WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 18, 476-487. Review Article ISSN 2277–7105

AN OVERVIEW OF REVIEW ARTICLE: DESIGN AND ASSESSMENT OF A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ETODOLAC AND THIOCOLCHICOSIDE

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Article Received on
28 July 2025,

Revised on 18 August 2025,
Accepted on 07 Sept. 2025

DOI: 10.20959/wjpr202518-38301

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ABSTRACT

The aim of this work is to develop and validate a simple, sensitive, rapid and accurate and less time consuming validated RP-HPLC method for Etodolac and Thiocolchicoside in pharmaceutical dosage form. The method was developed and validated for various parameters as per ICH guidelines. The results obtained were within the acceptance criteria. The proposed method was applied for the determination of Etodolac and Thiocolchicoside in marketed formulation. The assay results confirm with the label claim of formulation. Hence, the proposed method was found to be satisfactory and could be used for the routine analysis of Etodolac and Thiocolchicoside in combined tablet dosage forms.

KEYWORDS: Dosage forms, Etodolac, RP-HPLC, Thiocolchicoside.

INTRODUCTION

ANALYTICALCHEMISTRY

It is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparations.

There are two main types of analysis

Qualitative analysis*:* It reveals the chemical identity of a substance. Various qualitative tests are detection of evolved gases, formation of precipitates, limit tests, color change reactions, melting point and boiling point tests etc. Qualitative analysis is necessary to be carried out before quantitative analysis.

Quantitative analysis: It determines the amount or concentration of constituents in the sample. The techniques are based on measurement of spectroscopic properties, electrical measurement, chemical reaction etc.

CHROMATOGRAPHY

Chromatography is the method of separation that finds applications in all branches of science. The separated species appeared as colored bands on the column hence the name of the process (Greek *Chroma* meaning "color" and *graphein* meaning "writing"). It was first invented by **M. Tswettin** 1906.Since then, has undergone tremendous modifications and now a day's various types of chromatographic techniques have been developed.

Chromatography may be defined as a non-destructive procedure for separating mixture of components into individual components through equilibrium distribution between two phases.

Classification of Chromatographic Process

Adsorption Chromatography

- Gas-solid chromatography
- Liquid-column chromatography
- High performance liquid chromatography
- Thin- layer chromatography

Partition Chromatography

- Gas-liquid chromatography
- Super critical fluid chromatography
- Liquid-liquid chromatography
- Paper chromatography
- High performance liquid chromatography

Permeation Chromatography

- Size exclusion chromatography

Affinity Chromatography

- DNA affinity chromatography

Electrophoresis

- Capillary electrophoresis
- In the modern pharmaceutical industry, HPLC is a major analytical tool applied at all stages of drug discovery, development and production. In HPLC one phase is fixed (stationary phase) and other is mobile (mobile phase). The mobile phase passes over the stationary phase and transport components of the mixture at different speeds in the direction of flow of mobile phase. The separation of components is a result of differential affinity of the components for the mobile phase and stationary phase.

HPLC is also known as high performance, high pressure and high speed liquid chromatography.

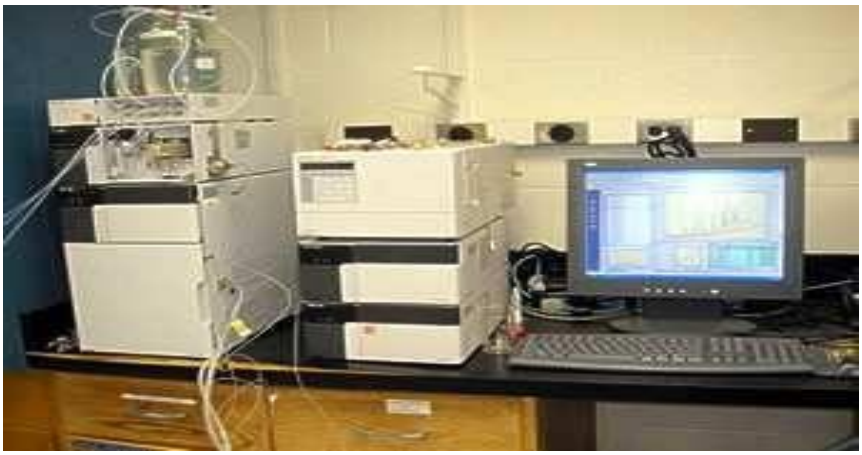

Fig. 1: Diagram of HPLC.

ANALYTICAL METHOD VALIDATION

Method validation can be defined as (International Conference of Harmonization) "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

Steps followed for validation procedures

1. Proposed protocols or parameters for validations are established.
2. Experimental studies are conducted.
3. Analytical results are evaluated.
4. Statistical evaluation is carried out.
5. Report is prepared documenting all the results.

OBJECTIVE

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH, typical analytical performance characteristics that should be considered in the validation of the types of methods are.

1. Accuracy
2. Precision
3. Specificity
4. Limit Of Detection
5. Limit Of Quantification
6. Linearity
7. Ruggedness
8. Robustness
9. System suitability

AIM AND OBJECTIVE

AIM

To develop and validate a simple, sensitive, rapid and accurate and less time consuming validated RP-HPLC method for Etodolac and Thiocolchicoside in pharmaceutical dosage form.

OBJECTIVE

- To develop new, simple, sensitive, accurate and economical analytical method for simultaneous estimation of Etodolac and Thiocolchicoside.
- To validate the proposed method.
- To apply the proposed method for analysis of these drugs in pure and combined dosage form.

DRUG PROFILE

Etodolac

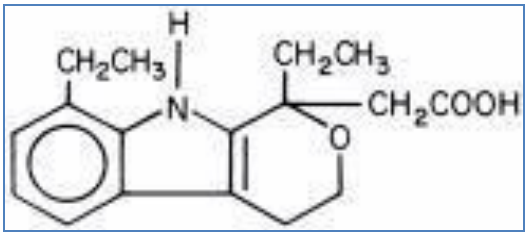

Fig. 2: Chemical structure of etodolac.

IUPAC NAME: (1,8- Diethyl - 1,3,4,9- tetra hydro pyrano[3,4 –b] indol - 1- yl) acetic acid

MOLECULAR FORMULA: $C_{17}H_{21}NO_3$.

MOLECULAR WEIGHT: 287.35 g/ mol

DESCRIPTION: White crytstalline powder

CATEGORY: Non-steriodal anti- inflammatory agents

MELTING POINT: 145-148°C

pKa: 4.65

PARTITION COEFFICIENT: 11.4 at pH 7.4.

SOLUBILITY: Soluble in alcohols, chloroform, DMSO

STORAGE: Tablets should stored in room temperature.

Adverse Effect

- The common sideeffects of Etodolac are Dyspepsia, abdominal pain, Diarrhoea, Flatulence, Nausea, Constipation, Gastritis, melena, vomiting.
- **Mechanism of action:** Similar to other NSAIDS, the inflammatory effects of etodolac results from inhibition of the enzyme cyclooxygenase (COX). This decreases the synthesis of peripheral prostaglandins involved in mediating inflammation. Etodolac binds to the upper portion of the COX inhibitor, but it is now known to be 5-50 times more selective for COX-2 than COX-1

Pharmacokinetic data

Bioavailability: 80%

Protein binding: >99% bound to plasma proteins

Half life: 6.4 hours

Absorption: The systemic bioavailability of Etodolac is 100% as compared to solution and at least 80% as determined from mass balance studies. Etodolac is well absorbed and had a

relative bioavailability of 100% when 200 mg capsules were compared with a solution of Etodolac. Based on mass balance studies, the systemic availability of Etodolac from either the tablet or capsule formulation is at least 80%.

Distribution: The mean apparent volume of distribution (Vd/F) of Etodolac is approximately 390 mL/kg. Etodolac is more than 99% bound to plasma proteins, primarily to albumin. The free fraction is less than 1% and is independent of Etodolac total concentration over the dose range studied.

Metabolism: Etodolac is extensively metabolized in the liver. The role, if any, of a specific cytochrome P450 system in the metabolism of Etodolac is unknown. Several Etodolac metabolites have been identified in human plasma and urine.

Excretion: The mean oral clearance of Etodolac following oral dosing is 49 (±16) mL/h/kg. Approximately 1% of an Etodolac dose is excreted unchanged in the urine with 72% of the dose excreted into urine as parent drug plus metabolite.

Drug interactions

ACE-inhibitors

Reports suggest that NSAIDs may diminish the antihypertensive effect of ACE-inhibitors. This interaction should be given consideration in patients taking NSAIDs concomitantly with ACE-inhibitors.

Antacids

The concomitant administration of antacids has no apparent effect on the extent of absorption of Etodolac. However, antacids can decrease the peak concentration reached by 15% to 20% but have no detectable effect on the time-to-peak.

Aspirin

When Etodolac is administered with aspirin, its protein binding is reduced, although the clearance of free Etodolac is not altered. The clinical significance of this interaction is not known; however, as with other NSAIDs, concomitant administration of Etodolac and aspirin is not generally recommended because of the potential of increased adverse effects.

Cyclosporine, Digoxin, Methotrexate

Etodolac, like other NSAIDs, through effects on renal prostaglandins, may cause changes in the elimination of these drugs leading to elevated serum levels of cyclosporine, digoxin, methotrexate, and increased toxicity. Nephrotoxicity associated with cyclosporine may also be enhanced. Patients receiving these drugs who are given Etodolac, or any other NSAID, and particularly those patients with altered renal function, should be observed for the development of the specific toxicities of these drugs.

Diuretics

Etodolac has no apparent pharmacokinetic interaction when administered with furosemide or hydrochlorothiazide. Nevertheless, clinical studies, as well as post marketing observations have shown that Etodolac can reduce the uretic effect of furosemide and thiazides in some patients. This response has been attributed to inhibition of renal prostaglandin synthesis. During concomitant therapy with NSAIDs, the patient should be observed closely for signs of renal failure (see WARNINGS, as well as to assure diuretic efficacy.

Uses

Etodolac is used to relieve pain from various conditions. It also reduces pain, swelling, and joint stiffness from arthritis. This medication is known as a nonsteroidal anti-inflammatory drug (NSAID). It works by blocking your body's production of certain natural substances that cause inflammation.

Brand Names

- Etogesic (India)
- Etova (India)
- Lodin (India)
- Etodin (South Korea)
- Etofree (India)

THIOCOLCHICOSIDE

Chemical structure

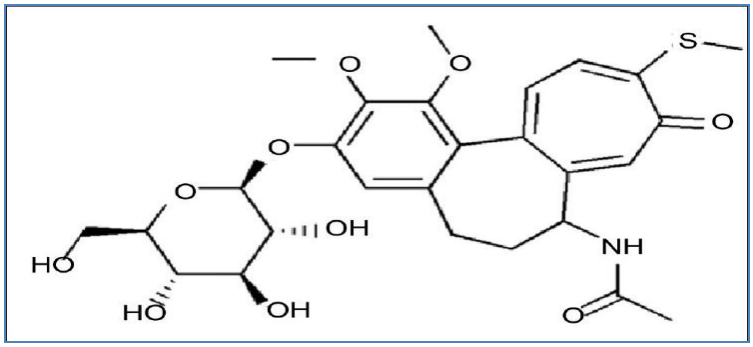

Fig.3: Chemical Structure Of Thiocolchicoside.

IUPAC NAME: N-[(7S) - 3- (beta-D - glucopyranosyloxy) - 1,2–dimethoxy - 10 - (methylsulfanyl) - 9 oxo– 5,6,7,9 - tetra hydro benzo [a] heptalen -7- yl] acetamide.

MOLECULER FORMULA: $C_{27}H_{33}NO_{10}S$

MOLECULAR WEIGHT: 563.618 g/ mol

DESCRIPTION: Light yellowish powder

CATEGORY: Non-steriodal anti- inflammatory agents

MELTING POINT: 145-148°C

pKa: 4.65 and an n-octanol: water partition of coefficient of 11.4 at pH 7.4.

SOLUBILITY: Soluble in alcohols, chloroform, DMSO

STORAGE: Tablets should store in room temperature.

Mechanism of Action

Thiocolchicoside exhibits a selective affinity for the inhibitory gamma-amino butyric acid and glycinergic receptors. It has an agonistic action at the spinal-strychnine-sensitive receptors that could mediate its myorelaxanteffect. However, experimental and clinical evidence strongly suggest a proconvulsant action for thiocolchicoside. Interaction with glycine receptors does not explain the convulsant action of the molecule. It has been suggested that thiocolchicoside might preferentially interact with a cortical subtype of the gamma-amino butyric acid type A (GABAA) receptor that expresses low-affinity binding sites for GABA. The low-affinity recognition site seems to be an antagonist-binding site. This explains the proconvulsant effect of thiocolchicoside. This is in contrast to earlier studies that suggested a GABA mimetic effect which would explain its muscle relaxant property.

Pharmacokinetic data

- **Bio availability**: Oral bioavailability is ~25%.
- **Protein binding**: The binding of thiocolchicoside to serum proteins is 12.8 +/- 5.3%, predominantly albumin. Thiocolchicoside bind erythrocytes in whole blood at 3.4 +/- 0.8%.
- **Half life**: Following oral administration the half lives of 3-O-glucurono-demethylcolchicine and 3-demethylcolchicine are 3.2-7 hours and 0.8 hours, respectively. Following IM injection the half lives of thiocolchicoside and 3-O-glucurono-demthylcolchicine are 1.5-1.9 hours and 9 hours, respectively.

Absorption: After IM administration, thiocolchicoside Cmax occur in 30 min and. reach values of 113 ng/mL after a 4 mg dose and 175 ng/mL after a 8 mg dose. The corresponding values of AUC are respectively 283 and 417 ng.h/mL. The pharmacologically active metabolite SL18.0740 is also observed at lower concentrations with a Cmax of 11.7 mg/mL occurring 5 h post dose and an AUC of 83 ng.h/mL. No data are available for the inactive metabolite SL59.0955. - After oral administration, no thiocolchicoside is detected in plasma. Only two metabolites are observed: For both metabolites, maximum plasma concentrations occur 1hour after thiocolchicoside administration.

Distribution: The apparent volume of distribution of thiocolchicoside is estimated around 42.7 L after an IM administration of 8 mg. No data are available for both metabolites.

Metabolism: After oral administration, thiocolchicoside is first metabolized in the aglycon 3-demethyltiocolchicine or SL59.0955. This step mainly occurs by intestinal metabolism explaining the lack of circulating unchanged thiocolchicoside by this route of administration. SL59.0955 is then glucuroconjugated into SL18.0740 which has equipotent pharmacological activity to thiocolchicoside and thus supports the pharmacological activity after oral administration of thiocolchicoside. SL59.0955 is also demethylated into didemethyl-thiocolchicine.

Excretion: - After IM administration the apparent t1/2 of thiocolchicoside is 1.5h and the plasma clearance 19.2 L/h. - After oral administration, total radioactivity is mainly excreted in feces (79%) while urinary excretion represents only 20%. No unchanged thiocolchicoside is excreted either in urine or feces. SL18.0740 and SL59.0955 are found in urine and feces while the didemethyl-thiocolchicine is only recovered in feces. After oral administration of

thiocolchicoside, the SL18.0740 metabolite is eliminated with an apparent t1/2 ranging from 3.2 to 7 hours and the metabolite SL59.0955 has a t1/2 averaging 0.8h.

Uses

Centrally acting muscle relaxants have been used in the treatment of painful muscle spasm associated with local tissue trauma or muscle strains, neurological disorders such as multiple sclerosis, cerebral palsy and stroke. Thiocolchicoside is indicated for the symptomatic treatment of painful muscle spasms. However, with the recent evidence of development of aneuploidy, the European Medicines Agency's Committee on Human Medicinal Products has recommended that the authorized uses for thiocolchicoside containing medicines for use by mouth or injection should be restricted.

Adverse Effects

The following undesirable effects have been described in the product literature. anaphylactic reactions, such as pruritus, urticaria, angioneurotic oedema; anaphylactic shock following intramuscular injection, somnolence, vasovagal syncope, usually occurring in the minutes following the intramuscular injection; diarrhoea, gastralgia, nausea, vomiting and allergic skin reaction.

Drug Interactions

Thiocolchicoside Aceclofenac Tablet is used for Muscles stiffness in spinal, Muscles stiffness in muscle diseases, Muscles stiffness in the joint diseases, Muscles stiffness in nerve diseases, Rheumatoid arthritis, Pain caused by nonaticular rheutism and other conditions. Thiocolchicoside Aceclofenac Tablet may also be used for purposes not listed in this medication guide.

Thiocolchicoside Aceclofenac Tablet works by reduces the swelling and pain; blocking the action of cyclooxygenase in the body.

Brand names

- Coltramyl
- Coltrax
- Miorel
- Musco-Ril
- Muscoril
- Neoflax

MATERIALS AND METHODS

The various materials and equipment's used for the present study are summarized follows.

CHEMICALS/REAGENTS AND SOLVENTS

Table 1: List of various materials used.

S. No	Materials	Grade	Company
1.	Water	HPLC	Fischer Scientific
2.	Acetonitrile	HPLC	Fischer Scientific
3.	Methanol	AR	Sd-fine chemicals

Table 2: Working Standard / Reference Standard/ API.

S. No	Working standard	Company
1.	Etodolac	Yarrow chem products, Hyderabad
2.	Thiocolchicoside	Yarrow chem products, Hyderabad

Table 3: Test Sample.

S.NO	Brand Name	Composition
1	Etova-MR	Etodolac-400 mg & Thiocolchicoside-4 mg
2	Etogesic- MR	Etodolac-400 mg & Thiocolchicoside-4 mg

Table 4: List of various Equipment used.

INSTRUMENT	SPECIFICATION
HPLC instrument	Waters HPLC2695 series with Quaternary pumps, Photo Diodearray detector
Injector	Auto sampler(robotic injector) integrated with empower 2software
Column	BDS 250mm x 4.6 mm, 5µ particle size.
UV spectrophotometer	Lab India UV double beam spectrophotometer with UVwin5.
pH meter	Lab India
Electronic balance	Denver
Ultra sonicator	Lab India
Pipettes, burettes, beakers	Borosil

ACKNOWLEDGEMENT

I would like to express my sincere appreciation to the Bhaskara institute of pharmacy and individuals whose contributions and support have greatly enhanced the quality and rigout of this research. First and foremost, I am grateful to my primary advisor DR.K.Rajesh sir for his unwavering guidance, insights, and constant encouragement throughout the research period. His expertise and wisdom were an invaluable asset to this project. I am grateful to the Bhaskara institute of pharmacy for offering facilities and resources for this project. Their support facilitated the smooth execution of the research. I extend my appreciation to my friends and colleagues, who have been supportive throughout and provided a stimulating

academic environment. Their encouragement was immensely motivating during my challenging research journey. Lastly, I am thankful to my family for their understanding, encouragement, and support.

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