
**“DEVELOPMENT AND VALIDATION OF NEWER
ANALYTICAL TECHNIQUES FOR SELECTED NON
STEROIDAL ANTI-INFLAMMATORY DRUGS”**

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**For the award of the degree of
Doctor of Philosophy
In the Faculty of Pharmacy**

By

Mr. RUPESH SHRIPAD KULKARNI M. Pharm.

(Registration No: KLEU/Ph.D./2020-21 /DO1220049)

Under the Guidance of

Dr. MAHESH S. PALLED M. Pharm, Ph.D.

Professor and Head

Department of Pharmaceutical Analysis

**KLE College of Pharmacy, Belagavi,
KLE Academy of Higher Education and Research,
Belagavi-590010.**

FEBRUARY 2025

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Mr. RUPESH S. KULKARNI M. Pharm.
Part-time Ph.D. Research scholar,
Reg. No: DO1220049
KLE College of Pharmacy,
KAHER, Belagavi-590010.

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Mr. Rupesh S. Kulkarni
Part-Time Ph.D. Scholar, 2020-21 Batch
KLE College of Pharmacy,
Faculty of Pharmacy, KAHER
Belagavi.

Cc to :

- 1) The Principal, KLE College of Pharmacy, Belagavi.
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✉️ info@kledeemeduniversity.edu.in 🌐 www.kledeemeduniversity.edu.in

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Mr. RUPESH S. KULKARNI M. Pharm.

Part time Ph.D. Research Scholar

Reg. No: DO1220049

KLE College of Pharmacy,
KAHER, Belagavi-590010.

Dr. MAHESH S. PALLED M. Pharm, Ph.D.

Professor and Head,

Department of Pharmaceutical Analysis.

KLE College of Pharmacy,
KAHER, Belagavi-590010.

Place: Belagavi

Date: 20/02/2025

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Date: 20/02/2025

Mr. RUPESH S. KULKARNI M. Pharm.

Part-time Ph.D. Research scholar,

Reg. No: DO1220049

KLE College of Pharmacy,

KAHER, Belagavi-590010.

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Place: Belagavi

Date: 20/02/2025

Dr. V. S. MASTIHOLIMATH M. Pharm, Ph.D.

Dean,

KLE College of Pharmacy,

KAHER, Belagavi-590010.

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Dr. MAHESH S. PALLED, Professor and Head, Department of
Pharmaceutical Analysis, KLE College of Pharmacy, Belagavi.*

Date: 20/02/2025
Place: Belagavi



Prof. (Dr). SUNIL S. JALALPURE M. Pharm, Ph.D.
Principal,
KLE College of Pharmacy,
KAHER, Belagavi-590010.

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guidance.*

Date: 20/02/2025

Place: Belagavi

A handwritten signature in black ink, appearing to read 'Mahesh S. Palled', is written over a circular stamp.

Prof. (Dr.) MAHESH S. PALLED

Professor and Head

Department of Pharmaceutical Analysis

KLE College of Pharmacy,

KAHER, Belagavi-590010.

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LIST OF ABBREVIATIONS

µg	:	Microgram
µL	:	Microliter
µm	:	Micro molar
ANOVA	:	Analysis of Variance
API	:	Active Pharmaceutical Ingredient
ATP	:	Analytical Target Profile
BBD	:	Box-Behnken Design
CMAs	:	Critical Material Attributes
COX	:	Cyclooxygenase
CPPs	:	Critical Process Parameters
CQAs	:	Critical Quality Attributes
DoE	:	Design of Expert
EMA	:	European Medicines Agency
FDA	:	Food and Drug Administration
GC	:	Gas Chromatography
H₂O₂	:	Hydrogen Peroxide
HCl	:	Hydrochloric Acid
HPLC	:	High-Performance Liquid Chromatography
ICH	:	International Council for Harmonisation
LC-MS/MS	:	Liquid Chromatography-Tandem Mass Spectrometry
LOD	:	Limit of Detection
LOQ	:	Limit of Quantification
NaOH	:	Sodium Hydroxide

NSAIDs	:	Non-Steroidal Anti-Inflammatory Drugs
OPA	:	Orthophosphoric Acid
PA	:	Peak Area
PAT	:	Process Analytical Technology
PDA	:	Photodiode Array
QbD	:	Quality by Design
QC	:	Quality Control
QTPP	:	Quality Target Product Profile
R & D	:	Research and Development
r²	:	Correlation Coefficient
RA	:	Risk Assessment
RSD	:	Relative Standard Deviation
RT	:	Retention Time
S	:	Slope
SD	:	Standard Deviation
TF	:	Tailing Factor
TP	:	Theoretical Plates
TQP	:	Target Quality Profile
UHPLC	:	Ultra-High-Performance Liquid Chromatography
UV	:	Ultra-Visible

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ABSTRACT

Background: The increasing complexity of pharmaceutical formulations necessitates precise and robust analytical methods to ensure quality, safety, and efficacy. Loxoprofen sodium, Baclofen, Etodolac, and Thiocolchicoside possess anti-inflammatory, muscle relaxant, and analgesic properties. However, their simultaneous quantitative estimation in formulations poses significant challenges. This study addresses the need for advanced UV-spectrometric and chromatographic methods optimized for the individual and simultaneous evaluation of these drugs, adhering to regulatory standards.

Objectives: The primary goal was to develop and validate robust analytical methods—UV-spectrometry, reverse-phase high-performance liquid chromatography (RP-HPLC), and ultra-high-performance liquid chromatography (RP-UHPLC) for the quantitative estimation of these compounds using a Quality by Design (QbD) approach.

Methods: Loxoprofen sodium was analyzed via UV-spectrophotometry at $\lambda_{\text{max}} = 223$ nm in distilled water, validated for linearity, precision, and sensitivity. Further, RP-HPLC analysis was performed using an Agilent C18 column with a methanol-orthophosphoric acid (75:25, v/v) mobile phase at 1 mL/min and detection at 223 nm. Baclofen was quantified using RP-HPLC with a methanol-orthophosphoric acid (50:50, v/v) mobile phase at 220 nm. Forced degradation under acidic, basic, oxidative, photolytic, and thermal conditions evaluated method stability. Simultaneous analysis of Etodolac and Thiocolchicoside employed RP-UHPLC with acetonitrile-phosphate buffer as the mobile phase. Critical chromatographic parameters were optimized using a Box-Behnken Design (BBD).

Results: The UV method for Loxoprofen sodium demonstrated excellent linearity (5–30 µg/mL, $r^2 = 0.999$), sensitivity (LOD = 1.10 µg/mL; LOQ = 3.71 µg/mL), and robustness. The RP-HPLC method exhibited comparable linearity (5–25 µg/mL, $r^2 = 0.9998$), precision (RSD < 2%), and recovery (98–102%). Baclofen analysis showed high linearity (5–25 µg/mL, $r^2 = 0.9991$) and accuracy (recovery = 100.16–101.04%). Stability studies confirmed the stability-indicating nature of the methods. The QbD-optimized RP-UHPLC method for Etodolac and Thiocolchicoside achieved precise retention times, linearity ($r^2 > 0.999$), and robustness.

Conclusion: These validated UV-spectrometric and chromatographic methods are reliable, sensitive, and cost-effective, providing robust tools for quality control and stability studies in pharmaceutical industries. Incorporating QbD significantly enhanced the RP-UHPLC method's robustness and precision for simultaneous quantification.

Keywords: Non Steroidal Anti-Inflammatory Drugs, Ultraviolet- Visible Spectroscopy, High-Performance Liquid Chromatography, Ultra High-Performance Liquid Chromatography, Quality by Design.

INTRODUCTION

1.1 BACKGROUND

A drug is a biologically active chemical compound obtained from various natural, synthetic, or biotechnological sources. It is designed for use in the treatment, management, diagnosis, prevention, or cure of a wide array of diseases and disorders affecting humans and animals ^[1]. Drugs may be administered either internally, such as through oral or injectable routes, or externally, such as topical applications, and are delivered in carefully formulated dosage forms tailored to ensure efficacy, safety, and patient compliance ^[2].

Pharmaceutical companies manufacture medications in numerous dosage forms, including tablets, capsules, liquids, and injectables, among others. Before any pharmaceutical product is approved for market release, it undergoes rigorous quality assessment to ensure it meets predefined safety, efficacy, and stability standards ^[3]. The quality evaluation of pharmaceuticals, whether in bulk raw materials or finished dosage forms, is a critical process. This evaluation is carried out using a variety of sophisticated analytical methodologies within industrial research and development (R&D) and Quality Control (QC) laboratories. These methodologies encompass titrimetric analysis, spectrophotometric techniques, chromatographic methods such as HPLC and GC, and biological assays, each chosen based on the specific requirements of the drug product being tested ^[4].

The Importance of Drug Testing and the Role of Analytical Methods

Drug testing is a cornerstone of modern healthcare and pharmaceutical development, ensuring the safety, efficacy, and quality of medicines. From preclinical research to post-marketing surveillance, it plays a vital role in safeguarding public

health. Central to this process are analytical methods, which enable the precise quantitative and qualitative assessment of drugs and their formulations ^[5]. These methods underpin critical aspects of pharmaceutical development, including quality control, pharmacokinetics, stability studies, and regulatory compliance, highlighting their indispensable role in the industry ^[6].

Ensuring Patient Safety

The primary aim of pharmaceutical testing is to ensure patient safety by verifying that medications are devoid of hazardous impurities, contaminants, or insufficient doses. Advanced analytical methodologies, including high-performance liquid chromatography (HPLC), mass spectrometry, and spectroscopic techniques, offer exceptional precision in identifying and quantifying active pharmaceutical ingredients (APIs), impurities, and degradation by products. For instance, impurities introduced during synthesis or degradation during storage can compromise drug efficacy or lead to adverse effects. Advanced analytical approaches ensure these impurities are maintained within permissible limits, thereby safeguarding patient health ^[7, 8].

Therapeutic Efficacy and Consistency

Another critical aspect of drug testing is verifying therapeutic efficacy. Analytical methods confirm that the actual content of APIs in drug formulations matches the labeled quantities, adhering to pharmacopeial standards ^[9]. This ensures consistency in drug performance, which is vital for achieving the desired pharmacological response, especially in chronic conditions requiring long-term treatment. Inadequate quantification can lead to therapeutic failure or the emergence of resistance, as seen with antibiotics. Techniques such as ultraviolet-visible (UV-Vis)

spectroscopy and chromatographic methods ensure the precision required to maintain therapeutic consistency ^[10].

Pharmacokinetics and Bioavailability

Drug testing also plays a pivotal role in pharmacokinetics and bioavailability studies, which are integral to drug development. These studies evaluate how a drug is absorbed, distributed, metabolized, and excreted, providing crucial insights into dosing regimens and administration strategies ^[11]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is extensively utilized for quantifying plasma drug levels, owing to its exceptional sensitivity and specificity. The data generated from such studies help optimize therapeutic regimens, reduce side effects, and enhance clinical outcomes ^[12].

Stability and Shelf Life

Stability testing is another critical area supported by analytical methods. Stability studies ensure that pharmaceutical products retain their intended efficacy, safety, and quality throughout their shelf life ^[13]. Analytical techniques are used to monitor degradation under various environmental conditions, such as temperature, humidity, and light exposure. These tests enable manufacturers to establish proper storage conditions and accurate expiration dates, ensuring the drug's reliability until its final use ^[14].

Regulatory Compliance

From a regulatory perspective, drug testing is an essential requirement for obtaining market approval. Authorities such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), and International

Council for Harmonisation (ICH) require rigorous analytical validation to demonstrate that testing methods are reliable and fit for their intended purpose ^[15]. Analytical techniques must meet stringent criteria for accuracy, precision, specificity, linearity, and sensitivity. Compliance with these global standards not only facilitates market approval but also ensures consistency and quality across international markets ^[16].

UV-Visible spectroscopy

UV-Visible spectroscopy is an essential tool in drug testing, widely employed for the qualitative and quantitative analysis of pharmaceutical compounds ^[17]. Its ability to measure the absorbance of ultraviolet (UV) and visible light by chemical substances provides critical insights into the identity, concentration, and purity of drugs. Operating within the wavelength range of 200–800 nm, UV-Visible spectroscopy is based on the principle that molecules with chromophores absorb light, leading to electronic transitions from lower to higher energy states. The resulting absorption spectrum serves as a fingerprint, enabling the identification of specific drug molecules.

In drug testing, UV-Visible spectroscopy plays a pivotal role in quality control, ensuring that pharmaceutical products meet regulatory standards. It is used to confirm the presence and quantify the concentration of active pharmaceutical ingredients (APIs) in formulations, ensuring consistency and therapeutic efficacy. The technique is particularly valuable for detecting impurities and degradation products that may arise during manufacturing or storage, safeguarding patient safety. Additionally, UV-Visible spectroscopy is integral to dissolution testing, a critical step in evaluating drug release profiles for oral dosage forms ^[18].

The technique is widely favored for its simplicity, rapid analysis, and non-destructive nature, allowing repeated testing without altering the sample. However, it is most effective for compounds with chromophores that absorb UV or visible light, limiting its application for certain drugs. Despite this, UV-Visible spectroscopy remains a cornerstone in pharmaceutical analysis, offering reliable and precise data that underpin drug development, regulatory compliance, and the overall assurance of medication quality ^[19].

Chromatographic techniques

Chromatographic methods, especially High-Performance Liquid Chromatography (HPLC), play a critical role in pharmaceutical analysis, providing outstanding precision and dependability for the qualitative and quantitative evaluation of drug substances ^[20]. HPLC functions by separating components of a mixture based on their interactions with a stationary phase and a mobile phase under elevated pressure. This technique serves as a robust tool for analyzing active pharmaceutical ingredients (APIs), impurities, degradation products, and excipients across various dosage forms.

In pharmaceutical quality control, HPLC is extensively employed for evaluating the purity, potency, and stability of drug formulations. By accurately separating and quantifying APIs, the method ensures compliance with regulatory standards for drug quality and safety. Its utility in impurity profiling is particularly noteworthy, as it enables the detection and quantification of trace impurities to confirm that they remain within acceptable limits, thus safeguarding patient health. Additionally, HPLC is integral to stability testing, providing insights into drug degradation under varied environmental conditions, including temperature, humidity,

and light exposure. This information is crucial for determining a product's shelf life and appropriate storage conditions ^[21].

The versatility of HPLC lies in its ability to analyze a diverse range of analytes using different detection methods, such as ultraviolet (UV) absorption, fluorescence, and mass spectrometry (HPLC-MS). UV detection is widely favored for its sensitivity and broad applicability to pharmaceutical compounds. Moreover, HPLC plays a key role in pharmacokinetic studies, facilitating the precise measurement of drug concentrations in biological matrices like plasma ^[22].

HPLC offers several advantages, including high reproducibility, robustness, and suitability for complex mixtures. Effective application of HPLC requires careful optimization of critical parameters, including mobile phase composition, flow rate, and column selection. Despite these complexities, HPLC continues to be the gold standard in pharmaceutical analysis, guaranteeing the safety, efficacy, and quality of therapeutic compounds ^[23].

Ultra-High-Performance Liquid Chromatography (UHPLC) represents a significant advancement in chromatographic technology, offering enhanced resolution, speed, and sensitivity compared to traditional liquid chromatography (LC) methods. Building upon the principles of High-Performance Liquid Chromatography (HPLC), UHPLC utilizes columns packed with sub-2 μm particles and operates under elevated pressures, often exceeding 15,000 psi. These refinements enable superior analytical performance, making UHPLC a valuable tool for modern analytical chemistry. Similar to HPLC, UHPLC relies on the interaction of analytes with a liquid mobile phase and a stationary phase within the column to achieve separation. However, the reduced particle size in UHPLC columns provides a markedly higher surface area, significantly enhancing the interactions between analytes and the

stationary phase. This enhancement results in sharper, well-defined chromatographic peaks and improved resolution, even for complex mixtures ^[24, 25].

Moreover, the smaller particles in UHPLC columns minimize the diffusion path length, promoting more efficient mass transfer. This feature not only improves separation efficiency but also allows for faster analysis times without compromising the quality of the results. The combination of high resolution, rapid analysis, and sensitivity positions UHPLC as a cornerstone of modern analytical techniques, particularly in applications demanding high throughput and precision ^[26].

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) are a widely used class of medications known for their analgesic, anti-inflammatory, and antipyretic effects. These drugs alleviate pain and inflammation through the inhibition of cyclooxygenase (COX) enzymes, which are central to the biosynthesis of prostaglandins. Prostaglandins are lipid mediators that play a critical role in amplifying inflammatory responses, pain, and fever ^[27].

There are two primary isoforms of COX enzymes: COX-1 and COX-2. COX-1 is constitutively expressed in most tissues and is involved in maintaining essential physiological functions, such as protecting the gastric mucosa, supporting platelet aggregation, and regulating renal blood flow ^[28]. In contrast, COX-2 is inducible and primarily associated with inflammation and pain. Traditional NSAIDs, including ibuprofen, naproxen, Loxoprofen sodium, and aspirin, inhibit both COX-1 and COX-2. While this dual inhibition effectively reduces pain and inflammation, it also leads to adverse effects, such as gastrointestinal irritation and an increased risk of bleeding ^[29].

Selective COX-2 inhibitors, such as celecoxib, Etodolac, and etoricoxib, were developed to mitigate these side effects by specifically targeting COX-2 while sparing COX-1. Despite their targeted mechanism of action, the prolonged use of COX-2 inhibitors has been associated with increased cardiovascular risks, including hypertension and myocardial infarction, highlighting the need for cautious use and individualized risk assessments ^[30]. NSAIDs are commonly prescribed for the management of various conditions, including osteoarthritis, rheumatoid arthritis, gout, dysmenorrhea, and acute musculoskeletal injuries. Additionally, aspirin, a distinctive NSAID, is used at low doses for its antiplatelet effects in the prevention of cardiovascular events, such as myocardial infarction and stroke ^[31].

Quality by Design (QbD)

Quality by Design (QbD) represents a systematic, proactive strategy in pharmaceutical development, emphasizing the integration of quality throughout every phase of product creation and manufacturing. This approach ensures that quality is not an afterthought or solely a matter of post-production testing, but an intrinsic aspect of the product's design from the very beginning. By embedding quality into the development process, QbD aims to foster a deeper understanding of both the product and the underlying manufacturing processes, thereby facilitating the consistent production of high-quality pharmaceuticals ^[32].

Core Principles of QbD

The central tenet of QbD is that quality cannot be merely tested into a product after production; it must be designed into the product from its inception. To achieve this, a Quality Target Product Profile (QTPP) is first established, which serves as a blueprint outlining the desired attributes of the final pharmaceutical product. This

profile encompasses critical factors such as the product's safety, efficacy, and overall performance. Building upon the QTPP, Critical Quality Attributes (CQAs) are identified—these are the physical, chemical, or biological properties of the product that must be controlled and maintained within specified limits to ensure the final product meets the desired quality standards. The identification and control of CQAs are essential for the development of consistent, reliable pharmaceutical products ^[33].

Understanding Variability

Another fundamental principle of QbD is the identification and management of Critical Process Parameters (CPPs) and Critical Material Attributes (CMAs). These parameters and attributes play a pivotal role in influencing the CQAs throughout the manufacturing process ^[34]. By thoroughly understanding how various factors such as raw material characteristics, process conditions, and equipment variables impact product quality, manufacturers can design robust, efficient processes that minimize variability. This approach not only helps in maintaining consistency across batches but also in mitigating potential risks associated with variations. A comprehensive risk assessment, coupled with the application of statistical tools, is integral to identifying, quantifying, and controlling sources of variability. This proactive strategy ensures that potential issues are addressed before they affect product quality, ultimately leading to more reliable manufacturing processes ^[35].

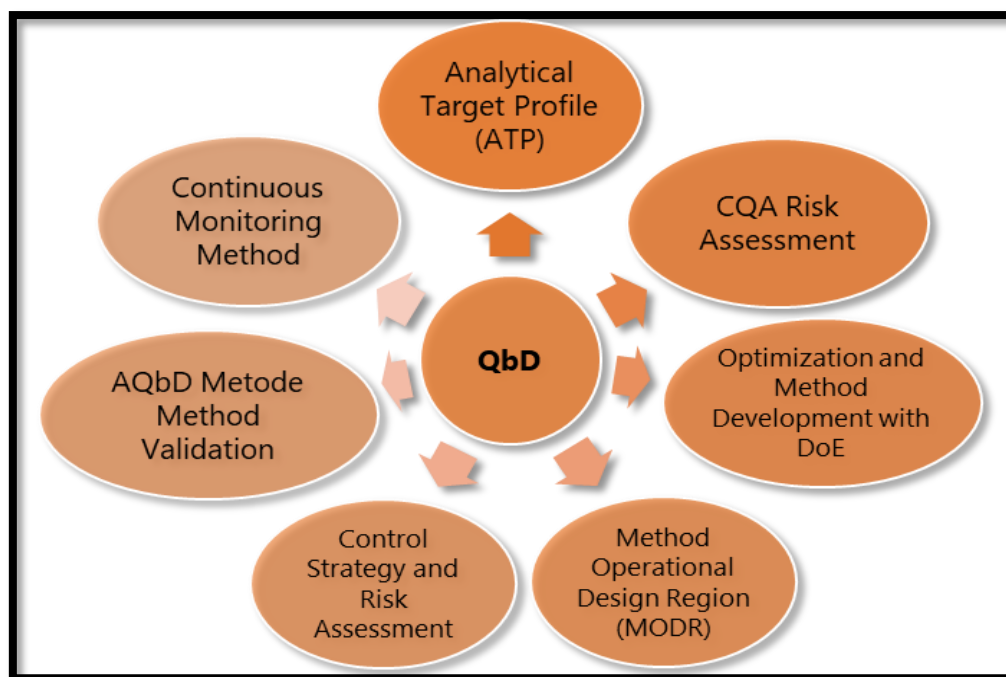


Figure 1. Elements of Quality by Design approach

Implementation of QbD in Analytical Methods

Quality by Design (QbD) principles are instrumental in the development of analytical methods. By adopting QbD in method development, analytical teams can gain a deeper understanding of how various method parameters—such as temperature, pH, flow rate, detection wavelength or reagent concentration—affect the method's performance and reproducibility ^[36]. This structured approach allows for the optimization of method parameters, ensuring that analytical procedures are both robust and capable of consistently meeting predefined quality standards. Furthermore, Process Analytical Technology (PAT) is an essential component of QbD in analytical methods. PAT facilitates real-time monitoring of critical process parameters and quality attributes during the manufacturing process, enabling operators to make informed decisions and adjustments as needed. The integration of PAT ensures that quality control is continuous and not limited to post-production testing, enhancing the overall reliability and consistency of pharmaceutical manufacturing ^[37].

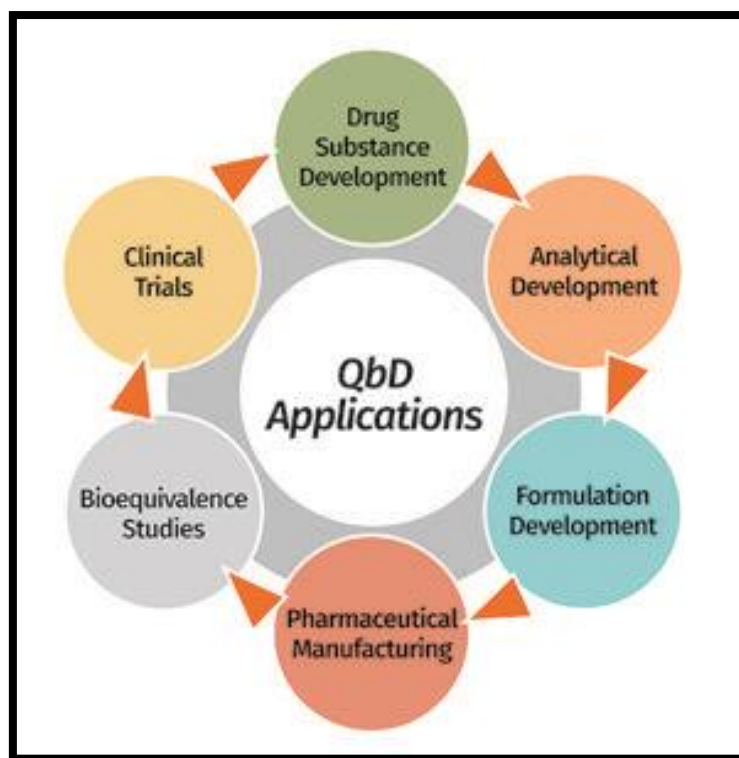


Figure 2.Applications of Quality by Design

1.2 REVIEW OF LITERATURE

DRUGS PROFILE

The list of NSAIDs selected for the proposed research work is summarized in Table 1. Detailed drug profiles for the selected drugs are provided below, outlining their category, mechanisms of action, and relevance to the study objectives.

Table 1. List of selected NSAIDs in proposed research

Sr. No.	Drug name	Category	Mechanism of action
1	Loxoprofen sodium	NSAID	Inhibits cyclooxygenase (COX) enzymes, reducing prostaglandin synthesis and alleviating pain and inflammation.
2	Baclofen	Skeletal Muscle Relaxant	Acts as a GABA-B receptor agonist, inhibiting synaptic transmission in the spinal cord to reduce muscle spasticity.
3	Etodolac	NSAID	Selectively inhibits COX-2 enzymes, decreasing prostaglandin synthesis and providing anti-inflammatory and analgesic effects.
4	Thiocolchicoside	Muscle Relaxant	Binds to GABA-A receptors and glycine receptors, exerting muscle relaxant and anti-inflammatory effects.

Loxoprofen Sodium

Loxoprofen sodium, an NSAID (non-steroidal anti-inflammatory drug), is extensively used for its potent analgesic, anti-inflammatory, and antipyretic properties^[38]. **Figure 3** illustrates its chemical structure. It functions by inhibiting prostaglandin synthesis through cyclooxygenase (COX) enzyme blockade, thereby alleviating inflammation and pain. Loxoprofen sodium is primarily administered for conditions such as rheumatoid arthritis, osteoarthritis, and postoperative pain management. It is commonly available as an oral tablet and also in topical formulations. Potential side effects include gastrointestinal discomfort, headaches, or dizziness. The drug is contraindicated in individuals with severe hepatic, renal, or cardiac impairments, as well as in those with a history of peptic ulcers or hypersensitivity to NSAIDs^[39].

Chemical Name: Sodium 2-[4-(2-Oxocyclopentylmethyl) phenyl] propanoate.

Molecular Formula: C₁₅H₁₇NaO₃

Molecular Mass: 268.29 g/mol

pKa: 4.0 (carboxylic group)

Solubility: Freely soluble in water and methanol

Appearance: Loxoprofen sodium appears as a white to pale yellow crystalline powder.

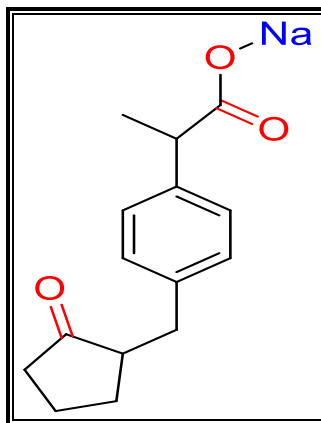


Figure 3. Chemical structure of Loxoprofen sodium

Baclofen

Baclofen, illustrated in **Figure 4**, is a centrally acting muscle relaxant and antispasmodic agent commonly prescribed for managing spasticity associated with conditions such as multiple sclerosis, spinal cord injuries, and cerebral palsy ^[40]. Its mechanism of action involves activating GABAB receptors in the central nervous system, leading to decreased excitatory neurotransmitter release and suppression of reflex hyperexcitability. Baclofen is available in both oral and intrathecal formulations. Frequently reported side effects include drowsiness, dizziness, and gastrointestinal disturbances. Abrupt discontinuation can result in severe complications, including seizures or hallucinations. It is contraindicated in individuals with hypersensitivity to Baclofen or significant renal impairment ^[41].

Chemical Name: 4-Amino-3-(4-chlorophenyl)butanoic acid

Molecular Formula: C₁₀H₁₂ClNO₂

Molecular Mass: 213.66 g/mol

pKa: 3.87 (carboxylic group)

Solubility: Slightly soluble in water; soluble in methanol

Appearance: Baclofen is a white to off-white crystalline powder.

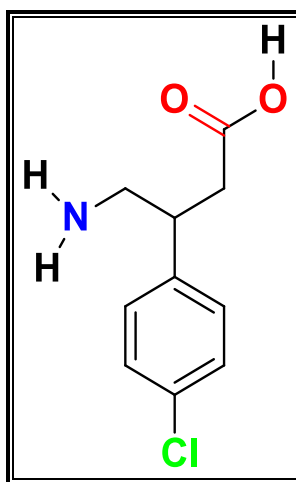


Figure 4. Chemical structure of Baclofen

Etodolac

Etodolac, depicted in **Figure 5**, is a NSAID primarily utilized for managing pain and inflammation in conditions such as osteoarthritis, rheumatoid arthritis, and acute pain ^[42]. Its therapeutic effect is achieved by selectively inhibiting cyclooxygenase-2 (COX-2), leading to reduced prostaglandin synthesis, which mediates inflammation and pain. Etodolac is commonly administered orally in tablet or capsule form. Typical side effects include gastrointestinal discomfort, dizziness, and headaches, while long-term use may elevate the risk of cardiovascular complications and gastrointestinal bleeding. It is contraindicated in patients with active peptic ulcers, significant hepatic or renal impairment, or hypersensitivity to NSAIDs ^[43].

Chemical Name: 1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid

Molecular Formula: C₁₇H₂₁NO₃

Molecular Mass: 287.35 g/mol

pKa: 4.65 (carboxylic group)

Solubility: Sparingly soluble in water, but soluble in ethanol and methanol.

Appearance: Etodolac is a white to off-white crystalline powder.

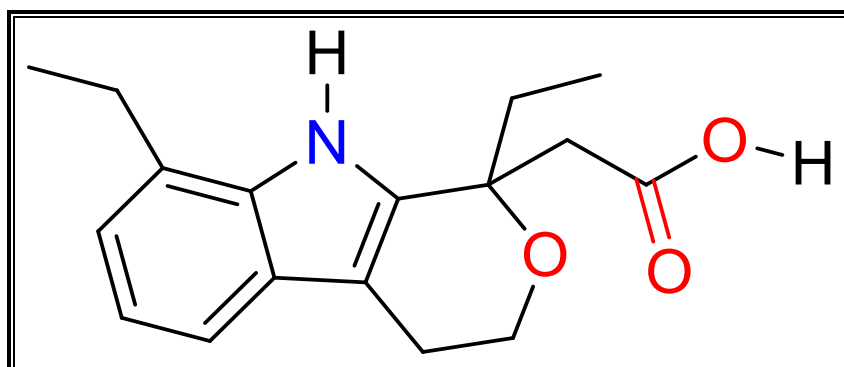


Figure 5. Chemical structure of Etodolac

Thiocolchicoside

Thiocolchicoside, shown in **Figure 6**, is a semi-synthetic muscle relaxant with anti-inflammatory and analgesic properties ^[44]. It is commonly used to alleviate painful muscular spasms associated with conditions such as low back pain, cervical pain, and post-traumatic muscle stiffness. Thiocolchicoside acts as a GABAA receptor agonist, promoting muscle relaxation by modulating neurotransmitter activity in the central nervous system. It is available in oral, injectable, and topical formulations. Common side effects include gastrointestinal discomfort, drowsiness, and allergic reactions. Prolonged or high-dose use may lead to potential adverse effects on fertility and should be avoided during pregnancy and lactation ^[45].

Chemical Name: N-[1,2-Dimethoxy-10-(methylthio)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl] acetamide.

Molecular Formula: C₂₇H₃₃NO₁₀S

Molecular Mass: 563.62 g/mol

pKa: 12.74

Solubility: Freely soluble in water and methanol

Appearance: Thiocolchicoside appears as a yellow crystalline powder.

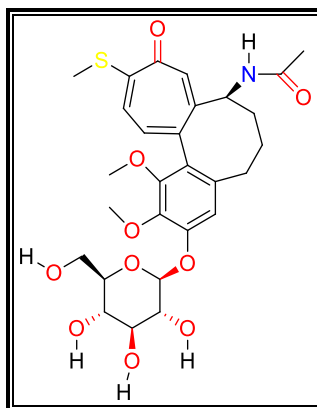


Figure 6. Chemical structure of Thiocolchicoside

Dongala et al. (2019) reported the development of a novel, stability-indicating HPLC method for the simultaneous quantification of 14 impurities in Excedrin tablets—a combination drug formulation containing acetaminophen, aspirin, and caffeine. Notably, the study identified and confirmed a previously unreported impurity formed due to aspirin degradation under accelerated stability conditions involving elevated temperatures. The impurity was characterized and structurally confirmed using advanced LC-MS techniques, demonstrating the method's robustness and appropriateness for monitoring impurities in complex pharmaceutical formulations ^[46].

Hemlata et al. (2016) developed a stability-indicating RP-HPLC method for the simultaneous determination of paracetamol (PCM), pantoprazole (PPZ), and ibuprofen (IBU) amidst their degradation products. The study investigated the degradation behavior of PCM, PPZ, and IBU under various stress conditions, including those mimicking the gastrointestinal environment, by employing simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) studies. This method demonstrates potential applicability for analyzing samples generated during extended accelerated stress degradation studies, offering a reliable tool for stability assessment in pharmaceutical formulations ^[47].

Sharma et al. (2017) emphasized the critical role of forced degradation studies in the stability testing of pharmaceutical products. Their review discusses the degradation pathways of drug substances and products, providing valuable insights into excipient compatibility assessments and aiding early-stage drug development. Although the impurities generated during stress studies may not always be detected in real-time stability studies, this approach serves as a foundational step for developing stability-indicating analytical methods. The authors advocate for initiating forced degradation

studies at the earliest stages of development to assess the inherent stability of drug candidates, thereby enhancing formulation design and optimizing manufacturing processes^[48].

Runje *et al.* (2016) developed a stability-indicating UHPLC method for nepafenac, suitable for routine analysis of production and stability samples. The degradation behavior of nepafenac was thoroughly investigated under stress conditions as outlined by ICH guidelines. The method effectively separated all degradation and process-related impurities from the drug substance, showcasing its robust stability-indicating capabilities. Compared to conventional HPLC methods, the developed UHPLC method demonstrated superior sensitivity, reduced analysis time, and minimized resource consumption, making it a more efficient alternative for quality control applications^[49].

Lipiec- Abramska *et al.* (2014) developed a HPLC method equipped with a UV spectrophotometric detector for the chemical purity analysis and assay of nepafenac. The method involved optimizing the mobile phase composition, which included 10 mM ammonium formate at pH 4.1, and the choice of HPLC column, specifically the Phenomenex Gemini-NX C18, during method development. Four major impurities were validated for quantitative analysis, while the remaining impurities were validated using a limit test, following ICH Q2(R1) guidelines. The method demonstrated accuracy with recovery values ranging from 90–108% for chemical purity and 99–101% for assay studies. The detection limit was highly sensitive, at 15–30 ng/mL, and the method's linearity met all statistical requirements, ensuring its reliability for pharmaceutical quality control^[50].

Jadhav *et al.* (2015) developed a stability-indicating RP-HPLC method for the simultaneous estimation of atorvastatin calcium and celecoxib in both bulk and niosomal formulations. The analysis was conducted using a Cosmosil-C18 column (4.6 mm × 250 mm, 5 µm) maintained at 25 °C. The mobile phase comprised acetonitrile, an ammonium acetate buffer (pH 5.0), and methanol in a 50:25:25 (v/v/v) ratio, with detection at 277 nm and a flow rate of 1.0 mL/min. Retention times for atorvastatin calcium and celecoxib were noted at 6.195 and 3.989 minutes, respectively. The method was validated according to ICH guidelines, demonstrating its reliability and suitability for stability and routine quality control analysis ^[51].

Venkataraman *et al.* (2018) investigated the degradation pathways and performed structural elucidation of degradation products formed under forced degradation conditions. The study emphasized the importance of forced degradation experiments in determining the specificity of stability-indicating methods. These experiments are crucial for identifying storage conditions and optimizing the manufacturing process of pharmaceutical formulations, thereby ensuring product stability and quality throughout its shelf life ^[52].

Analytical methods for estimation of Loxoprofen sodium

Muhammad Farooq *et al.* (2018) developed a high-performance liquid chromatography (HPLC) method for the analysis of Loxoprofen (LXP) in both mobile phase and human plasma. Chromatographic separation was achieved using a Mediterranean Sea C18 column (250 × 4.6 mm, 5 µm), with a mobile phase consisting of acetonitrile and 0.01 M sodium dihydrogen phosphate buffer in a 55:45 (v/v) ratio, adjusted to pH 6.5. The flow rate was maintained at 1 mL/min. The method exhibited a linear calibration range of 0.1–10 ppm with a high correlation coefficient ($R^2 = 0.999$) based on linear regression analysis. The lower limit of quantification (LLOQ)

was established at 0.1 ppm. The proposed method demonstrated the stability of LXP with an accuracy error of less than 10%, ensuring its reliability for bioanalytical and pharmaceutical applications ^[53].

Meng Li *et al.* (2019) developed a reverse-phase high-performance liquid chromatography (HPLC) method for the enantiomeric separation of nine profens, including ibuprofen, indoprofen, pranoprofen, flurbiprofen, ketoprofen, carprofen, naproxen, Loxoprofen, and Etodolac. The study systematically investigated the influence of critical parameters such as the selection of chiral columns and mobile phase compositions on enantiomeric resolution. The research primarily focused on chiral profens, exploring their enantiomeric separation using HPLC and capillary electrophoresis (CE), as well as their pharmacokinetic profiling in plasma through HPLC-MS/MS analysis. This comprehensive approach provides valuable insights into the chiral analysis and pharmacokinetics of profens ^[54].

Maya S. Eissa *et al.* (2013) developed and validated stability-indicating HPTLC and HPLC-UV methods for the determination of Loxoprofen in the presence of its forced degradation products. The HPTLC-densitometric method achieved separation using silica gel F254 plates and a mobile phase of toluene: acetone: acetic acid (1.8:1.0:0.1, v/v/v), with densitometric detection at 220 nm. For the HPLC-UV method, separation was performed on a C18 analytical column using an isocratic mobile phase comprising acetonitrile and 0.15% triethylamine (pH 2.2) in a 50:50 (v/v) ratio, with a flow rate of 1.0 mL/min and UV detection at 220 nm. Both methods were validated according to ICH guidelines and were successfully applied for the determination of Loxoprofen in its pharmaceutical formulations, demonstrating robustness and reliability ^[55].

Aslam I *et al.* (2013) developed an HPLC-UV method for the determination of Loxoprofen sodium in healthy humans. The method utilized a Hypersil BDS C18 column (250 × 4.6 mm, 5 µm, Thermo Scientific, Germany). The mobile phase consisted of acetonitrile and water in a 40:60 ratio, adjusted to pH 3.0 with orthophosphoric acid, flowing at 1 mL/min. The analysis was conducted at room temperature with UV detection set at 225 nm. This method enabled rapid quantification of Loxoprofen in blood samples across a range of 100 to 10,000 ng/mL, with a total run time of approximately 15 minutes. In comparison to previously described HPLC-UV methods that required at least 30 minutes and involved column switching techniques, this method proved to be significantly faster and more efficient ^[56].

Hea-Young Cho *et al.* (2006) developed a column-switching liquid chromatography method for the direct and simultaneous analysis of Loxoprofen and its metabolites (trans- and cis-alcohol metabolites) in human serum. The method involved direct injection of the serum sample into the system, followed by deproteinization and trace enrichment on a Shim-pack MAYI-ODS pretreatment column (10 mm × 4.6 mm i.d.). The eluent used for this step was a mixture of 20 mM phosphate buffer (pH 6.9)/acetonitrile (95/5, v/v) with 0.1% formic acid. The drug retained on the pretreatment column was subsequently introduced to the Shim-pack VP-ODS analytical column (150 mm × 4.6 mm i.d.) using an elution solvent of acetonitrile/water (45/55, v/v) containing 0.1% formic acid upon switching the 6-port valve. Ketoprofen served as the internal standard for the analysis, which was monitored using a UV detector at 225 nm. This column-switching approach provided enhanced sensitivity and specificity for the simultaneous detection of Loxoprofen and its metabolites in human serum ^[57].

R. Nanthakumar *et al.* (2016) developed a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of Loxoprofen sodium dihydrate (LSD) in tablet formulations. The analysis was conducted using a Phenomenex Luna C18(2) column (250 × 4.6 mm, 5 µm). The compound was separated with a mobile phase consisting of a mixture of 0.4% orthophosphoric acid and acetonitrile in a 45:55 (v/v) ratio. The flow rate was set at 1.0 mL/min, and UV detection was performed at 210 nm. The run time per sample was 8 minutes, with a retention time of 4.9 minutes. System suitability parameters, including theoretical plate count, tailing factor, and %RSD between six standard injections, were within acceptable limits, confirming the method's reliability and efficiency for routine quality control analysis of Loxoprofen sodium dihydrate in tablet formulations ^[58].

Analytical methods for estimation of Baclofen

Patharevirendra Sopan *et al.* (2022) developed an HPLC method for the separation of Baclofen and its impurities according to the latest regulatory requirements. The mobile phase comprised 0.1% trifluoroacetic acid (TFA) and acetonitrile in a 65:35 ratio, using an Inertsil ODS 3V column (150 × 4.6 mm, C18) with a detector wavelength set at 225 nm. The flow rate was maintained at 0.6 mL/min, with the column temperature at 27 °C. The diluent used was a mixture of acetonitrile and water in a 50:50 (v/v) ratio. The injection volume was 10 µL. The method demonstrated good linearity for Baclofen across a concentration range of 0.05 mg/mL, with a linear regression correlation coefficient (R) of 0.999. The Baclofen sample solution and mobile phase were found to be stable for at least 48 hours. The relative standard deviation (% RSD) for assay content in six sample solutions was 0.25, indicating high precision and robustness of the method ^[59].

Marwa S. Falih *et al.* (2018) developed a spectrophotometric method for the estimation of Baclofen utilizing the area under the curve (AUC) technique. The proposed method was applied over a broad concentration range of 50–450 mg/L. The detection limits for the AUC1 and AUC2 techniques were 0.451 and 0.520, respectively, indicating good sensitivity. The limit of quantitation was found to be 1.366 for AUC1 and 1.576 for AUC2. The results were statistically analyzed using recovery studies and relative standard deviation (RSD) studies, which demonstrated the reliability and precision of the methods. These findings suggest that the spectrophotometric AUC technique is a robust and reliable method for the determination of Baclofen in various concentrations ^[60].

KB Gabhane *et al.* (2014) developed a simple, sensitive, precise, and economical spectrophotometric method for the analysis of Baclofen in its bulk form. The method utilized water as the solvent, with Baclofen demonstrating maximum absorbance at 220 nm. The absorbance was observed to increase linearly with increasing concentrations of Baclofen, which was corroborated by the calculated correlation coefficient ($r^2 = 0.999$). The linear regression analysis for the calibration plot demonstrated a robust linear relationship within the concentration range of 10–100 $\mu\text{g/mL}$. The limits of detection (LOD) and quantitation (LOQ) were determined to be 1.25316 $\mu\text{g/mL}$ and 3.797468 $\mu\text{g/mL}$, respectively. This method was rigorously tested and validated according to International Conference on Harmonization (ICH) guidelines. The results indicated high accuracy, precision, and reproducibility (RSD <2%), confirming its reliability for Baclofen analysis ^[61].

Kamaldeep Singh *et al.* (2013) developed a simple and sensitive RP-HPLC method for the quantitative estimation of Baclofen in both bulk drug and pharmaceutical formulations. Chromatographic separation was achieved using a mobile phase

consisting of a mixture of Methanol: Water: Acetic Acid (100:100:0.5) with a flow rate of 1.0 mL/min. The method demonstrated linearity over the concentration range of 10–50 µg/mL, with a high correlation coefficient ($r^2 = 0.999$). Recovery studies were performed by adding known quantities of Baclofen solution (at 80%, 100%, and 120% levels) to the powdered sample of the tablet formulation, yielding high recovery rates for Baclofen (100.03%, 100.18%, and 100.05%). The robustness of the method was evaluated by analyzing the sample under varied conditions, such as changes in flow rate, detection wavelength, and column temperature. The method demonstrated excellent robustness with a tailing factor consistently less than 2, confirming its reliability for routine analysis ^[62].

G. Sowjanya *et al.* (2017) developed and validated a RP-HPLC method for the quantification of Baclofen in injection formulations. Chromatographic separation was achieved using a Symmetry C18 column (250 x 4.6 mm, 5 µm). The mobile phase comprised a binary mixture of potassium dihydrogen phosphate and pentane-1-sulfonic acid sodium salt buffer (pH 3.0), mixed with acetonitrile and methanol, in an optimized gradient mode. The method demonstrated linearity within the concentration range of 0.0050 – 0.0150 mg/mL. Precision was validated with relative standard deviations (RSDs) of less than 2% for both intra-day and inter-day variation studies, confirming high reproducibility of the method. The recovery of Baclofen ranged from 98.6% to 100.5%, confirming the method's accuracy ^[63].

Venkata Narasimha Rao G *et al.* (2018) developed an UPLC method for the simultaneous quantification of Baclofen and its potential impurities in a pre-filled syringe. Chromatographic separation was performed on an Acquity BEHC18 column (100 x 2.1 mm, 1.7 µm) using a simple and cost-effective buffer composed of potassium phosphate (0.01 M), along with a mixture of acetonitrile and methanol as

the mobile phase. The mobile phase was pumped at a flow rate of 0.3 mL min⁻¹ using a linear gradient elution mode. Sample components were detected with a UV-Visible (UV-VIS) detector set at 225 nm. A high linear correlation coefficient of 0.999 was achieved across the concentration range from the limit of quantification (LOQ) to 150% of the target specifications ^[64].

Analytical methods for estimation of Etodolac

Chintala Parimala Devi *et al.* (2020) conducted a comparative study of validation parameters between marketed Etodolac tablets (Etova 200 mg) and its active pharmaceutical ingredient (API) using UV spectroscopy. The absorbance was measured within the wavelength range of 275-279.5 nm. A solvent mixture of methanol and water in a 9:1 ratio was employed for the validation of both the marketed formulation and the API. The method was validated statistically through the analysis results and recovery studies. The validation results revealed higher linearity for the Etodolac tablets compared to the crude form of Etodolac, suggesting improved precision and consistency in the tablet form. Notably, there was no interference from impurities or excipients during the validation process, which ensures the specificity and reliability of the analytical method used ^[65].

Sabyasachi Biswal *et al.* (2019) developed a sensitive and validated analytical method using both RPLC and UV-spectrophotometry for the determination of Etodolac in both bulk and pharmaceutical dosage forms. The UV-spectrophotometric method involved measuring absorbance at its maximum wavelength of 226 nm using a solvent mixture of methanol and water (70:30). The drug conformed to Beer-Lambert's law within the concentration range of 2-20 µg/mL, with a high regression coefficient of 0.9998 at 226 nm. In the chromatographic method, optimization was achieved using a Phenomenex Luna C18 column with UV detection at 226 nm. The

mobile phase comprised a mixture of acetonitrile and methanol in a 65:35 ratio, with a flow rate of 1.0 mL/min. Recovery studies yielded results in the range of 98% to 102%, and the method's LOD and LOQ were determined to be 0.08 µg/mL and 0.26 µg/mL, respectively ^[66].

K. Rajeswar Dutt *et al.* (2017) developed a visible spectrophotometric method for the quantitative estimation of Etodolac in both bulk and pharmaceutical dosage forms. This method involves measuring absorption at its maximum wavelength of 549 nm using methanol as the solvent. The drug conformed to Beer-Lambert's law within the concentration range of 0-60 µg/mL, exhibiting a high regression coefficient of 0.999 at 549 nm. The method demonstrated excellent precision, with inter-day and intra-day % RSDs of 0.576 and 0.48, respectively, well below the 2% threshold, indicating high reproducibility. The results of the analysis were validated in accordance with ICH guidelines, confirming the method's accuracy and reliability ^[67].

Goel H *et al.* (2021) developed and validated a RP-HPLC method for the simultaneous estimation of Etodolac and 5-Fluorouracil (5-FU) in both bulk and coated tablet dosage forms. Standard instrument parameters were optimized using a C18 reverse phase Inertsil® ODS column (250 × 4.6 mm, 5 µm particle size) with a system equipped with a photodiode array (PDA) detector. An optimized mobile phase ratio of 60:40 (v/v) acetonitrile and di-potassium hydrogen phosphate buffer (pH 6.4, 25 mM) under low-pressure gradient mode was employed. This setup provided well-resolved, sharp peaks with adequate molar absorptivity at a λ_{max} of 280 nm, exhibiting retention times of 4.3 ± 0.2 minutes for 5-FU and 11.5 ± 0.3 minutes for Etodolac ^[68].

Rahman SN, et al. (2024) developed a systematically optimized RP-HPLC method for the estimation of Etodolac and Cyclosporin A. The method involved identifying the isosbestic point and a scouting step to select the optimal conditions. The maximum wavelength (λ_{max}) of 205 nm and an acetonitrile-water mixture in the ratio of 74:26 were selected as the optimal conditions for the mobile phase. An artificial neural network (ANN) topology (3:10:4) was generated from 20 trials produced by a face-centered central composite design (CCD) model ^[69].

Abdelhameed AS, et al. (2014) developed a simple, sensitive, and accurate HPLC-DAD method for the simultaneous determination of Pantoprazole (PTZ) and Etodolac (ETD) in rat plasma as a tool for therapeutic drug monitoring. Optimal chromatographic separation of the analytes was achieved using a Waters Symmetry C18 column. The mobile phase consisted of a phosphate buffer (pH ~ 4.0) as eluent A and acetonitrile as eluent B in a ratio of 55:45 (v/v) for a total run time of 6 minutes, pumped isocratically at a flow rate of 0.8 mL min⁻¹. The eluted analytes were monitored using a PDA, quantified at 254 nm. The method exhibited excellent linearity, with correlation coefficients (r^2) of 0.9999 for PTZ and 0.9995 for ETD across the concentration ranges of 0.1–15 µg/mL for PTZ and 5–50 µg/mL for ETD, respectively ^[70].

Analytical methods for estimation of Thiocolchicoside

Kumar S et al. (2011) developed an RP-HPLC method for the simultaneous estimation of Etoricoxib and Thiocolchicoside in tablet dosage forms. The method utilizes a photodiode array (PDA) detector set at 245 nm. The analysis was conducted over concentration ranges of 20–160 ppm for Etoricoxib and 1–10 ppm for Thiocolchicoside. Chromatographic separation was achieved using a PuritasEximius C18 column (250 × 4.6 mm, 5 µm) with an analyzer column temperature of 25°C ±

0.5°C. The mobile phase consisted of acetonitrile and 0.1% acetic acid in water, mixed in a 70:30 volume-to-volume composition ^[71].

Sanjay Shesha Shetgar *et al.* (2022) developed and validated a RP-UPLC method for the simultaneous quantification of Etoricoxib and Thiocolchicoside in tablet formulations. Chromatographic separation was achieved on a Waters Acquity UPLC system using a Hibar C18 column (100 × 2.1 mm, 1.8 µm). The mobile phase consisted of a mixture of 0.1% orthophosphoric acid (pH 2.5) and acetonitrile in a 90:10 (v/v) ratio. The method employed a flow rate of 0.3 mL/min and an injection volume of 2 µL. The column was maintained at 30°C, and detection was performed at 256 nm using an Acquity Tunable UV detector. Data acquisition and processing were managed using Waters Empower 2 software ^[72].

Dhiware AD, *et al.* (2012) developed and validated a simple, sensitive, rapid, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Etodolac and Thiocolchicoside in pharmaceutical dosage forms. Chromatographic separation was performed on a C18 column with a particle size of 5 µm. The retention times were recorded as 2.777 minutes for Etodolac and 2.380 minutes for Thiocolchicoside. The method provided quantitative estimations with satisfactory results, showing a recovery of 99.66% for Etodolac and 99.3% for Thiocolchicoside. The correlation coefficients were exceptionally high, with r^2 of 0.9999 and 0.9998 for Etodolac and Thiocolchicoside, respectively ^[73].

Silvio A, *et al.* (2012) developed a stability-indicating HPLC method for the determination of Thiocolchicoside (TCC) and its primary degradation products—Thiocolchicoside S-oxide (D1SO) and 3-O-demethylthiocolchicine (D3)—in both liquid and solid formulations. The method utilized a Synergi™ 4 µm Polar-RP 80 Å column (150 × 4.6 mm, Phenomenex) for effective separation and quantification. A

mobile phase consisting of eluent A: 20 mM sodium acetate buffer (pH 5.0) and eluent B: a mixture of methanol and acetonitrile (20:80, v/v) was employed in gradient mode. Detection was performed at 254 nm. The validated method demonstrated its stability-indicating capability by ensuring effective separation of TCC and its degradation products, facilitating accurate quantification in pharmaceutical formulations under various stress conditions ^[74].

Jadhav SD, et al. (2015) developed a robust stability-indicating RP-HPLC method for the simultaneous determination of Thiocolchicoside and diclofenac potassium at a single wavelength (258 nm). Chromatographic separation was achieved using a Zorbax SB CN column (250 mm × 4.6 mm, 5 µm) with a gradient elution technique. The mobile phase comprised solvent A, a 5 mM sodium dihydrogen phosphate buffer (pH 2.5), and solvent B, methanol. A flow rate of 1.0 mL/min was maintained throughout the analysis. This method was validated as stability-indicating, effectively separating both analytes under various stress conditions, ensuring reliable quantification in pharmaceutical formulations ^[75].

1.3 JUSTIFICATION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed medications worldwide, offering effective relief from a variety of inflammatory and pain-related conditions. These conditions include chronic diseases such as rheumatoid arthritis, osteoarthritis, and gout, as well as acute pain from conditions like headaches, postoperative recovery, and orthopedic fractures. Given their extensive use, the development of accurate, efficient, and reliable methods for the qualitative and quantitative analysis of NSAIDs and their combinations is crucial to ensuring the safety and efficacy of these therapeutic agents.

Several liquid chromatographic techniques have been developed for the individual and combined analysis of NSAIDs. These methods offer various advantages, such as sensitivity and specificity, and have been employed to monitor the active pharmaceutical ingredients (APIs) in NSAID formulations. However, despite the existence of numerous methodologies, there are significant limitations in the current approaches, highlighting the need for further advancement in analytical methods for NSAIDs.

One of the major concerns with existing liquid chromatographic methods is the use of high concentrations of organic solvents, often exceeding 50%. The reliance on organic solvents in such high volumes not only raises concerns regarding the environmental impact but also introduces significant cost inefficiencies. For routine pharmaceutical industry analyses, the cost of solvents can become prohibitively high, undermining the sustainability and affordability of the analytical process. Additionally, the use of excessive organic solvents may result in the precipitation of buffer salts at these high concentrations, which could have detrimental effects on the performance and lifespan of HPLC columns. This issue requires immediate attention,

as it compromises the reliability of the method and increases the frequency of equipment maintenance, further escalating costs.

Another significant drawback of many existing chromatographic methods is the unsatisfactory peak shapes and the issue of peak tailing. These issues can lead to inaccuracies in quantification, particularly when the analytes are present in low concentrations, as in certain therapeutic formulations or during pharmacokinetic studies. A well-defined and sharp peak is essential for the precise determination of the drug concentration, and poor peak resolution or tailing can complicate the interpretation of chromatographic data, leading to potential errors in analysis.

Furthermore, while stability-indicating methods are essential for evaluating the integrity of pharmaceutical formulations under various stress conditions (such as heat, light, and oxidation), many existing methods fail to adequately demonstrate the stability of NSAIDs and their combinations. The absence of detailed stress degradation studies and clear chromatographic representation of degradation products compromises the ability of the method to guarantee the stability and shelf-life of these drugs. Stress degradation studies are particularly crucial for regulatory compliance, as they provide information on the drug's behavior under extreme conditions, ensuring the formulation's safety and effectiveness over time.

Given these limitations, there is a clear and pressing need for the development of novel, stability-indicating analytical methods that address these shortcomings. These methods should aim to minimize the use of organic solvents, enhance chromatographic performance by improving peak shape and resolution, and provide comprehensive stress degradation profiles for NSAIDs and their combinations. Furthermore, with the growing emphasis on Quality by Design (QbD) principles in pharmaceutical development, the development of analytical methods that align with

QbD will allow for a more robust, efficient, and reproducible approach to the analysis of NSAIDs, facilitating regulatory compliance and ensuring the production of high-quality pharmaceutical products.

This study is needed to advance analytical techniques for NSAIDs, focusing on enhancing both the efficiency and reliability of these methods. The study addresses the limitations of existing approaches with the goal of contributing to the development of more sustainable, accurate, and cost-effective analytical methods. These methods are essential for ensuring the quality, safety, and efficacy of NSAID formulations.

1.4 AIM AND OBJECTIVES**Aim**

To develop and validate analytical techniques for selected non steroidal anti-inflammatory drugs.

Objectives

1. To develop suitable analytical methods for selected anti-inflammatory drugs.
2. To validate the newly developed analytical methods for selected NSAIDs in pharmaceutical dosage forms.

MATERIALS AND METHODS

Active pharmaceutical ingredients (API)

Loxoprofen sodium, Baclofen, Etodolac, and Thiocolchicoside were chosen as the model NSAIDs for the proposed study. These active pharmaceutical ingredients (APIs) were utilized for the development and validation of the analytical methods. Loxoprofen sodium, with a purity of 99%, was graciously provided as a complimentary sample by Dr. Reddy's Laboratories, Hyderabad. Similarly, Etodolac, Thiocolchicoside, and Baclofen, each with a purity of 99%, were generously supplied as gift samples by Unichem Laboratories Ltd., Goa. These high-purity APIs served as reference standards to ensure the precision and reliability of the analytical procedures established during the study.

Chemicals and reagents

The chemicals and reagents utilized for the analytical studies were of analytical grade and sourced from the college's chemical store. HPLC-grade methanol, acetonitrile, formic acid, and o-phosphoric acid were also of analytical quality and were acquired from Merck India Ltd.

Marketed formulations

To verify the suitability of the developed analytical method, commercially available formulations containing the selected NSAIDs were obtained from the local pharmacy market. The analysis included Loxoprofen sodium tablets (label claim: 60 mg), Baclofen tablets (label claim: 10 mg), and Etodolac and Thiocolchicoside tablets (label claim: 400 mg). These formulations were used to evaluate the performance and applicability of the developed technique.

Instruments and apparatus

UV-spectrophotometric methods were developed using a Shimadzu UV-1800 spectrophotometer, ensuring high sensitivity and accuracy for the analysis. These methods were optimized to achieve precise quantification of the selected NSAIDs in various formulations. For the optimization and validation of HPLC and UHPLC techniques, advanced chromatographic systems were utilized. These included the Agilent 1220 HPLC system with ChemStation software and the Shimadzu Prominence-i LC-2030C 3D Plus HPLC system, integrated with Shimadzu Prominence software and Lab Solutions Version 5.97 SP. In addition to the spectrophotometric and chromatographic equipment, essential laboratory apparatus was utilized to support the analytical work. An electronic analytical balance from Sartorius was used for precise weighing of analytes, while solutions were homogenized using an Ultrasonic Bath Sonicator-Ultrasonic Cleaner. Furthermore, Millipore water, critical for accurate analysis, was collected using a Direct-Q UV water purification system. These instruments collectively ensured the reliability and accuracy of the developed analytical techniques.

General methodology

In the proposed research methodology, a systematic approach was adopted for analytical method development and validation, adhering to standard protocols. The newly developed and optimized method underwent rigorous validation following the guidelines established by the ICH. This validation process ensured the reliability, accuracy, and precision of the method for its intended application. To demonstrate the practical applicability and robustness of the developed method, marketed pharmaceutical formulations containing the selected NSAIDs were analyzed. The

analysis of these formulations provided critical insights into the method's performance under real-world conditions, further substantiating its utility for routine quality control purposes. **Figure 7** illustrates the general steps involved in the proposed research methodology, providing a comprehensive overview of the workflow from method development to validation and application.

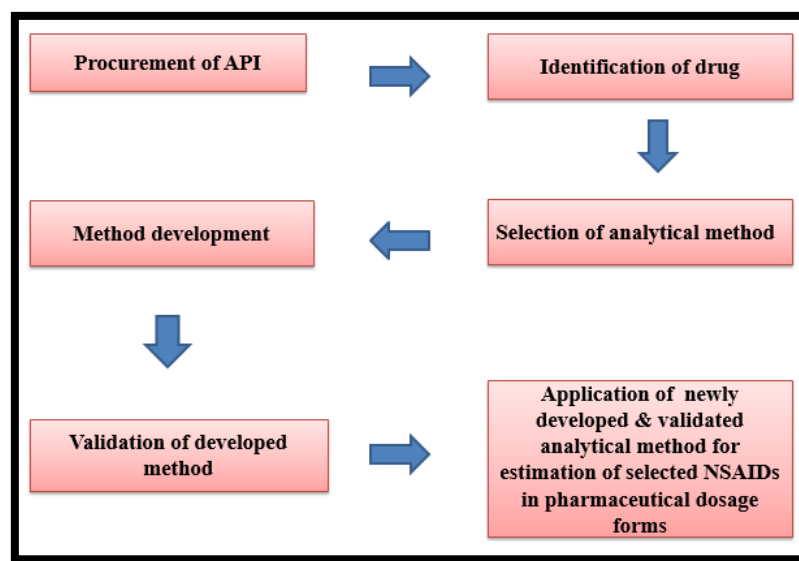


Figure 7. General steps involved in proposed research methodology

In this research, we focused on the development and evaluation of analytical methods for four NSAIDs: Loxoprofen sodium, Baclofen, Etodolac, and Thiocolchicoside. For Loxoprofen sodium, we established analytical techniques, including UV-spectroscopy and RP-HPLC. Similarly, a RP-HPLC method was developed for the accurate determination of Baclofen. Furthermore, forced degradation studies were conducted for Loxoprofen sodium and Baclofen to assess their stability under various stress conditions, providing critical insights into their degradation pathways. In addition, we employed a QbD approach to optimize and validate a simultaneous RP-UHPLC method for the combined analysis of Etodolac and Thiocolchicoside.

DEVELOPMENT AND VALIDATION OF UV-SPECTROMETRIC METHOD FOR ESTIMATION OF LOXOPROFEN SODIUM

Instrument and apparatus

The analyte was accurately weighed using a Sartorius analytical balance to ensure precision in measurement. Absorbance readings were recorded using a Shimadzu 1800 UV-Visible spectrophotometer, renowned for its reliability and accuracy in spectroscopic analysis. Additionally, sample sonication was performed using an ultrasonic bath sonicator, providing efficient and uniform mixing to ensure complete dissolution or dispersion of the analyte.

Selection of solvent and wavelength analysis

The development of a UV spectrophotometric method for Loxoprofen sodium commenced with the selection of an appropriate solvent and the determination of the compound's maximum absorbance wavelength (λ_{max}). The solubility of Loxoprofen sodium was assessed experimentally across various solvents and corroborated through a comprehensive literature review. The survey indicated that Loxoprofen sodium is soluble in both water and methanol. To finalize the choice of solvent, multiple trials were conducted in different media. Notably, most published studies employed methanol as the solvent for UV spectroscopic analysis. However, in this study, distilled water was selected as the solvent for the estimation of Loxoprofen sodium, aiming to ensure simplicity and environmental compliance. To determine the wavelength of maximum absorbance (λ_{max}), a 10 $\mu\text{g/mL}$ solution of Loxoprofen sodium was scanned across the ultraviolet (UV) spectrum within the range of 200–400 nm. This approach enabled the precise identification of the optimal analytical wavelength for subsequent quantitative evaluations.

Preparation of standard stock solutions

A standard stock solution of Loxoprofen sodium was prepared at a concentration of 1000 µg/mL by accurately weighing 10 mg of the substance and dissolving it in a 10 mL volumetric flask using distilled water as the diluent. The solution was thoroughly mixed to ensure complete dissolution and then brought to volume with distilled water. For the preparation of a working solution with a concentration of 100 µg/mL, an appropriate aliquot of the stock solution was diluted with distilled water to achieve the desired concentration, ensuring homogeneity in the final solution. To generate a calibration curve, the stock solution was further diluted in a stepwise manner to produce a series of working standards with concentrations ranging from 5 to 30 µg/mL.

Analytical method validation ^[76, 77]**Specificity and selectivity**

The specificity and selectivity of the method were evaluated by obtaining the UV spectrum with distilled water in both the blank and sample positions. This assessment ensured that the solvent did not produce any interfering absorbance in the spectral region of interest during the routine analysis of the drug sample. The absence of significant interference confirmed the method's capability to selectively measure Loxoprofen sodium in the presence of the solvent.

Linearity and range

To evaluate the linearity and range of the developed UV spectrophotometric method, a series of Loxoprofen sodium solutions with concentrations ranging from 5 µg/mL to 30 µg/mL were prepared by appropriately diluting the stock solution with

distilled water. Each concentration was analyzed in six replicates (n = 6) to ensure reproducibility and reliability of the measurements.

LOD & LOQ

The LOD and LOQ for the developed method were determined using statistical calculations based on the standard deviation of the response (σ) and the slope of the calibration curve (S). These parameters were calculated using the following formulas:

$$\text{LOD} = \frac{3.3 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slope of the calibration}}$$

$$\text{LOQ} = \frac{10 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slope of the calibration}}$$

Precision

The precision of the developed method was assessed by measuring the absorbance of Loxoprofen sodium solutions at three distinct concentrations: 5 $\mu\text{g/mL}$, 15 $\mu\text{g/mL}$, and 25 $\mu\text{g/mL}$. Intraday precision was evaluated by conducting the analyses at three different time intervals within the same day, while interday precision was determined by performing the analysis across three consecutive days. Absorbance values were recorded for each concentration and at each time point, and the RSD was subsequently calculated.

Ruggedness

The ruggedness of the developed method was evaluated by analyzing Loxoprofen sodium solutions at concentrations of 5 $\mu\text{g/mL}$, 15 $\mu\text{g/mL}$, and 25 $\mu\text{g/mL}$, each prepared in six replicates, by two different analysts under identical experimental

conditions. The absorbance values obtained by the analysts were compared, and the relative standard deviation (%RSD) was calculated for each concentration.

Accuracy

Recovery studies were conducted at three distinct levels—50%, 100%, and 150%—to assess the accuracy of the developed method. Known amounts of Loxoprofen sodium standard solution were spiked into pre-analyzed samples at each level, and the resulting mixtures were analyzed using the UV spectrophotometer. The percentage recovery was calculated to determine how effectively the method quantifies the analyte without interference.

Forced degradation studies

The stability of pure drug samples was evaluated using a newly developed UV-spectroscopic method. The drug was subjected to various stress conditions, including acidic, basic, and oxidative environments, according to standard procedures to assess its degradation profile.

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF LOXOPROFEN SODIUM

Chromatographic conditions

The analysis was performed using an Agilent 1100 HPLC system (LC-20AD, Japan) equipped with a quaternary pump with degasser (G7111A), an auto-injector (G7129A), and a photodiode array (PDA) detector (G13148). Data acquisition and processing were carried out using ChemStation software. Chromatographic separation of Loxoprofen sodium was achieved on an Agilent C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase, optimized for the analysis, consisted of methanol and 0.05% orthophosphoric acid (OPA) in a 75:25 (v/v) ratio. This mixture was delivered at a constant flow rate of 1 mL/min. During the analysis, a fixed injection volume of 20 µL was utilized, and the detection wavelength was set at 225 nm. All experiments were conducted under ambient temperature conditions to ensure method stability and reproducibility.

Preparation of standard stock solutions

A standard stock solution of Loxoprofen sodium was prepared at a concentration of 1000 µg/mL by accurately weighing 10 mg of the drug and dissolving it in a 10 mL volumetric flask with methanol as the diluent. To prepare a working solution with a concentration of 100 µg/mL, an appropriate aliquot of the stock solution was further diluted using the mobile phase (methanol: 0.05% orthophosphoric acid (OPA), 75:25 v/v), ensuring consistency and uniformity in the final concentration. Additional working solutions of Loxoprofen sodium were prepared through serial dilutions of the working solution using the same mobile phase as the diluent. These solutions, spanning a series of concentrations, were used for the

chromatographic analysis. Before injection, all samples were filtered through a 0.22 µm syringe filter to remove particulate matter, ensuring smooth operation of the chromatographic system and accuracy in analysis.

Method development

Preliminary experimental trials were conducted to evaluate various combinations of mobile phases and stationary phases to identify the optimal conditions for the analysis of Loxoprofen sodium. A comprehensive literature review indicated that acetonitrile is a commonly used mobile phase in combination with buffer solutions for the chromatographic analysis of Loxoprofen sodium. However, methanol was chosen as the mobile phase for this study due to its cost-effectiveness and comparable efficiency. Based on the results of the initial experiments, a C18 stationary phase column (250 mm × 4.6 mm, 5 µm particle size) was selected for further investigations, as it demonstrated excellent performance in achieving satisfactory separation and peak resolution ^[78].

Analytical method validation

The newly developed RP-HPLC method for the analysis of Loxoprofen sodium was validated in accordance with current regulatory guidelines to ensure its reliability and applicability for routine analysis. The method validation process included the evaluation of various parameters such as linearity and range, LOD and LOQ, system suitability, precision, accuracy, robustness, and forced degradation studies ^[79-81].

Linearity and range

To assess the linearity and range of the developed HPLC method, a series of Loxoprofen sodium solutions with concentrations ranging from 5 µg/mL to 25 µg/mL were prepared by accurately diluting the stock solution with the mobile phase (methanol: 0.05% orthophosphoric acid (OPA), 75:25 v/v). Each prepared solution was analyzed in six replicates (n = 6) under identical chromatographic conditions to ensure the reproducibility and reliability of the measurements.

LOD & LOQ

The LOD and LOQ for the developed HPLC method were calculated using statistical approaches based on the standard deviation of the response (σ) and the slope of the calibration curve (S). The calculations were performed using the following formulas:

$$\text{LOD} = \frac{3.3 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slop of the calibration}}$$

$$\text{LOQ} = \frac{10 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slop of the calibration}}$$

System suitability

System suitability parameters are essential in HPLC method validation to confirm the proper functioning of the chromatographic system and ensure the reliability of analytical results. For the developed method, system suitability was assessed by analyzing Loxoprofen sodium samples (15µg/mL) six times under the optimized chromatographic conditions.

Precision

Precision of an analytical procedure reflects the closeness of agreement, or the degree of scatter, among a series of measurements obtained from multiple samplings of a single homogeneous sample under prescribed conditions. Precision was evaluated by assessing system precision, intraday precision (measured three times within the same day), and interday precision (measured on three consecutive days) at varying concentrations of 5 µg/mL, 15 µg/mL, and 25 µg/mL. The study focused on determining the reproducibility of results both within the same day and across different days. The %RSD was calculated for the obtained data and was consistently found to be less than 2%, indicating high precision.

Accuracy

Recovery was evaluated using the standard addition method. A sample solution of Loxoprofen sodium (5 µg/mL) was spiked with standard concentrations equivalent to 80%, 100%, and 120% of the nominal value. The recovered amounts were quantified, and the percentage recovery along with the %RSD were calculated to verify the accuracy and reliability of the analytical method.

Robustness

The robustness of the developed method was evaluated by introducing controlled, minor perturbations in chromatographic conditions. These variations encompassed modifications to the mobile phase composition (74:26% and 76:24%), flow rate settings (0.9 and 1.1 mL/min), and detection wavelengths (224 nm and 226 nm).

Forced degradation studies

Forced degradation studies were conducted to evaluate the stability of the drug under a range of stress conditions, including acidic, basic, oxidative, neutral, and photolytic environments, as well as to evaluate the potential interference of degradation products. These studies are crucial for determining the intrinsic stability of the active pharmaceutical ingredient (API) and for identifying potential degradation pathways and products.

Acid degradation

A 0.3 mL aliquot of the standard drug solution (25 µg/mL) was transferred into a 10 mL volumetric flask, followed by the addition of 5 mL of 0.1 N HCl. The volume was adjusted to the mark with the mobile phase. After an incubation period of 1 hour under acidic conditions, the solution was neutralized using 0.1 N NaOH. A 20 µL aliquot of the resulting solution was then injected for subsequent analysis.

Basic degradation

A 0.3 mL aliquot of the standard drug solution (25 µg/mL) was transferred into a 10 mL volumetric flask, to which 5 mL of 0.1 N NaOH was added. The volume was then adjusted to the mark with the mobile phase. Following an hour of exposure to alkaline conditions, the solution was neutralized with 0.1 N HCl. A 20 µL aliquot of the resulting solution was subsequently injected for further analysis.

Oxidative degradation

A 0.3 mL aliquot of the standard drug solution (25 µg/mL) was transferred into a 10 mL volumetric flask, followed by the addition of 5 mL of a 3% H₂O₂ solution. The volume was adjusted to the mark with the mobile phase. After an hour

of exposure to oxidative conditions, the solution was directly injected (20 μ L) for subsequent analysis.

Neutral degradation

A 0.3 mL aliquot of the standard drug solution (25 μ g/mL) was transferred into a 10 mL volumetric flask, to which 5 mL of water was added. The volume was then adjusted to the mark with the mobile phase. After 1 hour of incubation, the solution was injected (20 μ L) for further analysis.

Photolytic degradation

Fifty milligrams of the drug was weighed accurately and exposed to sunlight for 24 hours. After exposure, the drug was dissolved, and appropriate dilutions were made to prepare a 25 μ g/mL solution. The resulting solution was then injected into the HPLC system for analysis.

Estimation of Loxoprofen sodium in marketed tablet

The assay test was conducted to determine the quantity of Loxoprofen sodium in the marketed formulation. Twenty tablets of Loxoprofen sodium (Roxonin 60 mg) were taken and triturated^[82]. A powdered sample equivalent to 5 mg of the drug was accurately weighed and transferred into a 10 mL volumetric flask. The sample was dissolved in methanol, and the solution was further diluted with the mobile phase to achieve a final concentration of 25 μ g/mL. A 0.5 mL aliquot of the test solution was injected into the HPLC system. This procedure was repeated three times, and the percentage assay was calculated based on the results obtained from each replicate.

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF BACLOFEN

Chromatographic conditions

The analysis was conducted using an Agilent 1100 HPLC system (LC-20AD, Japan), equipped with a quaternary pump with a degasser (G7111A), an auto-injector (G7129A), and a photodiode array (PDA) detector (G13148). Data acquisition and processing were managed using ChemStation software. Chromatographic separation of Baclofen was achieved on an Agilent C18 column (250 mm × 4.6 mm, 5 µm particle size). The optimized mobile phase consisted of methanol and 0.01% orthophosphoric acid (OPA) in a 50:50 (v/v) ratio, delivered at a constant flow rate of 0.7 mL/min. The injection volume was fixed at 20 µL, with detection performed at a wavelength of 225 nm. All analyses were conducted under ambient temperature conditions to ensure the stability and reproducibility of the method.

Preparation of standard stock solutions

A standard stock solution of Baclofen (1000 µg/mL) was prepared by accurately weighing 10 mg of the drug and dissolving it in methanol in a 10 mL volumetric flask. A working solution at a concentration of 100 µg/mL was obtained by diluting an appropriate aliquot of the stock solution with the mobile phase, which consisted of methanol and 0.01% OPA in a 50:50 (v/v) ratio, to ensure uniformity and consistency. Additional working solutions were prepared through serial dilutions of the primary working solution using the same mobile phase. These solutions, covering a range of concentrations, were utilized for chromatographic analysis. Prior to injection, all samples were filtered through a 0.22 µm syringe filter to remove particulate matter, ensuring optimal chromatographic performance and accuracy of the analysis.

Method development

Preliminary experimental trials were performed to explore various combinations of mobile and stationary phases to establish optimal chromatographic conditions for the analysis of Baclofen ^[83]. A thorough review of the literature revealed that acetonitrile, in combination with buffer solutions, is commonly employed as a mobile phase for Baclofen analysis. Nonetheless, methanol was selected for this study, as it offers a cost-effective alternative with comparable chromatographic efficiency. The initial trials indicated that a C18 stationary phase column (250 mm × 4.6 mm, 5 µm particle size) provided superior performance, achieving satisfactory separation and peak resolution, making it the preferred choice for subsequent investigations.

Analytical method validation

The newly developed RP-HPLC method for the analysis of Baclofen was rigorously validated in accordance with current regulatory guidelines to ensure its reliability and suitability for routine application. The validation process involved comprehensive assessments of critical parameters including linearity, range, LOD and LOQ, system suitability, precision, accuracy, robustness, and forced degradation studies ^[84, 85].

Linearity and range

The linearity and range of the developed HPLC method were evaluated by preparing a series of Baclofen solutions with concentrations ranging from 5 µg/mL to 25 µg/mL. These solutions were obtained by precisely diluting the stock solution using the mobile phase (methanol: 0.01% orthophosphoric acid, 50:50 v/v). Each concentration was analyzed in six replicates (n = 6) under identical chromatographic conditions to ensure the reproducibility and reliability of the measurements.

LOD & LOQ

The LOD and LOQ for the developed HPLC method were determined using statistical calculations based on the standard deviation of the response (σ) and the slope of the calibration curve (S). The calculations were performed using the following formulas:

$$\text{LOD} = \frac{3.3 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slop of the calibration}}$$

$$\text{LOQ} = \frac{10 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slop of the calibration}}$$

System suitability

System suitability parameters play a pivotal role in HPLC method validation, serving as a benchmark to verify the performance of the chromatographic system and ensure the consistency and reliability of analytical outcomes. To evaluate the developed method's robustness, system suitability was assessed by injecting six replicates of Baclofen (15 $\mu\text{g/mL}$) under the optimized chromatographic conditions. This evaluation confirms the adequacy of the system for precise and reproducible analysis.

Precision

The precision of an analytical method is a measure of the agreement, or the lack of scatter, among repeated measurements of a homogeneous sample under specified conditions. In this study, precision was assessed through system precision, intraday precision (evaluated three times within a single day), and interday precision (evaluated over three consecutive days) at concentrations of 5 $\mu\text{g/mL}$, 15 $\mu\text{g/mL}$, and 25 $\mu\text{g/mL}$. This approach ensured a comprehensive evaluation of reproducibility both

within a single day and across multiple days. The calculated %RSD values for all measurements were consistently below 2%, demonstrating excellent precision and reproducibility of the method.

Accuracy

The accuracy of the analytical method was assessed using the standard addition method. Baclofen sample solutions at a concentration of 5 µg/mL were spiked with known quantities of standard Baclofen at levels corresponding to 80%, 100%, and 120% of the nominal concentration. The spiked samples were analyzed, and the recovered amounts were quantified. Percentage recovery and %RSD were calculated for each level to confirm the method's accuracy and reliability.

Robustness

The robustness of the developed method was evaluated by deliberately introducing minor variations in critical chromatographic parameters. These included altering the mobile phase composition to 49:51% and 51:49%, modifying the flow rate to 0.6 mL/min and 0.8 mL/min, and adjusting the detection wavelength to 224 nm and 226 nm. The impact of these changes on the method's performance was carefully examined to ensure its reliability under slightly altered experimental conditions.

Forced degradation studies

Forced degradation studies were conducted to evaluate the stability of the drug under a range of stress conditions, including acidic, basic, oxidative, neutral, and photolytic environments. These studies are essential for assessing the intrinsic stability of the active pharmaceutical ingredient (API) and for identifying potential degradation pathways and products. Additionally, the potential interference of

degradation products with the analytical method was investigated, ensuring the robustness and reliability of the method for routine analysis.

Acid degradation

A 0.3 mL aliquot of the standard drug solution (25 µg/mL) was transferred into a 10 mL volumetric flask. To this, 5 mL of 0.1 N HCl was added. The volume was then adjusted to the mark with the mobile phase. The flask was incubated for 1 hour under acidic conditions. Subsequently, the solution was neutralized with 0.1 N NaOH. After neutralization, a 20 µL aliquot of the prepared solution was injected for analysis.

Basic degradation

A 0.3 mL aliquot of the standard drug solution (25 µg/mL) was transferred into a 10 mL volumetric flask, to which 5 mL of 0.1 N NaOH was added. The volume was adjusted to the mark with the mobile phase. The solution was incubated for 1 hour under alkaline conditions. After this incubation period, it was neutralized with 0.1 N HCl. Finally, a 20 µL aliquot of the resulting solution was injected for subsequent analysis.

Oxidative degradation

A 0.3 mL aliquot of the standard drug solution (25 µg/mL) was transferred into a 10 mL volumetric flask, followed by the addition of 5 mL of 3% H₂O₂ solution. The volume was adjusted to the mark with the mobile phase. The solution was incubated for 1 hour under oxidative conditions. After this period, a 20 µL aliquot of the treated solution was directly injected for further analysis.

Neutral degradation

A 0.3 mL aliquot of the standard drug solution (25 µg/mL) was transferred into a 10 mL volumetric flask, to which 5 mL of water was added. The volume was adjusted to the mark with the mobile phase. The solution was incubated for 1 hour. After this incubation period, a 20 µL aliquot of the resulting solution was injected for further analysis.

Photolytic degradation

Fifty milligrams of the drug were weighed accurately and exposed to sunlight for 24 hours. Following the exposure, the drug was dissolved, and appropriate dilutions were made to prepare a 25 µg/mL solution. The resulting solution was then injected into the HPLC system for analysis.

Estimation of Baclofen in marketed tablet

The assay test was performed to determine the quantity of Baclofen in a marketed formulation. Twenty Baclofen tablets (Intas Pharma, Brand: Baclof, Strength: 10 mg) were taken and triturated. A powdered sample equivalent to 5 mg of the drug was weighed and transferred to a 10 mL volumetric flask. The sample was dissolved in methanol to prepare a stock solution. This stock solution was further diluted with the mobile phase to achieve a test solution of 25 µg/mL. A 0.5 mL aliquot of the test solution was injected into the HPLC system. The procedure was repeated three times, and the percentage assay was calculated based on the results obtained.

DEVELOPMENT AND VALIDATION OF RP-UHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ETODOLAC AND THIICOLCHICOSIDE USING QUALITY BY DESIGN APPROACH

Chromatographic conditions

The analytical method was executed using an Agilent 1100 UHPLC system (LC-20AD, Japan), equipped with a quaternary pump and degasser (model G7111A), an auto-sampler (model G7129A), and a photodiode array (PDA) detector (model G13148). Chromatographic data acquisition and processing were facilitated using ChemStation software. The separation of Etodolac and Thiocolchicoside was achieved on an Agilent C18 column (100 mm × 4.6 mm, 2 µm particle size). The mobile phase was optimized to a mixture of methanol and 0.1% (v/v) OPA in a ratio of 94.30:5.70 (v/v), delivered at a steady flow rate of 0.96 mL/min. An injection volume of 20 µL was employed, with detection occurring at an isosbestic wavelength of 256 nm. All experiments were conducted at a controlled column temperature of 33°C to ensure method stability and reproducibility.

Preparation of standard stock solutions

A stock solution containing Etodolac (4000 µg/mL) and Thiocolchicoside (40 µg/mL) was prepared by accurately weighing 400 mg of Etodolac and 4 mg of Thiocolchicoside, which were then dissolved in 100 mL of methanol. Sequential dilutions of this stock solution were carried out using the mobile phase to obtain working concentrations ranging from 40 to 200 µg/mL for Etodolac and 0.4 to 2.0 µg/mL for Thiocolchicoside. Before injection, all samples were passed through a 0.22 µm syringe filter to eliminate particulate matter, thereby ensuring precise chromatographic performance and reliable analytical results.

Preliminary method development studies

A comprehensive literature survey indicated that methanol is a commonly used organic solvent in combination with buffer solutions for the analysis of Etodolac. To optimize the chromatographic conditions, both methanol and acetonitrile were evaluated as the organic phases. Methanol was selected due to its superior solubility for both Etodolac and Thiocolchicoside. Initially, water was tested as the aqueous phase; however, it resulted in poor peak shapes, necessitating further optimization. The addition of 0.1% OPA to the aqueous phase significantly improved peak sharpness and visibility, thereby enhancing the overall chromatographic performance. The method development was conducted using a reversed-phase approach on a C18 column (100 mm × 4.6 mm, 2 µm particle size), ensuring efficient separation and reliable analytical performance.

Determination of Isosbestic point by UV Spectroscopy

To determine the isosbestic point, a spectral scan was performed across the wavelength range of 200–400 nm to identify the maximum absorbance (λ_{max}) for the analytes. Based on the spectral data, 256 nm was identified as the isosbestic point, where both analytes exhibited significant absorbance overlap. Consequently, all subsequent analyses were carried out at this wavelength to ensure accurate and simultaneous quantification^[86, 87].

Quality by Design approach

The QbD-based method optimization was executed systematically through a series of well-defined steps to ensure robust and reliable method development^[88-90].

Risk assessment

The primary objective of this study was to systematically investigate the various factors influencing the Critical Quality Attributes (CQAs). Critical Analytical Attributes (CAAs) were utilized to map the relationship between method parameters and the CQAs, providing a comprehensive understanding prior to conducting a formal risk assessment. The specific goals included categorizing potential causes of challenges in meeting the desired quality attributes and identifying underlying reasons for deficiencies, discrepancies, and defects in the analytical method. Additionally, the study aimed to pinpoint the critical factors essential for achieving the CQAs, thereby ensuring robust method performance and consistent quality outcomes.

The factors influencing the target responses were systematically classified into three levels: low (-1), medium (0), and high (+1). Initially, seven factors were identified and assessed for their risk levels. A thorough evaluation allowed the identification of critical factors, narrowing the selection down to four key variables. These critical factors were further optimized using experimental design approaches. The study established the Target Quality Profile (TQP), emphasizing retention time (RT), peak area (PA), theoretical plates (TP), and tailing factor (TF) as the primary dependent variables. These parameters were prioritized to ensure method robustness, accuracy, and reproducibility, aligning with the analytical objectives.

Optimization by DoE

The Analytical Quality by Design (AQbD) approach was employed to systematically evaluate the impact of independent variables on multiple critical responses. To facilitate this, a BBD was selected using Design Expert software (version 13.0), due to its ability to efficiently generate optimal results with relatively

few experimental trials. A three-factor, three-level BBD was applied to refine the analytical method. The independent variables considered included: (A) the percentage of methanol in the mobile phase, (B) flow rate (mL/min), and (C) UV wavelength (nm). Their individual and interactive effects were evaluated for the following responses: Y_1 (retention time, R_t , min), Y_2 (peak area, PA), Y_3 (theoretical plates, TP), and Y_4 (tailing factor, Tf, %). A total of 14 experimental runs were conducted to identify the optimal combination of variables. The experimental data were fitted to various kinetic models, including linear, second-order, and quadratic models, to determine the most suitable model. Polynomial equations and three-dimensional response surface plots were employed to elucidate the effects of the variables on the responses, thereby guiding the method optimization process.

Table 2. Variables and their levels used in Box-Behnken design for simultaneous analysis Thiocolchicoside & Etodolac

Factor	Levels used, actual (coded)	
	Low (-1)	High (+1)
Independent variables		
A: Solvent conc. in mobile phase (% v/v)	81.5 (-1)	98.4(+1)
B: Flow rate (ml/min)	0.8 (-1)	1.1 (+1)
C: Wavelength (nm)	255.3	258
Dependent variables		
Y ₁ & Y ₅ : Retention time	Minimize	
Y ₂ & Y ₆ : Peak area	In the range	
Y ₃ & Y ₇ : Theoretical Plate	Maximize	
Y ₄ & Y ₈ : Tailing factor	Minimize	

Statistical analyses, such as analysis of variance (ANOVA) and regression analysis, were conducted to evaluate the significance of the models and identify the best fit. The F-test from ANOVA confirmed the significance of the second-order quadratic polynomial model (Eqn. 1) as the most appropriate representation of the experimental data. The model's reliability and predictive power were further validated through diagnostic checks and statistical parameters.

$$Y = \beta_0 + \beta^1 A + \beta^2 B + \beta^2 C + \beta^{12} AB + \beta^{13} AC + \beta^{23} BC + \beta^{12} A^2 + \beta^{22} B^2 + \beta^{33} C^2$$

Where, Y represents the selected response, while β_0 denotes the intercept. The regression coefficients, β_1 through β_{33} , correspond to the individual contributions of the factors to the response. The factors under investigation are represented by A, B, and C, which denote the percentage of methanol in the mobile phase, flow rate (mL/min), and UV wavelength (nm), respectively.

The model, developed through the design process, was thoroughly evaluated and compared using several statistical parameters, including the correlation coefficient, adjusted correlation coefficient, predicted correlation, and adequate precision. To further understand the model's behavior, response surface analysis was performed, which included the generation of 3D response surface plots. A numerical optimization method was employed to predict the optimal analytical conditions. These optimized conditions were then mapped within the designated Analytical Design Space, which was visually represented as an overlay plot. This plot incorporated a flag denoting all relevant parameters, providing a comprehensive visualization of the optimized conditions.

Analytical method validation ^[91, 92]

The newly developed RP-UHPLC method for the simultaneous analysis of Etodolac and Thiocolchicoside was validated in accordance with current regulatory guidelines, ensuring its reliability and suitability for routine analytical applications. The validation process included the assessment of key performance parameters such as linearity, range, LOD, LOQ, system suitability, precision, accuracy, and robustness.

Linearity and range

The linearity and range of the developed UHPLC method were evaluated by preparing a series of standard solutions at concentrations ranging from 40 µg/mL to 400 µg/mL for Etodolac and 0.4 µg/mL to 20 µg/mL for Thiocolchicoside. These solutions were prepared by sequentially diluting the stock solution with the mobile phase, composed of methanol and 0.1% orthophosphoric acid (94.30:5.70, v/v). Each solution was analyzed in six replicates (n = 6) under identical chromatographic conditions to ensure the reproducibility and reliability of the measurements.

LOD & LOQ

The LOD and LOQ for the developed UHPLC method were determined using statistical approaches based on the standard deviation of the response (σ) and the slope of the calibration curve (S). The calculations were performed using the following formulas:

$$\text{LOD} = \frac{3.3 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slope of the calibration}}$$

$$\text{LOQ} = \frac{10 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slope of the calibration}}$$

System suitability

System suitability parameters are essential in UHPLC method validation to verify the performance of the chromatographic system and ensure the reliability of analytical results. For the developed method, system suitability was evaluated by analyzing Etodolac (200 µg/mL) and Thiocolchicoside (0.8 µg/mL) in six replicates under optimized chromatographic conditions.

Precision

The precision of an analytical procedure reflects the closeness of agreement, i.e., the degree of scatter, among a series of measurements obtained from multiple samplings of a single homogeneous sample under specified conditions. Precision was evaluated through system precision, intraday precision (assessed three times within a single day), and interday precision (assessed over three consecutive days) at varying concentrations: 40 µg/mL, 120 µg/mL, and 400 µg/mL for Etodolac; and 0.4 µg/mL, 1.2 µg/mL, and 2 µg/mL for Thiocolchicoside. The study aimed to establish the reproducibility of results both within the same day and across different days. The percentage relative standard deviation (%RSD) for the obtained data was consistently less than 2%, demonstrating excellent precision and reliability of the method.

Accuracy

Accuracy was evaluated using the standard addition method. A sample solution containing Etodolac (40 µg/mL) and Thiocolchicoside (0.4 µg/mL) was spiked with standard concentrations corresponding to 80%, 100%, and 120% of the nominal value. The recovered amounts were quantified, and the percentage recovery, along with the percentage %RSD, was calculated to assess the accuracy and reliability of the analytical method.

Robustness

The robustness of the developed method was systematically assessed by making deliberate, minor modifications to the chromatographic parameters. Variations included adjustments to the mobile phase composition (comprising

93.3:6.7% and 95.3:4.7% compositions), flow rate (0.86 mL/min and 1.06 mL/min), and detection wavelength (255 nm and 257 nm).

Estimation of Etodolac and Thiocolchicoside in marketed tablet

The assay was performed to determine the quantity of Thiocolchicoside and Etodolac in a marketed formulation ^[93]. Twenty tablets of ETOTAG (manufactured by IKON Remedies Pvt. Ltd., strength: 4 mg Thiocolchicoside and 400 mg Etodolac) were weighed and finely triturated. A powdered sample equivalent to 4 mg of Thiocolchicoside and 400 mg of Etodolac (total weight of 503 mg) was accurately measured and transferred into a 100 mL volumetric flask. The sample was dissolved and subsequently diluted with methanol to prepare the stock solution. From the prepared stock solution, 0.3 mL was further diluted to a final volume of 10 mL using the mobile phase to achieve final concentrations of 1.2 µg/mL for Thiocolchicoside and 120 µg/mL for Etodolac. A 20 µL aliquot of this diluted solution was then injected into the UHPLC system. This procedure was repeated three times to assess reproducibility. The % assay for each analyte was calculated based on the resulting chromatographic data.

RESULTS AND DISCUSSION

DEVELOPMENT AND VALIDATION OF UV-SPECTROMETRIC METHOD FOR ESTIMATION OF LOXOPROFEN SODIUM

Method development

After extensive experimentation, distilled water was identified as the optimal solvent system for the estimation of Loxoprofen sodium using a UV spectrophotometric method.

Under these conditions, Loxoprofen sodium exhibited maximum absorbance at 223 nm, as illustrated in **Figure 8**. The optimized spectrometric parameters and conditions are summarized in **Table 3**.

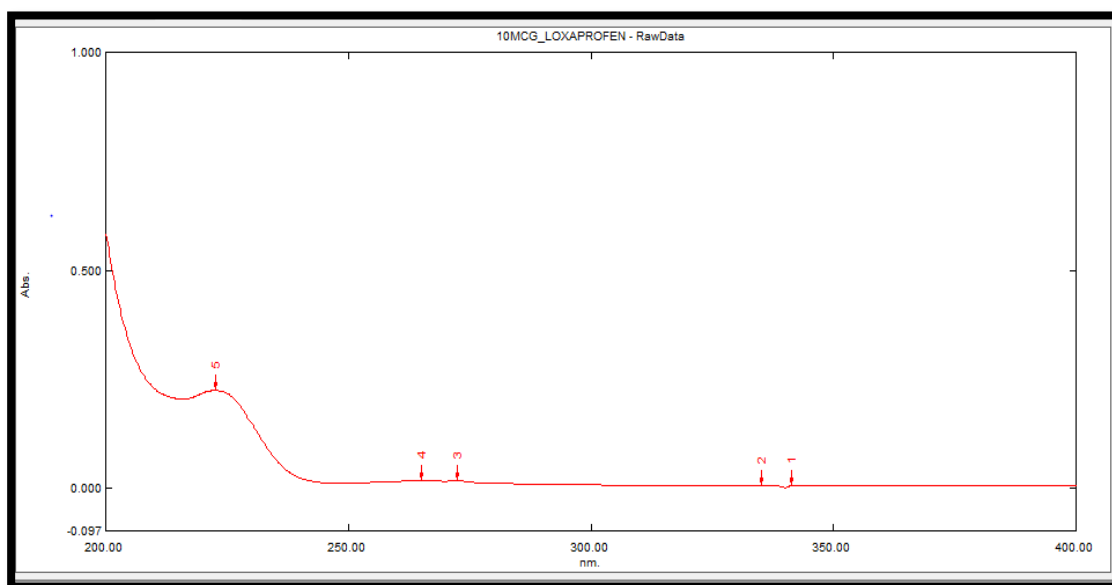


Figure 8. UV-Spectrum of Loxoprofen sodium at maximum absorbance 223 nm

Table 3. Optimized UV-Spectrometric conditions for Loxoprofen sodium

Sr. No.	Parameter	Specification
1	Method	UV-Spectrophotometric Method
2	Instrument	UV-Spectrophotometer
3	Model	Shimadzu
4	Make	UV-1800
5	Software	UV-Probe
6	Analyte	Loxoprofen sodium
7	Wavelength Run	400-200nm
8	Solvent	Distilled water
9	λ_{max}	223nm

Analytical method validation**Specificity and selectivity**

The spectrum obtained using distilled water as the blank showed no absorbance at the drug's maximum absorbance wavelength of 223 nm. These results confirm the absence of solvent interference, demonstrating the method's specificity and selectivity for accurately estimating the drug.

Linearity and range

The standard calibration curve was constructed by plotting concentration versus absorbance, based on a series of linear dilutions of Loxoprofen sodium. The resulting plot, as illustrated in **Figure 9**, demonstrates linearity across the concentration range of 5–30 µg/mL, with a correlation coefficient (r^2) of 0.999 (refer to **Table 4** for detailed data). These findings confirm that the method exhibits excellent linearity within the evaluated range and overlay spectrum of linearity (5-30 µg/mL) was shown in **Figure 10**.

Table 4. Linearity data of Loxoprofen sodium by UV-Spectroscopic method

Sr. No.	Concentration	Absorbance
1	5µg/ml	0.150
2	10µg/ml	0.322
3	15µg/ml	0.492
4	20µg/ml	0.639
5	25µg/ml	0.804
6	30µg/ml	0.955
7	Slope	0.160
8	r^2	0.999

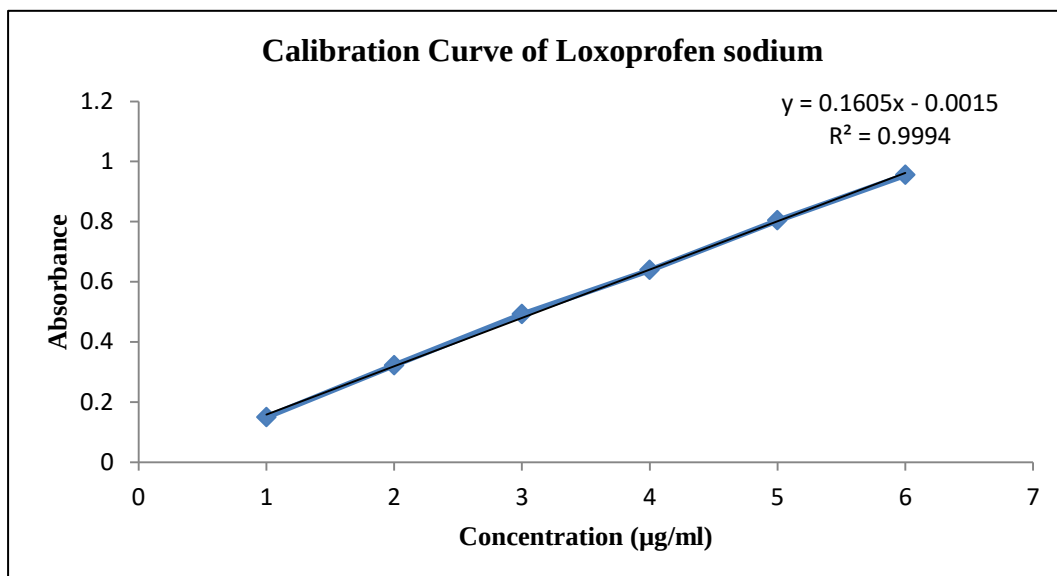


Figure 9. Standard calibration curve of Loxoprofen sodium by UV method

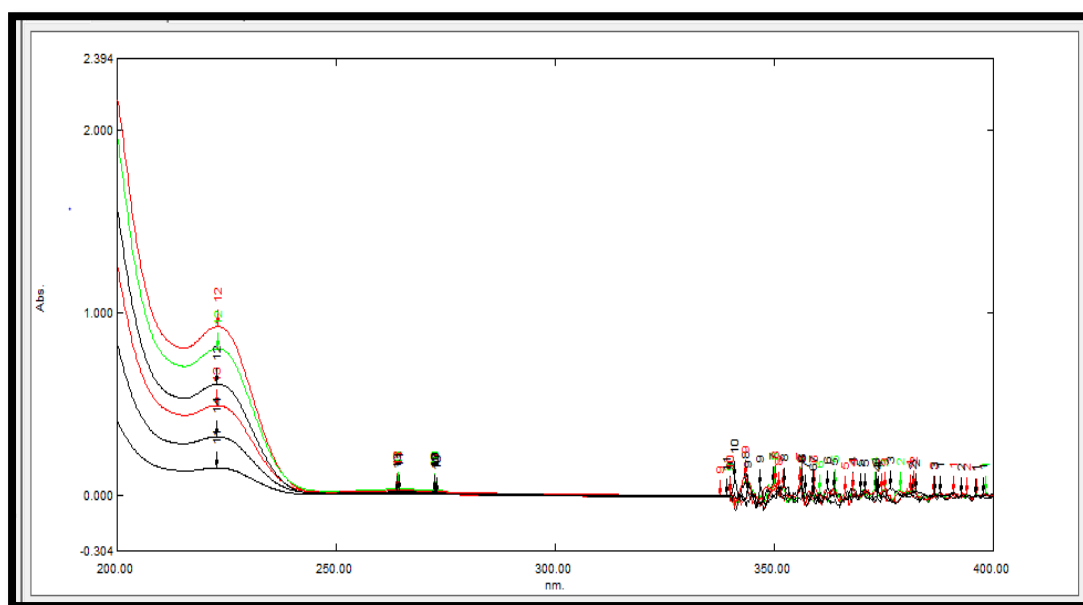


Figure 10. Overlay linearity spectrum of Loxoprofen sodium (5-30 µg/mL)

LOD and LOQ

The LOD and LOQ for Loxoprofen sodium were determined using linear regression data from the calibration curve. The calculated LOD was 1.10 µg/mL, while the LOQ was 3.71 µg/mL.

Precision

The method exhibited precision, as the % RSD for all sample solutions was found to be less than 2.0%, thereby meeting the established acceptance criteria. The detailed results are provided in **Table 5**.

Table 5. Precision data of Loxoprofen sodium by UV method

Type	Intra-day precision			Inter-day precision		
Conc. (µg/mL)	5	15	25	5	15	25
Mean abs.	0.156	0.475	0.806	0.159	0.470	0.810
SD	0.001	0.003	0.001	0.012	0.003	0.102
%RSD	0.942	0.712	0.217	0.652	0.742	0.987

Ruggedness

The results of the ruggedness study are summarized in **Table 6**. The % RSD for replicate sample solutions was below 2.0%, meeting the acceptance criteria for ruggedness under conditions involving a change in the analyst.

Table 6. Ruggedness data of Loxoprofen sodium by UV method

Ruggedness by change in analyst	15 µg/ml	
Replicates	Absorbance (Analyst I)	Absorbance (Analyst II)
1	0.482	0.474
2	0.471	0.482
3	0.478	0.474
4	0.473	0.480
5	0.480	0.479
6	0.482	0.478
Mean	0.477	0.477
SD	0.004	0.003
%RSD	0.979	0.680

Accuracy

The method was confirmed to be accurate, with recovery values at all levels falling within the acceptable range of 99.11% to 100.01%. The accuracy data are presented in **Table 7**.

Table 7. Accuracy data of Loxoprofen sodium by UV method

Levels (%)	Amount of sample($\mu\text{g/ml}$)	Amount of standard($\mu\text{g/ml}$)	Total amount ($\mu\text{g/ml}$)	Mean amount recovered ($\mu\text{g/ml} \pm \text{SD}$)	Mean % recovery $\pm$ SD
50	5	10	15	14.89 $\pm$ 0.01	99.78 $\pm$ 0.04
100	10	10	20	15.02 $\pm$ 0.02	100.01 $\pm$ 0.02
150	15	10	25	14.36 $\pm$ 0.12	99.11 $\pm$ 0.15

Forced degradation study

Forced degradation studies were conducted for Loxoprofen sodium under various conditions, including acidic, basic, and oxidative environments. The results showed significant degradation of the drug under basic and oxidative conditions, while it was comparatively more stable in acidic conditions. The percentage degradation under each condition, as determined by the UV method, is presented in

Table 8.

Table 8. Degradation data of Loxoprofen sodium by UV method

Condition	Initial abs.	Final abs.	% degradation
Acid	0.428	0.420	1.86
Base	0.359	0.319	11.14
Oxidative	0.602	0.540	10.29

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF LOXOPROFEN SODIUM

Method development

The chromatographic process was optimized by systematically varying key parameters, such as the composition of the mobile phase, flow rate, pH, column oven temperature, and detection wavelength. A series of experiments were conducted to identify an ideal combination capable of producing well-defined and resolved peaks. Different mobile phase compositions were tested, incorporating methanol, acetonitrile, water, and their mixtures.

Following extensive trials, methanol was identified as the optimal organic phase for the RP-HPLC analysis of Loxoprofen sodium, with 0.05% orthophosphoric acid (OPA) selected as the aqueous phase. The flow rate was adjusted to 1.0 mL/min, and the detection wavelength was set at 225 nm to ensure precise quantification. Various ratios of methanol to 0.05% OPA, including 80:20 (v/v), 70:30 (v/v), and 60:40 (v/v), were evaluated. However, these ratios did not produce well-resolved peaks with satisfactory characteristics, as illustrated in **Figures 11–13**.

Ultimately, a ratio of 75:25 (v/v) methanol to 0.05% OPA yielded optimal resolution, with a retention time of 4.06 minutes, as shown in **Figure 14**. This ratio provided the most distinct and well-separated chromatographic peaks, ensuring reliable and reproducible analysis of Loxoprofen sodium.

The optimized spectrometric parameters and conditions are summarized in **Table 9**.

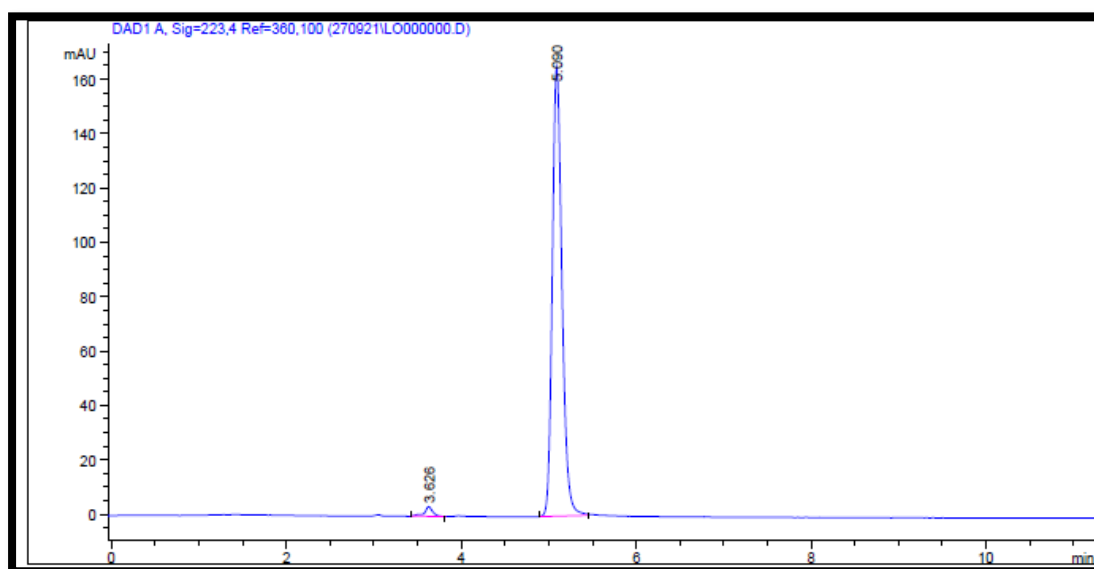


Figure 11. Chromatogram of Loxoprofen sodium (80:20 v/v, Methanol: 0.05% OPA)

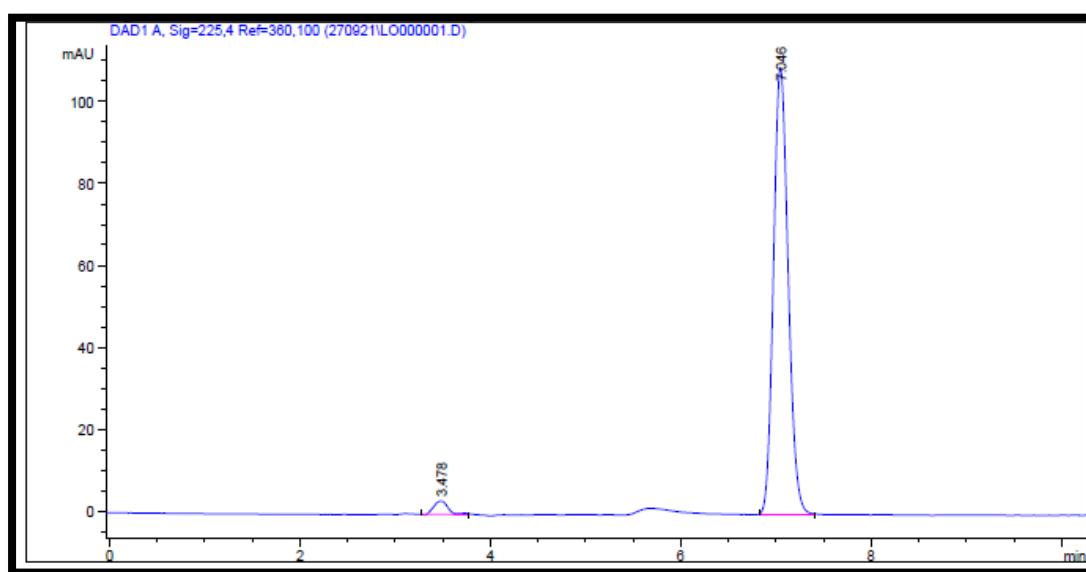


Figure 12. Chromatogram of Loxoprofen sodium (70:30 v/v, Methanol: 0.05% OPA)

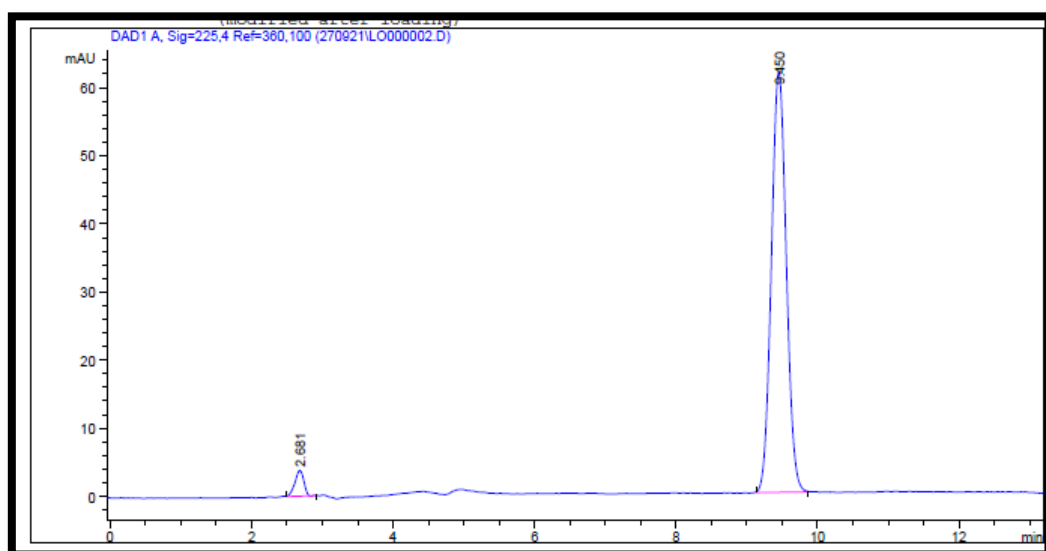


Figure 13. Chromatogram of Loxoprofen sodium (60:40 v/v, Methanol: 0.05% OPA)

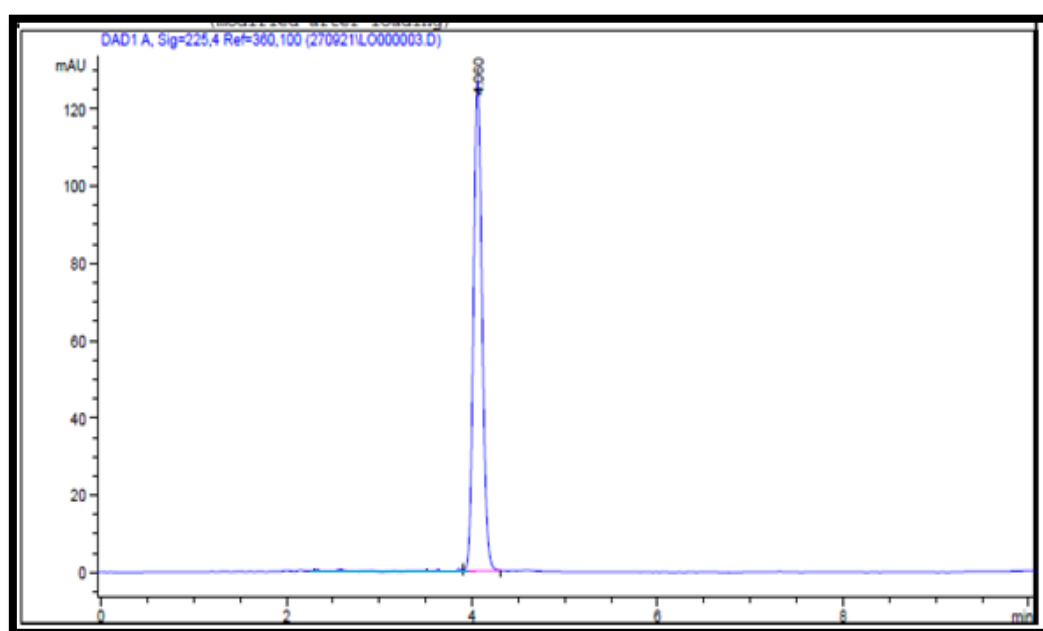


Figure 14. Chromatogram of Loxoprofen sodium (75:25 v/v, Methanol: 0.05% OPA)

Table 9. Optimized chromatographic conditions of Loxoprofen sodium by HPLC method

Sr. No.	Parameter	
1	Mobile Phase	METHANOL: 0.05%OPA (75:25, v/v)
2	Wavelength used	225nm
3	Flow Rate	1 ml/min
4	Temperature	Ambient
5	Sample size	20µl
6	Retention time	4.060
7	Area	819.61066
8	Theoretical plates	9551
9	Resolution	1.31
10	Height of peak	126.79233
11	Width of peak	0.0978
12	Selectivity	1.09

Analytical method validation

Linearity

The calibration curve for Loxoprofen sodium demonstrated excellent linearity across the tested concentration range of 5–25 µg/mL. Regression analysis demonstrated a high degree of consistency, with %RSD values below 2.0 across all concentration levels. The resulting correlation coefficient ($R^2 = 0.999$) confirmed the reliability and robustness of the method within the specified range (**Figure 15, Table 10**).

Table 10. Linearity and range data of Loxoprofen sodium by HPLC method

Sr. No.	Parameters	Results
1	Linearity range	5-25 (µg/mL)
2	Correlation coefficient	0.9998
3	Calibration equation	$y = 38.397x - 8.155$
4	Slope	38.397x
5	Intercept	8.155

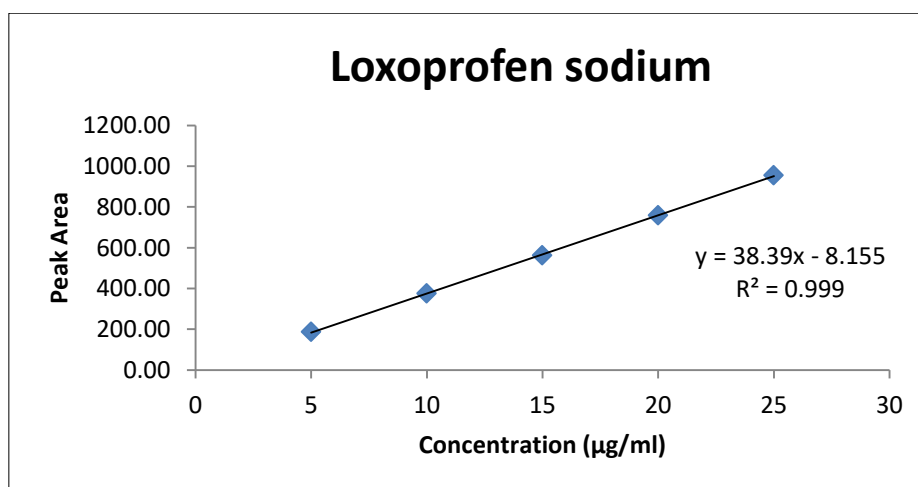


Figure 15. Calibration curve of Loxoprofen sodium by HPLC method.

LOD and LOQ

The LOD and LOQ for Loxoprofen sodium were determined using the linear regression data derived from the calibration curve. The calculated LOD was 0.91 µg/mL, while the LOQ was 2.78 µg/mL.

System suitability

The system suitability of the developed method was evaluated by calculating the percent relative standard deviation (%RSD) for key parameters, including peak area, retention time (RT), theoretical plates, and tailing factor (**Table 11**). The %RSD for all these parameters was found to be less than 2.0%, confirming the method's robustness and compliance with established acceptance criteria. These findings underscore the method's reliability for routine analytical applications.

Table 11. System suitability data of Loxoprofen sodium by HPLC

Sr. No.	System suitability	Loxoprofen sodium	
		Mean ± SD	%RSD
1	Peak area (% RSD)	562.0 ± 0.19	0.72
2	Retention time (% RSD)	4.02±0.36	0.58
3	Theoretical plates (%RSD)	9551±0.02	0.47
4	Tailing factor (%RSD)	1.09±0.07	0.81

Precision

The analytical method exhibited excellent precision, as evidenced by the % RSD values, which were consistently below 2.0% for all sample solutions. These results are in compliance with the established acceptance criteria, highlighting the method's reproducibility and reliability. Detailed precision data are summarized in **Table 12**.

Table 12. Precision data of Loxoprofen sodium by HPLC method

Type	Intra-day precision			Inter-day precision		
Conc. (µg/mL)	5	15	25	5	15	25
Mean area	372.84	560.60	767.60	378.8	582.45	770.65
SD	0.59	0.81	0.69	2.36	1.60	1.30
%RSD	0.16	0.15	0.09	0.62	0.27	0.16

Robustness

Robustness studies were performed by introducing minor variations in chromatographic conditions, such as the mobile phase ratio, flow rate, and detection wavelength. The results showed that the percent relative standard deviation (%RSD) remained consistently below 2.0% across all tested conditions, highlighting the method's reliability and its ability to withstand minor modifications. These findings, presented in **Tables 13-15**, substantiate the robustness of the developed analytical method.

Table 13. Robustness (Change in flow rate) data of Loxoprofen sodium by HPLC method.

Sr No.	Conc. µg/ml	FLOWRATE 0.9 ml/min	FLOWRATE 1.1 ml/min
		Area	Area
1	10	419.9682	355.9712
2	10	424.0482	349.6631
3	10	423.014	350.62
Mean		422.01	352.82
SD		2.88	4.46
%RSD		0.68	1.26

Table 14. Robustness (Change in mobile phase ratio) data of Loxoprofen sodium by HPLC method.

Sr No.	Conc. µg/ml	Mobile Phase Methanol and 0.05% OPA buffer (74:26)	Mobile Phase Methanol and 0.05% OPA buffer (76:24)
		Area	Area
1	10	374.6719	371.1599
2	10	370.2668	366.7215
3	10	371.082	367.841
Mean		372.5	368.94
SD		3.11	3.14
%RSD		0.84	0.85

Table 15. Robustness (Change in wavelength) data of Loxoprofen sodium by HPLC method.

Sr No.	Conc. µg/ml	224nm	226nm
		Area	Area
1	10	381.9029	360.5017
2	10	389.6729	364.6039
3	10	383.582	361.8425
Mean		385.8	362.55
SD		5.49	2.90
%RSD		1.42	0.80

Accuracy

Accuracy of the developed method was assessed through recovery studies. A reference solution of Loxoprofen sodium (5 µg/mL) was spiked with a composite sample at three concentration levels—low (80%), medium (100%), and high (120%) of the specified concentration. The spiked solutions were analyzed using the established method, yielding mean percentage recoveries ranging from 97.91% to 101.07%. The low %RSD values and high recovery rates, as presented in **Table 16**, confirm the method's excellent accuracy and its suitability for quantitative analysis of Loxoprofen sodium.

Table 16. Accuracy data of Loxoprofen sodium by HPLC method

Levels (%)	Amount of sample (µg/ml)	Amount of standard (µg/ml)	Total amount (µg/ml)	Mean amount recovered (µg/ml ±SD)	Mean % recovery ± SD
80	4	5	9	8.92±0.012	97.91±0.29
100	5	5	10	9.99±0.019	99.79±0.37
120	6	5	11	11.06±0.006	101.07±0.03

Forced degradation studies

Table 17 summarizes the results of the forced degradation study, highlighting the robustness and specificity of the developed method. The study demonstrated adequate separation between the drug peak and the peaks of its degradation products under various stress conditions, providing strong evidence for the method's reliability in distinguishing Loxoprofen sodium from its degradation products. The chromatograms corresponding to these observations are presented in Figures 16 to 20, further supporting the method's applicability for stability-indicating analysis.

Table 17. Degradation data Loxoprofen sodium by HPLC method

Sr. No.	Condition	% Undegraded drug	% Degradation
1	Acid Degradation	83.72	16.28
2	Basic Degradation	85.95	14.05
3	H ₂ O ₂ Degradation	80.24	19.76
4	Neutral	99.38	0.62
5	Photolytic	98.48	1.52

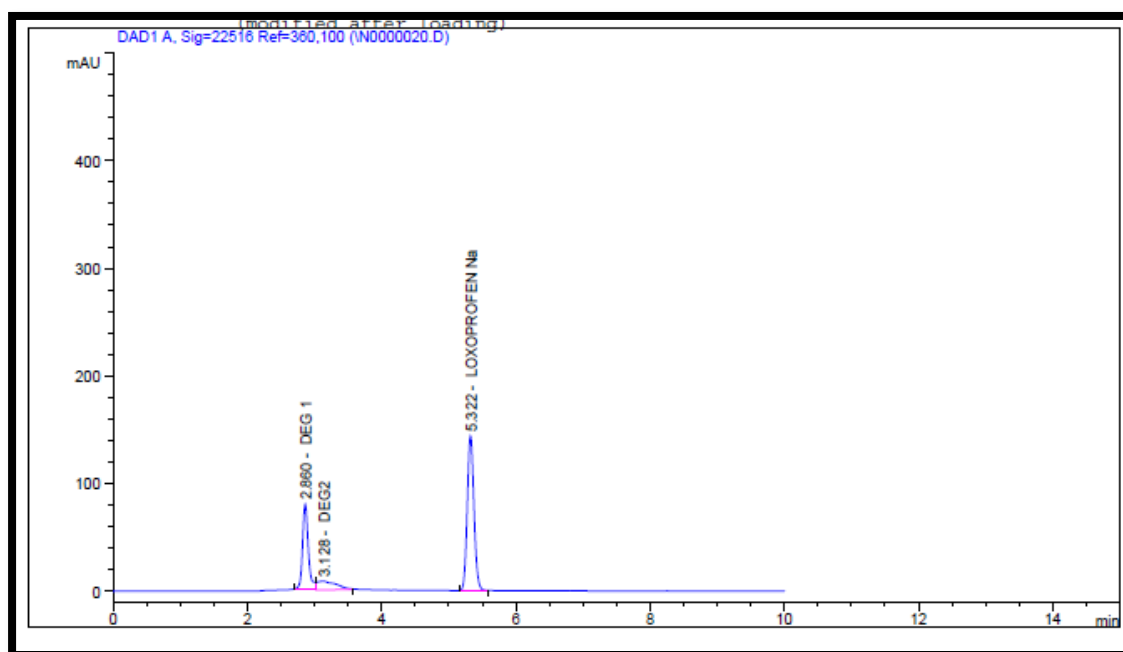


Figure 16. Chromatogram of Loxoprofen sodium under acid degradation condition

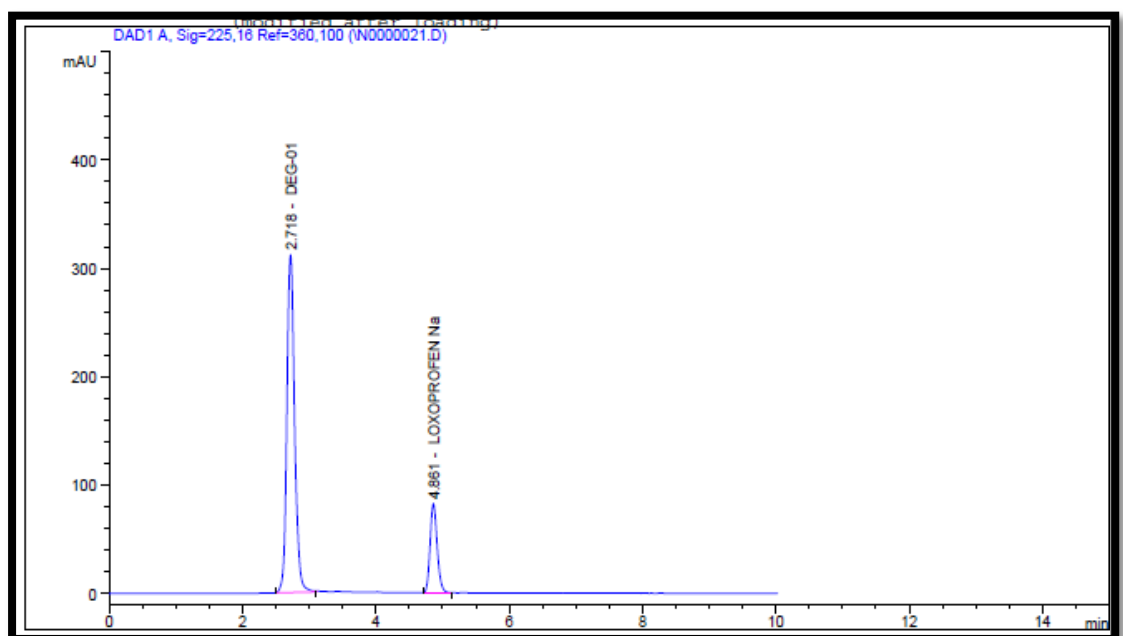


Figure 17. Chromatogram of Loxoprofen sodium under basic degradation condition

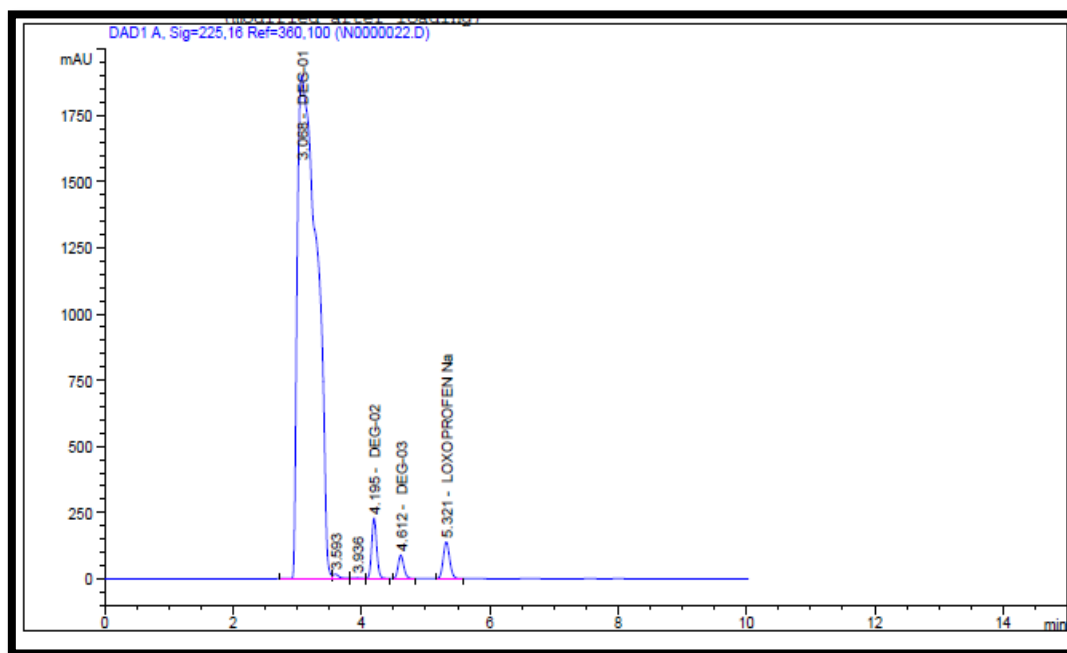


Figure 18. Chromatogram of Loxoprofen sodium under oxidative degradation condition.

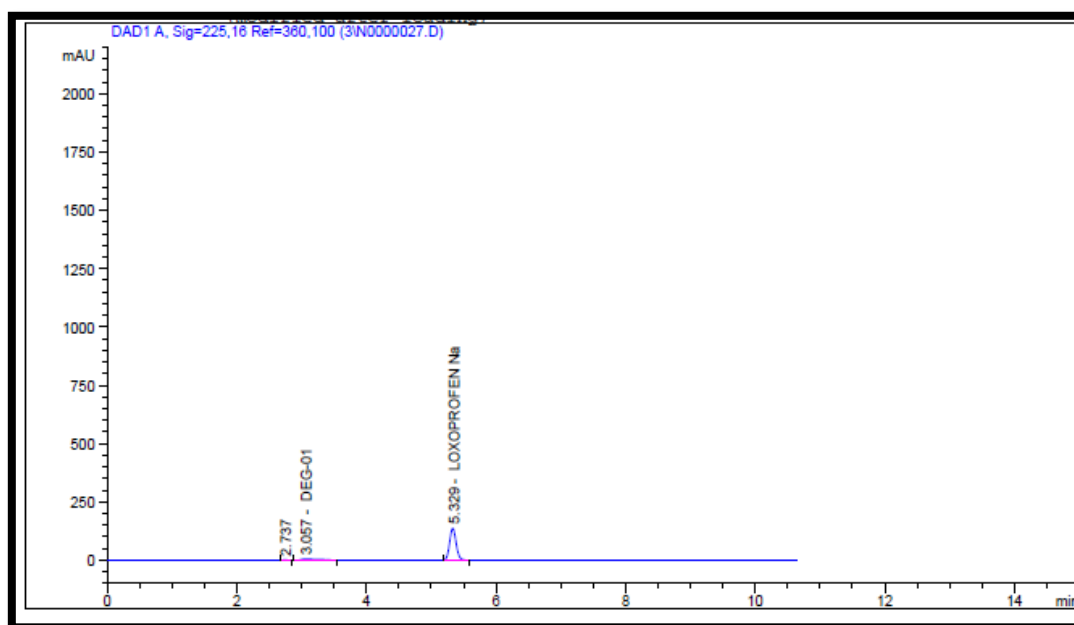


Figure 19. Chromatogram of Loxoprofen sodium under neutral degradation condition.

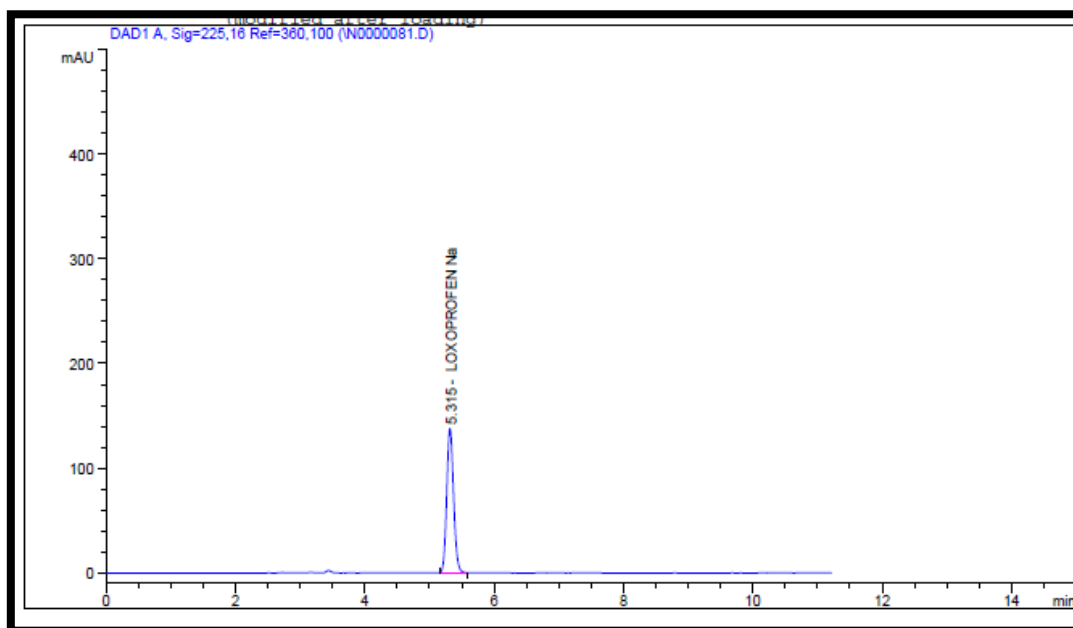


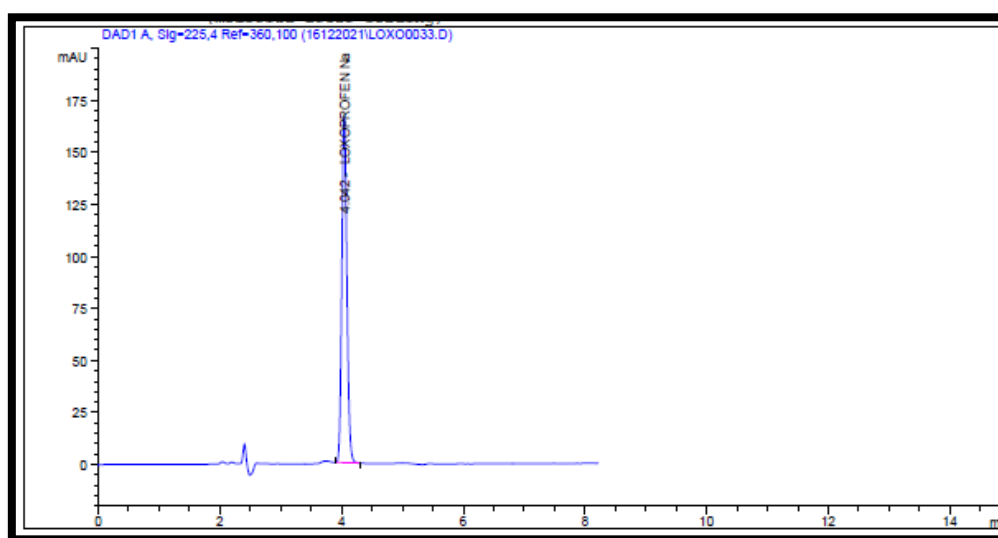
Figure 20. Chromatogram of Loxoprofen sodium under photolytic degradation condition.

Assay of Loxoprofen sodium in marketed tablet

The assay test was performed to quantify Loxoprofen sodium in a marketed formulation at a known concentration of 25 µg/mL. Results presented in **Table 18** indicate an excellent % mean recovery of $101.85 \pm 0.090\%$. Quantitative analysis revealed a distinct and prominent peak for Loxoprofen sodium, underscoring the method's high sensitivity, accuracy, and precision. These findings confirm the reliability of the analytical method, as further illustrated in **Figure 21**.

Table 18. % Assay of Loxoprofen sodium in marketed tablet

Sr. no	Area	Amount found	% Recovery
1	968.6907	25.44532	101.78
2	969.9154	25.47722	101.91
3	965.4521	25.1245	101.80
Mean	969.30	25.46	101.85
SD	0.866	0.023	0.090
%RSD	0.089	0.089	0.089

**Figure 21. Chromatogram of Loxoprofen sodium marketed tablet formulation.**

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF BACLOFEN

Method development

The chromatographic conditions for the analysis of Baclofen were meticulously optimized by systematically adjusting critical parameters, including the mobile phase composition, flow rate, pH, column oven temperature, and detection wavelength. A comprehensive series of experiments was performed to determine the optimal conditions that would result in well-defined and resolved chromatographic peaks. The evaluation included various combinations of methanol, acetonitrile, water, buffer solutions, and their mixtures as components of the mobile phase.

Through extensive trials, methanol emerged as the most suitable organic phase for the RP-HPLC analysis of Baclofen. The aqueous phase was optimized to consist of 0.01% orthophosphoric acid (OPA). The flow rate was maintained at 0.7 mL/min, and the detection wavelength was precisely set at 225 nm to ensure accurate and reliable quantification. Ratios of methanol to 0.01% OPA, such as 70:30 (v/v), 65:35(v/v), and 60:40 (v/v) were systematically tested. These ratios, however, failed to produce chromatograms with well-resolved peaks due to issues such as peak splitting, excessive peak broadening, and suboptimal peak widths, as depicted in **Figures 22–24**.

The breakthrough was achieved with a mobile phase composition of 50:50 (v/v) methanol to 0.01% OPA, which provided optimal resolution and yielded distinct and well-separated peaks. This composition delivered a retention time of 4.35 minutes, demonstrating its suitability for reproducible and accurate analysis of Baclofen, as presented in **Figure 25**. The finalized chromatographic parameters are summarized in **Table 19**.

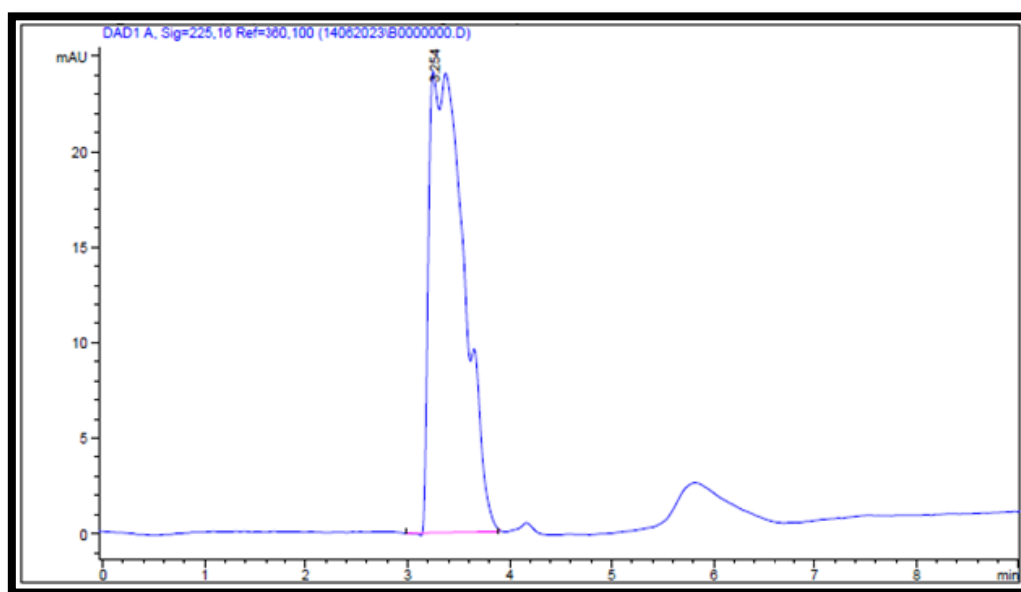


Figure 22. Chromatogram of Baclofen (70:30 v/v, Methanol: 0.01% OPA)

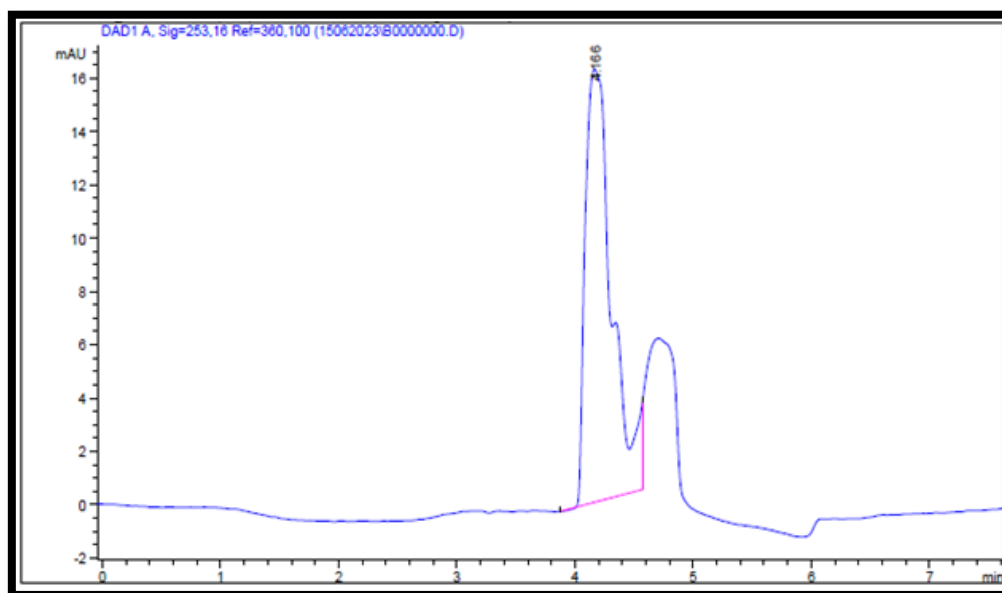


Figure 23. Chromatogram of Baclofen (65:35 v/v, Methanol: 0.01% OPA)

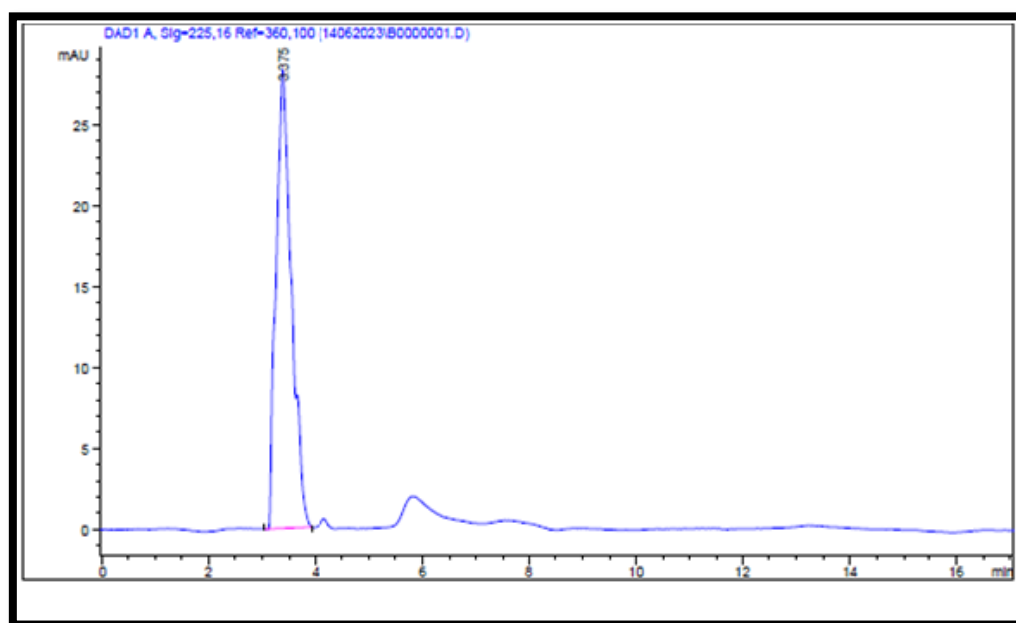


Figure 24. Chromatogram of Baclofen (60:40 v/v, Methanol: 0.01% OPA)

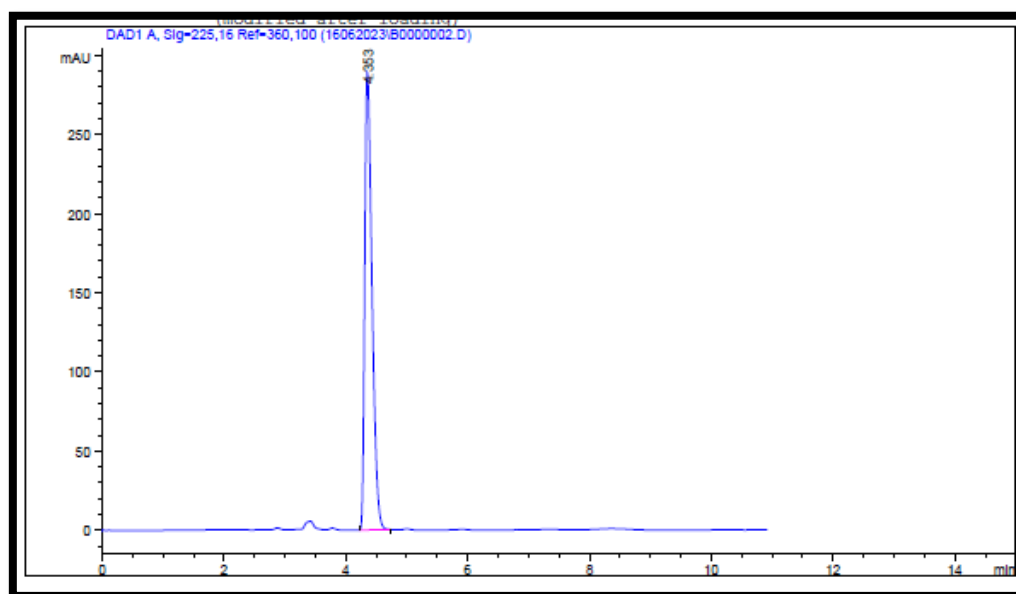


Figure 25. Chromatogram of Baclofen (50:50 v/v, Methanol: 0.01% OPA)

Table 19. Optimized chromatographic conditions of Baclofen by HPLC method

Sr. No.	Parameter	
1	Mobile Phase	METHANOL: 0.01%OPA (50:50, v/v)
2	Wavelength used	225nm
3	Flow Rate	0.7ml/min
4	Temperature	Ambient
5	Sample size	20µl
6	Retention time	4.353
7	Area	2399.05200
8	Theoretical plates	6543
9	Height of peak	289.49646
10	Width of peak	0.1267

Analytical method validation

Linearity

The calibration curve for Baclofen demonstrated outstanding linearity across the concentration range of 5–25 µg/mL, as indicated by regression analysis. The method exhibited excellent precision, with %RSD values consistently below 2.0 at all concentration levels, underscoring its reliability. The high correlation coefficient ($R^2 = 0.999$) further validated the robustness and accuracy of the analytical method within the specified range (**Figure 26, Table 20**). An overlay of the linearity chromatograms is provided in **Figure 27**, illustrating the consistent and reproducible peak responses across the evaluated concentration levels.

Table 20. Linearity and range data of Baclofen by HPLC method.

Sr. No.	Parameters	Results
1	Linearity range	5-25 (µg/mL)
2	Correlation coefficient	0.9991
3	Calibration equation	$y = 46.484x + 153.52$
4	Slope	46.484x
5	Intercept	153.52

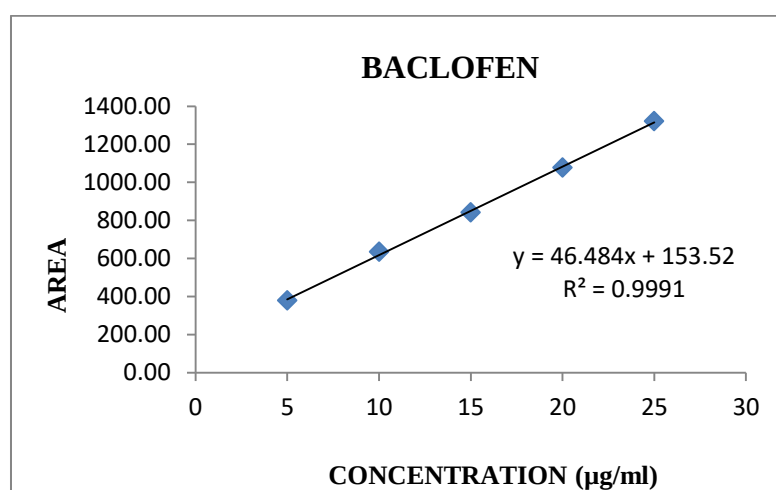


Figure 26. Calibration curve of Baclofen by HPLC method.

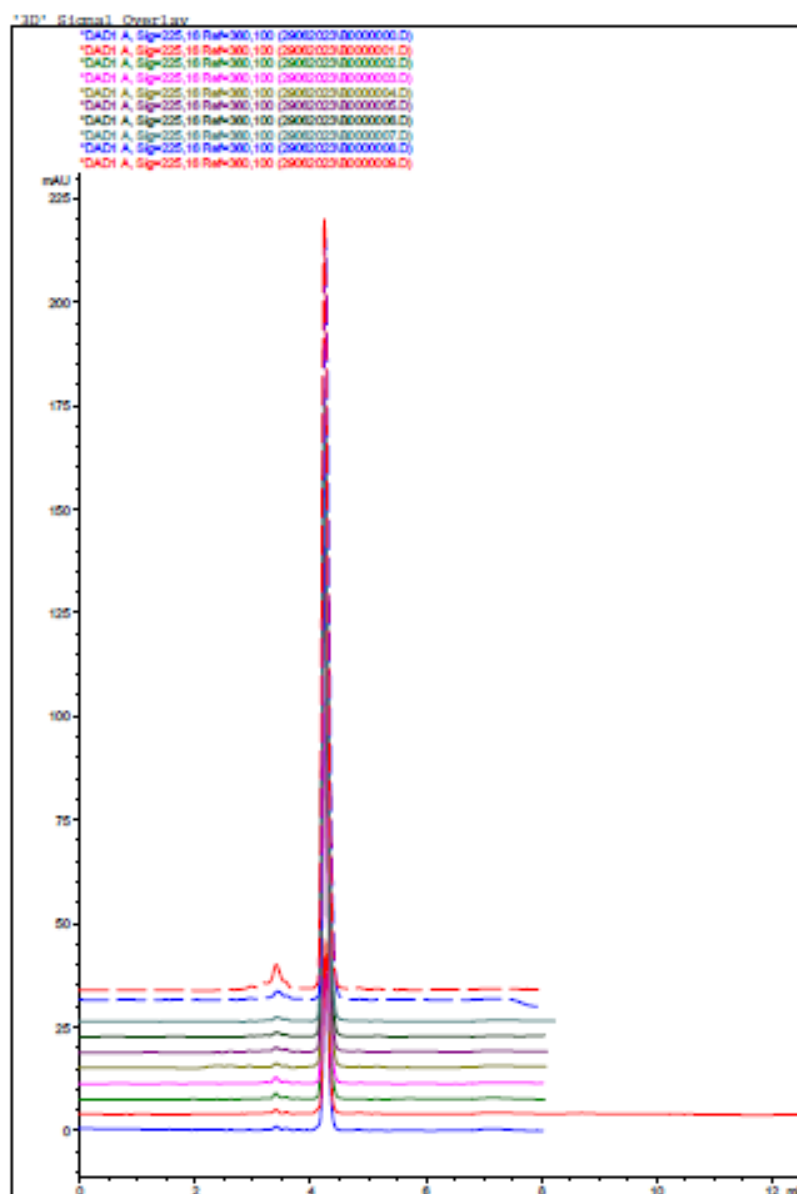


Figure 27. Overlay linearity chromatogram of Baclofen

LOD and LOQ

The LOD and LOQ for Baclofen were determined based on the linear regression data obtained from the calibration curve. The calculated LOD was 1.50 $\mu\text{g/mL}$, demonstrating the method's sensitivity in detecting minimal quantities of the analyte. The LOQ was established at 4.84 $\mu\text{g/mL}$, reflecting the method's capability to quantify Baclofen with accuracy and precision at low concentrations.

System suitability

The system suitability of the developed method was evaluated by analyzing critical parameters such as peak area, retention time (RT), theoretical plates, and tailing factor, as detailed in Table 21. The %RSD for all these parameters consistently remained below 2.0%, indicating excellent precision and consistency. This adherence to established acceptance criteria validates the robustness of the method. These results emphasize its reliability and suitability for routine analytical applications, ensuring consistent performance during repeated analyses.

Table 21. System suitability data of Baclofen by HPLC

Sr. No.	System suitability	Baclofen	
		Mean $\pm$ SD	%RSD
1	Peak area (% RSD)	2399.05 $\pm$ 0.03	0.35
2	Retention time (% RSD)	4.351 $\pm$ 0.06	0.78
3	Theoretical plates (%RSD)	6543 $\pm$ 0.07	0.89
4	Tailing factor (%RSD)	0.67 $\pm$ 0.12	0.92

Precision

The analytical method displayed excellent precision, as reflected by %RSD values consistently below 2.0% across all sample solutions. This adherence to established acceptance criteria underscores the method's high reproducibility and reliability for quantitative analysis. A comprehensive summary of the precision data is provided in **Table 22**, further validating the robustness of the method for routine analytical applications.

Table 22. Precision data of Baclofen by HPLC method

Type	Intra-day precision			Inter-day precision		
Conc. ($\mu\text{g/mL}$)	5	15	25	5	15	25
Mean area.	621.20	853.86	1088.21	378.8	582.45	770.65
SD	3.52	4.09	14.57	2.36	1.60	1.30
%RSD	0.57	0.48	1.34	0.62	0.27	0.16

Robustness

Robustness studies were performed by introducing minor deliberate variations in chromatographic conditions, such as the mobile phase composition, flow rate, and detection wavelength. Despite these changes, the %RSD values consistently remained below 2.0% across all tested conditions, affirming the method's reliability and resilience. These results, summarized in **Tables 23–25**, highlight the method's robustness and suitability for routine analytical use, even under slightly altered operational settings.

Table 23. Robustness (Change in flow rate) data of Baclofen by HPLC method

Sr No.	Conc. µg/ml	FLOWRATE- 0.6 ml/min	FLOWRATE- 0.8 ml/min
		Area	Area
1	25	1556.777	1168.654
2	25	1564.315	1168.287
3	25	1560.304	1168.301
Mean		1560.55	1168.47
SD		5.33	0.26
%RSD		0.34	0.02

Table 24. Robustness (Change in mobile phase ratio) data of Baclofen by HPLC method.

Sr No.	Conc. µg/ml	Mobile Phase Methanol and 0.01% OPA buffer (49:51)	Mobile Phase Methanol and 0.01% OPA buffer (51:49)
		Area	Area
1	25	1334.293	1334.132
2	25	1335.01	1333.866
3	25	1333.58	1333.528
Mean		1334.7	1334.00
SD		0.51	0.19
%RSD		0.04	0.01

Table 25. Robustness (Change in wavelength) data of Baclofen by HPLC method.

Sr No.	Conc. µg/ml	224nm	226nm
		Area	Area
1	25	1443.694	1222.342
2	25	1439.878	1218.607
3	25	1439.257	1220.417
Mean		1441.8	1220.47
SD		5.49	2.64
%RSD		1.42	0.22

Accuracy

The accuracy of the developed method was assessed through recovery studies by spiking a reference Baclofen solution (5 µg/mL) with a composite sample at three concentration levels: low (80%), medium (100%), and high (120%) of the specified concentration. The spiked samples were analyzed using the optimized method, yielding mean percentage recoveries ranging from 100.16% to 101.04%. The low %RSD values, coupled with the high recovery rates, indicate the method's excellent accuracy and reliability for quantitative analysis. Detailed results are provided in **Table 26**, further validating the method's suitability for Baclofen quantification and overlay chromatogram was shown in **figure 28**.

Table 26. Accuracy data of Baclofen by HPLC method

Levels (%)	Amount of sample (µg/ml)	Amount of standard (µg/ml)	Total amount (µg/ml)	Mean amount recovered (µg/ml ±SD)	Mean % recovery ± SD
80	4	5	9	9.04±0.004	100.92±0.01
100	5	5	10	10.04±0.013	100.86±0.25
120	6	5	11	11.01±0.006	100.24±0.11

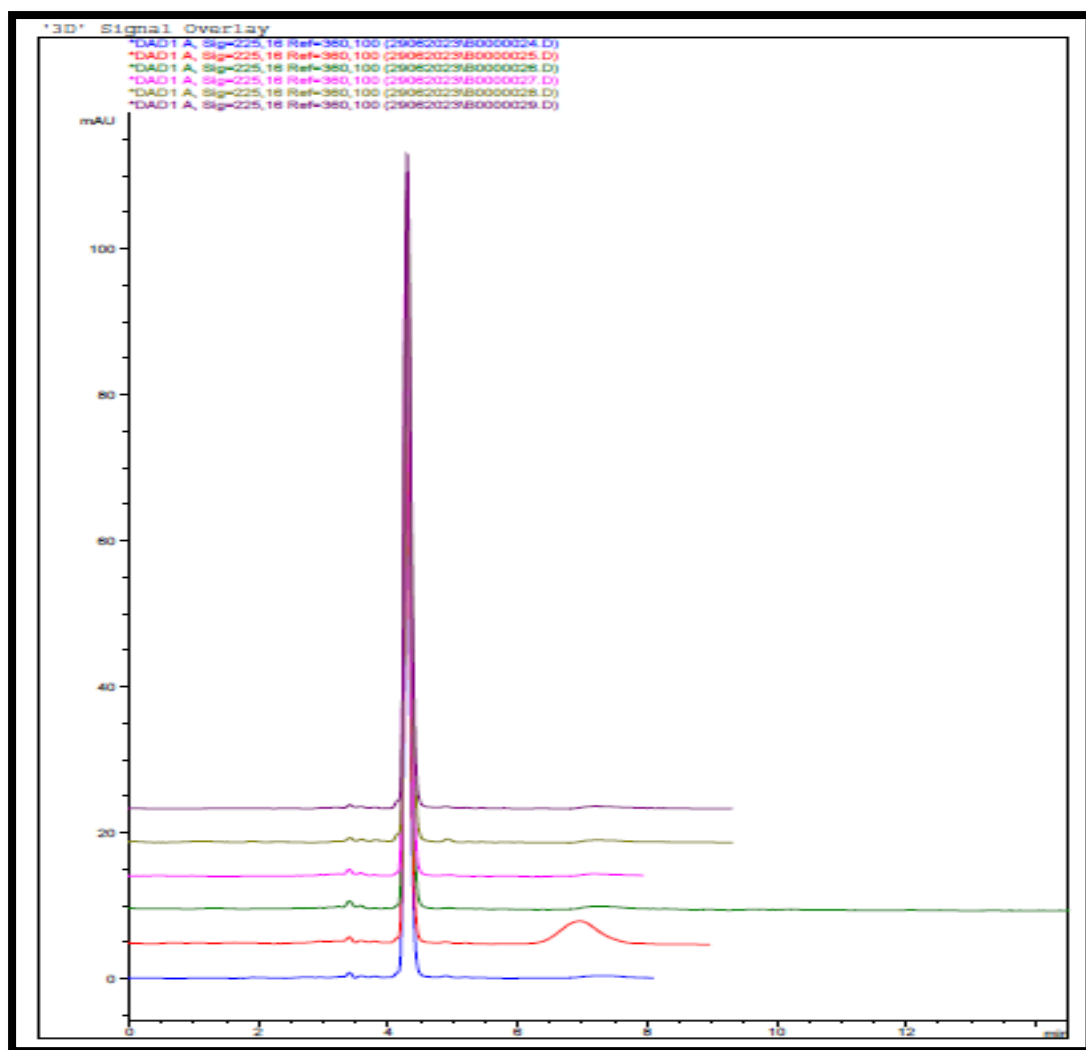


Figure 28.Overlay accuracy chromatogram of Baclofen

Forced degradation studies

Table 27 presents the results of the forced degradation study, which highlight the robustness and specificity of the developed analytical method. The study demonstrated effective separation between the Baclofen peak and its degradation products under various stress conditions, such as exposure to acidic, basic, oxidative, thermal, and photolytic environments. This separation confirms the method's reliability in distinguishing the drug from its degradation products, ensuring its accuracy in stability studies. The chromatograms illustrating these findings are displayed in **Figures 29 to 34**, providing visual evidence of the method's capability as a stability-indicating tool. These results highlight the method's applicability for monitoring Baclofen's stability and ensuring the integrity of the analysis under challenging conditions.

Table 27. Degradation data Baclofen by HPLC method

Sr. No.	Condition	% Undegraded drug	% Degradation
1	Acid Degradation	89.77	10.23
2	Basic Degradation	95.57	4.43
3	H ₂ O ₂ Degradation	105.76	-5.7
4	Neutral	99.73	0.27
5	Photolytic	99.55	0.45

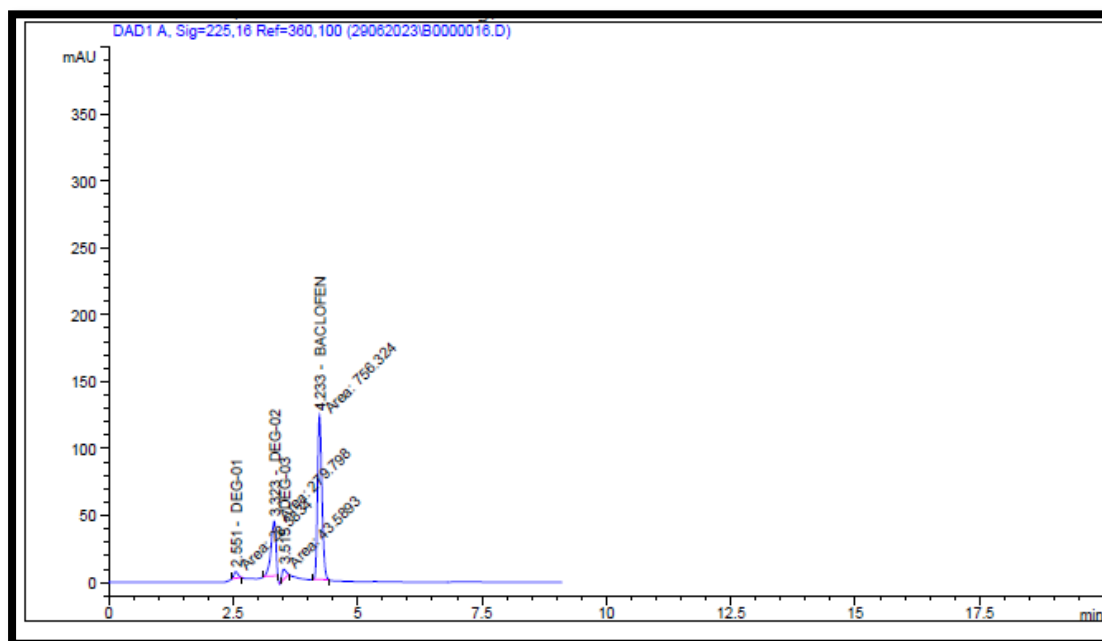


Figure 29. Chromatogram of Baclofen under acid degradation condition

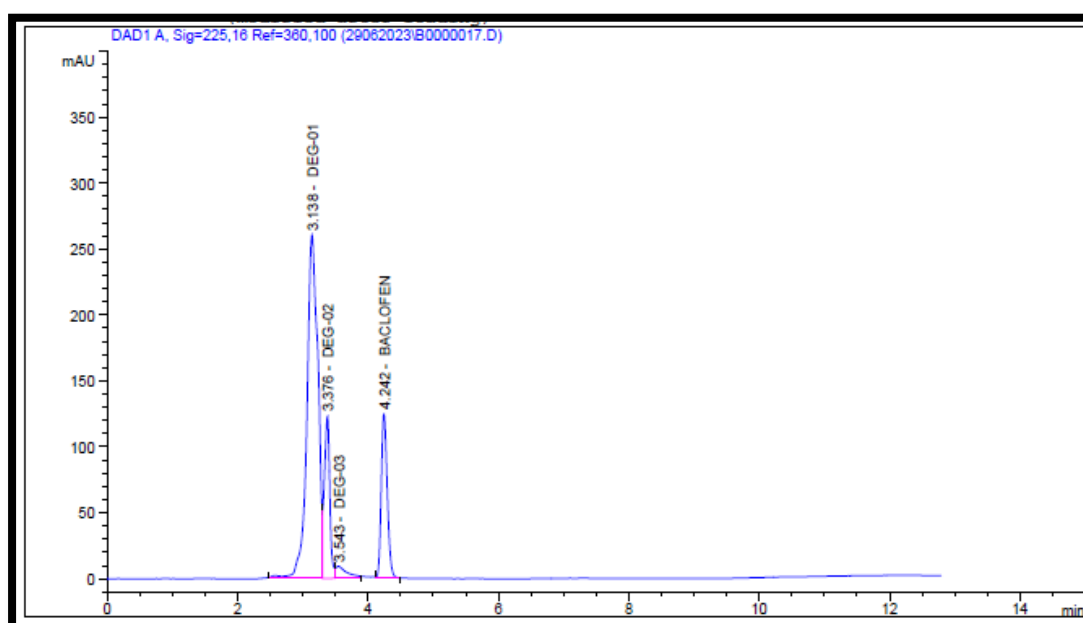


Figure 30. Chromatogram of Baclofen under basic degradation condition

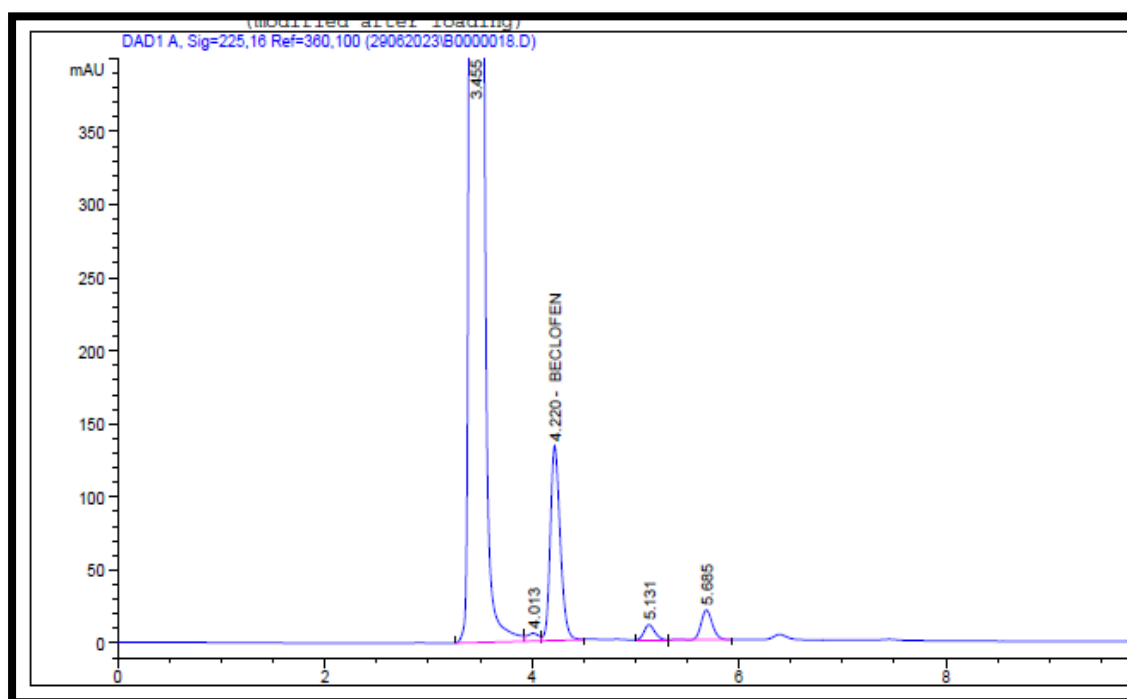


Figure 31. Chromatogram of Baclofen under oxidative degradation condition

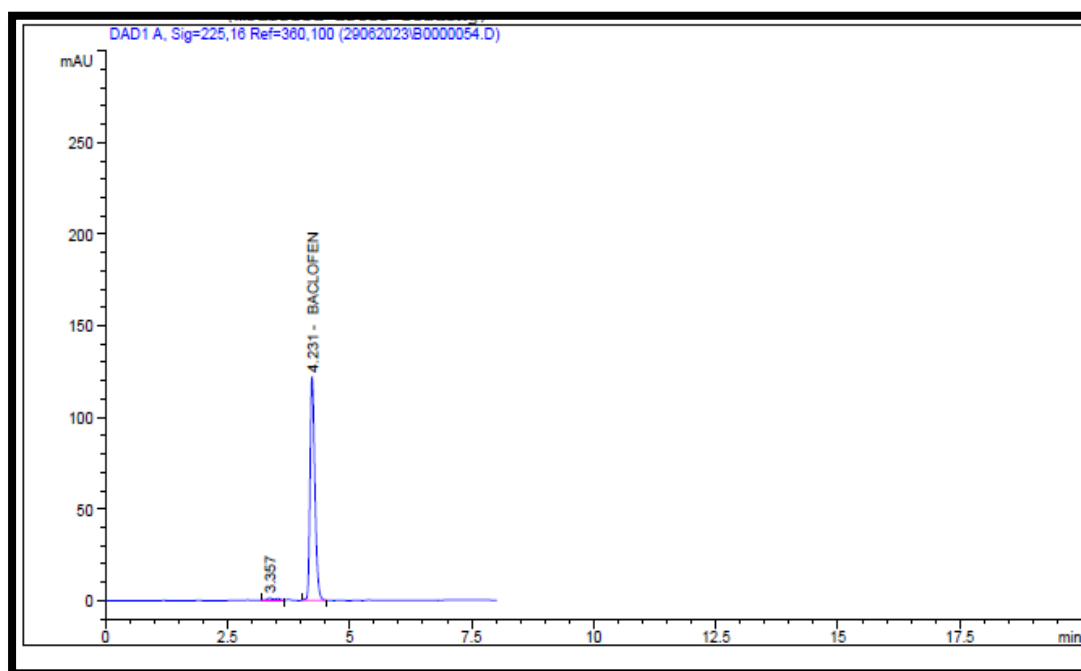


Figure 32. Chromatogram of Baclofen under neutral degradation condition

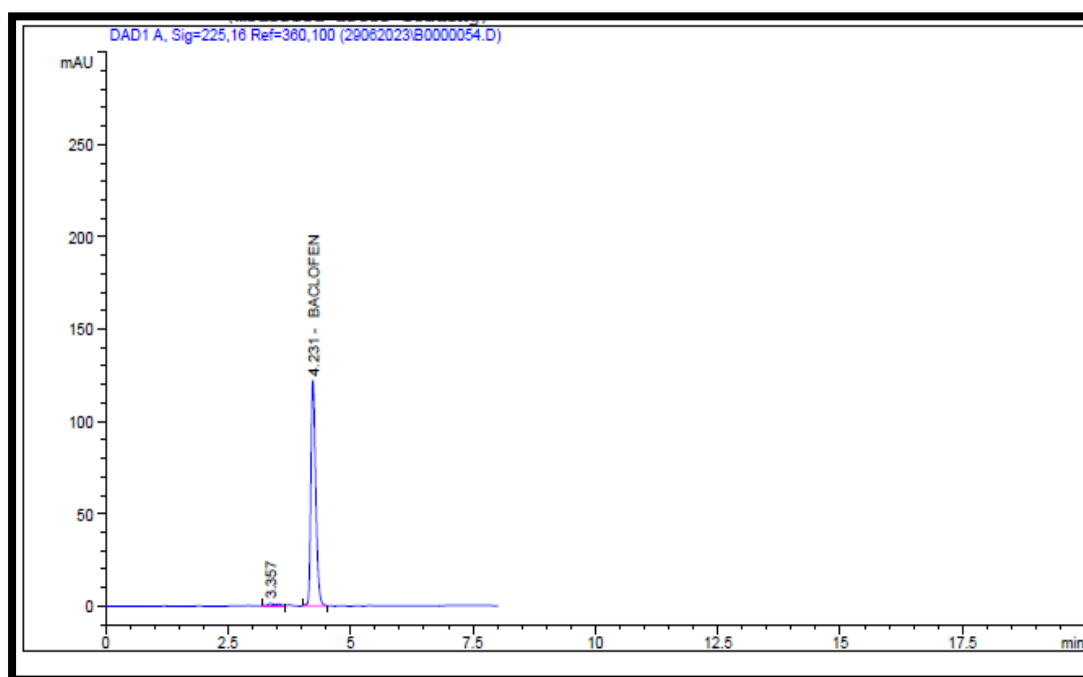


Figure 33. Chromatogram of Baclofen under photolytic degradation condition

Assay of Baclofen in marketed tablet

The assay test was conducted to quantify Baclofen in a marketed formulation with a known concentration of 25µg/mL. The results, summarized in **Table 28**, demonstrated an exceptional % mean recovery of $101.23 \pm 0.561\%$, highlighting the method's accuracy and reliability. The quantitative analysis produced a distinct and well-defined peak for Baclofen, underscoring the high sensitivity, precision, and robustness of the developed method. These findings further validate the method's suitability for routine quality control, as visually supported in **Figure 34**.

Table 28. % Assay of Baclofen in marketed tablet

Sr. No.	Area	Amount found	% Recovery
1	1325.22	25.20918	100.84
2	1334.4343	25.40736	101.63
3	1331.851	25.0741	101.80
Mean	1329.83	25.3101	101.23
SD	6.514	0.140	0.561
%RSD	0.490	0.554	0.554

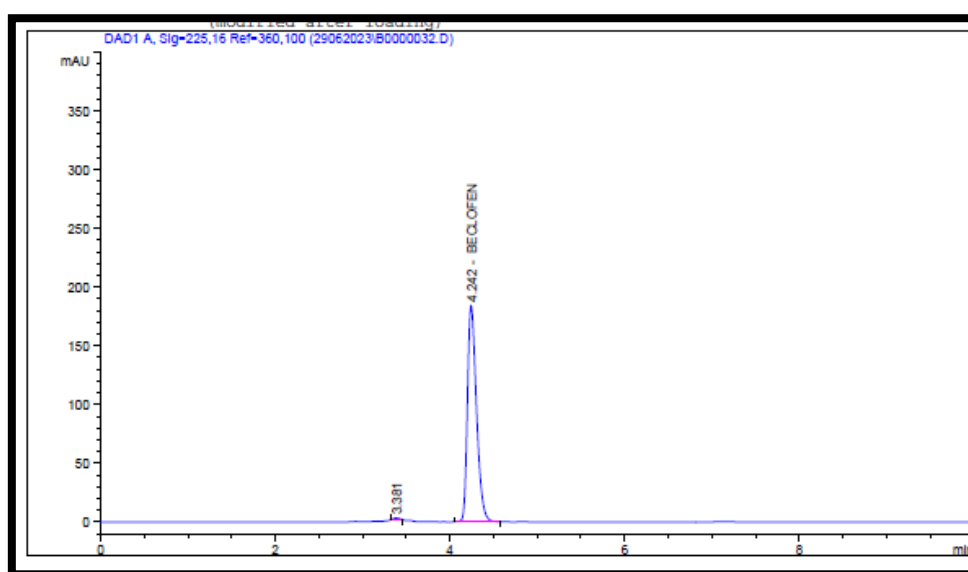


Figure 34. Chromatogram of Baclofen marketed tablet formulation.

DEVELOPMENT AND VALIDATION OF RP-UHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ETODOLAC AND THIOCOLCHICOSIDE USING QUALITY BY DESIGN APPROACH

Method development

The chromatographic conditions for the concurrent analysis of Etodolac and Thiocolchicoside were systematically refined by evaluating key variables, including the composition of the mobile phase, flow rate, pH, column temperature, and detection wavelength. A series of methodical experiments was conducted to establish optimal conditions, ensuring well-resolved and sharply defined chromatographic peaks. The optimization process involved evaluating various combinations of methanol, acetonitrile, water, and buffer solutions as mobile phase components.

Initially, methanol was tested as the organic phase in combination with water as the aqueous phase. However, no peaks were observed even after 20 minutes of analysis. Subsequently, water was replaced with 0.1% orthophosphoric acid (OPA) in the mobile phase, leading to peak formation. Despite this improvement, the peaks lacked the desired characteristics for accurate quantification and resolution. The chromatographic conditions were finalized using a Quality by Design (QbD)-driven Design of Experiments (DoE) methodology. This enabled systematic exploration of the factor interactions and optimization of critical variables, ultimately achieving robust and reproducible separation of Etodolac and Thiocolchicoside.

Determination of isosbestic point by UV spectroscopy

The isosbestic point was identified using UV spectroscopy, with the maximum absorbance (λ_{max}) observed at 256 nm within the detection range of 200–400 nm.

The corresponding UV spectrum is presented in **Figure 35**.

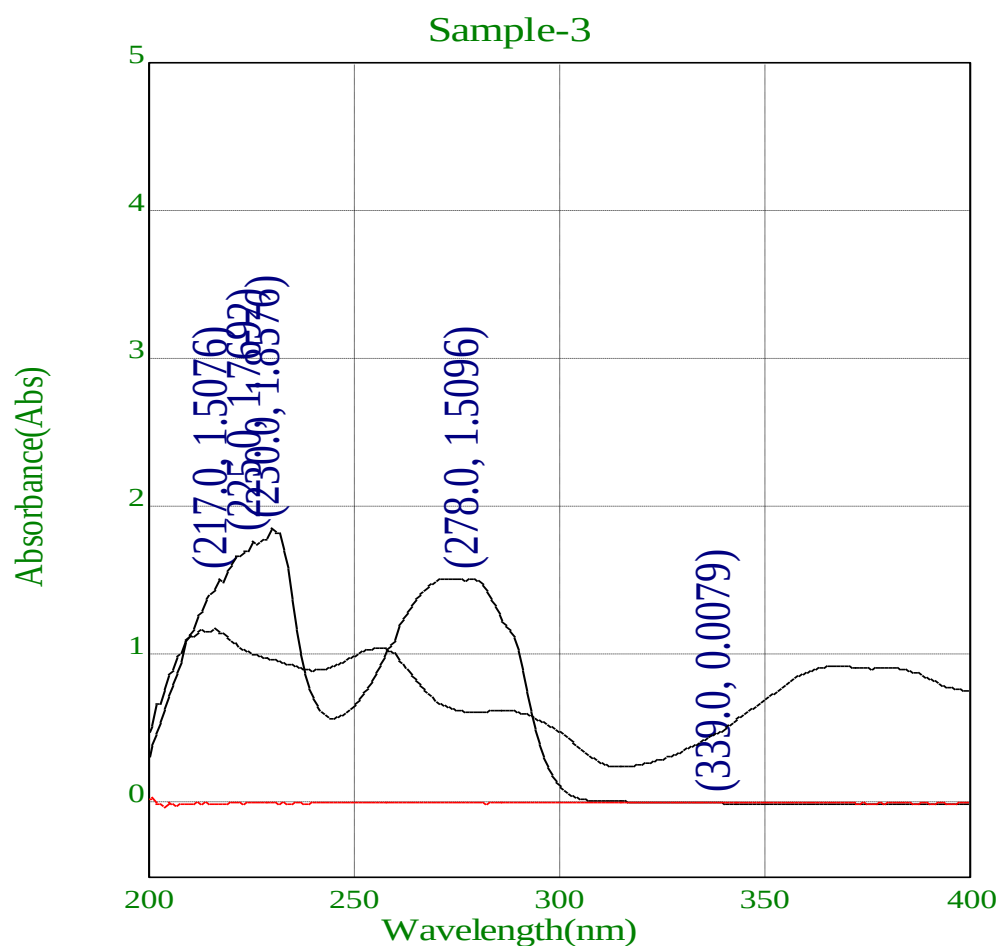


Figure 35. Isosbestic point of Etodolac and Thiocolchicoside.

Method optimization by QbD approach

Optimization was performed using the Box-Behnken Design (BBD), which targeted three independent variables identified through preliminary factor screening. These variables—organic phase percentage, flow rate, and detection wavelength—were systematically evaluated at five levels. The dependent response variables were: retention time (RT) for Thiocolchicoside (Y1) and Etodolac (Y5), peak area (PA) for Thiocolchicoside (Y2) and Etodolac (Y6), theoretical plates (TP) for Thiocolchicoside (Y3) and Etodolac (Y7), and tailing factor (TF) for Thiocolchicoside (Y4) and Etodolac (Y8).

The DoE approach was utilized to determine the critical parameter values necessary for achieving optimal outcomes, providing comprehensive insights into the experimental conditions. Model validation was performed using ANOVA in DoE software version 13.0, which also enabled the creation of an experimental matrix for response surface methodology. A statistically significant model, as confirmed by ANOVA, was the target outcome. The DoE approach yielded a total of 14 experimental runs for both drugs, with the data summarized in **Tables 29 and 30**.

Table 29. Box-Behnken design (BBD) based independent variable and their effect on dependent variables for Thiocolchicoside.

Thiocolchicoside							
Run	Independent Variables			Dependent variables			
	A	B	C	RT	PA	TP	TF
1	95	0.9	258	2.137	117.18	3078	0.88
2	85	1.1	256	1.877	127.86	4796	0.98
3	85	0.9	258	2.288	106.62	5392	0.89
4	95	1.1	258	1.744	103.08	3818	0.97
5	90	1	258.682	2.002	83.75	4007	0.95
6	81.591	1	257	2.092	112.61	5454	0.98
7	95	0.9	256	2.134	116.32	3114	0.87
8	85	1.1	258	1.876	91.57	4860	0.95
9	95	1.1	256	1.925	151.31	2909	0.98
10	98.409	1	257	1.888	131.32	3924	0.89
11	90	1	255.318	2.001	175.01	4003	0.96
12	90	1.16818	257	1.725	103.62	4002	0.83
13	90	0.831821	257	2.398	146.24	4714	0.79
14	85	0.9	256	2.282	156.40	5810	0.78

Table 30. Box-Behnken design (BBD) based independent variable and their effect on dependent variables for Etodolac.

Etodolac							
Run	Independent Variables			Dependent variables			
	A	B	C	RT	PA	TP	TF
1	95	0.9	258	3.710	2171.30	10152	0.88
2	85	1.1	256	4.357	1624.89	8796	0.89
3	85	0.9	258	5.303	2184.30	9459	0.87
4	95	1.1	258	3.050	1779.31	8616	0.91
5	90	1	258.682	3.897	2663.68	9389	0.88
6	81.591	1	257	5.696	1876.71	9186	0.86
7	95	0.9	256	3.702	2168.32	10023	0.89
8	85	1.1	258	4.365	1796.49	8619	0.89
9	95	1.1	256	3.334	1774.38	9446	0.87
10	98.409	1	257	3.144	1868.52	8776	0.89
11	90	1	255.318	3.899	1700.64	9138	0.88
12	90	1.16818	257	3.372	1613.37	8606	0.79
13	90	0.831821	257	4.662	2246.19	10854	0.77
14	85	0.9	256	5.270	1983.70	10007	0.80

The influence of the independent variables on the selected responses was further clarified through three-dimensional (3D) response surface plots, offering a detailed visual interpretation of variable interactions and their effects. These graphical representations proved invaluable for analyzing the relationships among the organic phase percentage, flow rate, and detection wavelength in relation to the targeted responses.

For retention time (RT; Y_1 and Y_4), the response surface plots revealed a significant inverse relationship, as increasing the organic phase concentration and flow rate resulted in a marked decrease in RT. Conversely, for peak area (PA; Y_2 and Y_5), the composition of the mobile phase and flow rate exerted minimal impact, indicating that these variables had a negligible effect on the peak area for both analytes.

In contrast, theoretical plates (TP; Y_3 and Y_7) were significantly influenced by the organic phase concentration and flow rate. An increase in these variables resulted in a proportional enhancement of the TP values for both drugs, indicating improved column efficiency. Regarding the tailing factor (TF; Y_4 and Y_8), both the organic phase concentration and flow rate displayed a direct impact; as these variables increased, the tailing factor also showed an upward trend. However, the detection wavelength exerted only a minimal effect on all the studied responses. The detailed interactions and trends are visually represented in the 3D response surface plots, illustrated in **Figures 36 to 39**, which provide critical insights into the optimization of method parameters.

Optimized method conditions

Using Design of Experiments (DoE) software (version 13.0, Stat-Ease, Inc.), all responses successfully met the predefined criteria, yielding satisfactory outcomes. Among the optimization solutions suggested by the software, those with a desirability score of 1 or close to 1 were prioritized for implementation. The shaded yellow region in the overlay plot (**Figure 40**) delineates the design space, highlighting the operational ranges that fulfill all specified constraints and response goals. This visualization serves as a critical tool for identifying robust and reliable conditions for the optimized method.

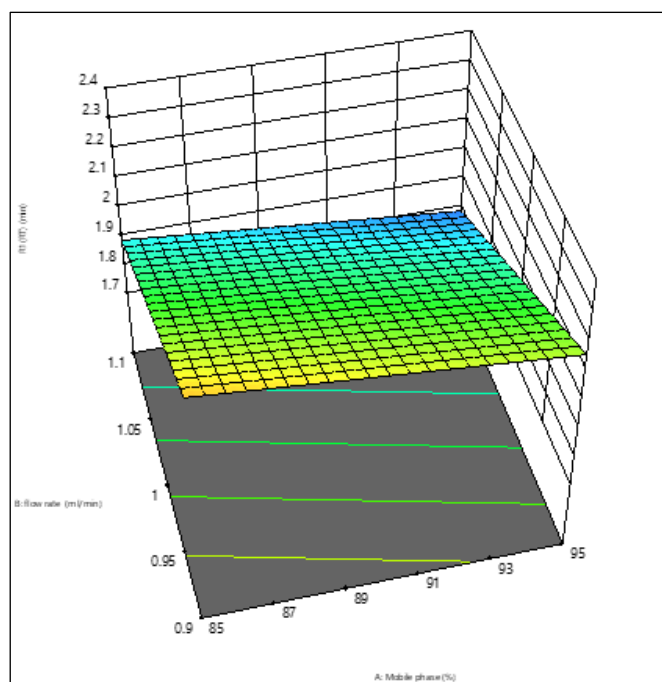


Figure 36. The response surface plot illustrates the influence of the organic phase percentage (A), flow rate (B), and detection wavelength (C) on retention time.

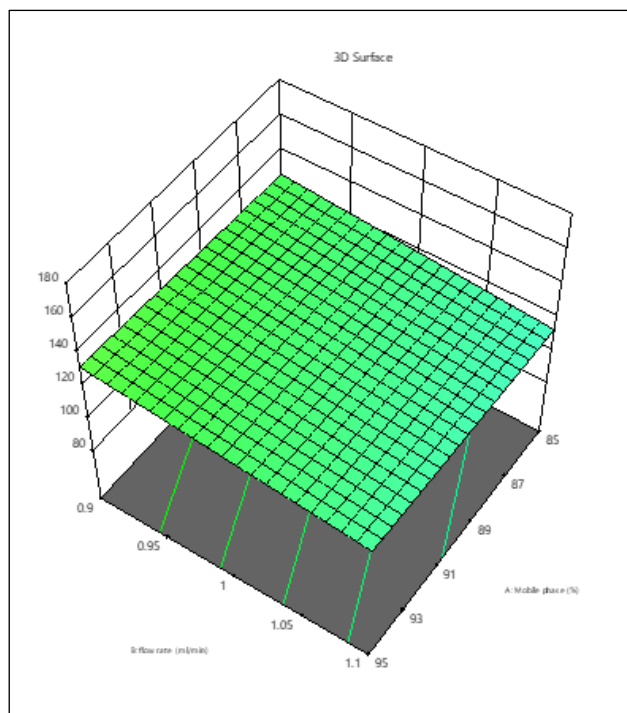


Figure 37.The response surface plot depicts the effects of organic phase percentage (A), flow rate (B), and detection wavelength (C) on peak area.

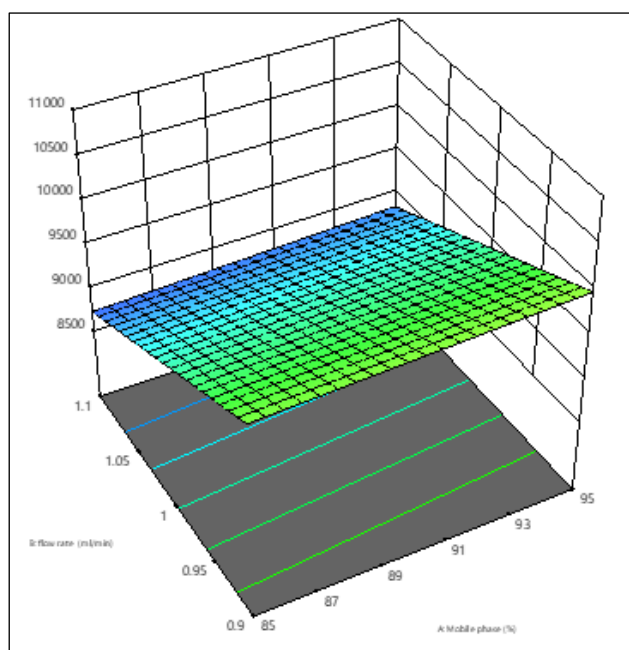


Figure 38.The response surface plot illustrates the effects of organic phase percentage (A), flow rate (B), and detection wavelength (C) on theoretical plates (TP).

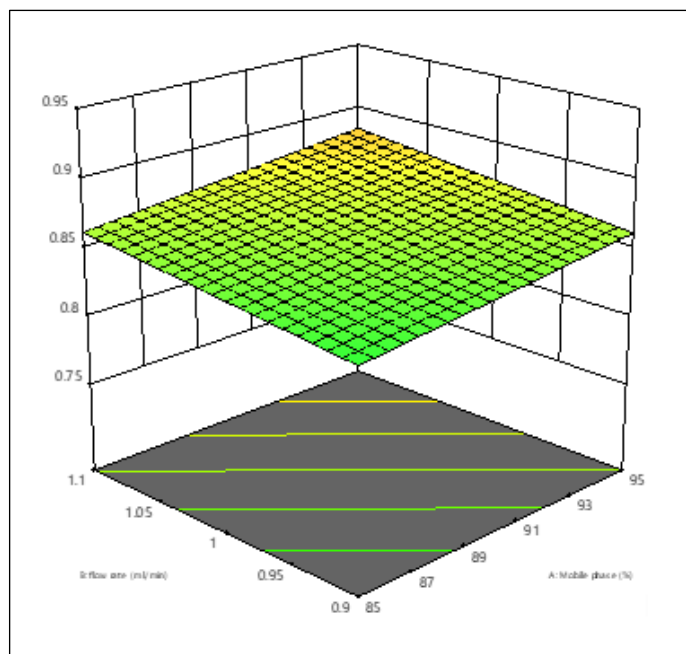


Figure 39. The response surface plot demonstrates the impact of organic phase percentage (A), flow rate (B), and detection wavelength (C) on the tailing factor.

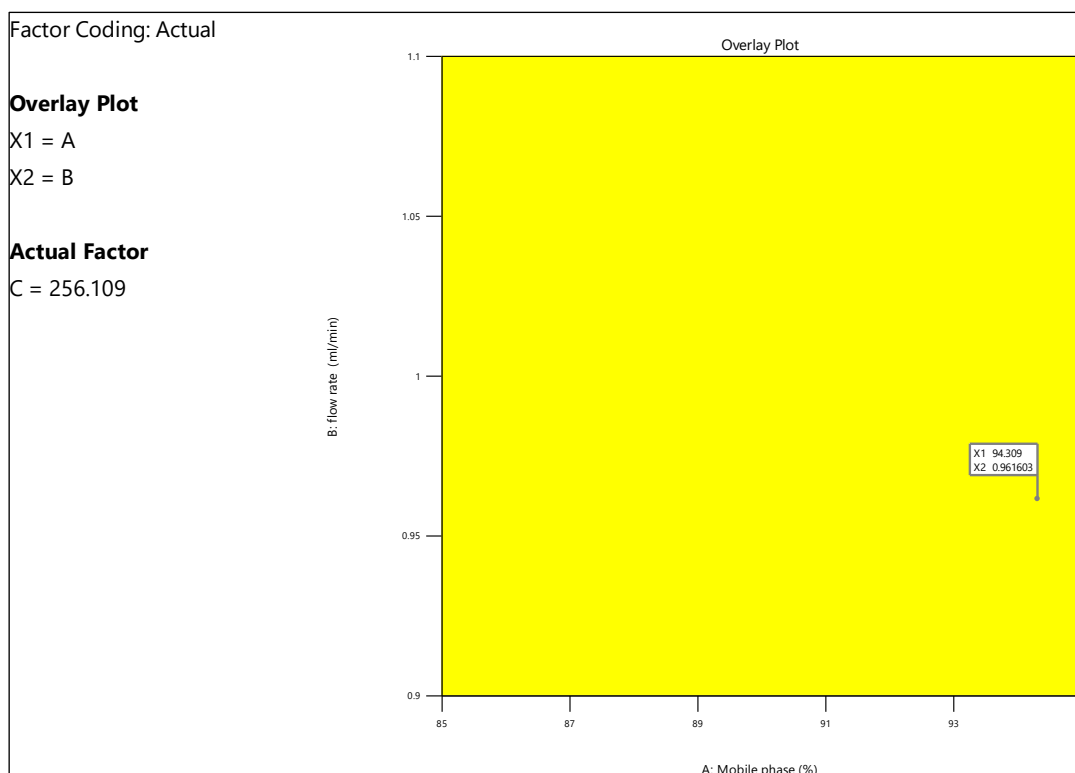


Figure 40. Design space overlay plot

The optimized chromatographic conditions were identified as 94.30% (v/v) organic phase composition, a flow rate of 0.96 mL/min, and a detection wavelength of 256 nm. To validate these parameters, six replicate injections ($n = 6$) were performed using Thiocolchicoside and Etodolac solutions at concentrations of 0.4 $\mu\text{g/mL}$ and 40 $\mu\text{g/mL}$, respectively. The observed values for both drugs demonstrated a deviation of less than 5% from the predicted values, thereby confirming the accuracy and reliability of the selected conditions. Under these optimized parameters, the chromatographic separation of both analytes was highly efficient, as depicted in **Figure 41**.

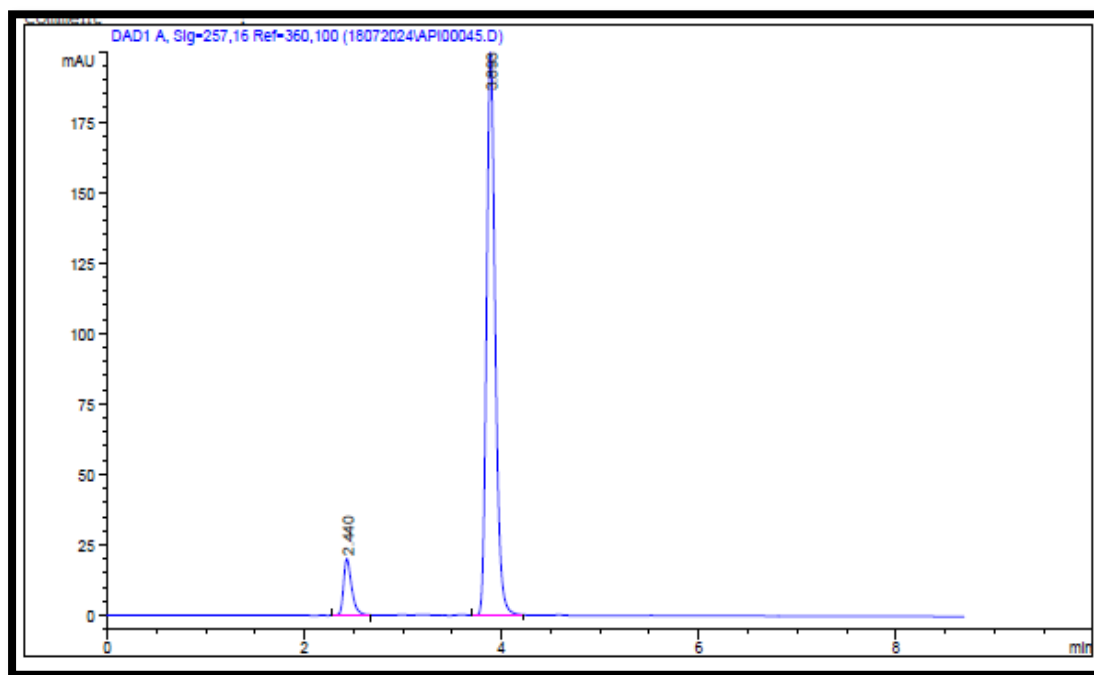


Figure 41. Simultaneous chromatogram of Thiocolchicoside and Etodolac.

Analytical Method validation**Linearity**

The calibration curves for Etodolac and Thiocolchicoside demonstrated exceptional linearity within the concentration ranges of 40–200 µg/mL for Etodolac and 0.4–2.0 µg/mL for Thiocolchicoside, as confirmed by regression analysis. The method exhibited remarkable precision, with %RSD values consistently below 2.0 across all concentration levels, highlighting its reliability. A high correlation coefficient ($R^2 = 0.999$) for both drugs further substantiated the accuracy and robustness of the analytical method within the specified ranges. **Figures 42 and 43**, along with **Tables 31 and 32**, provide a detailed representation of the calibration data, supporting the method's validity. Additionally, an overlay of the linearity chromatograms, presented in **Figure 44**, illustrates consistent and reproducible peak responses across the evaluated concentration levels, reinforcing the method's reliability.

Table 31. Linearity and range data of Etodolac by UHPLC method.

Sr. No.	Parameters	Results
1	Linearity range	40-200 µg/mL
2	Correlation coefficient	0.9997
3	Calibration equation	$y = 21.039x + 2925.1$
4	Slope	21.039x
5	Intercept	2925.1

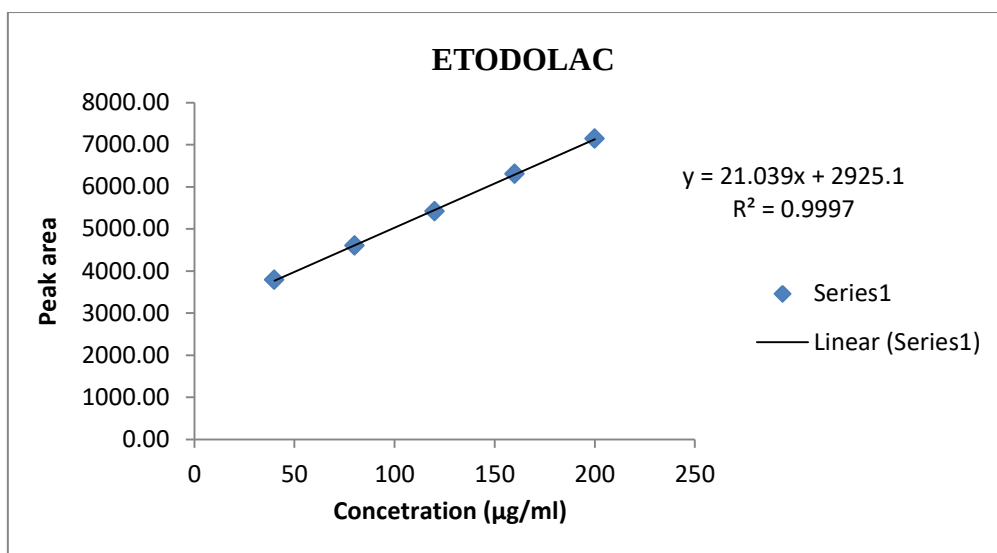


Figure 42. Calibration curve of Etodolac by UHPLC method.

Table 32. Linearity and range data of Thiocolchicoside by UHPLC method.

Sr. no.	Parameters	Results
1	Linearity range	0.4-2 µg/mL
2	Correlation coefficient	0.999
3	Calibration equation	$y = 259.79x + 401.79$
4	Slope	259.79x
5	Intercept	401.79

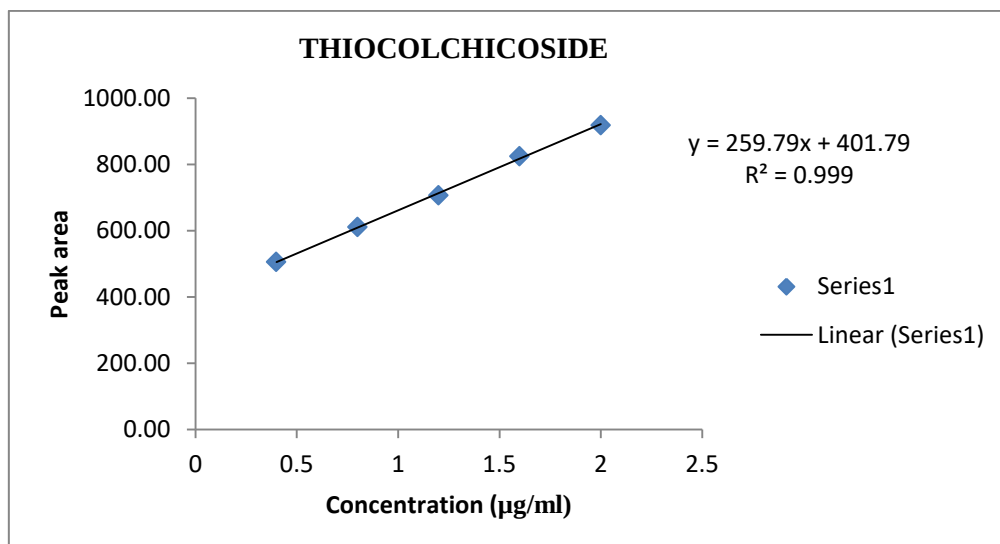


Figure 43. Calibration curve of Thiocolchicoside by UHPLC method.

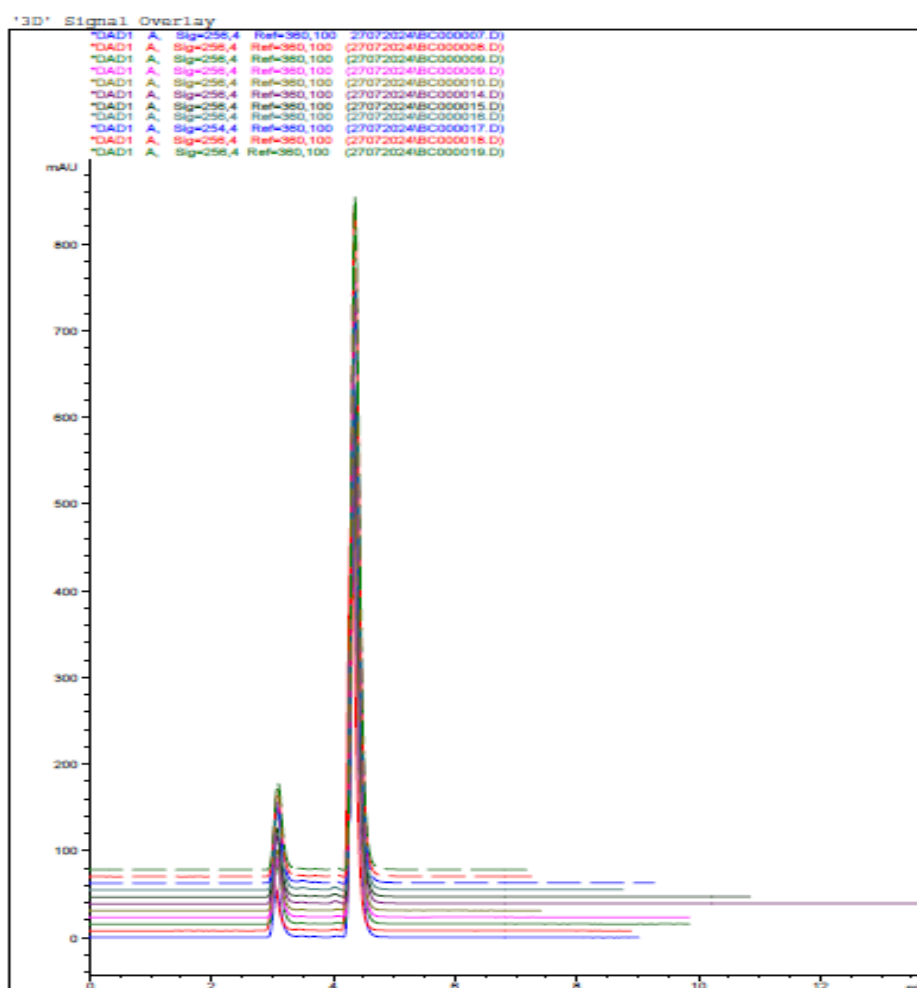


Figure 44. Overlay linearity chromatogram of Etodolac and Thiocolchicoside

LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) for Etodolac and Thiocolchicoside were determined using linear regression data obtained from the calibration curves. The LOD values were calculated to be 1.57 µg/mL for Etodolac and 0.051 µg/mL for Thiocolchicoside, indicating the method's high sensitivity for detecting trace concentrations of these analytes. Similarly, the LOQ values were established at 4.84 µg/mL for Etodolac and 0.157 µg/mL for Thiocolchicoside, demonstrating the method's capability to accurately and precisely quantify these compounds at low concentration levels.

System suitability

The system suitability of the developed method was evaluated by analyzing key parameters, including peak area, retention time (RT), theoretical plates, and tailing factor for both drugs, as summarized in **Table 33**. The %RSD values for all evaluated parameters consistently remained below 2.0%, indicating excellent precision and consistency. This adherence to established acceptance criteria validates the robustness of the method. These findings highlight the method's reliability and suitability for routine analytical applications, ensuring consistent and reproducible performance across repeated analyses.

Table 33. System suitability data of Etodolac and Thiocolchicoside by UHPLC

Sr. No.	System suitability	Etodolac		Thiocolchicoside	
		Mean $\pm$ SD	%RSD	Mean $\pm$ SD	%RSD
1	Peak area (% RSD)	2184.30 $\pm$ 0.02	0.68	103.08 $\pm$ 0.47	0.87
2	Retention time (% RSD)	4.30 $\pm$ 0.01	1.14	3.03 $\pm$ 0.78	1.57
3	Theoretical plates (%RSD)	9459 $\pm$ 0.25	0.54	5810 $\pm$ 1.14	0.624
4	Tailing factor (%RSD)	0.88 $\pm$ 0.35	1.45	0.78 $\pm$ 0.68	0.32

Precision

The analytical method demonstrated exceptional precision, with %RSD values for both drugs consistently remaining below 2.0% across all sample solutions. These results comply with established acceptance criteria, underscoring the method's reproducibility and reliability. A comprehensive summary of the precision data is presented in **Tables 34 and 35**.

Table 34. Precision data of Etodolac by UHPLC method

Type	Intra-day precision			Inter-day precision		
Conc. (µg/mL)	40	120	200	40	120	200
Mean area	3767.61	5424.07	7136.83	3785.18	5458.64	7174.27
SD	2.83	2.83	1.41	4.78	3.38	0.88
%RSD	0.075	0.05	0.025	0.13	0.06	0.01

Table 35. Precision data of Thiocolchicoside by UHPLC method

Type	Intra-day precision			Inter-day precision		
Conc. (µg/mL)	0.4	1.2	2.0	0.4	1.2	2.0
Mean area.	504.91	717.07	924.39	3785.18	5458.64	7174.27
SD	2.82	0.70	0.57	0.48	0.71	1.41
%RSD	0.56	0.09	0.54	0.109	0.10	0.15

Robustness

Robustness studies were conducted by introducing minor, deliberate variations in chromatographic conditions, including adjustments to mobile phase composition, flow rate, and detection wavelength. Despite these intentional changes, the %RSD values consistently remained below 2.0% under all tested conditions, demonstrating the method's reliability and resilience. These findings, detailed in **Tables 36–41**, confirm the robustness of the method and its suitability for routine analytical applications, even when subjected to slightly alter operational settings.

Table 36. Robustness (Change in flow rate) data of Etodolac by UHPLC method.

Sr No.	Conc. µg/ml	FLOWRATE- 0.86 ml/min	FLOWRATE- 1.06 ml/min
		Area	Area
1	100	6299.71143	4792.485
2	100	6292.2356	4782.524
3	100	6292.8755	4782.812
Mean		6295.97	4787.50
SD		5.29	7.04
%RSD		0.08	0.15

Table 37. Robustness (Change in mobile phase ratio) data of Etodolac by UHPLC method.

Sr No.	Conc. µg/ml	Mobile phase ratio Methanol and 0.1% OPA buffer 93.3:6.7	Mobile phase ratio- Methanol and 0.1% OPA buffer 95.3:4.7
		Area	Area
1	100	5521.981	5508.045
2	100	5520.786	5502.786
3	100	5520.785	5501.84
Mean		5521.4	5505.42
SD		0.85	3.72
%RSD		0.02	0.07

Table 38. Robustness (Change in wavelength) data of Etodolac by UHPLC method.

Sr No.	Conc. µg/ml	255nm	257nm
		Area	Area
1	100	5141.12	5998.743
2	100	5147.236	5995.452
3	100	5145.68	5993.78
Mean		5144.2	5997.10
SD		4.32	2.33
%RSD		0.08	0.04

Table 39. Robustness (Change in flow rate) data of Thiocolchicoside by UHPLC method.

Sr No.	Conc. µg/ml	FLOWRATE- 0.86 ml/min	FLOWRATE- 1.06 ml/min
		Area	Area
1	1.2	839.30939	643.3512
2	1.2	840.5623	640.7856
3	1.2	840.254	641.55
Mean		839.94	642.07
SD		0.89	1.81
%RSD		0.11	0.18

Table 40. Robustness (Change in mobile phase ratio) data of Etodolac by UHPLC method.

Sr No.	Conc. µg/ml	Mobile phase ratio- Methanol and 0.1% OPA buffer 93.3:6.7	Mobile phase ratio- Methanol and 0.1% OPA buffer 95.3:4.7
		Area	Area
1	1.2	697.9546	735.02582
2	1.2	695.2342	733.4286
3	1.2	695.25	734.251
Mean		696.6	734.23
SD		1.92	1.13
%RSD		0.28	0.15

Table 41. Robustness (Change in wavelength) data of Thiocolchicoside by UHPLC method.

Sr No.	Conc. µg/ml	255nm	257nm
		Area	Area
1	1.2	884.2449	787.39154
2	1.2	880.1263	785.6523
3	1.2	880.1555	786.8425
Mean		882.2	786.52
SD		2.91	1.23
%RSD		0.33	0.16

Accuracy

The accuracy of the developed method was evaluated through recovery studies by spiking reference solutions of Etodolac and Thiocolchicoside (40 µg/mL and 0.4 µg/mL, respectively) into a composite sample at three concentration levels: low (80%), medium (100%), and high (120%) of the target concentration. The spiked samples were analyzed using the optimized method, yielding mean percentage recoveries ranging from 100.60% to 101.85%. The consistently low %RSD values, combined with the high recovery rates, confirm the method's excellent accuracy and reliability for quantitative analysis. A detailed summary of the results is presented in Tables 42 and 43.

Table 42. Accuracy data of Etodolac by UHPLC method

Levels (%)	Amount of sample (µg/ml)	Amount of standard (µg/ml)	Total amount (µg/ml)	Mean amount recovered (µg/ml ±SD)	Mean % recovery ± SD
80	40	32	72	72.56	101.74
100	40	40	80	80.31	100.76
120	40	48	88	88.29	100.60

Table 43. Accuracy data of Thiocolchicoside by UHPLC method

Levels (%)	Amount of sample (µg/ml)	Amount of standard (µg/ml)	Total amount (µg/ml)	Mean amount recovered (µg/ml ±SD)	Mean % recovery ± SD
80	0.4	0.32	0.72	0.72	100.23
100	0.4	0.4	0.8	0.81	100.76
120	0.4	0.48	0.88	0.88	100.60

Assay of Etodolac and Thiocolchicoside in marketed tablet

The assay test was performed to quantify Etodolac and Thiocolchicoside in a marketed formulation with known concentrations of 120 µg/mL for Etodolac and 1.2 µg/mL for Thiocolchicoside. The results, summarized in **Tables 44 and 45**, revealed an exceptional % mean recovery of 99.65% for Etodolac and 100.92% for Thiocolchicoside, demonstrating the method's accuracy and reliability. The quantitative analysis yielded distinct and well-defined peaks for both drugs, underscoring the method's high sensitivity, precision, and robustness. These findings validate the method's appropriateness for routine quality control applications. Visual representations of the chromatograms are provided in **Figure 45**.

Table 44. % assay data of Etodolac in marketed tablet formulation

Sr. No.	Area	Amount found	% Recovery
1	5449.53516	120.0445	100.04
2	5430.08154	119.1194	99.27
3	5430.2945	119.1475	100.01
Mean	5439.81	119.58	99.65
SD	13.756	0.654	0.545
%RSD	0.253	0.547	0.547

Table 45. % assay data of Thiocolchicoside in marketed tablet formulation

Sr. No.	Area	Amount found	% Recovery
1	715.7099	1.209126	100.76
2	716.712	1.212984	101.08
3	715.892	1.205892	100.98
Mean	716.21	1.21	100.92
SD	0.709	0.003	0.227
%RSD	0.099	0.225	0.225

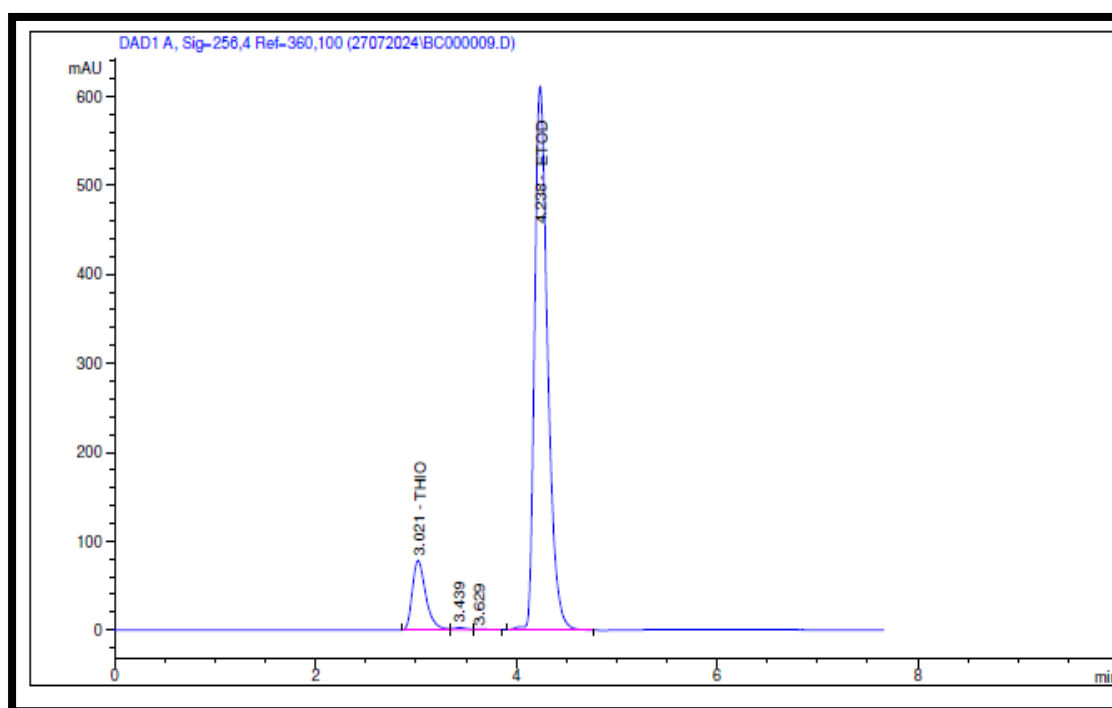


Figure 45. Chromatogram of Etodolac and Thiocolchicoside in marketed tablet formulation.

SUMMARY

Pharmaceutical development relies heavily on comprehensive drug testing and analytical methodologies to ensure the safety, efficacy, and quality of medications. These processes, encompassing techniques such as UV-Visible spectroscopy, HPLC, UHPLC, and LC-MS/MS, play pivotal roles across various stages of drug development, from quality control to regulatory compliance. UV-Visible spectroscopy, while simple and cost-effective, is ideal for compounds with chromophores, offering qualitative and quantitative analysis. In contrast, HPLC and UHPLC excels in impurity profiling, stability testing, and pharmacokinetics due to its precision and adaptability across a range of analytes. Advanced tools like LC-MS/MS further enhance sensitivity, particularly in bioavailability studies.

The integration of QbD principles elevates these methodologies, emphasizing a proactive and systematic approach to drug development. By identifying critical quality attributes and understanding the variability in manufacturing processes, QbD ensures that quality is built into products from the outset, enhancing their reliability and performance. The application of QbD to analytical methods optimizes parameters and enhances robustness, aligning with global regulatory requirements. Additionally, insights into drug mechanisms, as demonstrated in NSAID action, highlight the importance of tailored therapeutic strategies to minimize adverse effects while maximizing efficacy. Together, these approaches underscore the intersection of innovative testing methods, quality frameworks, and regulatory rigor in advancing pharmaceutical science and public health.

Summary of the UV-Spectrometric Method for Loxoprofen Sodium

The UV-spectrometric method developed for the estimation of Loxoprofen sodium demonstrates robust performance, meeting the required validation criteria. The selection of distilled water as a solvent emphasizes environmental compliance and method simplicity, with the λ_{max} identified at 223 nm ensuring precise quantification. The method's specificity and selectivity were validated by the absence of solvent interference. Linearity was excellent within the range of 5–30 $\mu\text{g/mL}$, as evidenced by an r^2 value of 0.999, indicating strong correlation and reliability.

The LOD) and LOQ values of 1.10 $\mu\text{g/mL}$ and 3.71 $\mu\text{g/mL}$, respectively, demonstrate the method's high sensitivity. Precision and ruggedness assessments resulted in %RSD values consistently below 2%, confirming the method's reproducibility across various conditions. Accuracy, validated through recovery studies at multiple levels, exhibited recovery rates near 100%, demonstrating the method's reliability for quantitative analysis. Forced degradation studies revealed significant degradation under basic and oxidative conditions but stability in acidic environments, confirming the method's capability to monitor drug stability effectively.

Summary of the RP-HPLC Method for Loxoprofen Sodium

The development and validation of a robust RP-HPLC method for the estimation of Loxoprofen sodium was successfully achieved using optimized chromatographic conditions. The analysis was performed on an Agilent 1100 HPLC system equipped with a photodiode array (PDA) detector and controlled using ChemStation software. Chromatographic separation was achieved on an Agilent C18 column (250 mm $\times$ 4.6 mm, 5 μm particle size) utilizing a mobile phase comprising methanol and 0.05% orthophosphoric acid (OPA) in a 75:25 (v/v) ratio, delivered at a

flow rate of 1 mL/min. The detection wavelength was set at 225 nm, with an injection volume of 20 μ L under ambient conditions. A standard stock solution of 1000 μ g/mL was prepared in methanol and diluted to the required working concentrations for analysis. Samples were filtered through a 0.22 μ m syringe filter to ensure system integrity and reproducibility.

Method development involved evaluating various mobile and stationary phase combinations to achieve optimal separation. Methanol was selected over acetonitrile due to its cost-effectiveness and efficiency. The final optimized conditions yielded a retention time of 4.06 minutes with excellent peak resolution. The validation of the developed method was conducted in accordance with regulatory guidelines, covering essential parameters including linearity, LOD, LOQ, system suitability, precision, accuracy, robustness, and forced degradation studies. The method demonstrated linearity in the range of 5–25 μ g/mL, with a correlation coefficient (R^2) of 0.9998, confirming excellent linearity and reliability. The LOD and LOQ were calculated as 0.91 μ g/mL and 2.78 μ g/mL, respectively, using standard statistical approaches.

System suitability was assessed by evaluating parameters such as peak area, retention time, theoretical plates, and tailing factor, all of which exhibited %RSD values below 2%, indicating high system performance. Precision studies revealed %RSD values of less than 2% for intra- and inter-day analyses, confirming the reproducibility of the method. Accuracy was established through recovery studies using the standard addition method, with recoveries ranging between 98% and 102%. Robustness was evaluated by introducing minor variations in flow rate, mobile phase composition, and detection wavelength, with all %RSD values remaining below 2%, confirming method reliability under varied conditions.

Forced degradation studies were conducted under acidic, basic, oxidative, neutral, and photolytic conditions to evaluate the stability of Loxoprofen sodium and to elucidate potential degradation pathways. Study revealed that significant degradation under acid, basic, and oxidative conditions but stability in thermal and photolytic environments, confirming the method's capability to monitor drug stability effectively. Additionally, the method was successfully applied to quantify Loxoprofen sodium in marketed tablets, demonstrating its suitability for routine quality control analysis. Collectively, the developed and validated RP-HPLC method proved to be reliable, precise, and robust, making it a valuable tool for the estimation of Loxoprofen sodium in pharmaceutical formulations.

Summary of the RP-HPLC Method for Baclofen

The comprehensive optimization of chromatographic conditions for Baclofen analysis using RP-HPLC has established a reliable and robust method for routine quality control. The systematic evaluation of parameters such as mobile phase composition, flow rate, pH, and detection wavelength enabled the identification of an optimal mobile phase ratio of 50:50 (v/v) methanol and 0.01% orthophosphoric acid. This ratio resulted in well-resolved chromatographic peaks with a retention time of 4.35 minutes, ensuring precision and reproducibility.

The validation of the method confirmed its accuracy, linearity, and robustness. Linearity was demonstrated over a concentration range of 5–25 µg/mL, with a correlation coefficient (R^2) of 0.9991, underscoring the method's reliability. Recovery studies yielded mean recoveries within the range of 100.16%–101.04%, further supporting the method's accuracy. Precision and system suitability tests produced %RSD values consistently below 2.0%, highlighting the method's reproducibility and consistency. The method's robustness was validated under varied conditions,

maintaining precision with minor variations in mobile phase ratio, flow rate, and wavelength. Forced degradation studies demonstrated its stability-indicating capability, effectively separating Baclofen from its degradation products under stress conditions. Study revealed that significant degradation under acid conditions but stability in other studied environments, confirming the method's capability to monitor drug stability effectively. The method's applicability for quantifying Baclofen in marketed formulations was confirmed with a mean recovery of 101.23%, reaffirming its utility for quality control and regulatory compliance.

Summary of the RP-UHPLC Method for Etodolac and Thiocolchicoside

The development and validation of an RP-UHPLC method for the simultaneous estimation of Etodolac and Thiocolchicoside were achieved using a systematic QbD approach. The comprehensive application of DoE ensured robust optimization of chromatographic conditions, yielding a reliable and reproducible method. Critical parameters, such as mobile phase composition, flow rate, and detection wavelength, were systematically evaluated, leading to precise and efficient separation of both analytes.

Method development began with an extensive trial of mobile phases, initially testing methanol and water combinations, which failed to yield satisfactory chromatograms. The incorporation of 0.1% orthophosphoric acid significantly improved peak formation. The QbD approach allowed a structured evaluation of factor interactions using a Box-Behnken Design (BBD). By optimizing these parameters, the method achieved well-resolved peaks, as indicated by the excellent system suitability results with %RSD values below 2.0%. The optimized conditions comprised a mobile phase of 94.30% organic phase, a flow rate of 0.96 mL/min, and a detection wavelength of 256 nm. These parameters provided robust chromatographic

performance, evidenced by precise retention times, high theoretical plate counts, and acceptable tailing factors for both Etodolac and Thiocolchicoside. Response surface plots highlighted the intricate relationships between variables, offering insights into their effects on retention time, peak area, column efficiency, and peak symmetry.

Analytical method validation demonstrated the method's reliability, precision, and sensitivity. The calibration curves for both drugs exhibited exceptional linearity ($R^2 > 0.999$) across their respective concentration ranges, with low LOD and LOQ, demonstrating high sensitivity of the method. The method exhibited strong intra- and inter-day precision, with %RSD values well below 2.0%. Robustness studies confirmed the method's ability to withstand minor variations in operating conditions, further validating its utility for routine analysis. Overall, the QbD-based method development provided a systematic and scientifically sound framework for achieving a highly reliable analytical method. The incorporation of advanced statistical tools ensured comprehensive optimization and validation, making this method suitable for simultaneous quantification of Etodolac and Thiocolchicoside in pharmaceutical formulations.

CONCLUSION

The developed and validated analytical methods for the estimation of Loxoprofen sodium, Baclofen, Etodolac, and Thiocolchicoside in pharmaceutical formulations demonstrate excellent performance in terms of precision, reliability, and robustness. The UV-spectrometric method for Loxoprofen sodium, employing distilled water as the solvent, demonstrated remarkable linearity, sensitivity, and accuracy. Its successful application in forced degradation studies underscores its utility in evaluating drug stability under various stress conditions. The RP-HPLC methods for Loxoprofen sodium, Baclofen, and RP-UHPLC method for Etodolac, and Thiocolchicoside were systematically optimized, ensuring excellent peak resolution, retention times, and overall reliability. The Loxoprofen sodium RP-HPLC method exhibited commendable linearity, precision, and accuracy, confirming its suitability for routine quality control in pharmaceutical formulations. Similarly, the Baclofen RP-HPLC method validated with high precision and recovery rates affirms its compliance with regulatory standards. The RP-UHPLC method for Etodolac and Thiocolchicoside, developed using a QbD approach and optimized via DoE, demonstrated enhanced robustness and reproducibility. This method exhibited excellent linearity, sensitivity, and successful application in forced degradation studies, further validating its efficacy for simultaneous quantification of both drugs in complex pharmaceutical matrices. These analytical methods, encompassing both UV-spectrometry, RP-HPLC, and RP-UHPLC possess key attributes of accuracy, sensitivity, and reproducibility. Their robustness across varied conditions further ensures their practicality and suitability for quality control and stability testing in pharmaceutical manufacturing. These methods, thus, serve as reliable tools for routine pharmaceutical analysis, ensuring the integrity and quality of drug products..

BIBLIOGRAPHY

1. Hanson GR, Venturelli PJ, Platteborze P. Drugs and society. Jones & Bartlett Learning; 2024.
2. Langer R. Drugs on target. Science. 2001 Jul 6;293(5527):58-9.
3. Allen L, Ansel HC. Ansel's pharmaceutical dosage forms and drug delivery systems. Lippincott Williams & Wilkins; 2013 Dec 23.
4. ul Hasan IM, Ashraf R, Tahir MS. Advanced instrumentation approaches for quantification of pharmaceuticals in liquid and solid samples. In: Pharmaceuticals in aquatic environments. CRC Press; 2024. p. 85-100.
5. Kabir M. The role of quality assurance in accelerating pharmaceutical research and development: strategies for ensuring regulatory compliance and product integrity. J Angiother. 2024 Dec 8;8(12):1-9. <https://doi.org/10.25163/angiotherapy.81210102>
6. Kumar A, Pandey M, Pal AK. Balancing innovation and safety: the regulatory journey in drug development. Int J Pharm HealthcInnov. 2023 Dec 2.
7. Tapkir N, Soni F, Sahu AK, Jadav T, Tekade RK, Sengupta P. A comprehensive review on assessment and key control strategies for impurities in drug development with a special emphasis on post-marketing surveillance. J Pharm Innov. 2021:1-20. <https://doi.org/10.1007/s12247-021-09607-9>
8. Reddy AV, Jaafar J, Umar K, Majid ZA, Aris AB, Talib J, Madhavi G. Identification, control strategies, and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: a comprehensive review. J Sep Sci. 2015 Mar;38(5):764-79. <https://doi.org/10.1002/jssc.201401143>

9. Ahuja S, Scypinski S. Modern pharmaceutical analysis: an overview. *Handb Mod Pharm Anal*. 2001 Jan 1;3:1-4.
10. Riley CM, Nguyen KL, editors. *Specification of drug substances and products: development and validation of analytical methods*. Elsevier; 2024 Sep 22.
11. Ruiz-Garcia A, Bermejo M, Moss A, Casabo VG. Pharmacokinetics in drug discovery. *J Pharm Sci*. 2008 Feb 1;97(2):654-90. <https://doi.org/10.1002/jps.21009>
12. Shushan B. A review of clinical diagnostic applications of liquid chromatography–tandem mass spectrometry. *Mass Spectrom Rev*. 2010 Nov;29(6):930-44. <https://doi.org/10.1002/mas.20295>
13. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. *J Pharm Biomed Anal*. 2002 Jun 15;28(6):1011-40. [https://doi.org/10.1016/S0731-7085\(02\)00047-X](https://doi.org/10.1016/S0731-7085(02)00047-X)
14. Sengupta P, Chatterjee B, Tekade RK. Current regulatory requirements and practical approaches for stability analysis of pharmaceutical products: A comprehensive review. *Int J Pharm*. 2018 May 30;543(1-2):328-44. <https://doi.org/10.1016/j.ijpharm.2018.04.007>
15. Van Norman GA. Drugs and devices: comparison of European and US approval processes. *JACC Basic Transl Sci*. 2016 Aug;1(5):399-412.
16. Zhou M. *Regulated bioanalytical laboratories: technical and regulatory aspects from global perspectives*. John Wiley & Sons; 2011 Mar 31.
17. Kumar AP, Kumar D. Determination of pharmaceuticals by UV-visible spectrophotometry. *Curr Pharm Anal*. 2021 Oct 1;17(9):1156-70. <https://doi.org/10.2174/1573412917999200909144354>

18. Mandru A, Mane J, Mandapati R. A review on UV-visible spectroscopy. J Pharm Insights Res. 2023 Dec 1;1(2):091-6. <https://jopir.in/index.php/journals/article/view/42>
19. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: a review. Arab J Chem. 2017 Feb 1;10:S1409-21. <https://doi.org/10.1016/j.arabjc.2013.04.016>
20. Shinde S, Rajurkar V. Advances in high-performance liquid chromatography (HPLC) method development and validation: a comprehensive review. J Drug Deliv Biother. 2024 Mar 15;2(03):16-26.
21. Siddique I. High-performance liquid chromatography: comprehensive techniques and cutting-edge innovations. Eur J Adv Eng Technol. 2023 Sep 30;10(9):66-70.
22. D'Atri V, Fekete S, Clarke A, Veuthey JL, Guillarme D. Recent advances in chromatography for pharmaceutical analysis. Anal Chem. 2018 Nov 8;91(1):210-39. <https://doi.org/10.1021/acs.analchem.8b05026>
23. Kanu AB. Recent developments in sample preparation techniques combined with high-performance liquid chromatography: A critical review. J Chromatogr A. 2021 Sep 27;1654:462444. <https://doi.org/10.1016/j.chroma.2021.462444>
24. Nováková L, Svoboda P, Pavlík J. Ultra-high performance liquid chromatography. In: Liquid chromatography. Elsevier; 2017 Jan 1. p. 719-69. <https://doi.org/10.1016/B978-0-12-805393-5.00029-4>
25. Tudela R, Ribas-Agusti A, Buxaderas S, Riu-Aumatell M, Castellari M, Lopez-Tamames E. Ultrahigh-performance liquid chromatography (UHPLC)–tandem mass spectrometry (MS/MS) quantification of nine target indoles in sparkling

- wines. J Agric Food Chem. 2016 Jun 15;64(23):4772-6.
<https://doi.org/10.1021/acs.jafc.6b01254>
26. Schmitt K, Woiwode U, Kohout M, Zhang T, Lindner W, Lämmerhofer M. Comparison of small size fully porous particles and superficially porous particles of chiral anion-exchange type stationary phases in ultra-high performance liquid chromatography: effect of particle and pore size on chromatographic efficiency and kinetic performance. J Chromatogr A. 2018;1569:149-59. <https://doi.org/10.1016/j.chroma.2018.07.056>
27. Day RO, Graham GG. Non-steroidal anti-inflammatory drugs (NSAIDs). BMJ. 2013 Jun 27;346:f3195. <https://doi.org/10.1136/bmj.f3195>
28. Zidar N, Odar K, Glavac D, Jerse M, Zupanc T, Stajer D. Cyclooxygenase in normal human tissues—is COX-1 really a constitutive isoform, and COX-2 an inducible isoform? J Cell Mol Med. 2009 Sep;13(9b):3753-63. <https://doi.org/10.1111/j.1582-4934.2008.00430.x>
29. Khan AA, Iadarola M, Yang HY, Dionne RA. Expression of COX-1 and COX-2 in a clinical model of acute inflammation. J Pain. 2007 Apr 1;8(4):349-54. <https://doi.org/10.1016/j.jpain.2006.10.004>
30. Kiefer W, Dannhardt G. COX-2 inhibition and pain management: a review summary. Expert Rev Clin Immunol. 2005 Sep 1;1(3):431-42. <https://doi.org/10.1586/1744666X.1.3.431>
31. Eren OT, Armağan R, Talmaç MA. Nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Musculoskeletal Research and Basic Science. 2016;683-93. https://doi.org/10.1007/978-3-319-20777-3_41
32. Grangeia HB, Silva C, Simões SP, Reis MS. Quality by design in pharmaceutical manufacturing: A systematic review of current status,

- challenges and future perspectives. *Eur J Pharm Biopharm.* 2020 Feb 1;147:19-37. <https://doi.org/10.1016/j.ejpb.2019.12.007>
33. Jain S. Quality by design (QBD): A comprehensive understanding of implementation and challenges in pharmaceuticals development. *Int J Pharm Pharm Sci.* 2014;6:29-35.
34. Benetti C, Benetti AA. Quality by Design in Formulation Development. In: *Introduction to Quality by Design (QbD) From Theory to Practice.* Singapore: Springer Nature Singapore; 2024 Apr 10. p. 139-59. https://doi.org/10.1007/978-981-99-8034-5_6
35. Lee SH, Kim JK, Jee JP, Jang DJ, Park YJ, Kim JE. Quality by Design (QbD) application for the pharmaceutical development process. *J Pharm Investig.* 2022 Nov;52(6):649-82. <https://doi.org/10.1007/s40005-022-00575-x>
36. Vogt FG, Kord AS. Development of quality-by-design analytical methods. *J Pharm Sci.* 2011 Mar 1;100(3):797-812. <https://doi.org/10.1002/jps.22325>
37. Orlandini S, Pinzauti S, Furlanetto S. Application of quality by design to the development of analytical separation methods. *Anal Bioanal Chem.* 2013 Jan;405:443-50. <https://doi.org/10.1007/s00216-012-6302-2>
38. Alqarni M, Namazi NI, Alshehri S, Naguib IA, Alsubaiyel AM, Venkatesan K, Elmokadem EM, Pishnamazi M, Abourehab MA. Solubility optimization of Loxoprofen as a nonsteroidal anti-inflammatory drug: Statistical modeling and optimization. *Molecules.* 2022 Jul 7;27(14):4357. <https://doi.org/10.3390/molecules27144357>
39. Inaba H, Yoshigai E, Okuyama T, Murakoshi M, Sugiyama K, Nishino H, Nishizawa M. Antipyretic analgesic drugs have different mechanisms for regulation of the expression of inducible nitric oxide synthase in hepatocytes

- and macrophages. Nitric Oxide. 2015 Jan 30;44:61-70.
<https://doi.org/10.1016/j.niox.2014.12.001>
40. de Sousa N, Santos D, Monteiro S, Silva N, Barreiro-Iglesias A, Salgado AJ. Role of Baclofen in modulating spasticity and neuroprotection in spinal cord injury. J Neurotrauma. 2022 Feb 1;39(3-4):249-58.
<https://doi.org/10.1089/neu.2020.7591>
41. Meythaler JM, Kowalski S. Pharmacologic management of spasticity: oral medications. Spasticity Diagnosis and Management. 2011:199-227.
42. Gaddala P, Choudhary S, Sethi S, Sainaga Jyothi VG, Katta C, et al., Etodolac utility in osteoarthritis: drug delivery challenges, topical nanotherapeutic strategies and potential synergies. Ther Deliv. 2024 Dec 1;15(12):977-95.
<https://doi.org/10.1080/20415990.2024.2405456>
43. Ribeiro H, Rodrigues I, Napoleão L, Lira L, Marques D, et al., Non-steroidal anti-inflammatory drugs (NSAIDs), pain and aging: Adjusting prescription to patient features. Biomed Pharmacother. 2022 Jun 1;150:112958.:
<https://doi.org/10.1016/j.biopha.2022.112958>
44. Umalkar AR, Bavaskar SR, Yewale PN. Thiocolchicoside as muscle relaxant: a review. History. 2011;8(9):11-2.
45. Medhi S, Narvekar S, Srivastav A. Repurposing of a muscle relaxant drug Thiocolchicoside as an anticancer agent. MGM J Med Sci. 2021 Jan 1;8(1):33-43.
46. Dongala T, Katakam LN, Palakurthi AK, Katari NK. RP-HPLC stability indicating method development and validation of pseudoephedrine sulfate and related organic impurities in tablet dosage forms, robustness by QbD approach.

- Anal Chem Lett. 2019 Sep 3;9(5):697-710.
<https://doi.org/10.1080/22297928.2019.1696701>
47. Hemlata SU, Saraf SK. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of NSAIDS-Antiulcer agent combination. *Int J Pharm Tech Res.* 2016;9(8):288-300.
48. Sharma MK, Murugesan M. Forced degradation study: an essential approach to develop stability indicating method. *J Chromatogr Sep Tech.* 2017;8(1):349.
49. Runje M, Babić S, Meštrović E, Nekola I, Dujmić-Vučinić Ž, Vojčić N. Forced degradation of nepafenac: Development and validation of stability indicating UHPLC method. *J Pharm Biomed Anal.* 2016 May 10;123:42-52.
<https://doi.org/10.1016/j.jpba.2016.02.003>
50. Lipiec-Abramska E, Jedynak Ł, Formela A, Roszczyński J, Cybulski M, Puchalska M, Zagrodzka J. Development and validation of high-performance liquid chromatography with a spectrophotometric detection method for the chemical purity and assay of Nepafenac. *J Pharm Biomed Anal.* 2014 Mar 25;91:1-6. <https://doi.org/10.1016/j.jpba.2013.12.009>
51. Jadhav PS, Jamkar PM, Avachat AM. Stability indicating method development and validation for simultaneous estimation of atorvastatin calcium and celecoxib in bulk and niosomal formulation by RP-HPLC. *Braz J Pharm Sci.* 2015 Jul;51:653-61. <https://doi.org/10.1590/S1984-82502015000300017>
52. Venkataraman S, Manasa M. Forced degradation studies: Regulatory guidance, characterization of drugs, and their degradation products—a review. *Drug Invent Today.* 2018 Feb 1;10(2).
53. Farooq M, Shoaib MH, Yousuf RI, Siddiqui F, Hanif M, Naz S, Bashir L. Development and validation of liquid chromatographic method for quantitative

- determination of Loxoprofen in mobile phase and in human plasma. Pak J Pharm Sci. 2018 Nov 1;31(6):2629-33.
54. Li MH, Ding XM, Liang JY. Reverse phase high-performance liquid chromatography (RP-HPLC) system for analysis of vitamin D3 soft capsules with soybean oil matrix. Fudan Univ J Med Sci. 2019 Jan 25;46(1):23-31. <https://doi.org/10.3969/j.issn.1672-8467.2019.01.004>
55. Eissa MS, Abd El-Sattar OI. Identification and structure elucidation of forced degradation products of the novel propionic acid derivative Loxoprofen: development of stability-indicating chromatographic methods validated as per ICH guidelines. J. Chromatogr. Sci. 2017 Apr 1;55(4):417-28. <https://doi.org/10.1093/chromsci/bmw196>
56. Aslam I, Iqbal SM, Mustafa R. HPLC-UV Detection Method for Quantification of Loxoprofen in Human Plasma with Liquid-Liquid Extraction Technique: Application in Pharmacokinetic Study. Lat. Am. J. Pharm. 2013;32(4):587-92.
57. Cho HY, Park CH, Lee YB. Direct and simultaneous analysis of Loxoprofen and its diastereometric alcohol metabolites in human serum by on-line column switching liquid chromatography and its application to a pharmacokinetic study. J Chromatogr B. 2006 May 1;835(1-2):27-34. <https://doi.org/10.1016/j.jchromb.2006.02.058>
58. Nanthakumar R, Prabakaran V, Chitra K, Reddy CU. Estimation of Loxoprofen sodium dihydrate in tablets by reverse phase high-performance liquid chromatography. Arab J Chem. 2016 Sep 1;9:S632-6. <https://doi.org/10.1016/j.arabjc.2011.07.010>

59. Patharevirendra S. Analytical method development and validation for Baclofen and its stages by high-performance liquid chromatography. *Int J Sci Res.* 2022;5(11):1852-5.
60. Falih MS, Abbas RF, Hussain AM, Hammed WM, Abdul Abass DA, Maryoosh AA, Abed MA. Development of spectrophotometric method for the estimation of Baclofen drug. *Int Res J Pharm.* 2018;9(11):37-41.
61. Gabhane KB, Jaiswal AM, Tapar KK. Simple and validated ultraviolet spectrophotometric method for the estimation of Baclofen in bulk form. *Res J Pharm Biol Chem Sci.* 2014;5(6):104-9.
62. Singh K, Singh GP, Sharma SK. Development and method validation of Baclofen by RP-HPLC in bulk drug and pharmaceutical formulation. *RRJP.* 2013;7(3):11-6.
63. Sowjanya G, Sankar DG, Rao JS. RP-HPLC method development and validation for the determination of Baclofen in injections. *World J Pharm Res.* 2017 Aug 8;6(12):821-35.
64. Venkata Narasimha Rao G, Muralee Krishna B, Ranjith Kumar Reddy B, Ravi Kumar Bellam C. Ultra performance liquid chromatographic separation and quantification of Baclofen and its potential impurities in an injection formulation. *IOSR J Pharm Biol Sci.* 2018;4(11):29-38.
65. Devi CP, Ameena A, Hemalatha B, Lakshmi Prasanna M, Sree L. Determination of evaluation parameters of Etodolac compared with standard by using UV-spectrophotometer. *World J Pharm Sci.* 2020;9(4):1073-87.
66. Biswal S, Mondal S, Mondal P. UV spectrophotometric and stability-indicating RP-HPLC assay methods for the estimation of Etodolac in bulk and tablet

- dosage form. Int J Pharm Investig. 2019;9(2):53-8.
<https://doi.org/10.5330/ijpi.2019.2.11>
67. Dutt KR, Yashoda P, Vasanthi R, Sundar PS, Raja MA, Rao KN, et al. Method development and validation of Etodolac by visible spectroscopy. Indo Am J Pharm. 2017;3(5):245-53.
68. Goel H, Singla R, Chawla R, Sahoo U, Tiwary AK, Ranjan Sinha V. Facile validated HPLC method using photodiode array detector for the combined analysis of Etodolac and 5-FU in bulk and tablet dosage form. Egypt J Chem. 2021 Mar 1;64(3):1601-14. <https://doi.org/10.21608/ejchem.2020.45336.2922>
69. Ruhina Rahman SN, Goswami A, Jala A, Venuganti A, Deka A, Borkar RM, et al. Studies on cationic ocular emulsions containing bipartitioned oil droplets to co-deliver cyclosporin A and Etodolac. Nanomedicine. 2024 Apr 30;1-6. <https://doi.org/10.2217/nnm-2023-0364>
70. Abdelhameed AS, Afifi SA. A validated HPLC-DAD method for simultaneous determination of Etodolac and pantoprazole in rat plasma. J Chem. 2014;2014(1):719801. <https://doi.org/10.1155/2014/719801>
71. Kumar S, Joshi A, Thakur RS, Pathak AK, Shah K. Simultaneous estimation of etoricoxib and Thiocolchicoside by RP-HPLC method in combined dosage forms. Acta Pol Pharm. 2011 Nov 1;68(6):839-43.
72. Shetgar SS, Dharmasoth R, Rao BM, Keloth B. RP-UPLC method development and validation for simultaneous estimation of etoricoxib and Thiocolchicoside in tablets. J Appl Pharm Sci. 2022 Feb 5;12(2):144-51. <https://doi.org/10.7324/JAPS.2021.120214>
73. Dhiware AD, Gandhi SV, Deshpande PB, Bhavnani VS. A simple and sensitive RP-HPLC method for simultaneous estimation of Etodolac and

- Thiocolchicoside in combined tablet dosage form. International journal of pharmacy and pharmaceutical sciences. 2012;4(4):214-6.
74. Silvio A, Giorgio G. Forced degradation study of Thiocolchicoside: characterization of its degradation products. J Pharm Biomed Anal. 2012 Mar 5;61:215-23. <https://doi.org/10.1016/j.jpba.2011.12.008>
75. Jadhav SD, Butle SR, Patil SD, Jagtap PK. Validated stability-indicating RP-HPLC method for simultaneous determination and in vitro dissolution studies of Thiocolchicoside and diclofenac potassium from tablet dosage form. Arab J Chem. 2015 Jan 1;8(1):118-28. <https://doi.org/10.1016/j.arabjc.2011.01.018>
76. Sarkar M, Khandavilli S, Panchagnula R. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for the quantitative estimation of antiretroviral drugs in pharmaceutical dosage forms. J Chromatogr B. 2006 Jan 18;830(2):349-54. <https://doi.org/10.1016/j.jchromb.2005.11.014>
77. Behera S, Ghanty S, Ahmad F, Santra S, Banerjee S. UV-visible spectrophotometric method development and validation of assay of paracetamol tablet formulation. J Anal Bioanal Tech. 2012 Oct 31;3(6):151-7.
78. Wang Y, Harrison M, Clark BJ. Optimizing reversed-phase liquid chromatographic separation of an acidic mixture on a monolithic stationary phase with the aid of response surface methodology and experimental design. J Chromatogr A. 2006 Feb 10; 1105 (1-2):199-207. <https://doi.org/10.1016/j.chroma.2005.11.101>
79. Urmi KF, Nawaz M, Islam SM. Analytical quality by design approach to RP-HPLC method development and validation for simultaneous estimation of

- esomeprazole and naproxen in modified-release dosage form. *FJPS*. 2022;8(1):1-6. <https://doi.org/10.1186/s43094-021-00396-z>
80. Peraman R, Chiranjeevi P, Reddy YP, Kumar KV, Vasantharaju SG, Nagappan K. Beta-Alanine and Tris-(hydroxyl methyl) Aminomethane as Peak Modifiers in the Development of RP–HPLC Methods Using Aceclofenac and Haloperidol Hydrochloride as Exemplar Drugs. *J. Chromatogr. Sci.* 2021;23. <https://doi.org/10.1093/chromsci/bmab010>
81. Pereira FJ, Rodríguez-Cordero A, López R, Robles LC, Aller AJ. Development and Validation of an RP-HPLC-PDA Method for Determination of Paracetamol, Caffeine and Tramadol Hydrochloride in Pharmaceutical Formulations. *Pharmaceuticals*. 2021;14(5):466. <https://doi.org/10.3390/ph14050466>
82. Venkatesan P, Janardhanan VS, Muralidharan C, Valliappan K. Improved HPLC Method with the Aid of Chemometric Strategy: Determination of Loxoprofen in Pharmaceutical Formulation. *Acta Chimica Slovenica*. 2012 Jun 1;59(2).
83. dos Santos J, Rosa P, Adams AI. Validation of a simple reversed phase-HPLC method for the determination of Baclofen in tablets. *Drug Anal Res.* 2018;2(2):37-43. <https://doi.org/10.22456/2527-2616.87929>
84. International Conference on Harmonization (ICH) Harmonized Tripartite Guideline: Validation of analytical procedures: text and methodology Q2(R1), 2005.
85. Da Silva MN, da Silva JV, da Fonsêca NF, Oshiro Junior JA, Dantas Medeiros AC. An Overview of Analytical Methods for the Identification and Quantification of Baclofen. *Current Pharmaceutical Analysis*. 2023;19(5):353-70.

86. Mohamed EH, Lotfy HM, Hegazy MA, Mowaka S. Different applications of isosbestic points, normalized spectra and dual wavelength as powerful tools for resolution of multicomponent mixtures with severely overlapping spectra. *Chem Cent J*. 2017;11:1-5. <https://doi.org/10.1186/s13065-017-0270-8>
87. Lotfy HM, Hegazy MA, Rezk MR, Omran YR. Comparative study of novel versus conventional two-wavelength spectrophotometric methods for analysis of spectrally overlapping binary mixture. *Spectrochim Acta A Mol Biomol Spectrosc*. 2015;148:328-37. <https://doi.org/10.1016/j.saa.2015.04.004>
88. Patil TS, Deshpande AS. Development of an innovative quality by design (QbD) based stability-indicating HPLC method and its validation for clofazimine from its bulk and pharmaceutical dosage forms. *Chromatographia*. 2019;82(2):579-90. <https://doi.org/10.1007/s10337-018-3660-8>
89. Alruwaili NK. Analytical quality by design approach of reverse-phase high-performance liquid chromatography of atorvastatin: Method development, optimization, validation, and the stability-indicated method. *Int J Anal Chem*. 2021; 2021. <https://doi.org/10.1155/2021/8833900>
90. Al-Kadhi NS, Mohamed MA, Ahmed HA, Nassar HF. Facile synthesis and eco-friendly analytical methods for concurrent estimation of selected pharmaceutical drugs in their solutions: application to quality by design, lean six sigma, and stability studies. *BMC chemistry*. 2023 Oct 10;17(1):136. <https://doi.org/10.1186/s13065-023-01028-8>
91. Ahmad W. Box–Behenken-Supported Development and Validation of UPLC Method for the Estimation of Eugenol in *Syzygium aromaticum*, *Cinnamomum tamala*, and *Myristica fragrance*. *Separations*. 2023 Apr 11;10(4):248. <https://doi.org/10.3390/separations10040248>

92. Narikimalli A, Galla R. A stability indicating UPLC method development and validation for the simultaneous estimation of nateglinide and metformin hydrochloride in bulk and tablet dosage form. FJPS. 2023 Jun 28;9(1):55. <https://doi.org/10.1186/s43094-023-00503-2>
93. Syamala S. Development and validation of new RP-HPLC method for simultaneous estimation of drug Thiocolchicoside and Etodolac in tablet dosage form. Indian J Res Pharm Biotechnol. 2016;4(4):180.

ANNEXURES

LIST OF RESEARCH PUBLICATIONS

1. Kulkarni RS, Palled MS. A Rapid, Sensitive and Cost-Effective RP-HPLC Method for Determination of Loxoprofen Sodium. Research Journal of Pharmacy and Technology. 2024 Jun 1;17(6):2889-94.
2. Kulkarni RS, Palled MS. Stability Indicating UV Spectrophotometric Method for Loxoprofen Sodium: Method Development and Validation. International Journal of Pharmaceutical Quality Assurance. 2023;14(4):874-878.

LIST OF POSTER PRESENTATIONS

1. Presented paper titled “Method development and validation of stability indicating UV-spectrometric method for Loxoprofen sodium” in the National Conference on Translational Research in Drug Development held between 2-4 Nov 2023, Jointly organized by department of Pharmaceutical Chemistry, Pharmacology, Pharmaceutical Biotechnology, Pharmacognosy, MCOPS, MAHE, Manipal, India.
2. Presented poster entitled as “A Rapid, Sensitive and Cost-Effective RP-HPLC Method for Determination of Loxoprofen Sodium” in the National Conference on Recent Advances in Drug Design organized by Annasaheb Dange College of B. Pharmacy, Ashta on 5th to 6th Feb 2024

ANNEXURE – I

LIST OF RESEARCH PUBLICATIONS

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**RESEARCH ARTICLE****A Rapid, Sensitive and Cost-Effective RP-HPLC Method for Determination of Loxoprofen Sodium****Rupesh S. Kulkarni^{1,2}, Mahesh S. Palled^{1*}**¹Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi, Karnataka, India.²KLES's College of Pharmacy, Nipani-591237, Karnataka, India.*Corresponding Author E-mail: rupesh.k88@gmail.com, pjalled@gmail.com**ABSTRACT:**

Aim: The present study deals with development and validation of accurate, sensitive and cost-effective RP-HPLC method for Loxoprofen sodium. **Method:** PRIMESIL C18 column (id 4.6 x 250 mm length) was used as stationary phase and a mobile phase used was mixture of methanol and 0.05% OPA buffer in ratios of 75:25 v/v. The 20µl samples were injected at a flow rate of 1 ml/min. The detector used is G-13148 (DAD) detector, with a wavelength observed of 225nm at ambient column temperature. Further, the method developed was validated as per ICH guidelines. **Results:** The method's linearity was studied at concentrations between 5 and 25µg/ml, wherein the results were found to be linear with correlation coefficient (r^2) values of 0.999. The limits of detection and quantitation for developed method were observed to be 0.09175 and 0.278032 µg/ml respectively. The intraday and interday precision results were analysed and found to be within 2%. The percentage assay of the marketed product was found to be 101.85±0.090%. The percentage recovery of the drug was ranged between 97.7 1% to 101.09%. during accuracy study.

KEYWORDS: ICH guidelines, Linearity, Loxoprofen sodium, RP-HPLC, Validation.**INTRODUCTION:**

NSAIDS are the most frequently prescribed drugs worldwide for the treatment of short-term pain relief (headache, toothache, etc.) as well as chronic conditions like osteoarthritis.¹⁻² Globally, non-steroidal anti-inflammatory medications are taken daily by more than 30 million people.³ The availability of over-the-counter NSAIDS also aids in the market's expansion. Throughout the forecast period of 2023–2030, it is anticipated that the global NSAIDS market would expand by around 6%.⁴ The growing interest in selective cyclooxygenase-2 (COX-2) inhibitors is one of the key market trends as it is used in the management of chronic pain.⁵ The development of novel cyclooxygenase-2 (COX-2) inhibitors is currently receiving major funding from numerous pharmaceutical firms. The rise in demand for NSAIDS increases the responsibility of pharmaceutical manufacturing industries to produce high quality NSAIDS at a low cost.

Pharmaceutical analysis is essential to ensure the quality of bulk medications and other pharma preparations. The quality of the drug depends on the analytical methods used.⁶

In the group of COX-2 inhibitors, Loxoprofen sodium was recently emerged derivative of propionic acid.⁷⁻⁸ It is used as a NSAID to relieve pain and inflammation related to musculoskeletal, joint disorders and operative procedures.⁹⁻¹¹

Loxoprofen acts as a prodrug. It is absorbed from GIT and get converted to an active metabolite.¹² Loxoprofen inhibits prostaglandin biosynthesis by acting on cyclooxygenase.¹³⁻¹⁵

The chemical name of Loxoprofen sodium is sodium-2-[4-(2-oxocyclopentyl)-1-methyl] phenyl] propionate dehydrate (Figure 1).

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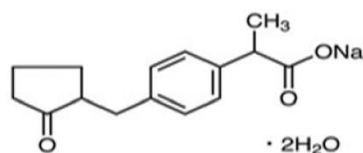


Figure 1: Chemical structure of Loxoprofen Sodium

It is recognized by the Japanese Pharmacopoeia as short acting NSAID.¹⁶⁻¹⁷ Loxoprofen is marketed in India under the brand name 'Loxomac'.¹⁸ A literature survey revealed that several HPLC methods are available for the analysis of Loxoprofen sodium. But we found some problems in those developed methods, which are as follows:

- The majority of techniques used acetonitrile (organic solvent) as mobile phase at concentration of more than 50%, which is not economical.
- More than 50% organic solvent in the mobile phase is hazardous to the HPLC column.
- In some methods, the peak shapes observed are not appropriate and have a higher retention time

To overcome these drawbacks, the study was undertaken with the objective of using a cost-effective mobile phase and developing a method with a short retention time.¹⁹ In the underlined study quick and reliable RP-HPLC method has been established by using methanol and buffer solution as mobile phase. Methanol is cost-effective, and the lack of interpretation behavior is an added advantage over existing mobile phases used to develop HPLC methods.²⁰

MATERIALS AND METHODS:

Materials:

Loxoprofen sodium, a pure drug, is obtained from Dr. Reddy Labs, Hyderabad, India, as a gift sample. Solvents (HPLC grade) required for chromatographic estimation were obtained from store house of KLE College of Pharmacy, Belagavi. Roxon in 60mg tablets (Mfg. Date: 03/06/2020, Exp. Date: 03/06/2024, Company: SAJA Pharmaceuticals) were purchased from a local pharmacy store.

Instruments:

A UV-Spectrophotometer of the make Shimadzu and model 1800 having UV probe software was used for the determination of λ max. The reverse phased HPLC

method was developed and validated using an Agilent Technologies 1100 series model, equipped with a G-13148 (DAD) detector. A computer connected to the instrument was used to capture the chromatograms using Chemstation software. Loxoprofen sodium was analysed by chromatography using a PRIMESIL C18 column (id 4.6 x 250mm length).

Preparation of Stock solution and working standard solutions:

The procedure used to prepare stock solution and working standard solutions is summarized in Figure 2.

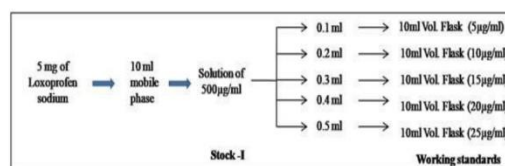


Figure 2: Flow chart of preparation of stock solution and working standard

Chromatographic Conditions:

The stationary phase used for the separation of Loxoprofen sodium is a PRIMESIL C18 column (id 4.6 x 250mm length). A mixture of methanol and 0.05% OPA buffer in the ratios of 75:25 v/v was selected as a mobile phase. The 20 µl samples were injected at a flow rate of 1 ml/min. The detector used is a G-13148 (DAD) detector, with a wavelength observed of 225nm at ambient column temperature.

RP-HPLC Method Development:

Methanol is selected as a mobile phase for the development of a novel RP-HPLC technique for analyzing Loxoprofen sodium. Four different trails were taken with different mobile phase combinations. The details of the trials are given in the Table No.1.

RP-HPLC Method Validation²¹⁻³¹

Based on the results obtained, trail 4 was found to be giving acceptable results. The method parameters in Trial 4 were validated with reference to ICH guidelines. The parameters studied during the validation of an analytical method include linearity, precision, accuracy, repeatability, specificity, selectivity, limit of detection, limit of quantification, and robustness of the method.

Table No. 1: Chromatographic Conditions of Trail run

Trial No.	Mobile phase composition	Ratio	Flow rate (ml/min)	Injection volume	Wavelength
1	Methanol and 0.05% OPA buffer	80: 20 %v/v	0.7ml/min	20µl	225nm
2	Methanol and 0.05% OPA buffer	70: 30 %v/v	0.7ml/min	20µl	225nm
3	Methanol and 0.05% OPA buffer	60: 40 %v/v	0.9ml/min	20µl	225nm
4	Methanol and 0.05% OPA buffer	75: 25 %v/v	1 ml/min	20µl	225nm

Linearity study:

Linearity is determined at 5 concentrations, viz., 5µg/ml to 25µg/ml of the analyte, under test conditions. The graph was plotted between the peak area and concentration in ppm. A further trend line was plotted, and the equation of the line and correlation coefficient (r^2) were determined.

Detection Limit and Quantitation Limit:

LOD and LOQ of method was computed using following formula.

$$\text{LOD} = \frac{3.3 \times \text{avgSD}}{\text{Slope}} \quad \text{LOQ} = \frac{10 \times \text{avgSD}}{\text{Slope}}$$

Precision:

The method's precision was assessed on the basis of intraday (Thrice a day) and interday (three times on consecutive days) precision. The reproducibility of results was observed at different time intervals on same day and also on different days. The percent RSD was calculated from the obtained results, and precision was determined.

Accuracy:

The recovery study was used to investigate the method's accuracy. In brief, to the known quantity (5mg) of standard drug, powdered samples of marketed tablets equivalent to 80% (4mg), 100% (5mg), and 120% (6 mg) were added. The mixture is diluted sufficiently, and the percent recovery of added quality was determined by a developed method.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, variations in method parameters and provides an indication of its reliability during normal usage.¹⁶ Several parameters

viz., flow rate (± 0.1 ml), composition (74:26v/v, 76:24v/v), and wavelength (± 1 nm) of the developed method were deliberately changed, and analysis was done to study the effect of these variations on results.

Assay of marketed formulation:

The assay test was carried out to access the quantity of Loxoprofen sodium in the marketed formulation. 20 tablets of Roxonin were taken and triturated. A powdered sample equivalent to 5mg of drug was weighed and transferred to a 10ml volumetric flask. The sample was diluted with the mobile phase. The obtained solution was further diluted to get a test solution of 25µg/ml. The 0.5ml of test solution was injected. The procedure was repeated twice. Based on the results obtained, the percentage assay was calculated.

RESULTS AND DISCUSSION:**RP-HPLC method development:**

In RP-HPLC, methanol and acetonitrile are the two most commonly utilised organic modifiers. A literature survey revealed that acetonitrile is the common mobile phase, which is widely used in combination with buffer solutions for the analysis of Loxoprofen sodium. Methanol is cost effective in comparison to acetonitrile. In this context, methanol is selected as a mobile phase to develop a novel RP-HPLC technique for analyzing Loxoprofen sodium. The observations of the trial runs are given in the Table No.2

The peak observed for the mobile phase in trial 4 was well defined with good resolution. Therefore, this chromatographic condition kept constant throughout the Loxoprofen sodium drug estimation. The optimized chromatographic condition are given in Table No.3 and chromatogram is shown in Figure 3.

Table No: 2: Observations of the Trial runs

Trial No.	Retention time	Area	Theoretical plates	Resolution	Height of peak	Width of the peak	Selectivity	Observations
1	5.090	1271.00781	10918	8.74	165.19722	0.1147	1.40	Peak height is more.
2	7.046	1166.55847	10101	12.96	109.04530	0.1650	2.03	Retention time is more.
3	9.450	904.70343	9629	21.99	61.72464	0.2267	3.52	Peak width & retention time is more.
4	4.060	819.61066	9551	1.31	126.79233	0.0978	1.09	Well defined peak with good resolution & low retention time.

Table No: 3 Optimized Chromatographic condition

Mobile Phase	Methanol : 0.05% OPA 75 : 25	Area	819.61066
Stationary Phase	Primesil C18 column	Theoretical plates	9551
Wavelength used	225nm	Resolution	1.31
Flow Rate	1 ml/min	Height of peak	126.79233
Temperature	Ambient	Width of peak	0.0978
Sample size	20µl	Selectivity	1.09
Retention time	4.060		

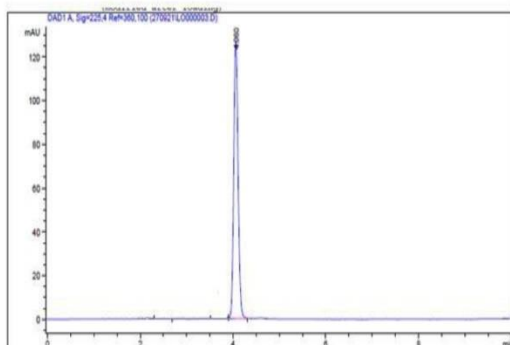


Figure 3: Chromatogram of Trial 4

RP-HPLC method validation:**Linearity:**

The method's linearity was investigated in a concentration range of 5 to 25 µg/ml, wherein the results were found to be linear with correlation coefficient (r^2) values of 0.999. The results of the linearity study are shown in Figure 4.

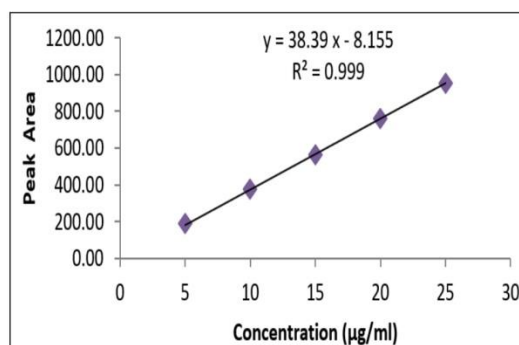


Figure 4: Standard calibration curve of Loxoprofen sodium

LOD and LOQ:

Based on the results obtained during the development of the calibration curve, limits of detection and quantitation for developed method were observed to be 0.09175 and 0.278032 µg/ml respectively.

Precision:

To determine intraday and interday precision, three distinct concentrations, viz. 10, 15 and 20 µg/ml, were analysed at three different times on same day (for intraday) and three different days (for interday). The percent RSD value for intraday study was ranged between 0.09% to 0.16%, while for interday study it was

ranged between 0.16% to 0.62%, which is below the acceptance limit of 2%. Thus, the developed method was found to be precise. The results of the precision study are as shown in Table No. 4

Table No. 4: Observation Table for Intraday and Interday Precision

Intraday Precision					
Conc (µg/ml)	Area I	Area II	Area III	Mean	% RSD
10	373.4038	372.2188	372.8834	372.84	0.16
15	559.7612	561.3866	560.6543	560.60	0.15
20	768.2559	766.879	767.6785	767.60	0.09
Interday Precision					
10	381.0605	376.3238	378.6745	378.68	0.62
15	580.777	583.9671	582.632	582.45	0.27
20	769.2219	771.7639	770.987	770.65	0.16

Accuracy:

The developed method's accuracy was assessed by the percent recovery method at three addition levels: 80%, 100%, and 120%. The present recovery for all the samples ranged between 97.71% to 101.09%. The results witnessed the accuracy of the method in predicting results. The results of the accuracy study are as shown in Table No. 5

Table No. 5: Results of Accuracy Study

Addition Level	Amount added (mg)	Area	Amount recovered	Mean Recovery (%)	% RSD
80%	4	333.838	3.91	97.91	0.21
	4	334.465	3.92		
	4	334.152	3.92		
100%	5	374.842	4.97	99.79	0.26
	5	375.851	5.00		
	5	375.346	4.98		
120%	6	416.542	6.06	101.07	0.02
	6	416.650	6.06		
	6	416.596	6.06		

Robustness:

In the robustness study, intentional minor changes in the optimised analytical parameters were made, viz., flow rate (± 0.1 ml). Mobile phase combination ($\pm 1\%$) and wavelength (± 1 nm). The findings of the robustness study revealed that the minor changes in the analytical parameters do not significantly affect the results (% RSD < 2). Thus, the method was found to be robust. The results of the robustness study are as shown in Table No. 6.

Table No. 6: Observation table for Robustness

FLOW RATE (0.9 ml/min)				FLOW RATE (1.1 ml/min)			
Conc. (µg/ml)	Area	Mean	%RSD	Area	Mean	% RSD	
10	419.9682	422.01	0.68	355.9712	352.82	1.26	
10	424.0482			349.6631			
MOBILE PHASE Methanol : 0.05% OPA buffer (74:26 v/v)				MOBILE PHASE Methanol : 0.05% OPA buffer (76:24 v/v)			
10	374.6719	372.5	0.84	371.1599	368.94	0.85	
10	370.2668			366.7215			
Change in Wavelength(224nm)				Change in Wavelength(226nm)			
10	381.9029	385.8	1.42	360.5017	362.55	0.80	
10	389.6729			364.6039			

Assay of marketed formulation:

The percent assay of the marketed formulation was determined based on the equation of the calibration curve. The % assay was found to be 101.85±0.090%, which witnesses that the tablet passes the compendial requirement. Thus, it can be ascertained that the method can be used to efficiently assess the quality of marketed formulations on a regular basis. The results of assay are given in Table No. 7.

Table No. 7 Observation table for Assay results

Concentration	Area	Amt Found	% Label Claim
25.00	968.6907	25.44532	101.78
25.00	969.9154	25.47722	101.91
25.00	969.3031	25.46127	101.85
Mean	969.30	25.46	101.85
SD	0.866	0.023	0.090
%RSD	0.089	0.089	0.089

CONCLUSION:

For the analysis of Loxoprofen sodium, a new RP-HPLC method was developed and validated. The developed method was accurate, sensitive, and precise. Methanol was used in the mobile phase, which is cost-effective and lacks any detrimental effects on the column. The developed method was validated according to ICH Q2(R1) guidelines. The outcomes of the validation study show that the method was sensitive for Loxoprofen sodium detection and quantification at low concentrations. The validation data further supports the method's linearity (over a range of 5µg/ml to 25µg/ml), and the fact that the RSD is less than 2% shows that the method is accurate and reliable. The shorter retention time of 4.06 minutes completes the analysis within a shorter duration of time with less consumption of chemicals. The outcomes of the study clearly demonstrate that method can be effectively utilized for rapid and economic estimation of Loxoprofen sodium in bulk and dosage form.

ABBREVIATIONS:

RP-HPLC: Reverse Phase High Performance Liquid Chromatography, **UV:** Ultra violet, **LOD:** Limit of detection, **LOQ:** Limit of quantification, **ICH:** International conference of harmonization, **RSD:** Relative Standard Deviation, **GIT:** Gastrointestinal Tract.

CONFLICT OF INTEREST:

The authors declare that there is no any conflict of interest.

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REFERENCES:

- Greig SL, Gamcock-Jones KP. Loxoprofen: A review in pain and inflammation. Clin Drug Investig. 2016; 36: 771-81. DOI 10.1007/s40261-016-0440-9.
- Venkatesan P, Janardhanan VS, Manavalan R, Valliappan K. Preformulation parameters characterization to design, development and formulation of loxoprofen loaded microspheres. Int. J. Pharm. Biomed. 2011; 3: 107-17.
- Singh G. Gastrointestinal complications of prescription and over-the-counter nonsteroidal anti-inflammatory drugs: a view from the ARAMIS database. Arthritis, Rheumatism, and Aging Medical Information System. Am J T. 2000; 7(2): 115-21.
- Non-steroidal Anti-Inflammatory Drugs (NSAIDs) Market Analysis – Industry Size, Share, Research Report, Insights, Covid-19 Impact, Statistics, Trends, Growth and Forecast 2023-2030. (Accessed on 10/04/2023). Available from: <https://markwideresearch.com/non-steroidal-anti-inflammatory-drugs-nsaids-market>.
- Non-steroidal Anti-Inflammatory Drugs (NSAIDs) Market Size, Share & Industry Analysis, By Disease Indication (Arthritis, Migraine, Ophthalmic Diseases, and Others), By Route of Administration (Oral, and Parenteral), By Distribution Channel (Hospital Pharmacy Retail Pharmacy, and Online Pharmacy), and Regional Forecast 2020-2027. (Accessed on 10/04/2023). Available from: <https://www.fortunebusinessinsights.com/non-steroidal-anti-inflammatory-drugs-nsaids-market-102823>.
- Shantier SW. Drug analysis. In Pharmaceutical Formulation Design-Recent Practices. IntechOpen; 2019; 1-18. DOI: <http://dx.doi.org/10.5772/intechopen.88739>.
- Riendeau D, Salem M, Styhler A, Ouellet M, Mancini JA, Li CS. Evaluation of loxoprofen and its alcohol metabolites for potency and selectivity of inhibition of cyclooxygenase-2. Bioorganic Med Chem Lett. 2004; 14(5): 1201-3. <https://doi.org/10.1016/j.bmcl.2003.12.047>.
- Cho HY, Park CH, Lee YB. Direct and simultaneous analysis of loxoprofen and its diastereometric alcohol metabolites in human serum by on-line column switching liquid chromatography and its application to a pharmacokinetic study. J. Chromatogr. B. 2006; 835(1-2): 27-34. doi:10.1016/j.jchromb.2006.02.058.
- Miki K, Ikemoto T, Hayashi K, Arai YC, Sekiguchi M, Shi K, Ushida T. Randomized open-label non-inferiority trial of acetaminophen or loxoprofen for patients with acute low back

- pain. *J Orthop Sci.* 2018; 23(3): 483-7. <https://doi.org/10.1016/j.jos.2018.02.007>.
10. Yamashita Y, Sano N, Shimohira D, Danjo A, Goto M. A parallel-group comparison study of celecoxib with loxoprofen sodium in third mandibular molar extraction patients. *Int J Oral Maxillofac Surg.* 2014; 43(12): 1509-13. <http://dx.doi.org/10.1016/j.ijom.2014.09.002>.
 11. Nourwali I, Namnakani A, Almutairi M, Alaifi A, Aljohani Y, Kassim S. Loxoprofen Sodium Versus Diclofenac Potassium for Post-Dental Extraction Pain Relief: A Randomized, Triple-Blind, Clinical Trial. *Dent J.* 2019; 8(1): 2. <http://dx.doi.org/10.3390/dj8010002>.
 12. Zhao D, Chen Z, Hu S, Lin J, Shao Z, Wang G, Xiao W, Zheng Y, Zhang Z, Shi Y, Li Z. Efficacy and safety of loxoprofen hydrogel transdermal patch versus loxoprofen tablet in Chinese patients with myalgia: a double-blind, double-dummy, parallel-group, randomized, controlled, non-inferiority trial. *Clin Drug Investig.* 2019; 39: 369-77. <https://doi.org/10.1007/s40261-019-00756-x>.
 13. Kanazawa H, Tsubayashi A, Nagata Y, Matsushima Y, Mori C, Kizu J, Higaki M. Stereospecific analysis of loxoprofen in plasma by chiral column liquid chromatography with a circular dichroism-based detector. *J Chromatogr A.* 2002; 948(1-2): 303-8. [https://doi.org/10.1016/S0021-9673\(01\)01312-7](https://doi.org/10.1016/S0021-9673(01)01312-7).
 14. Yoon TH, Lee E, Kim JM, Kim WS, Kim IH. The enrichment of loxoprofen enantiomer by 6-columns simulated moving bed chromatography. *Korean J Chem Eng.* 2008; 25: 285-90. <https://doi.org/10.1007/s11814-008-0050-1>.
 15. Li M, Liang X, Guo X, Di X, Jiang Z. Enantiomeric separation and enantioselective determination of some representative non-steroidal anti-inflammatory drug enantiomers in fish tissues by using chiral liquid chromatography coupled with tandem mass spectrometry. *Microchem. J.* 2020; 153: 104511. <https://doi.org/10.1016/j.microc.2019.104511>.
 16. Eissa MS, Abd El-Sattar OL. Identification and structure elucidation of forced degradation products of the novel propionic acid derivative loxoprofen: development of stability-indicating chromatographic methods validated as per ICH guidelines. *J Chromatogr Sci.* 2017; 55(4): 417-28. doi: 10.1093/chromsci/bmw196.
 17. Miki K, Ikemoto T, Hayashi K, Arai YC, Sekiguchi M, Shi K, Ushida T. Randomized open-label non-inferiority trial of acetaminophen or loxoprofen for patients with acute low back. *Microchem. J.* 2018; 23(3): 483-7. <https://doi.org/10.1016/j.jos.2018.02.007>.
 18. Bhuniya R, Nanda S. Asymmetric synthesis of the active form of loxoprofen and its analogue. *Tetrahedron: Asymmetry.* 2011; 22(10): 1125-32. <http://dx.doi.org/10.1016/j.tetasy.2011.06.019>.
 19. Kumar SD, Kumar DH. Importance of RP-HPLC in analytical method development: a review. *IJPSR.* 2012; 3(12): 4626.
 20. Pereira FJ, Rodríguez-Cordero A, López R, Robles LC, Aller AJ. Development and validation of an RP-HPLC-PDA method for determination of paracetamol, caffeine and tramadol hydrochloride in pharmaceutical formulations. *Pharmaceuticals.* 2021; 14(5): 466. <https://doi.org/10.3390/ph14050466>.
 21. Sayare AS, Undre PB, Ghode PD, Singh SV, Ghode SP. Development and validation of RP-HPLC method for the estimation of Clomiphene citrate in pharmaceutical dosage form. *Research Journal of Pharmacy and Technology.* 2021; 14(7): 3483-8. doi:10.52711/0974-360X.2021.00604.
 22. Singh M, Kashkhedikar SG, Soni L, Garg A, Gandhi T and Patel A. Development of RP-HPLC method for estimation of Carvedilol in tablet formulations. *Research Journal of Pharmacy and Technology.* 2008; 1(1): 18-22.
 23. Pawar HI, Kothapalli L, Thomas A, Nanda RK and Mare S. Simultaneous RP-HPLC method for estimation of Ezetimibe and Fenofibrate in synthetic mixture. *Research Journal of Pharmacy and Technology.* 2008; 1(1): 25-28.
 24. Chitlange SS, Imran M, Bagri K and Sakarkar DM. A stability-indicating reverse phase high performance liquid chromatography method for the simultaneous determination of Ramipril and Valsartan in pharmaceutical dosage form. *Research Journal of Pharmacy and Technology.* 2008; 1(3): 215-217.
 25. Kalyani Lingamaneni, Mukthinuthalapati Mathrusri Annapurna. Development and Validation of a New Stability Indicating Liquid Chromatographic Method for the Simultaneous quantification of Hydrochlorothiazide and Telmisartan. *Research Journal of Pharmacy and Technology.* 2021; 14(4): 2081-8. doi: 10.52711/0974-360X.2021.00369.
 26. Singh A, Dwivedi J, Gandhi SV. Development and validation of stability-indicating RP-HPLC method for determination of Metoprolol succinate and Olmesartan medoxomil in bulk and in formulation. *Research Journal of Pharmacy and Technology.* 2014; 7(12): 1368-73.
 27. Nagavalli D, Sankar AS, Anandakumar K, Vetrichelvan T, Balaji M. Estimation of Metaxalone in bulk and in tablet dosage form by RP-HPLC. *Research Journal of Pharmacy and Technology.* 2010; 3(2): 409-11.
 28. Manasa M, Aanandhi VM. Stability indicating method development and validation of semaglutide by RP-HPLC in pharmaceutical substance and pharmaceutical product. *Research Journal Of Pharmacy And Technology.* 2021; 14(3): 1385-9. doi:10.5958/0974-360X.2021.00247.X.
 29. Sridevi S, Vijayakumar R, Nalini CN. Method development and validation for the simultaneous estimation of Ascorbic acid, phenylephrine HCl, Paracetamol and Levocetirizine HCl using RP-HPLC. *Research Journal of Pharmacy and Technology.* 2020; 13(4): 1913-8. doi:10.5958/0974-360X.2020.00344.3.
 30. Pawar VT, Pawar SV, More HN, Kulkarni AS, Gaikwad DT. RP-HPLC method for simultaneous estimation of Cilnidipine and Chlorthalidone. *Research Journal of Pharmacy and Technology.* 2017; 10(11): 3990-6. doi:10.5958/0974-360X.2017.00724.7.
 31. International Conference on Harmonization (ICH) Harmonized Tripartite Guideline: Validation of analytical procedures: text and methodology Q2 (R1), 2005.

RESEARCH ARTICLE

Stability Indicating UV Spectrophotometric Method for Loxoprofen Sodium: Method Development and Validation

Rupesh Kulkarni, Mahesh Palled*

KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi and KLES's College of Pharmacy, Nipani, Karnataka, India.

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ABSTRACT

Loxoprofen sodium was analyzed using UV spectrophotometry, and the method was designed and validated in the present study in accordance with ICH Q2 recommendations. Loxoprofen sodium's stress degradation behavior was investigated using a newly discovered technique. Analysis was carried out at 223 nm by using distilled water as solvent. Stress degradation of drug was studied at acidic, basic and oxidative conditions. With a correlation coefficient of 0.999, linearity was found between 5 and 25 µg/mL. Intraday and interday precision studies showed RSD values of <2.0%, demonstrating the accuracy of the presented approach. Results obtained for LoD and LoQ are 0.012 and 0.037 µg/mL, respectively. For the ruggedness study, the absorbance value of six replicates was found to be 0.477 ± 0.004 with %RSD > 2.0% states that the developed method was found to be rugged. During the stress degradation study, it was observed that the drug is susceptible to basic and oxidative conditions with degradation of more than 10%.

Keywords: Loxoprofen sodium, Stress degradation, Method validation, UV-Spectrophotometric, Linearity.

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INTRODUCTION

Pharmaceutical analysis has its own importance in the maintenance of quality assurance and quality control of various pharmaceutical preparations. Loxoprofen sodium is a newly developed propionic acid derivative.¹ It is used as an NSAID drug to treat inflammation and reduce the pain in joints and muscles.² Loxoprofen is a precursor which shows its effects when absorbed in GIT by transforming to an active metabolite. Loxoprofen inhibits the synthesis of prostaglandin through its action on cyclooxygenase.³ IUPAC name of loxoprofen sodium is "sodium-2-[4-(2-oxocyclopentyl-1-methyl) phenyl] propionate dihydrate". In Japan, it is the most widely prescribed NSAID.⁴ The structure of loxoprofen sodium is given in Figure 1.

Stress testing of drug products plays very important role in understanding the stability of pharmaceutical products.⁵⁻⁷ It is studied at exaggerated conditions of temperature and humidity to understand the stability characteristics of drug products.⁸ A stability study is a very useful tool in determining storage conditions of various existing as well as newly developing pharmaceutical dosage forms.⁹

Various liquid chromatographic methods have been developed and reported for loxoprofen sodium.¹⁰ The reported

chromatographic methods are complex and require costly instrumentation set-up and skilled personnel to operate. Many laboratories in the developing country cannot afford these costly instruments. In contrast, UV-Spectrophotometry is considered as most convenient analytical technique in most quality control and quality assurance laboratories. Small-scale industries widely use spectrophotometry as the cost of the equipment is less and the results are reliable. Therefore, the purpose of this research was to create and verify an easy-to-use UV Spectrophotometric method for detecting loxoprofen sodium.

MATERIAL AND METHODS

Standard drug loxoprofen sodium is obtained from Dr. Reddy Labs, Hyderabad as a gift sample. Solvents required for UV Spectrophotometric estimation of selected drugs were obtained from store house of "KLE Society's College of Pharmacy, Belagavi". UV Spectrophotometer (Shimadzu-1800) was used.

Method Development

UV-Spectrophotometric method development began with solvent selection and $\lambda_{\max}$ determination. The solubility of ioxoprofen sodium was checked in various solvents. A literature survey revealed that loxoprofen is sodium soluble in

*Author for Correspondence: pjpalled@gmail.com

Method Development for Loxoprofen Sodium

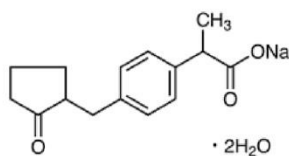


Figure 1: Chemical structure (Loxoprofen Sodium)

water as well as in methanol. In most of the studies, methanol is used as a solvent for UV spectroscopy. In our research work, we have used distilled water for estimation.

Selection of $\lambda_{\max}$

The medication was precisely weighed out at 10 mg, and then diluted with distilled water to reach the desired concentration of 25 $\mu\text{g/mL}$. To find the $\lambda_{\max}$, the solution was scanned from 200 to 400 nm.

Preparation of Standard Stock*Stock solution-I*

A total of 10 mg of ioxoprofen sodium were accurately weighed and poured into a 100 mL volumetric flask. The medication was dissolved in distilled water, and the final volume was adjusted accordingly. From stock solution pipetted out 0.5–3.0 mL solutions and transferred them into 10 mL of volumetric flasks. Volume was made by adding distilled water up to the mark to get solutions with concentrations of 5 to 30 $\mu\text{g/mL}$. The prepared solutions were scanned for absorbance at 223 nm. Based on the results obtained, the calibration curve was plotted, and the correlation coefficient (r^2) was determined. The parameters of the developed method are given in Table 1.

Method Validation

Validation of the developed method was carried out in conditions of various parameters as per the current regulatory requirements.¹¹ The parameters studied during the validation of an analytical method include selectivity, specificity, LoQ, LoD, precision and ruggedness.¹²

Specificity and selectivity

The spectrum was obtained by keeping distilled water at blank and the sample position to check if any interference of solvent during routine analysis of the drug sample.

LoD and LoQ

The following formulae were used to calculate LoD and LoQ:

$$\text{LoD} = \frac{3.3 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slope of the calibration}}$$

$$\text{LoQ} = \frac{10 \times \text{Standard Deviation of } Y \text{ intercept}}{\text{Slope of the calibration}}$$

Precision

In the intraday precision study, six replicates of drug solutions of 5, 15 and 25 $\mu\text{g/mL}$ were prepared, and their absorbance were determined at three different times within the same day at 223 nm. Based on the results obtained, %RSD was determined.

Table 1: Developed UV method parameters for loxoprofen sodium

Parameter	Details
Method	UV-spectrophotometric Method
Instrument	UV-spectrophotometer
Analyte	Loxoprofen sodium
Wavelength Run	400–200 nm
Solvent	Distilled water
$\lambda_{\max}$	223 nm
Model	Shimadzu(UV-1800)
Software	UV-Probe

Ruggedness

By repeating six replicates of a 15 $\mu\text{g/mL}$ solution by two different analysts, ruggedness was determined. Results obtained were compared, and %RSD was calculated.

Forced degradation studies

The stability of pure drug samples was accessed with a newly developed UV-spectroscopic method by exposing the drug to various stress conditions like acid, base and oxidation by using standard procedures.

RESULTS AND DISCUSSION

The UV spectrum obtained for loxoprofen sodium is as shown in Figure 2. The $\lambda_{\max}$ was found to be 223 nm which is in line with the available literature.

Method Validation*Linearity study*

Figures 3 and 4 depict various absorbance values for respective concentrations. Linearity was observed between concentration and absorbance values within the studied range viz. 5–30 $\mu\text{g/mL}$. The correlation coefficient (r^2) was found to be 0.999. Results of Linearity are given in Table 2.

Specificity and selectivity

The spectrum obtained by keeping distilled water at blank was not show any absorbance at the maximum absorbance wavelength of the drug at 223 nm. It was determined that the solvent did not affect the results, leading researchers to conclude that the established approach was both specific and selective for the measurement of the medication.

LoD and LoQ

The results obtained for LoD and LoQ are as shown in Table 3.

Precision

%RSD values for intraday and interday precision study were found to be <2.0% which highlighted that the developed method was found to be precise for regular analysis.

The obtained results are shown in Table 4 and 5.

Ruggedness

The results obtained for the ruggedness study are as presented in Table 6. The absorbance value of six replicates was found

Method Development for Loxoprofen Sodium

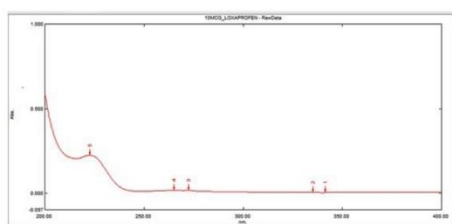
Figure 2: UV spectrum of loxoprofen sodium – λ^{max} at 223 nm

Table 2: Results of linearity and range study of loxoprofen sodium

S. No.	Concentration ($\mu\text{g/mL}$)	Absorbance
1	5	0.150
2	10	0.322
3	15	0.492
4	20	0.639
5	25	0.804
6	30	0.955
7	Slope	0.160
8	r^2	0.999

Calibration curve of Loxoprofen Sodium

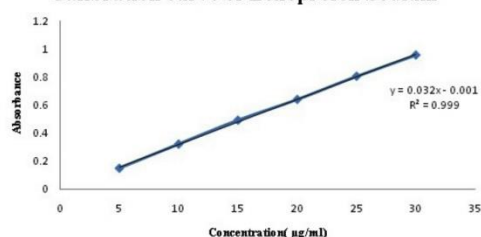


Figure 3: Standard calibration curve of loxoprofen sodium

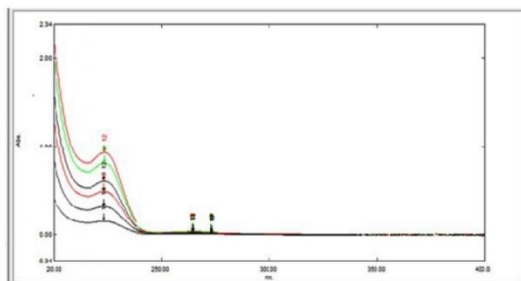
Figure 4: Overlay of UV-spectrum of loxoprofen sodium (5–30 $\mu\text{g/mL}$)

Table 3: LoD and LoQ data of loxoprofen sodium

S. No.	Parameter	Values ($\mu\text{g/mL}$)
1	LoD	0.012
2	LoQ	0.037

Table 4: Intraday-1, 2, 3 precision data of ioxoprofen sodium (15 $\mu\text{g/mL}$)

Precision			
	Intraday -1	Intraday -2	Intraday -3
Concentration ($\mu\text{g/mL}$)	Absorbance	Absorbance	Absorbance
15	0.480	0.480	0.474
15	0.472	0.472	0.475
15	0.479	0.479	0.474
15	0.473	0.473	0.479
15	0.473	0.478	0.480
15	0.475	0.478	0.479
Mean	0.475	0.476	0.476
SD	0.003	0.003	0.002
%RSD	0.712	0.697	0.584

Table 5: Interday-1, 2, 3 precision data of loxoprofen sodium (15 $\mu\text{g/mL}$)

Precision	Interday -1 precision	Interday -2 precision	Interday -3 precision
Concentration ($\mu\text{g/mL}$)	Absorbance	Absorbance	Absorbance
15	0.482	0.483	0.486
15	0.485	0.484	0.490
15	0.484	0.486	0.490
15	0.484	0.486	0.486
15	0.485	0.489	0.489
15	0.487	0.489	0.488
Mean	0.484	0.486	0.488
SD	0.001	0.002	0.001
%RSD	0.206	0.411	0.204

Table 6: Ruggedness data of loxoprofen sodium (15 $\mu\text{g/mL}$)

Ruggedness (change in analyst)		
15 $\mu\text{g/mL}$		
Replicates	Absorbance (Analyst I)	Absorbance (Analyst II)
1	0.482	0.474
2	0.471	0.482
3	0.478	0.474
4	0.473	0.480
5	0.480	0.479
6	0.482	0.478
Mean	0.477	0.477
SD	0.004	0.003
%RSD	0.979	0.680

to be 0.477 ± 0.004 with %RSD values less than 2.0%. Thus developed method was established to be rugged.

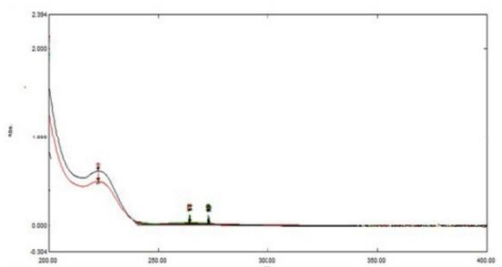
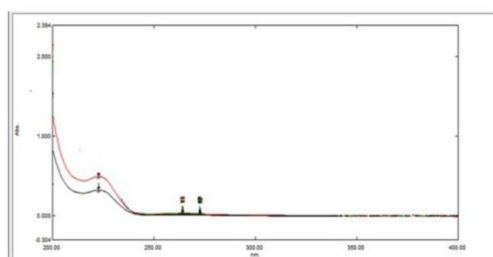
Forced degradation studies

Forced degradation study results obtained are shown in Table 7.

Method Development for Loxoprofen Sodium

Table 7: Forced degradation study data of loxoprofen sodium

Parameter	Loxoprofen sodium at 223 nm		
	Initial	Final	%degradation
Acid	0.428	0.420	1.86
Base	0.359	0.319	11.14
Oxidation	0.602	0.540	10.29

**Figure 5:** Overlay graph of loxoprofen sodium under basic condition**Figure 6:** Overlay graph of loxoprofen sodium under oxidation degradation condition

- *Acid degradation study*

There were no any considerable changes observed in the spectra of drug solution after 2 hours of heating in acidic conditions. Thus it can be considered that drug is stable at acidic pH conditions.

- *Base degradation study*

The alkaline degradation study has revealed that drug is susceptible to basic conditions and within 2 hours of heating at 80°C, >10% drug was degraded. Comparative spectra of Initial and final drug solutions are as shown in Figure 5.

- *Oxidation degradation study*

The oxidative degradation study with 30% H₂O₂ has revealed that the drug is susceptible to oxidation and within 2 in hours of heating the presence of H₂O₂ more than 10% of the drug was found to be degraded. Spectral changes are shown in Figure 6.

CONCLUSION

The simple and accurate UV-spectroscopic method for analysis of loxoprofen sodium was developed and validated. Results

obtained have highlighted the accuracy, specificity, linearity, precision and ruggedness of the method. Researchers looked at how loxoprofen sodium responded to a number of different types of stress and presented their findings. Studies on basic and oxidative degradation showed significant drug breakdown, but acidic conditions kept the medication stable. Small-scale industries might use the suggested method for monitoring the quality of bulk samples.

REFERENCES

1. Alqarni M, Namazi NI, Alshehri S, Naguib IA, Alsubaiyel AM, Venkatesan K, *et al.* Solubility Optimization of Loxoprofen as a Nonsteroidal Anti-Inflammatory Drug: Statistical Modeling and Optimization. *Molecules*. 2022; 27(14):4357.doi.org/10.3390/molecules27144357.
2. Ji C, Yu Y, Zhang M, Yu W, Dong S. Loxoprofen sodium alleviates oxidative stress and apoptosis induced by angiotensin II in human umbilical vein endothelial cells (HUVECs). *Drug Design, Development and Therapy*. 2020;14: 5087-96. doi.org/10.2147/dddt.s266175.
3. Kanazawa H, Tsubayashi A, Nagata Y, Matsushima Y, Mori C, Kizu J, *et al.* Stereospecific analysis of loxoprofen in plasma by chiral column liquid chromatography with a circular dichroism-based detector. *Journal of Chromatography A*. 2002;948(1-2):303-8. doi.org/10.1016/S0021-9673(01)01312-7.
4. Eissa MS, Abd El-Sattar OI. Identification and structure elucidation of forced degradation products of the novel propionic acid derivative loxoprofen: development of stability-indicating chromatographic methods validated as per ICH guidelines. *Journal of Chromatographic Science*. 2017;55(4):417-28.doi.org/10.1093/chromsci/bmw196.
5. Murakami T, Kawasaki T, Takemura A, Fukutsu N, Kishi N, Kusu F. Identification of degradation products in loxoprofen sodium adhesive tapes by liquid chromatography–mass spectrometry and dynamic pressurized liquid extraction–solid-phase extraction coupled to liquid chromatography–nuclear magnetic resonance spectroscopy. *Journal of Chromatography A*. 2008;1208:164-74. doi.org/10.1016/j.chroma.2008.08.076.
6. S.W. Baertschi, P.J. Jansen, in: S.W. Baertschi (Ed.), *Pharmaceutical Stress Testing: Predicting Drug Degradation*, Informa Healthcare, New York, USA, 2007, p. 13-19.
7. Uday TC, Shivabasappa PM, Sanjay SS, Maruti MS. Development and Validation of Stability Indicating UV-Spectrophotometric Method for the Simultaneous Estimation of Telmisartan and Metformin Hydrochloride in Bulk Drugs. *Indian journal of pharmaceutical education and research*. 2021;55(2):590-7.DOI: 10.5530/ijper.55.2.98.
8. Sneha K, Geetha M, Swapna B, Setty RS. Development and Validation of UV-spectrophotometric Method for Estimation of Gallic Acid in Acalypha indica Leaf Extract and its Cellulose Formulation. *Indian journal of pharmaceutical education and research*. 2022;56(3):881-7. DOI: 10.5505/ijper.56.3.141.
9. Kempwade A, Taranalli A, Jadhav K. Development and validation of UV Spectrophotometric method to study stress degradation behaviour of Rizatriptan Benzoate. *Spectroscopy and Spectral Analysis*. 2015;35(1):137-40. DOI: 10.3964/j.issn.1000-0593(2015)01-0137-04.
10. Farooq M, Shoaib MH, Yousuf RI, Siddiqui F, Hanif M, Naz S, Bashir L. Development and validation of liquid chromatographic

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- method for quantitative determination of Loxoprofen in mobile phase and in human plasma. Pak. J. Pharm. Sci. 2018;31(6):2629-33.
11. International Conference on Harmonization (ICH) Harmonized Tripartite Guideline: Validation of analytical procedures: text and methodology Q2 (R1), 2005.
12. Mujawar T, Ahmad S, Tajane P, Sable V, Gaikwad A, Tare H. Development and validation of ultraviolet spectrophotometric method for Saquinavir estimation in bulk and dosage form. Multidisciplinary Science Journal. 2023 Apr 9;5(2):2023020.

ANNEXURE – II

LIST OF POSTER PRESENTATIONS

 MANIPAL COLLEGE OF PHARMACEUTICAL SCIENCES MANIPAL (A constituent unit of MAHE, Manipal)	 125th BIRTH ANNIVERSARY Dr. TMA Pai Pharmaceutical Pioneer	Manipal College of Pharmaceutical Sciences Diamond Jubilee Commemorative National Conference on Translational Research in Drug Development 2 – 4 November 2023	Jointly Organized by Departments of Pharmaceutical Chemistry, Pharmacology, Pharmaceutical Biotechnology and Pharmacognosy MCOPS, MAHE, Manipal, India	<i>Certificate of Participation & Presentation</i>	This is to certify that <u>Mr Rupesh S Kulkarni</u> has participated and presented a paper titled <u>Method development and Validation of stability indicating UV-Spectrophotometric method for Loxoprofen Sodium</u> in the National Conference on Translational Research in Drug Development held between 2 – 4 November 2023.	Total Co-curricular Credits Earned - 02
 Dr. K Nandakumar Organising Secretary, Head, Department of Pharmacology MCOPS, MAHE, Manipal	 Dr. Krishnamurthy Bhat Co-Convenor, Vice-Principal MCOPS, MAHE, Manipal	 Dr. C Malikarjuna Rao Convener, Principal MCOPS, MAHE, Manipal	 LABEX An ISO 9001:2008 Certified Company	 ThermoFisher SCIENTIFIC	 Group Pharmaceuticals Ltd healthy smiles matter	 Schrodinger natural SOLUTIONS

