Development and Validation of Stability Indicating Assay for Thiocolchicoside & Etoricoxib by RP-HPLC Method

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Abstract :

Thiocolchicoside is a muscle relaxant widely used for managing painful muscle spasms. With additional anti inflammatory and analgesic properties, thiocolchicoside is often prescribed for patients suffering from chronic back pain, orthopedic disorders, and rheumatic conditions. Thiocolchicoside is a semi synthetic derivative of the colchicine, a natural anti inflammatory glycoside which originates from the flower seeds of Superba gloriosa. It is a muscle relaxant with anti inflammatory and analgesic effects. thiocolchicoside molecular formula C27H33NO10S & molecular weight 563.6 g/mol. Etoricoxib is a selective COX-2 inhibitor used to relieve moderate post-surgical dental pain as a short-term treatment and inflammatory and painful symptoms of various forms of arthritis. Etoricoxib is a member of the class of bipyridines that is 2,3'-bipyridine which is substituted at the 3, 5, and 6' positions by 4-(methylsulfonyl)phenyl, chlorine, and methyl groups, respectively. It has a role as a cyclooxygenase 2 inhibitor and a non-steroidal anti-inflammatory drug. It is a sulfone, a member of bipyridines and an organochlorine compound. Etoricoxib is a new COX-2 selective inhibitor. Current therapeutic indications are: treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis. Like any other COX-2 selective inhibitor, Etoricoxib selectively inhibits isoform 2 of cyclo-oxigenase enzyme (COX-2) to reduce the generation of pr ostaglandins (PGs) from arachidonic acid . Etoricoxib molecular formula $C_{18}H_{15}ClN_2O_2S$ and molecular weight 358.8 g/mol . the aim of present study is to developed and validation of a reverse phase

high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of Thiocolchicoside & Etoricoxib in pharmaceutical dosage forms Validation is essential to ensure the analytical procedure meets predetermined criteria for accuracy, precision, linearity, and robustness, as mandated by regulatory bodies like the FDA and ICH. The use of this method is essential for maintaining therapeutic efficacy and patient safety. **Keywords:** Thiocolchicoside& Etoricoxib, RP- HPLC, Validation ,ICH Guidelines

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1. Introduction

Thiocolchicoside is a semi synthetic derivative of the colchicine, a natural anti inflammatory glycoside which originates from the flower seeds of Superba gloriosa. It is a muscle relaxant with anti inflammatory and analgesic effects. thiocolchicoside molecular formula C27H33NO10S & molecular weight 563.6 g/mol and Etoricoxib is a selective COX-2 inhibitor used to relieve moderate post-surgical dental pain as a short-term treatment and inflammatory and painful symptoms of various forms of arthritis. Etoricoxib is a member of the class of bipyridines that is 2,3'-bipyridine which is substituted at the 3, 5, and 6' positions by 4-(methylsulfonyl)phenyl, chlorine, and methyl groups, respectively. It has a role as a cyclooxygenase 2 inhibitor and a non-steroidal anti-inflammatory drug. It is a sulfone, a member of bipyridines and an organochlorine compound. Etoricoxib is a new COX-2 selective inhibitor. Current therapeutic indications are: treatment of rheumatoid arthritis. combination of thiocolchicoside and etoricoxib represent a synergistic approach to management of musculoskeletal pain and inflammatory condition involving muscle spasms and joint pain .thiocholchicoside is muscle relaxant (semi - synthetic derivative of colchicoside) and its acts on the central nervous system by modulating GABA-A and glycine receptors, leading to muscle spasm associated with orthopedic and neurological disorders .etoricoxib is Selective COX-2 Inhibitor (NSAID) selectively inhibits cyclooxygenase - 2(COX-2) enzyme ,reducing the synthesis of prostaglanding responsible for pain and inflammation study have focus on development and validation of a reverse phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of Thiocolchicoside & Etoricoxib in pharmaceutical dosage forms is essential for ensuring quality control in drug manufacturing. One of the primary objectives is to establish a reliable analytical methodology that can

accurately quantify both active pharmaceutical ingredients (APIs) within a single run, thus enhancing efficiency and reducing costs associated with separate analyses. Furthermore, the validation process aims to confirm the method's specificity, precision, accuracy, linearity, and robustness according to regulatory guidelines. This ensures that the RP-HPLC method can be consistently applied across different batches of pharmaceuticals without significant variability in results.

2. Materials & Methods

Table 2.1: Chemicals and Drug

Material	Company name
Thiocolchicoside & Etoricoxib	Maan Pharmaceutical Pvt. Ltd
Water	Aquarch Pvt. Ltd
Methanol	Merck Specialties Pvt. Ltd
Acetonitrile	Merck Specialties Pvt. Ltd

Table 2.2: Instrumentation:

Instrumentation	Company
HPLC	Agilent 1120 compact LC system
C18 column	Agilent ODS UG 5 column, 250mm x 4.5mm
Software	Class VP series version 5.03 (Shimadzu)
UV Spectrophotometer	Shimadzu

Table 2.3: Chromatographic conditions

Parameters	Methods
Stationary phase (column)	C18 column
Mobile phase	Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 20:50:30, v/v
Flow rate	1 ml/min
Column temperature	Ambient
Volume of injection	20 µl
Retention time	5.7 min

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3.Method development

3.1 Method Development

3.1.1 Selection of Mode of separation:

The first consideration when developing an HPLC method is to determine the solubility of sample components. As Thiocolchicoside & Etoricoxib were soluble in polar solvents, RP-HPLC mode was chosen.

3.1.2 Selection of Mobile phase:

Standard solutions containing Thiocolchicoside & Etoricoxib were injected into the column and run using different mobile phases. Different mobile phases of different proportions of the organic phase and buffers of different pH were tried at different flow rates for the better elution and separation of the drugs. Each mobile phase was filtered through 0.45µ membrane filter and sonicated for 15 min before the trials. Standard solutions were injected into the column after obtaining a steady base line to get well resolved and stable peaks for both the drugs. After performing trials with different mobile phases, both the drugs were found to separate well with stable retention times when run with a mobile phase of combination acetonitrile, methanol and phosphate buffer of pH 3.0 in a ratio of 20:50:30, v/v at a flow rate of 1ml/min. so,, this mixture was selected

as the mobile 190 phase for the chromatographic method development because of the sharp symmetrical peaks and reproducible retention times obtained.

3.1.3 Selection of Stationary phase:

Selection of appropriate stationary phase helps to improve the efficiency of the method. In the present study, in order to get better peak resolution with less tailing factor and more theoretical plates, C18 column (Agilent ODS UG column, 250mm x 4.5mm) was selected.

4.0 Method Validation of Developed Method for Individual Drugs and Combined Dosage Form

Adjusting several UFLC settings (FDA, 1995, 1997, 2000, 1994, 1987; USP, 2000) confirmed the reliability of the UFLC approach. Precision, specificity, robustness, accuracy, and ruggedness were determined; in addition to linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, and limit of detection (LOQ). Minimum concentrations of these medications were used to establish the LOD and LOQ. “Microsoft Excel was used to conduct statistical analysis on the experimental data, including the calculation of confidence intervals, correlation coefficients, and the relative standard deviation (SD).” The correlation coefficients and relative standard deviations were calculated based on the calibration plots' linearity and the experimental points' low scatter. The wide range of experimental variables confirmed the method's reliability in determining the peak regions .

4.1 Linearity

Calibration plot least-squares linear regression analysis verified the UFLC method's linearity. Furthermore, the linearities of calibration plots (peak area versus quantity) were optimized and compared across various concentration ranges for standards. The UFLC was filled with 5.0 L of the usual as described above. Statement drug UFLC chromatograms were generated separately and in tandem.

4.2 Detection and Quantitation Limits

The limits of detection and quantification for the medicines mentioned were determined to be three and five epochs, respectively, above and below the baseline noise. The process adhered to the guidelines established by the United States Pharmacopoeia

4.3 Specificity

The process's sensitivity was determined by monitoring the HPLC findings for any alterations brought on by a few adulterations in the standard samples. Very little uncut drug data was published, enough to throw off the standard models

4.4 Precision

The described medications' precision values were determined at three different amounts or concentrations: 0.04, 0.05, and 0.06 mgmL-1. All three values were analyzed by HPLC using five resting-state tests

4.5 Accuracy

HPLC accuracy was evaluated using varying amounts of the probe molecules. The range of concentrations used was from 0.04-0.06 mgL-1. Five separate optimum HPLC runs were performed (n = 5). Interpreting peak regions from five replicates of these reported medications allowed us to determine their accuracy

4.6 Robustness

Robustness was determined by making a minor adjustment to variables like flow rate, temperature, eluent components, and max in chromatographic experiments. Peak area, peak form, and retention duration were compared between standard and slightly off-center experimental conditions .

4.7 Ruggedness

The method's durability was tested by introducing random variables into the experiments, such as various handlers and time intervals

Results and Discussion

5.1 Characterization of the Sample Drug

5.1.1 Organoleptic characterization and Solubility check of drug

In the field of pharmaceutical sciences, methods including solubility check, melting point measurement, and organoleptic characterization are crucial. These techniques offer important insights into the chemical and physical characteristics of medications, which are critical to their effectiveness and formulation. The sensory assessment of a medication using our five senses—sight, smell, taste, touch, and hearing—is known as organoleptic characterization. This technique aids in detecting any physical modifications brought on by handling or storage, such as discoloration, changes in odour, or changes in taste. A crucial component of drug research is the solubility assessment, which ascertains a drug's capacity to dissolve in a range of solvents. Designing dose forms that guarantee the drug's best absorption and bioavailability requires the use of this knowledge. To sum up, solubility testing, melting point analysis, and organoleptic characterization are critical instruments in pharmaceutical research that help guarantee the efficacy and quality of medications. These methods are essential for creating drugs that are both effective and safe for patients to taken into care (Table 3).

Table 3: Organoleptic characterization and Solubility check of drug

Characters	Observations	
	Thiocolchicoside	**Etoricoxib**
Colour	White to off-white	White to off-white
Texture	Crystalline powder	Crystalline powder
Taste	Tasteless	Tasteless
Odour	Odourless	Odourless
pH	Neutral	Weak base

Solubility Study		
Solvents	**Thiocolchicoside**	**Etoricoxib**
Water	Sparingly soluble	Insoluble
Ethanol	Freely soluble	Freely soluble
Methanol	Freely soluble	Freely soluble
Acetone	Soluble	Freely soluble

5.2 Melting Point Determination

Melting point determination is another essential technique used to assess the purity and identification of a medication. The melting point range of a solid substance can be found by measuring its temperature, and its identity can be verified by comparing the resultant value with known values. Thiocolchicoside and Etoricoxib shows a melting point of about 195.95±0.969°C and 184.87±0.534°C, respectively.

5.3 Solubility

Solubility check is also a key parameter in drug development as it determines the ability of a drug to dissolve in various solvents. Thiocolchicoside is a solid that exhibits varying solubility in different solvents. It is soluble in organic solvents like DMSO (5 mg/ml) and dimethyl formamide (1 mg/ml). While slightly soluble in methanol and water, it can be dissolved in aqueous buffers like PBS (pH 7.2) at a concentration of approximately 5 mg/ml. Etoricoxib is poorly soluble in water. It is considered a BCS Class II drug, meaning it has low solubility and high permeability. Pure etoricoxib is insoluble in water, phosphate buffer, and hydrochloric acid, but it is soluble in methanol. Solubility can be enhanced using techniques like solid dispersion, complexation, and co-crystallization.

5.4 Loss on Drying

The loss on drying of Thiocolchicoside and Etoricoxib was found to be 0.47 and 0.51 % ^{w}/W, respectively.

5.5 Partition Coefficient

The partition coefficient of Thiocolchicoside and Etoricoxib is found to be 2.10 and 3.05, respectively indicating that Thiocolchicoside and Etoricoxib are moderately lipophilic and has good permeability across membranes; this value suggests a relatively high ability to distribute between an oily phase (octanol) and water.

5.6 Calibration curve of Thiocolchicoside & Etoricoxib

Thiocolchicoside exhibits absorption maxima at 274 nm, while The wavelength selected for measurement is 285 nm as at this wavelength Etoricoxib exhibited maximun rate of change of absorbance with wavelength against wavelength. The calibration curve has been prepare for Thiocolchicoside and Etoricoxib at wavelength of 274 (Figure 8) and 285 nm (Figure 9), respectively. The calibration curve shown the regression coefficient of Thiocolchicoside & Etoricoxib were 0.994 and 0.993, respectively and found to be linear in the concentration range of 2 to 10 µg/ml.

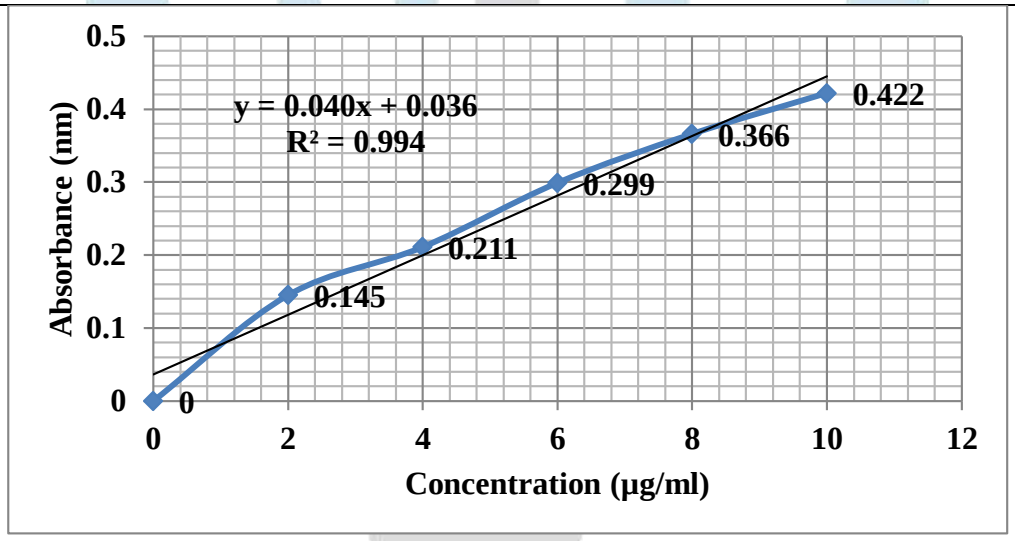

Figure 8: Calibration Curve of Thiocolchicoside (274 nm)

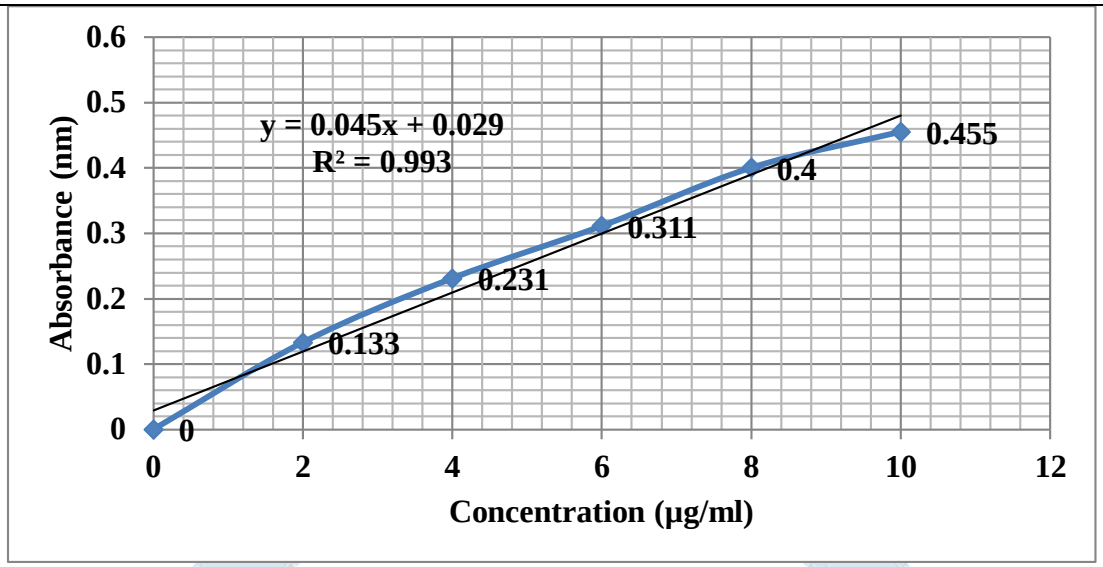

Figure 9: Calibration Curve of Etoricoxib (285 nm)

5.7 Selection of mobile phase:

Each mobile phase was filtered through Whatman filter paper No. 42. Peak, well resolved peaks with symmetry within limits and significant Based on sample solubility & stability, various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. The mobile phases tried are as follows:

Table 5: List of Mobile Phases Trials

S.NO.	Mobile Phase
1	Methanol(100)
2	Methanol : Water (90:10)
3	Methanol : Water (80:20)
4	Methanol : Water (70:30)
5	Methanol : Water (70:30)pH 5.5
6	Methanol : Water 65:35) pH5
7	Methanol : Water (65:35) pH4

From various mobile phases tried, mobile phase containing Methanol: Water (70:30) pH 5.5 was selected, since it gives sharp reproducible retention time for the drug.

6.0 Validation of the Method

Table 6 shows the results of the accuracy tests that were conducted using the usual addition method for recovery. A measurement series' standard deviation (S.D.) or root-mean-square (RMSD) is a measure of an analytical method's precision. Table 7 shows the results of the replicated drug estimations using the proposed technique. In order to determine the method's specificity, we looked at how effectively it could isolate the peak from the matrix component peaks. As a whole, the Thiocolchicoside & Etoricoxib retention time is 3.981 and 4.012. There was no interference from the matrix component since the values were so close to those in the standard laboratory mixture. Range and linearity: A concentration ranging from 80% to 120% of the test concentration was achieved by dissolving and diluting tablet powder according to USP standards, which is comparable to 80, 90, 100, 110, or 120% of the label claim. We documented the chromatograms of the solutions that came out of it. It was discovered that the Thiocolchicoside & Etoricoxib marketed formulation was linear within ± 20% of the drug's test concentration (Table 8). A slight change in the organic content of the mobile phase, wavelength, or flow rate had no effect on the chosen parameters, according to the robustness analysis. The results of the system's suitability should fall within the specified range. Therefore, the approach was reliable (Table 9). The minimum detectable concentration of an analyte in a sample that cannot be precisely measured is known as the limit of detection. According to Table 10, the limit of quantitation is the smallest concentration of analyte that can be accurately and precisely measured in a given sample. The experimental and results sections detail the steps used to generate the standard laboratory mixture and conduct the analysis once the chromatographic conditions had been established. It was expanded for medication estimate in commercialized tablet formulation and produced accurate and dependable results.

Table 6: Results and Statistical Data for Recovery study

S.NO.	Amount of Drug (mg/ml)	% of Recovery of Drug	
		Thiocolchicoside	Etoricoxib
1	0.5	99.56	98.98
2	1.0	98.78	98.79
3	1.5	99.03	99.99
4	2.0	99.99	95.99
5	2.5	94.65	99.77
6	3.0	98.89	99.86

Table 7: Results and Statistical Data of Precision Study

S.NO.	Amount of Drug (mg/ml)	% Label Claim	
		Thiocolchicoside	Etoricoxib
1	100.5	99.22	98.88
2	101.0	97.78	98.65
3	101.5	98.03	99.89
4	102.0	99.66	99.34
5	102.5	98.89	98.76
6	103.0	99.00	99.08

Table 8: Observations of Linearity and range study

S.NO.	Thiocolchicoside		Etoricoxib	
	% Label Claim	Peak Area	% Label Claim	Peak Area
1	99.22	49997.90	99.89	49876.04
2	97.78	50555.34	98.78	48999.76
3	98.03	47345.55	99.03	50098.84
4	98.88	44436.98	99.99	51098.45
5	98.65	51980.44	99.07	49837.64

Table 9: Result of Robustness study

Condition	Parameter	Thiocolchicoside	Etoricoxib
		RT	RT
Change in Wavelength	253 nm	4.23	4.32
	255 nm	4.55	4.77
	257 nm	5.01	4.89
Change in Temperature	30ºC	4.33	4.26
	25 ºC	4.66	4.89
	20 ºC	5.00	5.11
Change in Mobile Phase	75:25	4.23	4.34
	70:30	2.57	3.99
	65:35	4.99	5.00

Table 10: Limit of detection (LOD) and Limit of quantization (LOQ)

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Thiocolchicoside	0.056	1.004
Etoricoxib	0.044	1.134

Chapter 7: Conclusion

On conclusion, the development and validation of a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method is crucial for ensuring quality control in the pharmaceutical industry, particularly for determining Thiocolchicoside & Etoricoxib in pharmaceutical dosage forms. Traditional analytical methods may lack the specificity and sensitivity required for such analyses, highlighting the need for an advanced RP-HPLC approach. Validation is essential to ensure the analytical procedure meets predetermined criteria for accuracy, precision, linearity, and robustness, as mandated by regulatory bodies like the FDA and ICH. Establishing a validated RP-HPLC method enhances product quality, facilitates compliance with regulatory standards, and improves patient outcomes through effective medication monitoring.

Chapter 8: References

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