REVIEW ARTICLE

Exploring thiocolchicoside injection: Mechanism, formulation development, and analytical validation

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Abstract

Globally, musculoskeletal disorders – which include ailments such as osteoarthritis, lower back pain, and cervical spondylosis – are major contributors to pain and disability. To reduce related muscular spasms and discomfort, doctors frequently prescribe muscle relaxants, particularly those that target pathways in the central nervous system. Thiocolchicoside (THC), a semi-synthetic version of colchicine, is derived from the *Gloriosa superba* plant. It helps lower pain and inflammation and relaxes muscles mostly in the brain and spinal cord. The injectable version of THC is becoming more and more popular for the treatment of acute and severe musculoskeletal problems due to its low oral bioavailability and gastrointestinal side effects. The formulation creation of THC injection is the main topic of this work, which also addresses important issues including stability, isotonicity, sterility, and solubility optimization. To choose the best excipient and store it in the right method, the physicochemical characteristics – such as solubility, partition coefficient, and degradation pathways – are investigated. Franz diffusion cell-based *in vitro* permeability studies provide additional insight into the drug's absorption properties and possible parenteral delivery-based bioavailability increase. Furthermore, an examination of contemporary analytical techniques, such as reverse-phase high-performance liquid chromatography and ultraviolet spectrophotometry, enables International Council for Harmonization-compliant quality control and validation procedures. All things considered, the study offers thorough insights into the injectable form of THC, confirming its cliHnical effectiveness and providing a dependable substitute for oral administration in the treatment of musculoskeletal conditions.

Key words: Thiocolchoside , Thiocolchoside Injection, Analytical Method, Validation, HPLC, Validation

INTRODUCTION

Musculoskeletal Disorders and the Role of Muscle Relaxants

Musculoskeletal disorders encompass a broad spectrum of conditions affecting muscles, bones, and joints, leading to pain, stiffness, and impaired mobility. Common examples include osteoarthritis, low back pain, cervical spondylosis, and muscle spasms resulting from neurological conditions. These disorders significantly impact quality of life and often necessitate pharmacological intervention. Muscle spasms and the pain they cause are often treated with muscle relaxants. By modifying the central nervous system, they lessen discomfort and muscular tone. While effective, their use is typically limited to short-term treatment due to potential side effects such as dizziness, drowsiness, and the risk of drug dependence.[1]

In both working environments and the general community, pain is among the most commonly stated state of health problems. In medicine, effectively managing it continues to be a major, unsolved global concern.[2] Thiocolchicoside (THC) is a naturally occurring glycoside that can be extracted through the *Gloriosa superba* seeds flower along with is produced through a semi-synthetic process from colchicine. In clinical practice, it is frequently used as a muscle relaxant to treat musculoskeletal pain, particularly acute neck and lower back pain as well as additional disorders that cause

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Received: 31-05-2025
Revised: 24-06-2025
Accepted: 30-06-2025

discomfort and muscle stiffness.[3,4] THC, a muscle relaxant that was first used in 1959, is a chemical known as N-[3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10(methylthio)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide. Numerous studies have shown that it is beneficial for relaxing muscles and has good therapeutic efficacy.[5] It has been used as an analgesic, anti-inflammatory, and muscle relaxant in medical applications for more than 35 years. These results have led to the widespread use of THC in the treatment of a variety of rheumatologic, orthopedic, and traumatic disorders.[1]

THC Injection

THC injection is a muscle relaxant used to alleviate acute muscle spasms and painful musculoskeletal conditions, such as stiffness and muscle tension. It is typically administered intramuscularly (IM) under medical supervision. In India, brands such as Musoril, Myoril, Thioquest, and Albesyde offer THC injections.[6] THC, a semi-synthetic derivative of colchicoside, is a potent muscle relaxant that is commonly used to treat painful muscle spasms. It has analgesic and anti-inflammatory properties. Despite its efficacy, the study of alternative delivery systems, like injectable formulations, is required because to its poor gastrointestinal effects and restricted oral bioavailability. The formulation development of THC injection is highlighted in this paper, with an emphasis on maximizing sterility, stability, and solubility for parenteral administration. The selection of appropriate stabilizers, solvents, and isotonic agents is given special attention to guarantee physicochemical compatibility and improved therapeutic efficacy. The paper also examines *in vitro* permeability tests that evaluate the transmembrane transport properties of THC by employing several diffusion models (such as Franz diffusion cells). These investigations offer vital information about the drug's permeability profile, absorption kinetics, and potential for increased bioavailability through injection. All things considered, the creation of a stable and efficient THC injection and thorough permeability testing support its clinical use as a good substitute for oral formulations, particularly in cases of acute and severe muscle spasm.[7]

THC Injection's Physicochemical Characteristics

THC, which has the chemical formula C27H33NO10S and molecular weight of roughly 563.62 g/mol, is a glycosidic derivative of the naturally occurring alkaloid colchicoside. In terms of injectable formulation development, it is described as a yellow crystalline powder that is only weakly soluble in water but more soluble in alcohol and other organic solvents. To improve its water compatibility, THC is usually dissolved in its injectable form using cosolvents or solubilizing agents. To guarantee drug stability and reduce discomfort during administration, the formulation needs to maintain an ideal pH range, which is normally between 4.5 and 6.5.

Other crucial Physicochemical Factors

Moderate lipophilicity that permits a certain amount of membrane permeability is indicated by the partition coefficient (log P). Stability: Heat and light sensitivity necessitates storage in a dry, cool environment shielded from light (often between 2°C and 8°C). Parenteral use requires isotonicity and sterility, which are frequently accomplished using isotonic agents (such as sodium chloride) and sterilization methods such as autoclaving or filtration. Optimizing the injectable formulation to guarantee safety, effectiveness, and patient compliance requires an understanding of these characteristics.[8]

LITERATURE REVIEW

Rajani and Mukkanti (2014)

Rajani and Mukkanti (2014) developed a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of THC, a muscle relaxant, and lornoxicam, an anti-inflammatory drug, in tablet formulations. Using a gradient mobile phase of acetonitrile and phosphate buffer (pH 3.0) on a Hypersil BDS C-18 column, the method achieves rapid analysis with retention times of 2.3 and 6.1 min for each drug. The method demonstrates excellent linearity, accuracy (99.96% recovery for THC and 100.65% for Lornoxicam), and precision, making it ideal for routine quality control in the pharmaceutical industry. Its robustness ensures reliable results even with slight variations in experimental conditions, making it a valuable tool for ensuring the quality of combination therapies involving these drugs.[9]

Jadhav *et al*. (2021)

The present study by Jadhav *et al*. focuses on the development and analytical method validation of a novel ultraviolet (UV)-spectrophotometric method for the simultaneous estimation of THC and ibuprofen in tablet dosage form. THC is a semi-synthetic derivative of colchicine with muscle relaxant, anti-inflammatory, and analgesic properties, acting through selective gamma-aminobutyric acid (GABA)-A receptor binding. Ibuprofen, a widely used non-steroidal anti-inflammatory drug (NSAID), acts by non-selective inhibition of COX-1 and COX-2 enzymes, thereby reducing pain and inflammation. This research represents the 1st-time application of a UV absorbance ratio method for simultaneous quantification of these two drugs. Methanol was selected as the solvent due to its superior solubility properties for both compounds. λmax was determined at 256 nm for THC and 228 nm for ibuprofen, with an isoabsorptive point at 248.5 nm. The method demonstrated linearity in the range of 0.4–1.2 µg/mL for THC and 0.5–2.5 µg/mL for ibuprofen, with correlation coefficients (r^2) of 0.9988 and 0.944,

respectively. Validation parameters, as per International Council for Harmonization (ICH) guidelines, included accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness. Results confirmed high accuracy (92.60–102.69% for THC and 99.12–102.54% for Ibuprofen) and precision (relative standard deviation <2%), proving the method to be reproducible and reliable. Overall, the study successfully establishes a simple, rapid, and cost-effective analytical method, suitable for routine quality control of combined THC and Ibuprofen formulations.[10]

Suraj D. Jadhav *et al.* (2015)

In their study, Jadhav Suraj D. developed and validated a robust HPLC method for the simultaneous estimation of THC and diclofenac potassium in tablet formulations. The authors validated the method according to ICH Q2(R1) guidelines, assessing parameters such as accuracy, precision, and specificity. The study also involved forced degradation testing under oxidative, thermal, and photolytic conditions to evaluate the stability of both compounds in the formulation. The results indicated that the method was highly sensitive and stable, meeting the required specifications for use in pharmaceutical quality control.

Key findings

The validated method showed excellent linearity, precision, and robustness, with alow LOD and LOQ. The forced degradation studies demonstrated that the method could separate the drug from its degradation products.

Citation

Sharma, P., Gupta, N., & Rathi, R. (2023). Simultaneous estimation of THC and diclofenac potassium using RP-HPLC method. Journal of Pharmaceutical Analysis, 13(1), 12-20.[3]

Baiju Mathews* *et al.* 2016

Etodolac is a NSAID that inhibits cyclooxygenase (COX-1 and COX-2) enzymes, reducing prostaglandin synthesis to relieve pain and inflammation. THC is a muscle relaxant with both anti-inflammatory and analgesic effects, acting as a GABA-A receptor antagonist and also inhibiting glycine receptors. The combination of etodolac and THC is used for treating musculoskeletal pain, osteoarthritis, rheumatoid arthritis, and post-operative pain. Literature on the determination of etodolac and THC reveals a limited number of official methods for their individual estimation. RP-HPLC methods have been explored for the simultaneous quantification of both drugs in combination. Different mobile phases, such as acetonitrile and water or methanol with acetic acid, have been used to optimize the chromatographic separation. The newly developed method for their combined analysis was validated for accuracy, precision, and robustness, making it suitable for routine quality control in pharmaceutical formulations.[11]

CHEMISTRY

THC is a chemical compound derived from thiocolchicine, also known as 2-demethoxy-2-glucosidoxy thiocolchicine. Its molecular formula is C27H33NO10S, and it has a molecular weight of 563.62 g/mol. THC appears as a yellow crystalline powder and is soluble in water, methanol, 0.1N HCl, and 0.1N Sodium hydroxide (NaOH). The content of THC in a sample is guaranteed to be between 98.0% and 102.0% of its molecular weight.[11] Thiocolchicine is a chemical compound with the IUPAC name N-[(7S)-5,6,7,9-Tetrahydro-1,2,3-trimethoxy-10-(methylthio)-9-oxabenzo[a]heptalen-7-yl] acetamide. A modified form of thiocolchicine, known as THC, features slight structural changes, including the addition of a sugar-like structure. Its full chemical name is N-[(7S)-2-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-5,6,7,9-Tetrahydro-1,3-dimethoxy-10-(methylthio)-9-oxabenzo[a]heptalen-7-yl]acetamide. In simpler terms, THC is a derivative of thiocolchicine that includes a sugar-like component incorporated into the molecular structure, altering its properties.[12]

CHEMICAL STRUCTURE

Physicochemical Properties Thiocolchicoside

- Color: Light yellow to yellow solid powder
- Melting point: 190–198°C
- Boiling point: Approximately 929.6°C at 760 mmHg
- Density: Approximately 1.5 g/cm^3
- Solubility: Slightly soluble in methanol and water (when heated), phosphate buffer saline (PBS), Acetonitrile, Methanol, and n-Octanol
- Flash point: Approximately 516°C
- LogP (partition coefficient): –1.23, indicating hydrophilic character
- Type of Compound: Alkaloids.[14,15] Figures 1 and 2

Stability profile

THC exhibits stability under photolytic and certain hydrolytic conditions but degrades under acidic, basic, and oxidative stresses. Identified degradation products include D1SO, D1SO(2), D2, D3, and D4, each resulting from specific chemical modifications. For instance, D1SO arises from oxidation at the sulfur atom, while D3 involves demethylation at the 3-position. A comprehensive degradation pathway has been proposed based on these observations.[16]

Mechanism of action

Specific receptors in the body are targeted by THC, primarily the glycine and inhibitory GABA receptors. It has a relaxing effect on muscles by acting on specific receptors in the spinal cord that are sensitive to strychnine. However, both lab and clinical studies show that THC may also trigger seizures (proconvulsant

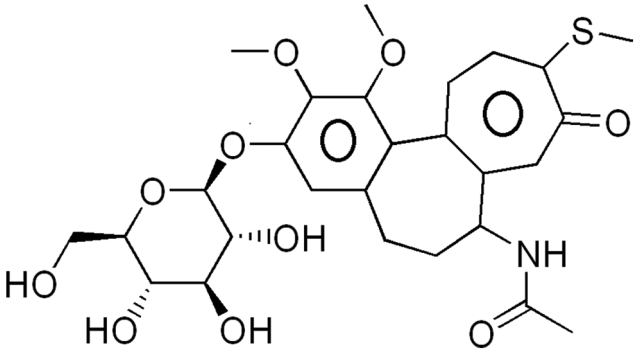

Figure 1: Thiocolchicoside chemical structure[13]

effect). While it interacts with glycine receptors, this alone does not explain why it might cause seizures. Researchers believe that THC could primarily interact with a particular type of GABA receptor in the brain that has low-affinity sites for GABA. These low-affinity sites might act as antagonist-binding sites, which could explain the seizure-promoting effects. This finding contrasts with earlier studies that thought THC mimicked GABA, which would have explained its muscle-relaxing effects. GABAB receptors do not seem to be affected by THC, so they are not involved in its muscle relaxation. Although the precise process causing the muscular relaxation is still unknown, inhibiting glycine receptors could be one explanation.[17,18]

Extraction of THC

A naturally occurring substance called THC is present in *G. superba* plant seeds. It has a connection to colchicine, another chemical. Researchers determine that the seeds of *G. superba* contain the highest concentration of colchicine using a technique known as HPLC.

To extract THC from the seeds:

1. First, 0.5 g of powdered plant material is taken
2. It is mixed with 25 mL of petroleum ether
3. The mixture is shaken well for 1 h, and this step is done twice
4. After shaking, the mixture is filtered, and the solid part is collected and air-dried
5. Then, 10 mL of dichloromethane is added to the dried solid, and the mixture is shaken again for 30 min at room temperature
6. The liquid is then vigorously agitated for ten minutes after adding 0.5 mL of a 10% ammonia solution
7. It is then left undisturbed for 30 min before being filtered
8. The collected solid is twice cleaned using 10 mL of dichloromethane, and the washings are then added to the filtrate
9. The combined organic liquid is evaporated to remove the solvent, leaving a dry substance
10. This dry residue is finally dissolved in 1 mL of 70% ethanol to get the final test sample.[5]

Synthesis of THC

In this process, acetonitrile is used as a solvent in a reaction flask. A mixture of 3-demethylthiocolchicine, D-glycopyranosyl fluoride, and 2,3,4,6-tetra-O-acetyl-α is suspended in acetonitrile in an inert environment at ambient temperature. Next, 1,1,3,3-tetramethylguanidine is added to the solution. Once the reagents dissolve, the solution turns red.

1. After that, boron trifluoride etherate is added, which changes the solution's color to a lighter shade. The reaction is stirred continuously, and its progress is monitored using thin-layer chromatography (TLC). Within 20 min, the starting materials are converted into the desired product.
2. To stop the reaction, a saturated solution of potassium bicarbonate is added. This results in two separate liquid layers. Ethyl acetate, or AcOEt, is used to extract the aqueous (water-based) layer. To get a crude product, the mixed organic layers are subsequently dried with magnesium sulfate ($MgSO_4$), filtered, and the solvent is eliminated.
3. This crude product is then dissolved in ethanol. NaOH is added while stirring continuously. The reaction is again monitored with TLC and is complete in about three hours. THC forms as crystals directly from the solution, giving a high yield of 97%.[16]

Synthesis of THC in flow chart

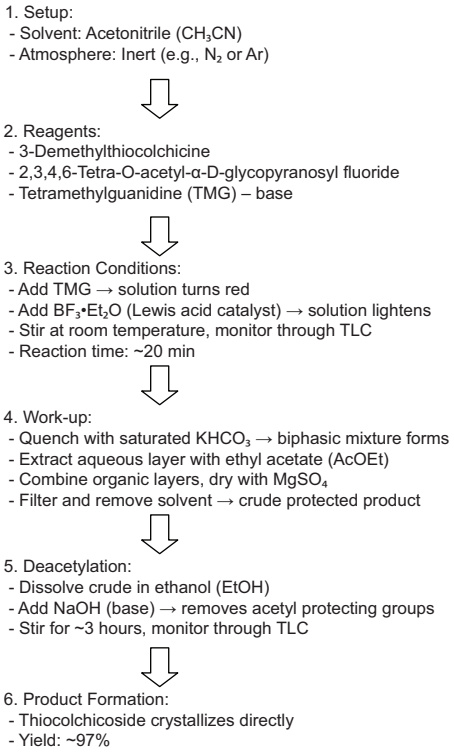

Figure 2: Process of synthesis of thiocolchicoside[18]

Validation of Analytical Methods

A key component of pharmaceutical quality assurance is analytical method validation, it confirms if an analytical technique is suitable for the purpose for which it is designed. It is a written procedure that guarantees the approach will continuously produce accurate, reproducible, and dependable outcomes within predetermined bounds. To guarantee the

integrity of data in medication research and manufacturing, regulatory bodies including the US food and drug administration (FDA), European Medicines Agency (EMA), World Health Organization, and the ICH need analytical technique validation.

Method Validation Goals

Proving that an analytical method is suitable for the purpose for which it is being used – whether that be the identification, quantification, or impurity profiling of active pharmaceutical ingredients, excipients, or final products – is the primary objective of method validation.[19]

Analytical Procedure Types

1. Identification examinations
2. Quantitative impurity testing
3. Impediment limit testing
4. Quantitative assessments of a medication product's active ingredients.

Each of these has specific validation requirements depending on their purpose and regulatory expectations.

Crucial Elements of Method Validation

The ICH Q2(R1) recommendations state that the following criteria are usually assessed:

1. Accuracy:- The degree to which the test result and the actual value accord. Usually, it is stated as a percentage of recovery.
2. Precision: The level of consistency between each test results following several method applications. It consists of:
   a. Repeatability (precise within a day)
   b. Inter-day, analyst-to-analyst, and intermediate precision
   c. Reproducibility (across various labs).
4. Particulars the ability to determine the analyte's final state in the presence of contaminants, degradants, or matrix components.
5. The concept of linearity the method's ability to produce results within a given range that are precisely proportionate to the analyte concentration 5. Range is the separation between the top and lower levels of the analyte that has been demonstrated to be determined with a suitable level of precision, accuracy, and linearity.
6. Detection limit (LOD): The minimum amount of analyte that is detectable but not always measurable.
7. Quantitative limit (LOQ):- The smallest amount of analyte that can be recognized quantitatively with a high degree of precision and accuracy.
8. Strongness the ability of the procedure to be insensitive to minor, intentional changes in variables such as pH, temperature, or the makeup of the mobile phase.
9. Testing for system suitability carried out before sample analysis to guarantee sufficient system performance. Resolution, repeatability, and theoretical plate number are among the parameters.[19]

Methods of Validation

i. Before the method's regular use, prospective validation is carried out
ii. Concurrent validation: Carried out during ordinary production
iii. Historical data from previous productions is the basis for retrospective validation.

The Regulatory Structure

The following regulations control the validation of analytical methods:

ICH Q2 (R1), "Validation of Analytical Procedures: Text and Methodology" "Validation of Compendial Procedures" is USP <1225>. FDA Guidance on Analytical Procedures and Methods Validation for Industry.[19]

These documents offer standardized standards for validation procedures used by international regulatory organizations.

Relevance to the Pharmaceutical Sector

Throughout the course of the medication lifecycle, pharmaceutical goods are guaranteed to be safe, effective, and of high quality thanks to validated analytical techniques. They are necessary for pharmaceutical industry

- Approval and compliance with regulations
- Stability testing and batch release
- Validation and management of processes
- Identification of fake or inferior goods.[19]

Prospects for the Future of THC Injection

The natural substance colchicoside is the source of THC, a muscle relaxant that has long been used to treat severe muscular diseases such as sciatica, low back pain, and other rheumatologic or orthopedic illnesses. Although topical and oral forms are still in use, the injection form has attracted a lot of attention due to new safety concerns, especially those related to genotoxicity and possible hazards to reproductive health.

Metabolites may cause chromosomal damage (aneugenic effect), the EMA issued a rule in 2013 that limited the drug's use, particularly when administered IM and intravenously. Consequently, the EMA advised that oral doses beyond 7 days be totally prohibited and that intramuscular injections be limited to a maximum of 5 days and a dose of 8 mg daily.

The precedent established by this regulatory action is having an impact on national regulatory bodies around the world.[20]

Outlook for Research and Development

In the future, THC injections will probably concentrate on resolving safety issues while maintaining therapeutic effectiveness. Numerous options are being investigated:

1. To improve safety profiles without sacrificing muscle relaxant effectiveness, structural changes to THC may lower genotoxic metabolites, leading to the development of safer analogues or prodrugs.
2. Localized and targeted delivery methods: Depot injections or nanocarriers may reduce the risk of systemic genotoxicity by limiting systemic exposure and localizing therapeutic effects.
3. Pharmacogenomic profiling: Knowing a patient's unique genetic predispositions may assist identify those who are more likely to experience side effects, allowing for more individualized treatment.
4. Combination therapies: THC may be used in fixed-dose combinations with other analgesics or anti-inflammatory medications to reduce dosage and improve therapeutic results while minimizing side effects.
5. Post-marketing monitoring and empirical research: Injectable THC risk-benefit analysis will continue to be guided by future pharmacovigilance data, particularly in populations where existing data are insufficient, such as youngsters and pregnant women and children.[21]

CONCLUSION

THC, a semi-synthetic derivative of colchicine, has shown promise as a potent muscle relaxant with additional analgesic and anti-inflammatory properties, making it a valuable therapeutic agent for the management of musculoskeletal disorders.

This study emphasizes its therapeutic importance, especially in injectable formulations that tackle issues related to gastrointestinal side effects and low oral bioavailability. With a focus on stability, sterility, solubility, and isotonicity, the formulation and physicochemical characterization of THC injection show that it is appropriate for parenteral use. Its potential for enhanced therapeutic efficacy through injectable methods is further supported by *in vitro* permeability investigations. Furthermore, THC's dependability and repeatability in pharmaceutical applications are supported by the synthesis, extraction, and analytical method validations.

All things considered, the thorough comprehension of THC's pharmacological profile, formulation techniques, and quality control procedures supports its ongoing applicability in the management of severe and excruciating musculoskeletal disorders.

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Source of Support: Nil. **Conflicts of Interest:** None declared.