC-2-20 $\mu$ g/mL	Selective	[25]
14	THC+ DXKET	Tablet	Absorbance correction		370 nm and 255 nm	THC 4-40 $\mu$ g/mL and DKET 5- 50 $\mu$ g/mL	Accurate and sensitive	[26]
			First order derivative		232 nm and 242 nm			
15	THC+ KET	Tablet	First order derivative		THC 233.0 nm and KET 243.0 nm	THC- 3-15 $\mu$ g/mL and KET-12-60 $\mu$ g/mL	Simple and selective	[27]
			Absorbance correction		THC 372.0 nm, KET 260.0 nm.	THC-1-5 $\mu$ g/mL and KET- 10-50 $\mu$ g/mL		
16	PCM+ THC+ ACE	Tablet	Multicomponent mode		PCM-256 nm, THC 258 nm, ACE 270 nm.	PCM- 1-25 $\mu$ g/mL, THC- 2.5-25 $\mu$ g/mL and ACE- 1-25 $\mu$ g/mL	Economical	[28]
17	PCM+ THC+ ACE	Tablet	Multicomponent mode		PCM 249 nm, THC 258 nm, ACE 276 nm.	PCM -5-25 $\mu$ g/mL THC-1-5 $\mu$ g/mL and ACE- 1-5 $\mu$ g/mL	Economical	[29]
18	THC	Injection	Spectrofluorimetry		289 nm and 366 nm.	THC-1-10 $\mu$ g/mL	Specific	[30]

373.0 nm (where DCFP does not interfere in absorbance of THC) were selected for analysis by proposed method, while the second method; involves formation of simultaneous equation at 260 and 275.5 nm, using 0.1 N NaOH as solvent [22].

A simple UV-Spectrophotometric absorption correction method has been reported, which is based upon determination of THC at 397.80 nm and DCF-P at 271.0 nm, in distilled water [23].

Only one AUC method is available for simultaneous analysis of THC with ACE. The method employed selection of area under curve in wavelength region of 264.5 - 254.5 nm for THC; 279.0 - 269.0 nm for Aceclofenac and solving the equations [24].

An absorbance ratio (Q-analysis) UV spectrophotometric method has been developed for the simultaneous determination of THC and ETD from the combined tablet dosage form using 232nm as isoabsorptive point and 0.1 N HCl as solvent [25]. Two methods, *i.e.* Absorbance correction and First order derivative spectroscopy are also reported for simultaneous estimation of THC with DXKET using methanol as solvent. The amount of drug determined by proposed methods ranges from 99.8 to 100.2 % for both. The method reports recovery study values of THC and DXKET 99.8% and 100.2%, respectively for both the methods with RSD less than 2 %. [26] Few other methods, First order derivative spectroscopic (% label claimed 99.94 % for THC and 99.60 % for KET) and Absorbance correction method (% label claimed 100.32 % for THC and 99.95 % for KET) is presented in literature for estimation of THC with KET using distilled water as solvent [27]. Two methods using multicomponent mode of analysis are also reported for THC in combined dosage form with PCM and ACE. The accuracy of the both methods was evaluated by percentage recovery (by standard addition method) of all three drugs and it was in the range of 99.84 - 101 % with values for SD and % RSD of < 2% [28, 29].

A single Spectrofluorometric method was reported for estimation THC in injection which is based on the oxidation of THC with cerium (IV) to produce cerium (III), whose fluorescence was monitored at 366 nm [30].

## Chromatography

### High-Performance Liquid-Chromatography (HPLC) [31-54]

About Twenty four HPLC methods were reported for determination THC in combined pharmaceutical formulations. Table 2. shows the summary of the reported HPLC methods indicating the mobile phase used for determination, sample matrix,  $\lambda_{\max}$  and linearity ( $r^2$ ).

Table 3. Summarizes the column with specifications for analysis by HPLC methods along with the conditions used such as flow rate, temperature, detector, analysis type *etc.*

### High-Performance Thin-layer Chromatography [55-63]

Nine simple HPTLC methods have been reported for simultaneous estimation of THC in combined dosage form with ACE, DCF, DXKET, LOR, ETR and ETD. Table 4. shows the summary of the reported HPTLC methods.

## Estimation of THC Using Hyphenated Techniques and in Biological Matrices

In Hyphenated techniques, Liquid-chromatography (LC), Gas-chromatography (GC) or capillary electrophoresis is linked to spectroscopic techniques, *e.g.* MS, FT-IR, NMR *etc.* resulting in new advanced techniques like CE-MS, GC-MS, MS-MS, LC-MS LC-MS-MS LC-NMR *etc.* Such techniques provide structural information and identification of drugs and degradation compounds. Amongst these analytical techniques, LC-MS is reported to be extensively used technique. An LC-ESI-MS<sup>n</sup> analysis is performed for forced degradation study of THC to assess its inherent chemical stability. Method reports five degradation products of THC and the degradants were characterized by MS, NMR and IR spectroscopic techniques confirming it by synthesis of degradants. The study elucidated that hydrolysis and oxidation are major degradation pathways for THC. Five degradation products were resulted and the DPs were: **D1SO**-(N-[1,2-dimethoxy-10-methylsulphoxide-9-oxo-3-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide), **D1SO2**-(N-[1,2-dimethoxy-10-methylsulphone-9-oxo-3-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide), **D2**([1,2-dimethoxy-10-methylsulphonyl-9-oxo-3-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amine), **D3** (N-[1,2-dimethoxy-3-hydroxy-10-methylsulphonyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide or 3-O-demethylthiocolchicine), **D4** ([1,2-dimethoxy-3-hydroxy-10-methylsulphonyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amine or N-deacetyl-3-O-demethylthiocolchicine). Products **D1SO** and **D3** could be considered as the indicators of THC stability and they will be introduced in the development and validation of HPLC-UV stability-indicating methods for determination of THC in drug substance and drug product [64].

Quantitative determination of drugs and their metabolites from biological matrices such as blood, plasma, serum, or urine play vital role in drug discovery and development. Chromatographic methods like HPLC or GC have been widely used for the bioanalysis of small molecules and Liquid-Chromatography coupled with Triple Quadrupole Mass Spectrometry (LC/MS/MS) is the single most commonly used technology amongst it. A highly sensitive Liquid-Chromatography-tandem Mass Spectrometry (LC-MS-MS) method has been illustrated for the determination of 3-desmethylthiocolchicine in human plasma to evaluate the bioequivalence of thiocolchicoside after oral administration. The study divulge that thiocolchicoside is rapidly converted to 3-desmethylthiocolchicine (possibly partially in the acidic stomach juices) during absorption and during the first-pass effect through the liver [65]. A radioimmunoassay technique is also reported in literature to study the single-dose bioavailability of thiocolchicoside by oral and intramuscular route. This method report to relative bioavailability of both oral formulations was approximately 25%, compared to the intramuscular formulation. An another study based on radioimmunoassay using cross-reacting colchicines- specific polyclonal antibodies and [<sup>3</sup>H] colchicines as marker is also depicted. In the study cross-reactivity tests were performed for some colchicine analogues, but not for

**Table 2. HPLC methods for Thiocolchicoside.**

Sr. No.	Name of drug	Sample matrix	Mobile phase composition	Detection ( $\lambda$ max) nm	Linearity	Rt (in. min)		Ref.
1	THC+GF	Tablet MIX-I	Methanol : 0.035 M phosphate buffer (50:50, v/v) pH 4.5	400	THC-0.2 – 2 $\mu$ g/mL GF and FN -20–200 $\mu$ g/mL	THC-2.56 GF-4.5	Good resolution with low Rt	[31]
	THC+FN	Tablet MIX-II	Methanol: 0.03M phosphate buffer (70:30, v/v), pH 4			THC-3.84 FN-5.88		
2	THC+ACE	Tablets	Acetonitrile: Water: Methanol (70:20:10, v/v)	260	THC-4 - 20 $\mu$ g/mL ACE-40-200 $\mu$ g/mL	THC-3.36 ACE-4.12	Low Rt and Robust	[32]
3	THC+ACE	Tablets	Acetonitrile: Water: 0.025M pot. dihydrogen orthophosphate buffer (pH adjusted to 3.0 with OPA) (70:10:20, v/v)	260	THC-1-6 $\mu$ g/mL ACE-25-150 $\mu$ g/mL	THC-2.70 ACE-4.76	Simple and sensitive	[33]
4	THC+ACE	Tablets	Phosphate buffer :Acetonitrile (40:60 v/v)	261	THC 2 - 12 $\mu$ g/mL ACE 25-150 $\mu$ g/mL	THC-2.17 ACE-4.80	Simple mobile phase	[34]
5	THC+ACE	Tablets	Acetonitrile: buffer of pH 6 (42:58, v/v)	261	ACE 25-125 $\mu$ g/mL THC 1-6 $\mu$ g/mL	THC-4.15 ACE-4.88	Low resolution	[35]
6	THC+DCF	Capsules	Acetonitrile: Methanol: Water (35:15; 50, v/v), pH adjusted to 3.5 with orthophosphoric acid.	286	THC 10-100 $\mu$ g/mL DCF- 20-100 $\mu$ g/mL	THC-3.3 DCF-4.0	Low resolution	[36]
7	THC+DCF	Tablet	Water (pH 9.2adjusted with di-Potassium hydrogen Phosphate) (60: 40, v/v)	223	THC 50-150 $\mu$ g/mL DCF- 50-150 $\mu$ g/mL	DCF-3.229 THC-4.999	Low resolution	[37]
8	THC+LOR	Tablets	Methanol: THF: acetate buffer (60: 10: 30, v/v); pH adjusted to 5.5 with glacial acetic acid	250	LOR 0.2 to 80 $\mu$ g/mL THC 0.1 to 40 $\mu$ g/mL	LOR- 4.08 THC- 3.36	Low resolution	[38]
9	THC+LOR	Tablet	Methanol: Acetate buffer (PH-4.6) :THF(50:35:15, v/v)	375	LOR -4-100 $\mu$ g/mL THC-2-50 $\mu$ g/mL	LOR-.400 THC- 2.92	Complex mobile phase	[39]
10	THC+LOR	Tablet	Ammonium Dihydrogen Phosphate buffer (pH 7.3 with TEA): Methanol (45:55, v/v)	290	LOR-0.24 - 120 $\mu$ g/mL THC-0.23 - 120 $\mu$ g/mL	LOR -9.40 THC-2.96	Time consuming	[40]
11	THC+LOR	Tablet	Sodium Phosphate buffer( pH 6.8 adjusted with NaOH): ACN (35:65% v/v)	298	LOR - 1- 50 $\mu$ g/mL THC-5 - 25 $\mu$ g/mL	LOR -4.50 THC -3.40	Low resolution	[41]
12	THC+KET	Tablet	Acetonitrile: Water: Phosphate buffer (pH 3.0) (60:30:10, v/v)	260	THC-4-20 $\mu$ g/mL KET-20-100 $\mu$ g/mL	THC-2.70 KET-4.90	Low resolution	[42]
13	THC+KET	Tablet	Acetonitrile: Water (60:40, v/v)	300	KET-80-120 $\mu$ g/mL THC-6.4-9.6 $\mu$ g/mL	THC-3.7 $\pm$ 0.1 KET-7.90 $\pm$ 0.1	Economical and good resolution	[43]
14	THC+DXKET	Tablet	Methanol: Sodium acetate buffer (pH 5 with Glacial acetic acid) (70:30, v/v)	265	DXKET-5-30 $\mu$ g/mL THC-1 –10 $\mu$ g/mL	THC-3.0133 DXKET 6.013	Economical And well separation	[44]
15	THC+DXKET	Tablet	Methanol: Phosphate buffer (pH adjusted to 4.5 with OPA) (65:35 v/v)	260	THC 4-24 $\mu$ g/mL DXKET-5-30 $\mu$ g/mL	THC-3.02 DXK 8.91	Economical and well separation	[45]
16	THC+ETR	Tablet	Trifluoroacetic acid buffer (pH 2.6): Acetonitrile (75:25, v/v)	220	ETR 20-160 $\mu$ g/mL THC 2 -16 $\mu$ g/mL	ETR-6.6 THC-3.1	Simple	[46]
17	THC+ETR	Tablet	1 mL TFA in 2 liter milli-Q water) and Acetonitrile (75:25 v/v)	258	ETR 1.598-90 $\mu$ g/mL THC 4.95-12 $\mu$ g/mL	THC-3.37 ETR-8.62	Simple	[47]

(Table 2) contd....

Sr. No.	Name of drug	Sample matrix	Mobile phase composition	Detection ( $\lambda$ max) nm	Linearity	Rt (in. min)		Ref.
18	THC+ETR	Tablet	Phosphate buffer (PH 6, adjusted with orthophosphoric acid) and Methanol (30:70 v/v)	255	ETR 40-80 $\mu$ g/mL THC 2-6 $\mu$ g/mL	THC-2.50 ETR-4.60	Robust	[48]
19	THC+ETD	Tablet	Methanol and Phosphate buffer pH 6, (85:15 v/v)	259	ETD 16-96 $\mu$ g/mL THC 4-24 $\mu$ g/mL	ETD4.39 $\pm$ 0.10 THC3.52 $\pm$ 0.10	Simple mobile phase but less separation	[49]
20	THC+ETD	Tablet	Acetonitrile: 20mM potassium dihydrogen phosphate buffer (65:35 v/v)	257	ETD 25-150 $\mu$ g/mL THC 0.5-10 $\mu$ g/mL	THC-2.240 ETD-7.141	Robust, Good resolution	[50]
21	THC+ETD	Tablet	Acetonitrile: Potassium dihydrogen phosphate buffer (pH 3.0) (50:50, v/v)	255	ETD 100-600 $\mu$ g/mL THC 1-6 $\mu$ g/mL	ETD-4.27 THC-2.6	high linearity values	[51]
22	THC+PCM	Tablet	Potassium Dihydrogen phosphate: Methanol (40:60, v/v)	247	PCM 250-750 $\mu$ g/mL THC 1-3 $\mu$ g/mL	PCM-3.27 THC-5.50	Economical with low Rt	[52]
23	THC+ACE+PCM	Tablet	Acetonitrile: Water (30: 70, v/v)	263	PCM-50-175 $\mu$ g/mL ACE-10-35 $\mu$ g/mL THC-0.40-1.4 $\mu$ g/mL	PCM- 2.51 THC-3.55 ACE -5.20	Simple and economical	[53]
24	THC+ACE+PCM		Buffer of pH 6.5 and Acetonitrile in Gradient elution.	300	PCM 1250-3750 $\mu$ g/mL, THC 20-60 $\mu$ g/ mL ACE 250-750 $\mu$ g/ mL	PCM- 2.70 THC-3.95 ACE -9.91	Well resolution	[54]

3-desmethylthiocolchicine (aglycone part of thiocolchicoside) [66]. Similarly, a capillary gas chromatography-mass spectrometry (GC-MS) method is presented for THC following enzymatic hydrolysis of thiocolchicoside to its aglycone (3-demethylthiocolchicine). The study reports oral bioavailability of the capsule formulation was 1.06  $\pm$  0.39 relative to the tablet formulation [67].

A bioequivalence study has been reported for fixed dose combination of thiocolchicoside and lornoxicam in twenty four healthy male volunteers using liquid chromatography-tandem mass spectrometry (LC-MS-MS) method [68]. Similarly, a bioanalytical method is also reported for simultaneous determination of THC and lornoxicam from pharmaceutical formulation in human plasma which uses an isocratic RP-HPLC method [69].

A bioanalytical method for the simultaneous estimation of active metabolite of thiocolchicoside (3-demythylthiocolchicine) and diclofenac in human plasma by means of LC-MS/MS is also reported. The method employed Reversed-Phase phenomenonex gemini C<sub>18</sub> column with a mobile phase containing Methanol: Water (containing 0.2% formic acid) (9:1, v/v). The calibration curves were linear over the range of 1 to 50 ng/mL for 3-demythylthiocolchicine and 25 to 2500 ng/mL for DCF with the lower limit of quantification validated at 0.5 ng/mL for 3-demythylthiocolchicine and 5 ng/mL for DCF [70].

One UV-spectrophotometric method was found for simultaneous estimation of THC with ETD using methanol as solvent by simultaneous equation and Q-absorbance ratio method in tablets and in spiked human urine. The authors

have also performed force degradation study for both drugs and reported that THC is only subjected to alkaline hydrolysis indicating change in  $\lambda_{\max}$  of THC. The authors also compared their proposed method with reference method from literature and concluded that, the present method is better than the existing reference method because 0.1 N NaOH is used in reference method which can be responsible for degradation of THC in alkaline medium and interfere with the  $\lambda_{\max}$  of THC [71].

#### Stability-Indicating Methods (SIM) for Determination of THC [72-83]

About thirteen stability-indicating methods have been reported for determination of THC in bulk substances and pharmaceutical formulations using different analytical techniques. Among it, three methods are for estimation of THC alone and ten of them described for stability-indicating nature of method for combined dosage form. Maximum of 14-15% were reported using HPLC, 3-4 % were based on HPTLC and 1 - 2% were LC/MS methods. Table 5. shows the reported stability-indicating methods for THC; illustrating sample matrix,  $\lambda_{\max}$ , linearity range and retention time/factor.

#### Official Methods for Analysis of Thiocolchicoside [84]

THC is official drug in Indian Pharmacopoeia 2010. Two HPLC methods are described for assay of THC from pharmaceutical formulations and its related substances.

**Table 3. HPLC chromatographic columns and optimized analytical parameters.**

Sr. No.	Name of drug	Column	Internal diameter and Partical Size	Temp.	Detector	Flow rate	Mode of analysis	Diluents	Ref.
1	THC+GF	C18 (Waters symmetry)	i.d.-250×4.6mm; 5-µm	RT	UV detector	1mL/min	Isocratic	Methanol	[31]
	THC+FN								
2	THC+ACE	C18 (Intersil)	250 mm x 4.6 mm, 20-µm	RT	Variable wave-length detector	1mL/min	Gradient	Methanol	[32]
3	THC+ACE	C18(Thermo)	i.d.-250x4.6 mm;5-µm	Ambient	UV detector	1mL/min	Isocratic	M.P	[33]
4	THC+ACE	C18 (Waters symmetry)	i.d.-150×4.6mm; 5-µm	RT	UV detector	1mL/min	Isocratic	Methanol	[34]
5	THC+ACE	C18 (Thermo Hypersil BDS)	i.d.-250X4.6 mm; 5µm	RT	diode array detector	0.6mL/min.	Isocratic	MP	[35]
6	THC+DCF	C18 (Intersil)	i.d.-250 x 4.6 mm;5µm	RT	Variable wave-length detector	1mL/min	Gradient	Water	[36]
7	THC+DCF	C18 (Waters Symmetry)	i.d.250X4.6 ;5µm	30°C	PDA detector	1mL/min	Isocratic	M.P	[37]
8	THC+LOR	C18 (Waters Symmetry)	i.d.-250 mm × 4.6 mm;5.0 µm	50 °C	PDA detector	0.75mL/min	Isocratic	MP	[38]
9	THC+LOR	C18(Varian)	i.d.-250 mm x 4.6 mm ; 5 µm	25± 3°C.	UV detector	0.5 mL/min	isocratic	Methanol	[39]
10	THC+LOR	C18 (Inertsil ODS 3V)	i.d.-250 x 4.6 mm; 5 µm	30°C	PDA detector	1.5 mL/min	isocratic	MP	[40]
11	THC+LOR	C8 (X terra),	i.d.-4.6 x 250mm; 5 µm,	Ambient	UV detector	1mL/min	isocratic	MP	[41]
12	THC+KET	C18 (Thermo scientific)	i.d.-250×4.6 mm; 5µm	25 ±1 °C	UV detector	1mL/min	Isocratic	MP	[42]
13	THC+KET	C18	i.d.-150 x 4.6 mm; 5µm	RT	UV detector	1mL/min.	Isocratic	MP	[43]
14	THC+DXKET	C18 (HS, HiQ sil)	i.d.-250 x 4.6 mm; 5µm	Ambient	PDA detector	1mL/min.	Isocratic	MP	[44]
15	THC+DXKET	RP-18e (Purosphere STAR)	i.d.-250× 4mm ;5µm	RT	UV detector	1.0 mL/min.	Isocratic	MP	[45]
16	THC+ETR	C-18 (BDS Hypersil)	i.d.-250 X4.6 mm; 5-µm.	Ambient	UV detector	1.5 mL/min	Isocratic	MP	[46]
17	THC+ETR	C18 (RP-select B Li-chrospher)	i.d.-250 x4.6mm;5 µm	---	PDA	1.5 mL/min	Isocratic	MP	[47]
18	THC+ETR	C18 (InertSil ODS-3)	i.d.-250x4.6 mm; 5µm	--	UV detector	1.2 mL/min	Isocratic	MP	[48]
19	THC+ETD	C-18 (Phenomenex)	i.d.-250 mm × 4.60 mm; 5 µm	--	UV detector	0.8mL/ min	Isocratic	MP	[49]
20	THC+ETD	C18 (HiQ sil HS)	i.d-250 x 4.6 mm; 5 µm	Ambient	UV detector	1mL/min	Isocratic	MP	[50]
21	THC+ETD	C18 (Symmetry)	i.d.-150X4.6 mm ;5µm	25 ±1°C	UV detector	1.0 mL/min	Isocratic	Methanol	[51]
22	THC+PCM	C18 (BDS hypersil)	i.d.-250mm × 4.6mm, 5µm	Ambient	UV detector	1.0 mL/min	Isocratic	MP	[52]
23	THC+ACE+PCM	C18 (HiQ Sil)	i.d.-250 mm × 4.6 mm, 5.0 µm	Ambient	UV detector	1.0 mL/min	Isocratic	Methanol	[53]
24	THC+ACE+PCM	C18 (inertsil ODS)	i.d-150x4.6mm ;5 µm	Ambient	PDA detector	1.0 mL/min	Gradient	MP	[54]



Table 4. HPTLC methods for determination of thicolchicoside.

Sr. No.	Name of Drug	Sample matrix	Mobile phase composition	Detection ( $\lambda_{max}$ ) nm	Linearity	Rf	Comments	Ref.
1	THC+DCF	Capsule	Toluene: Ethyl acetate: Methanol (5:3:2 v/v)	285	50-300 ng/band for both	THC-0.17 DCF-0.53	Simple and economical	[55]
2	THC+DCF	Capsule	Toluene: Acetone: Methanol: Formic acid (5:2:2:0.01 v/v/v/v)	280	THC 160-800 ng/band DCF 1000-5000 ng/band	THC-0.29±0.02 DCF-0.71±0.02	Complex mobile phase with high linearity values	[56]
3	THC+ACE	Tablet	Toluene: Ethyl acetate: Methanol: Glacial acetic acid (4: 6: 2: 0.5 v/v).	255	THC 6–21 ng/band ACE 10-35 ng/band	THC 0.16 ACE 0.79	High resolution	[57]
4	THC+ACE	Tablet	Methanol:Chloroform: Water 9.6:0.2:0.2 v/v)	254	30–180 ng/band THC 750–4500 ng/band ACE	THC 0.70 ± 0.05 ACE 0.83 ± 0.05	Solvents with diverse polarity	[58]
5	THC+ACE		Methanol: Chloroform: Water (9.6: 0.2: 0.2 v/v)	254	THC 30-180 ng/band ACE 750-4500 ng/band	THC 0.70 ± 0.05 ACE 0.83 ± 0.05	Low resolution	[59]
6	THC+DXKET	Tablet	Toluene: Methanol: Ethyl Acetate (6: 2.5: 0.5, v/v)	280	THC-100-800 ng/band DXKET-600-4800 ng/band	THC 0.33±0.011 DXKET 0.61 ± 0.007	High linearity range	[60]
7	THC+DXKET	Tablet	Toluene: Ethyl acetate: Methanol (5:3:2 v/v)	286	THC-50-350 ng/band DXKET-100-700 ng/band	THC 0.10 DXKET 0.40	Low Rf of THC (not acceptable)	[61]
8	THC+LOR	Tablet	Methanol:Chloroform: Water (9.6:0.2:0.2 v/v)	377	THC 30-180 ng/band LOR 60-360 9ng/band	THC 0.58±0.05 LOR 0.85±0.05	Water not suitable as solvent for NP	[62]
9	THC+ETR	Tablet	Ethyl acetate: Methanol (8 :2 v/v)	290	THC 100–500 ng/band ETR 50–250 ng/band	THC 0.17 ETR 0.70	Low Rf value for THC	[63]

Table 5. Stability-indicating HPLC and HPTLC methods for determination of thicolchicoside.

Sr.No.	Name of Drug	Sample matrix	Mobile phase	Detection ( $\lambda_{max}$ ) nm	Linearity	Rt/Rf	Comments	Ref.
1	THC	Capsule	Acetonitrile: Water (70:30)	286	THC- 0-10µg/mL	3.35 min.	Simple	[72]
2	THC	Capsule	Acetonitrile: Phosphate Buffer pH 3.5, (70:30 % v/v)	260	80-120 % of THC label claim	2.24 min.	All parameters of forced degradation are not explored	[73]
3	THC	Capsule	Methanol:Water (70 : 30 v/v)	377	100–600 ng per band.	0.60 ± 0.02	Simple and economical	[74]
4	THC+ETD	Tablet	Acetonitrile: Potassium dihydrogen phosphate buffer pH 6.8 (70:30% v/v)	260	THC-4.012 - 12.03 µg/mL ETD-249.09 - 747.29µg/mL	THC-3.05min ETD-2.10min.	Degradation not in detail	[75]
5	THC+ACE	Tablet	Potassium phosphate monohydrate buffer (pH-5.0): Acetonitrile: Methanol in (40:20:40 % v/v)	263	THC -7.5 to 25µg/mL ACE-100-300µg/mL	THC-2.8 min ACE-4.2 min	Not completely explored	[76]
6	THC+DCF	Tablet	Solvent A (5 mM sodium dihydrogen phosphate, pH 2.5) and Solvent B (Methanol)	258	THC-40.48–121.43 µg/mL DCF 24.91–74 µg/mL	THC-5.8 min. DCF-11.0 min.	Good resolution	[77]

(Table 5) contd....

Sr.No.	Name of Drug	Sample matrix	Mobile phase	Detection ( $\lambda$ max) nm	Linearity	Rt/Rf	Comments	Ref.
7	THC+DCF	Tablet	Methanol: Acetonitrile : Phosphate buffer (40:20:40 v/v at pH 5.0)	263	THC-7.5-25 $\mu\text{g/mL}$ DCF-100-300 $\mu\text{g/mL}$	THC-2.8 min ACE-4.2 min	Separation not suitable in case of impurities	[78]
8	THC+ACE	Tablet	Methanol: Acetonitrile: THF: Acetate buffer (56:4:10:30 v/v) pH adjusted to 6.5 with Acetic acid	312	THC-0.4- 24 $\mu\text{g/mL}$ ACE-10 -600 $\mu\text{g/mL}$	THC-4.7 min. ACE-6.3min.	Complex mobile phase	[79]
9	THC+ACE	Tablet	(A) 10 mM Ammonium acetate pH 5.00 buffer and (B) Acetonitrile: Water (70:30 v/v)	265	ACF-80-280 $\mu\text{g/mL}$ THC- 6.4–22.4 $\mu\text{g/mL}$	THC-13.29min ACF-2.20 min	Higher Rt	[80]
10	THC+ACE	Tablet	5% ammonium acetate buffer and methanol (40:60 v/v) pH 5 with OPA	276	THC-4.8 -7.2 $\mu\text{g/mL}$ ACE-63.8 -96 $\mu\text{g/mL}$	THC-0.697 min. ACE-1.125 min.	Very low Rt	[81]
11	THC+KET	Tablet	Methanol: Toluene: Benzene (2.5:3.5:4 v/v)	260	125-750 ng/band THC 1000-6000 ng/band KET	THC 0.35 and KET 0.65 min.	Well resolved components	[82]
12	THC+PCM+DCF	Capsule	Acetonitrile: Phosphate buffer adjusted pH 3 with OPA	228	100- 500 $\mu\text{g/mL}$ PCM, THC and DCF	PCM -5.3;THC-9.61;DCF-21.47	Economical but not suitable as stability indicating	[83]

## CONCLUSION

The present review epitomizes numerous analytical methods applied for the determination of THC. A great number of studies including UV/Vis-Spectrophotometry, Spectrofluorimetry, HPLC, HPTLC, LC-ESI-MS, LC-MS, GC-MS *etc.* were reported for analysis of thiocolchicoside in bulk and in its combined pharmaceutical formulations as well as in plasma. It has been found that Liquid-Chromatography with UV-detection is the most studied for the determination of THC in bulk as well as pharmaceutical dosage forms while LC-MS and LC-MS/MS are the widely used for its estimation from plasma and other biological fluids to determine its pharmacokinetics as well as for bioavailability and bioequivalence studies. Few chromatographic methods like HPTLC and GC-MS are also published in literature. Certain Spectrophotometric methods in UV-Visible as well as fluorimetry are most often used for quantification of THC.

## ABBREVIATIONS

ACN	=	Acetonitrile
ACE	=	Aceclofenac
CE	=	Capillary Electrophoresis
DCFS	=	Diclofenac Sodium
DCFP	=	Diclofenac Potassium
DPs	=	Degradation products
DXKET	=	Dexketoprofen
ESI-MS	=	Electron Spray Ionization Mass Spectrometry
ETD	=	Etodolac

ETR	=	Etoricoxib
FN	=	Floctafenine
GC	=	Gas Chromatography
GC-MS	=	Gas Chromatography- Mass Spectroscopy
GF	=	Glafenine
HPLC	=	High Performance Liquid Chromatography
HPTLC	=	High Performance Thin Layer Chromatography
IM	=	Intra-muscular
IR	=	Infrared
KET	=	Ketoprofen
LBP	=	Low Back Pain
LC	=	Liquid Chromatography
LOD	=	Limit of Detection
LOQ	=	Limit of Quantitation
LOR	=	Lornoxicam
M	=	concentration (mol/L)
MP	=	Mobile Phase
MS	=	Mass Spectrometry
nm	=	nanometer
NMR	=	Nuclear Magnetic Resonance
NSAIDs	=	Non-Steroidal Anti-inflammatory Drugs
OPA	=	Orthophosphoric Acid
PCM	=	Paracetamol

RP-HPLC = Reversed Phase-High Performance Liquid Chromatography  
 R.S.D. = Relative Standard Deviation  
 TEA = Triethylamine  
 TFA = Trifluoroacetic Acid  
 THC = Thiocolchicoside  
 TLC = Thin Layer Chromatography  
 UV-Vis = Ultraviolet-Visible

### CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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