**A REVIEW IN ANALYTICAL METHODS FOR DETERMINATION OF ANTI INFLAMMATORY AGENTS**

 **Practice school**



**Submitted to**

**THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY, CHENNAI.**

***In partial fulfillment for the award of the degree of***

**BACHELOR OF PHARMACY**

**NOVEMBER 2024**

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**EVALUATION CERTIFICATE**

This is to certify that the dissertation work entitled “**A Review in analytical Method for Determination of Anti inflammatory Agents”**

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**ACKNOWLEDGEMENT**

We Want to acknowledge with thanks and bring to light those who have helped us complete our practice school, without which this work has not reached its destination.

For most, we want to offer this endeavour to our "**LORD ALMIGHTY**" for the wisdom he bestowed upon us and for allowing us to work on his project.

We sincerely thank our Managementand our Secretary **Mr. M. PRUKINTHRAJ., M.E.**, Secretar, Arunai College of Pharmacy, for his constant support and speciality in performing our research works.

We sincerely thank our Principal, **Dr. S.K. SENTHIL KUMAR, M. Pharm., Ph.D.,** Arunai College of Pharmacy, Tiruvannamalai, for granting permission and providing the facilities to conduct our research successfully

.

We avail this opportunity to express our deep sense of gratitude and respect to our beloved guide, **Mr. TAMIL SELVAN R, M.Pharm., M.A(Psy).,** AssociateProfessor, Department of Pharmaceutical Analysis, Arunai College of Pharmacy, Tiruvannamalai, for suggesting the problem, persistent encouragement, constructive advice and inspiration that enabled us to carry out this work successfully.

Our heart goes out to **our beloved Parents and family members**. Their support, prayers, encouragement, love and care have enabled us to achieve this much in life for which we owe more than words and for which nothing can ever pay off. May almighty give them long life and prosperity.

Finally, we thank all our Teachingand Non-Teachingwho directly or indirectly supported us throughout the coursework.

At this ecstatic moment, we apologize to all other **unnamed** who helped us in various ways for the completion of our project.

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 **ABBREVIATIONS USED**

|  |  |
| --- | --- |
| IUPAC | International Union Of Pure And Applied Chemistry |
| HPLC | High Performance Liquid Chromatography |
| NP-HPLC | Normal Phase - High Performance Liquid Chromatography |
| RP-HLC | Reverse Phase - High Performance Liquid Chromatography |
| UV | Ultra-Violet |
| GC | Gas Chromatography |
| API | Active Pharmaceutical Ingredient |
| SD | Standard Deviation |
| LOD | Limit Of Detection |
| LOQ | Limit Of Quantification |
| RSD | Relative Standard Deviation |

 **INTRODUCTION**

 **1. INTRODUCTION**

**1.1** **INFLAMMATION**

 Inflammation is a complex biological response of the body's immune system to harmful stimuli, such as injury, infection, or damage. Inflammation is a normal response by the body to injury or infection that involves the release of chemicals to trigger an immune response. It's a protective response that helps to isolate and destroy the harmful agent, prepare the tissue for healing, and control infection. Some signs of inflammation include: Redness, Swelling, Pain, Warmth, and Loss of function. Inflammation can be caused by a number of factors, including: toxic chemicals, environmental agents, trauma, overuse, and infection. While inflammation is usually a normal and important process, it can sometimes be harmful. For example, inflammation that occurs in healthy tissues or lasts too long can be harmful.

 It is a protective mechanism that aims to:

 1. Eliminate the harmful stimulus

 2. Repair damaged tissue

 3. Restore normal function

Types of Inflammation

1. Acute Inflammation: Short-term, immediate response to injury or infection

2. Chronic Inflammation: Long-term, persistent inflammation lasting weeks, months, or years

3. Autoinflammatory Disorders: Excessive or inappropriate inflammation, e.g., rheumatoid

arthritis

**1.1.1 Etiology of Inflammation**

 1. Injury or Infection

 2. Autoimmune Disorders (e.g., Rheumatoid Arthritis)

 3. Genetic Predisposition

 4. Environmental Factors (e.g., Pollution)

 5. Metabolic Disorders (e.g., Diabetes)

**1.1.2 Pathophysiology of Inflammation**

 1. Release of Pro-Inflammatory Mediators (e.g., TNF-α, IL-1β)

 2. Activation of Immune Cells (e.g., Neutrophils, Macrophages)

 3. Increased Blood Flow and Permeability

 4. Tissue Damage and Repair

**1.1.3 Mechanisms of Anti-Inflammatory Agents**

 1. Inhibition of Pro-Inflammatory Mediators

 2. Suppression of Immune Cell Activation

 3. Reduction of Oxidative Stress

 4. Modulation of Gene Expression

**1.1.4 Causes of Inflammation**

 1. Injury (trauma, burns)

 2. Infection (bacterial, viral, fungal)

 3. Autoimmune disorders (rheumatoid arthritis, lupus)

 4. Environmental factors (pollution, UV radiation)

 5. Metabolic disorders (diabetes, obesity)

 6. Genetic predisposition

**1.1.5 Signs and Symptoms**

 1. Redness (rubor)

 2. Swelling (tumor)

 3. Heat (calor)

 4. Pain (dolor)

 5. Loss of function

**1.1.6 Stages of Inflammation**

 1. Initiation: Harmful stimulus triggers inflammation

 2. Amplification: Inflammatory mediators recruit immune cells

 3. Resolution: Inflammation subsides, tissue repair begins

**1.1.7 Consequences of Uncontrolled Inflammation**

 1. Tissue damage

 2. Organ dysfunction

 3. Chronic diseases (arthritis, diabetes, cardiovascular disease)

 4. Cancer

**1.1.8 Treatment and Management**

 1. Anti-inflammatory medications

 2. Lifestyle modifications (diet, exercise)

 3. Alternative therapies (acupuncture, herbal supplements)

**1.1.9 Classification of Anti-Inflammatory Agents**

|  |  |  |
| --- | --- | --- |
|  | **Drug class** | **Agent** |
|  Salicylates | Aspirin |
|  Anthranillic acid | Mefenamic acid, Flufenamic acid |
|  Indole acetic acid | Indomethacin |
|  Aryl propianic acids | Ibuprofen, Naproxen |
|  Hetero aryl acetic acids | Tolmetin, Diclofenac, Keterolac |
|  Oxicams | Piroxicam |
|  Pyrazolone & Pyrazolidinedione | Phenylbutazone, Antipyrine |
|  Pyrrole acetic acid | Zomepirac |
|  Para amino Phenol | Phenacetin, Acetaminophen  |

 **TABLE 1: Classification of Anti inflammatory agents**

**1.2 INTRODUCTION TO SALICYLATES:**

Salicylates are a class of drugs derived from salicylic acid. They are primarily known for their analgesic, anti-inflammatory, antipyretic, and antiplatelet properties. Aspirin is the most common and widely used salicylate. Common Salicylates: Acetylsalicylic acid (Aspirin). Salicylates are used to treat mild to moderate pain, fever, inflammation, and cardiovascular conditions requiring antiplatelet therapy.

**1.2.1 Mechanism of action:**

Salicylates inhibit the cyclooxygenase (COX) enzymes, primarily COX-1 and COX-2, leading to decreased synthesis of prostaglandins and thromboxanes. Anti-inflammatory and Analgesic Effects: Reduced prostaglandin production alleviates pain and inflammation. Antipyretic Effect: Acts on the hypothalamus to lower elevated body temperature. Antiplatelet Effect (Aspirin): Irreversible inhibition of COX-1 in platelets reduces thromboxane A2 synthesis, preventing platelet aggregation.

**1.2.2 Contraindications:**

1. Hypersensitivity: Allergy to salicylates.

 2. Peptic Ulcer Disease: Increased risk of gastric bleeding.

 3. Asthma: May trigger bronchospasm in sensitive individuals.

 4. Children with Viral Infections: Risk of Reye's syndrome.

 5. Severe Hepatic or Renal Impairment: Potential for toxicity.

 6. Pregnancy (Third Trimester): Risk of prolonged labor and fetal complications.

**1.2.3 Drug interactions:**

Anticoagulants (e.g., Warfarin) can increased bleeding risk, NSAIDS can additive gastrointestinal toxicity, Methotrexate can reduced renal clearance, increasing toxicity, Corticosteroids can increased risk of gastrointestinal bleeding, Antihypertensive Drugs can reduced efficacy due to sodium retention, Probenecid can interferes with uricosuric effects in gout treatment.

**1.3 INTRODUCTION TO ANTHRANILLIC ACID:**

Anthranilic acids, also known as fenamates, are a subclass of nonsteroidal anti-inflammatory drugs (NSAIDs). They are used for their analgesic, anti-inflammatory, and antipyretic properties. Examples include: Mefenamic acid, Flufenamic acid.

**1.3.1 Mechanism of Action:**

 Anthranilic acids inhibit the cyclooxygenase (COX) enzymes, reducing prostaglandin synthesis. This decreases inflammation, pain, and fever **.**

**1.3.2 Contraindications:**

1. Hypersensitivity to anthranilic acids or NSAIDs

 2. Peptic ulcer disease

 3. Severe renal or hepatic impairment

 4. Congestive heart failure

 5. Pregnancy (especially third trimester)

 6. Active bleeding disorders

**1.3.3 Drug Interactions:**

 Anticoagulants can increased bleeding risk, NSAIDs can sdditive gastrointestinal toxicity, Corticosteroids ca increased risk of ulcers and bleeding, Antihypertensives can reduced efficacy due to sodium retention, Lithium can increased serum lithium levels, Methotrexate can increased toxicity due to reduced clearance.

**1.4 INTRODUCTION TO INDOLE ACETIC ACID:**

 Indole acetic acids are nonsteroidal anti-inflammatory drugs (NSAIDs) belonging to the arylacetic acid derivative class. These compounds are known for their potent anti-inflammatory, analgesic, and antipyretic properties. They work by inhibiting cyclooxygenase (COX) enzymes, reducing prostaglandin synthesis, which mediates inflammation and pain. Examples: Indomethacin.

**1.4.1 Mechanism of Action:**

 These drugs inhibit the cyclooxygenase (COX) enzymes: COX-1 inhibition can reduces prostaglandins involved in protecting the gastric mucosa and maintaining renal function. COX-2 inhibition can reduces prostaglandins that mediate inflammation, pain, and fever. The primary therapeutic effects—anti-inflammatory, analgesic, and antipyretic—are due to COX-2 inhibition, while adverse effects like gastric irritation are linked to COX-1 inhibition.

**1.4.2 Contraindications:**

 1. Hypersensitivity: Patients with a known allergy to NSAIDs.

 2. Asthma: May precipitate bronchospasm in NSAID-sensitive asthma.

 3. Peptic ulcer disease: Due to increased risk of gastric bleeding and ulceration.

 4. Severe renal impairment: Risk of nephrotoxicity.

 5. Pregnancy (third trimester): May cause fetal complications.

 6. Severe hepatic dysfunction: Potential to exacerbate liver injury.

**1.4.3 Drug Interactions:**

 Anticoagulants (e.g., Warfarin) can increased risk of bleeding due to platelet function inhibition. ACE inhibitors and ARBs can reduced antihypertensive effects and increased risk of renal impairment. Diuretics (e.g., Furosemide) can may reduce diuretic efficacy. Lithium can increased serum lithium levels due to reduced renal clearance.

**1.5 INTRODUCTION TO ARYL PROPIONIC ACIDS:**

 Aryl propionic acids are a group of NSAIDs characterized by their arylpropionic acid structure. They are widely used for their anti-inflammatory, analgesic, and antipyretic effects. Common examples include: Ibuprofen, Naproxen.

**1.5.1 Mechanism of Action:**

 Aryl propionic acids inhibit cyclooxygenase (COX) enzymes, decreasing prostaglandin

synthesis. This results in reduced inflammation, pain, and fever. Their reversible inhibition of COX-1 and COX-2 provides therapeutic effects with fewer gastrointestinal side effects compared to other NSAIDs.

**1.5.2 Contraindications:**

 1. Hypersensitivity to NSAIDs

 2. History of peptic ulcers or gastrointestinal bleeding

 3. Severe renal or hepatic impairment

 4. Congestive heart failure

 5. Pregnancy (especially third trimester)

 6. Asthma triggered by NSAIDs

**1.5.3 Drug Interactions:**

 Anticoagulants (e.g., Warfarin) can increased risk of bleeding, Antihypertensives (e.g., ACE inhibitors) can reduced efficacy, Corticosteroids can enhanced gastrointestinal toxicity, Diuretics can reduced diuretic effect and increased renal risk.

**1.6 INTRODUCTION TO HETERO ARYL ACETIC ACIDS:**

 Heteroaryl acetic acids are a class of compounds that feature an aromatic ring containing at least one heteroatom (such as nitrogen, oxygen, or sulfur) attached to an acetic acid (−CH₂COOH) group. These compounds combine the structural properties of heteroaryl rings (such as pyridine, thiophene, or furane) with the functional group of acetic acid. Heteroaryl acetic acids are significant in medicinal chemistry because of their biological activity and potential therapeutic applications, including anti-inflammatory, analgesic, and anti-cancer properties.

**1.6.1 Mechanism of Action:**

 The mechanism of action of heteroaryl acetic acids is similar to other NSAIDs. They primarily work by inhibiting the cyclooxygenase (COX) enzymes, which are responsible for the production of prostaglandins—molecules that promote inflammation, pain, and fever. COX-1 Inhibition: Reduces the production of prostaglandins that are involved in normal physiological processes such as gastric protection and platelet aggregation. COX-2 Inhibition:Reduces the production of prostaglandins that mediate inflammation, pain, and fever. Some heteroaryl acetic acids have a preference for COX-2 inhibition, which may reduce the risk of gastrointestinal side effects.

**1.6.2 Contraindications:**

 Heteroaryl acetic acids share many of the contraindications seen in other NSAIDs: Gastrointestinal Disorders: Contraindicated in patients with active gastrointestinal bleeding, peptic ulcers, or a history of GI bleeding, as they can increase the risk of bleeding and ulceration. Renal Impairment: Caution in patients with renal insufficiency, as these drugs can impair renal function, especially with long-term use. Hypersensitivity: Should not be used in patients with known hypersensitivity to NSAIDs or any component of the drug. Pregnancy and Lactation: Generally avoided, especially in the third trimester of pregnancy, due to risks of fetal harm (e.g., premature closure of the ductus arteriosus).

**1.6.3 Drug Interactions:**

 Like other NSAIDs, heteroaryl acetic acids may interact with several drugs: Anticoagulants (e.g., warfarin) can increased risk of bleeding when used together. Other NSAIDs can using multiple NSAIDs together can increase the risk of gastrointestinal bleeding and renal toxicity. Diuretics and ACE Inhibitors are drugs may reduce the antihypertensive effects of diuretics and ACE inhibitors. Lithium are Heteroaryl acetic acids may increase lithium levels, increasing the risk of toxicity.

**1.7 INTRODUCTION TO OXICAMS:**

 Oxicams are a class of nonsteroidal anti-inflammatory drugs (NSAIDs) that are commonly used to treat pain, inflammation, and fever, particularly in conditions like arthritis (osteoarthritis and rheumatoid arthritis) and musculoskeletal disorders. They are known for their long half-life, which often allows for once-daily dosing. The most commonly prescribed oxicams include piroxicam, meloxicam, tenoxicam, and isoxicam.

**1.7.1 Mechanism of Action:**

 Oxicams exert their therapeutic effects by inhibiting the activity of cyclooxygenase enzymes (COX). There are two primary forms of this enzyme: COX-1: Generally found in most tissues and involved in the protection of the stomach lining, kidney function, and platelet aggregation. COX-2: Induced during inflammation and primarily responsible for producing prostaglandins that mediate pain, inflammation, and fever. Oxicams preferentially inhibit COX-2, reducing the production of pro-inflammatory prostaglandins, which helps alleviate pain and inflammation. However, at higher doses, they may also inhibit COX-1 to some extent, which is associated with side effects like gastrointestinal irritation.

**1.7.2 Contraindications:**

 Oxicams should be used cautiously or avoided in certain conditions due to potential risks. These contraindications include: Hypersensitivity to NSAIDs or oxicams: Patients who have had allergic reactions (such as anaphylaxis or bronchospasm) to other NSAIDs should avoid oxicams. Active peptic ulcer disease: Since NSAIDs inhibit COX-1, they can increase the risk of gastric ulcers or bleeding. Gastrointestinal bleeding or disorders: Patients with a history of gastrointestinal bleeding, Crohn's disease, or ulcerative colitis should avoid oxicams. Severe renal impairment: Oxicams can impair renal function, so they should be avoided in patients with significant kidney dysfunction. Severe hepatic impairment: Patients with advanced liver disease or cirrhosis may need to avoid oxicams due to the drug's metabolism in the liver.

**1.7.3 Drug Interactions:**

 Oxicams can interact with several types of medications, which may affect their effectiveness or increase the risk of adverse effects. Common drug interactions include: Anticoagulants (e.g., warfarin, heparin): Oxicams may enhance the anticoagulant effect, increasing the risk of bleeding. Other NSAIDs: Concurrent use with other NSAIDs (including aspirin) can increase the risk of gastrointestinal ulcers, bleeding, and renal toxicity. Antihypertensive drugs (e.g., ACE inhibitors, diuretics, beta-blockers): Oxicams may reduce the effectiveness of antihypertensive medications and increase the risk of renal impairment. Lithium: Oxicams may reduce the renal clearance of lithium, increasing its blood levels and the risk of toxicity.

**1.8 INTRODUCTION TO PYRAZOLONE & PYRAZOLIDINEDIONE:**

 Pyrazolones and pyrazolidinediones are classes of drugs primarily used for their analgesic, anti-inflammatory, and antipyretic properties. Some members also have uricosuric effects, making them useful in gout management. Examples: Phenylbutazone, Antipyrine.

**1.8.1 Mechanism of Action:**

 Both classes inhibit the cyclooxygenase (COX) enzymes, reducing prostaglandin synthesis, which alleviates pain, inflammation, and fever. Some agents like sulfinpyrazone also inhibit uric acid reabsorption in the kidneys, providing uricosuric effects.

**1.8.2 Contraindications:**

 Hypersensitivity to the drug, severe hepatic or renal impairment, peptic ulcer disease, blood dyscrasias (e.g., agranulocytosis, aplastic anemia),pregnancy and lactation, congestive heart failure.

**1.8.3 Drug Interactions:**

 Anticoagulants can increased bleeding risk, NSAIDs can additive gastrointestinal toxicity, Sulfonylureas can enhanced hypoglycemic effect, Methotrexate can increased toxicity, Diuretics can reduced efficacy in combination, Alcohol van increased risk of gastrointestinal bleeding.

**1.9 INTRODUCTION TO PYRROLE-ACETIC ACID:**

 Pyrrole acetic acid derivatives are a class of NSAIDs used for their anti inflammatory, analgesic, and antipyretic properties. These drugs are particularly effective in managing inflammation and pain in musculoskeletal and rheumatic conditions. Example: Zomepirac.

**1.9.1 Mechanism of Action:**

 Pyrrole acetic acid derivatives inhibit cyclooxygenase (COX) enzymes, reducing the synthesis of prostaglandins. This leads to decreased inflammation and swelling, relief from pain, reduction in fever.

**1.9.2 Contraindications:**

 Hypersensitivity to pyrrole acetic acid derivatives or other NSAIDs, history of peptic ulcers or gastrointestinal bleeding, severe hepatic or renal impairment, asthma exacerbated by NSAIDs, pregnancy (especially third trimester), active bleeding disorder.

**1.9.3 Drug Interactions:**

 Anticoagulants can increased bleeding risk, ACE inhibitors/ARBs can reduced antihypertensive effect and potential renal impairment, Corticosteroids can increased gastrointestinal toxicity, Lithium can elevated serum lithium levels due to reduced clearance.

**1.10 INTRODUCTION TO PARA-AMINO PHENOL:**

Para-aminophenol is an organic compound that is commonly used as a precursor

in the synthesis of drugs such as acetaminophen (paracetamol). It is also used in the preparation of some hair dyes and as a developing agent in photographic processes. It is a colorless, crystalline compound that is known for its analgesic and antipyretic properties when used in the form of acetaminophen.

**1.10.1 Mechanism of Action:**

 Para-aminophenol, when metabolized in the body, is converted into acetaminophen (paracetamol), which exerts its pharmacological effects primarily in the central

nervous system. The exact mechanism is not entirely clear, but it is believed to involve:

Inhibition of Cyclooxygenase (COX) enzymes: Acetaminophen inhibits COX enzymes,

particularly COX-2 in the brain, which reduces the synthesis of prostaglandins (chemical

mediators involved in pain and fever). This is why it has analgesic (pain-relieving) and

antipyretic (fever-reducing) effects. Action on the Endocannabinoid System: There is evidence suggesting that acetaminophen's active metabolite may interact with the endocannabinoid system, contributing to its analgesic effects. Effect on Serotonergic Pathways: Some studies have suggested that acetaminophen may enhance serotoninergic signaling, which could also play a role in its analgesic effects.

**1.10.2 Contraindications:**

 Hypersensitivity: Individuals who are allergic to acetaminophen or any other component of the drug formulation should avoid it. Liver Disease: Para-aminophenol and its derivative, acetaminophen, are metabolized in the liver. Therefore, individuals with liver impairment or those with active liver disease (such as cirrhosis or hepatitis) should avoid this drug, as excessive doses can lead to liver toxicity. Chronic Alcoholism: Chronic alcohol consumption can induce liver enzymes that increase the metabolism of acetaminophen, increasing the risk of liver damage.

**1.10.3 Drug Interactions:**

 Alcohol: Concurrent use of acetaminophen with alcohol can increase the risk of liver toxicity due to the enhanced production of toxic metabolites. Anticoagulants (e.g., Warfarin): Chronic use of acetaminophen can increase the anticoagulant effect of warfarin, leading to an increased risk of bleeding. This is especially true when acetaminophen is taken at high doses over a long period. Isoniazid: This tuberculosis drug can increase the risk of liver toxicity when used together with acetaminophen.

1. **INSTRUMENTATIONS**

**2.1 Introduction to Spectroscopy**

 Spectroscopy is the measurement and interpretation of electromagnetic radiation (EMR) absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. This change may be from ground state to excited state or excited state to ground state. The energy of a molecule is sum of rotational, vibrational, and electronic energies In other words, spectroscopy measures the changes in rotational, vibrational, and electronic energies. Electromagnetic radiation is made up of discrete particles called photons. EMR has got both wave characteristics as well as particle characteristics. This means that it can be travel in vacuum also. The different types of EMR are visible radiation, UV radiation, IR radiation, micro waves. Radio waves, X-rays, Y-rays, or cosmic rays. As these radiations have different wave length or frequency or energy, they are conveniently named also.

The energy of an electromagnetic radiation can be given by the following equation:

 E=h*v*

 Where,

 E=Energy of radiation

 h=plank's constant (6.624$×$10-34 J sec)

 v=frequency of radiation.

**2.2 Ultra Violet Spectroscopy:**

 Ultra violet spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200nm to 340nm. Compounds which are colored, absorb radiation from 340nm to 900nm. But Compounds which are colourless absorb radiation is the UV region. In both UV as well as visible spectroscopy, only the valence electrons absorb the energy, there by the molecule undergo transition from ground state to excited state. This absorption is characteristic and depends on the nature of the electron present. The intensity of absorption depends upon the concentration and path lengths as given by Beers-Lambert's law.

The type of electrons present in any molecule may be conveniently classified as

1. **'**$σ$**' electrons**: These are the ones present in saturated compounds, such electrons don't absorb Vacuum or UV radiation ($<$2000nm).
2. **'II' electrons**: These electrons are present in unsaturated compounds

 Ex: Double or Triple bonds ex: >c=<, -c=c-.

 3. **'n' electrons**: These are non bonded electrons which are not involved in any bonding

 Ex: lone pan of electrons like in S, O, N, and Halogens(x).

**2.3 Introduction to Chromatography**

 Techniques related to Chromatography have been used for centuries to separate materials such as dyes extracted from plants. Russian botanist ***Tswett*** is credited with the discovery of Chromatography. In 1903 he succeeded in separating leaf pigments using a solid polar stationary phase. It was not until 1930s that this technique was followed by Kuhn and Lederer as well as Reichstein and Van Euw for the separation of natural products. Martin and Synge were awarded the Noble Prize for their work in 1941 in which they described liquid-liquid partition chromatography. Martin and Synge applied the concept of theoretical plates as a measure of chromatographic efficiency. The term “*Chromatography*” (Color-Writing, derived from the Greek for Color-Chroma and Write-Graphein).

**2.3.1 High Performance Liquid Chromatography**

 HPLC is separation technique based on the difference in the distribution of the components between two immiscible phases of which one is called as liquid mobile phase and the other is a solid support called as stationary phase. High Performance Liquid Chromatography (HPLC) is one of the most used analytical techniques. Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase HPLC utilizes a liquid mobile phase to separate the components of a mixture. The stationary phase can be a liquid or a solid phase.The amount of resolution is important, and is dependent upon the extent of interaction between the solute components and the stationary phase. The stationary phase is defined as the immobile packing material in the column The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases. As a result, HPLC acquires a high degree of versatility not found in other chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures.

**Reverse phase chromatography**

 In 1960s, chromatographers started modifying the polar nature of silanol group by chemically reacting silica with organic silanes. In reverse phase technique, a non-polar stationary phase is used. The mobile phase is polar in nature. Hence polar compound get eluted first and non- polar compounds are retained for a longer time. Since most of the drugs and pharmaceuticals are polar in nature, they are not retained for a longer time and eluted faster which is advantageous. The retention decreases in the following order: aliphatic greater than induced dipoles (i.e. CCl4) > permanent dipoles (e.g. CHCl3) > weak lewis bases (ethers, aldehydes and ketones)> strong lewis bases (amines) > weak lewis acids (alcohols and phenols)> strong lewis acids (carboxylic acids). The retention increases as the number of the carbon atoms increases.

**2.4 Introduction to Thin Layer Chromatography:**

 Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation TLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC is to obtain well defined, well separated spots. Once the procedure gets completed, different spots can be found on the stationary surface at distinct levels, reflecting various elements of the mixture. Basically, the compounds that are more attracted towards the stationary phase secure their position at lower levels while others move towards the higher levels of the surface. So their spots can be seen accordingly.

**LITRATURE REVIEW**

 **3. LITERATURE REVIEW**

 Latif D.Jamadar et al. described Analytical Method Development And Validation For Aspirin. A sensitive, specific, precise and cost effective High Performance Liquid Chromatographic method of analysis for aspirin in presence of its degradation products is developed and validated The method employed Hypersil BDS C18 (100 x 4.6 mm 5μ) column as stationary phase.The mobile phase consisted of sodium perchlorate buffer (pH 2.5): acetonitrile: isopropyl alcohol (85:14:1 % v/v). It is pumped through the chromatographic system at a flow rate of 1.5 ml min−1.The UV detector is operated at 275 nm. This system was found to give good resolution between aspirin and its degradation products. Method was validated as per ICH guidelines. [1]

 Ashira et al. described Review For Analytical Methods For The Determination Of Mefenamic Acid. Mefenamic acid (MFA) is a non-steroidal anti-inflammatory drug that belongs to the anthranilic acid derivative family. It is used to relieve mild to moderate pain. The present review article includes a compilation of articles on the various properties along with an extensive literature survey on the reported analytical methods of MFA. Using a comprehensive computer assisted literature review; this article discusses the analytical methodologies for quantifying MFA both in active pharmaceutical ingredient and pharmaceutical dosage forms. This is the first review article in this series with focus on the analytical profile of MFA. Although, several methods like High Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), spectrophotometry, fluorimetry, turbidimetry, Atomic Absorption Spectroscopy (AAS), Mass Spectroscopy (MS) and electro analytical methods were reported in the literature, HPLC stands out first for the quantification of MFA. [2]

 Karima Fadhil Ali et al. described New assay method UV spectroscopy for determination of Indomethacin in pharmaceutical formulation. New, simple and rapid method indicating UV spectroscopy was developed and validated for the estimation of Indomethacin(IND)in pure form, and in respective formulations. (0.1N) KOH solution was used as a solvent to decompose IND to p-Chlorobenzoic acid and 5-methoxy-2-methyl-3-indoleacetic acid .The adequate drug solubility and maximum assay sensitivity was found asp-Chlorobenzoic acid, the maximum absorbance of p-Chlorobenzoic acid was measured at (228nm)in the wavelength range of (200‐650 nm), the linear calibration curve was obeyed in the concentration range of (1-10 ppm)show regression equation (Y =0.1263X -0.002), and correlation Coefficient (R2=0.9949). This method was validated and applied to the determination of IND in capsules from different companies in Iraqi market, no interference was found from capsule excipients at the selected wavelength and analysis conditions. It was concluded that the developed method is accurate, sensitive, precise, and reproducible; as well as itcan be applied directly for the estimation of p-Chlorobenzoic acid and indirectly for the estimation of IND content in pharmaceutical formulations. [3]

Sanjay Jain et al. described Development and validation of simple UV Spectrophotometric method of quantization of indomethacin in solid dosage formulation using mixed solvency concept. Organic solvents are most frequently employed in spectrophotometric analyses. They may be sources of pollution. Some of them may be toxic while others may be costlier. Volatility may be a source of inaccuracy in spectrophotometric estimations. In the present investigation, it was proposed to solubilize Indomethacin by use of mixed solvency concept. Indomethacin shows maximum absorbance in the concentration range of 10-50μg/ml at 320 nm. Method of analyses have been validated for different parameters like linearity, accuracy, precision, LOD and LOQ. The percent drug estimated in tablet formulation of Batch-I and of Batch-II were 101.25±0.05 and 100.78±0.10 respectively. The range of percent recoveries varied from 100.61±0.349 to 101.50±0.330.Sodium Benzoate, Niacinamide and sodium Caprylate do not interfere above300 nm. The analytical method was found to be simple, safe (free from toxicity), economic and eco-friendly*.* [4]

 Sagar Bashyal Described Ibuprofen And Its Different Analytical And Manufacturing Methods: A Review. Ibuprofen is a nonsteroidal anti-inflammatory drug, and many of its similar class includes aspirin, indomethacin (Indocin), naproxen (Aleve), nabumetone (Relafen), and many others. This drug is used in moderate pain, fever, and inflammation, which is promoted by the release in the body of chemicals called prostaglandins. According to the IUPAC, it is (RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid. The original synthesis of ibuprofen by the Boots Group started with the compound 2-methylpropyl benzene. Ibuprofen blocks the enzyme that makes prostaglandins (cyclooxygenase), resulting in lower levels of prostaglandins that help in reducing inflammation, pain, and fever. This review is focused on various chemical and functional properties and experimental studies of ibuprofen including various detection methods such as potentiometric, ultraviolet spectrophotometric, gas chromatography, high-performance liquid chromatography (HPLC), and reverse-HPLC which can also be used for the extraction, quantification, and quality analysis. [5]

 A.A. Gouda et al. described Non-steroidal anti-inflammatory drugs (NSAIDs) are the group most often used in human and veterinary medicine, since they are available without prescription for treatment of fever and minor pain. The clinical and pharmaceutical analysis of these drugs requires effective analytical procedures for quality control and pharmacodynamic and pharmacokinetic studies. An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the instrumental analytical methods which were developed and used for determination of some non-steroidal anti-inflammatory, coxibs, arylalkanoic acids, 2-arylpropionic acids (profens) and N-arylanthranilic acids (fenamic acids) in bulk drugs, formulations and biological fluids have been reviewed. This review covers the time period from 1985 to 2010 during which 145 spectrophotometric methods including UV and derivative; visible which is based on formation of metal complexation, redox reactions, ion pair formation, charge-transfer complexation and miscellaneous; flow injection spectrophotometry as well as spectrofluorometric methods. [6]

 K.A. Al-Rashood *Et Al. described* Spectrophotometric Determination Of Tolmetin Sodium In Capsule Dosage Form. Two different U.V. spectrophotometric modes, zero-order and first derivative, have been appied for the quantization of tolmetin sodium (Tolectin® 200 mg) in bulk form and in its pharmaceutical formulation. Direct U.V.-measurement of aqueous solution of the drug at 325 nm exhibits significant linearity at the concentration range 0.1-1.5 mg% with

a coefficient of variation (C.V.) 0.34%. The first derivative (d'A) spectrophotometric measurements at 342 nm yield results with a C.V. 0.29%. Drug assay of the capsule gives percent contents of 100A2 ± 0.34 and 100.28 ± 0.29 by adopting zero-order and d'Aspectrophotometry respectively. The reproducibility and accuracy the two proposed methods have been assessed by employing standard additions technique. Accordingly the percent recoveries obtained were 99.60 ± 0.22 and 100.16 ± 0.26 for the zero order and the d’A-spectrophotometry respectively. [7]

 Fu-An Chen et al. described Quantitation Of Tolmetin By High-Performance

Liquid Chromatography And Method Validation. A high-performance liquid chromatographic (HPLC) assay method for assessing the degradation of tolmetin (TLM) is developed and validated under acidic, basic, and photoirradiated conditions. The HPLC method includes an Inertsil 5 ODS-3V column (250- 4.6- mm i.d.), guard column of Inertsil 7 ODS-3V (50- 4.6-mm i.d.), mobile phase of CH3OH–1% HOAc (64:36, v/v), and UV detection at 254 nm. The developed method satisfies the system suitability criteria, peak integrity, and resolution for the parent drug and its degradants. The established assay method exhibits good selectivity and specificity suitable for stability measurements. From the intraand interday tests of six replicates, the coefficients of variation are between 0.20% and 1.77% for the former, and 0.12% and 3.40%

 for the latter. Recoveries are found to be 98.7–101.7%. TLM is determined to be more reactive when exposed to light and acidic conditions, yet TLM is stable in a basic medium. A kinetic study of the photodegradation of TLM shows that it follows an apparent first-order reaction in three alcoholic solvents. [8]

 Dinesh K Sawant1\*, P P Ige1 described Development and Validation of Simple UV-Spectrophotometric Method for Estimation Diclofenac Sodium in Bulk and Tablet Dosage Form. A simple, efficient, precise and accurate spectroscopic method has been developed and validated for quantitative estimation of diclofenac sodiumin bulk and pharmaceutical dosage form. Diclofenac standard solution was scanned in the UV rang (400-200 nm) in a 1 cm quartz cell in a double beam UV spectrophotometer. The absorption maxima of diclofenac were found to be 276.20 nm. The method obeys beers law in the concentration range from 10- 30 μg/ml. The correlation coefficient was found to be 0.9995 and regression of the curve was found y = 0.0324x + 0.0021 with excellent recovery 98.72-99.86%. Limit of detection and limit of quantitation were found to be 1.03 mg/ml and 3.12 mg/ml respectively. The method was validated for several parameters like accuracy, precision as per ICH guidelines. Value of % RSD and % recovery was found satisfactory, hence the proposed method is precise, accurate and economical hence can be used for routine analysis of diclofenac sodium. [9]

 S. Muralidharan et al. described Simple and sensitive method for the analysis of ketorolac in human plasma using high-performance liquid chromatography. To develop a simple and sensitive method of ketorolac in drug free human plasma using high-performance liquid chromatographic (HPLC). Ketorolac from blank plasma was extracted using protein precipitation method. Trichloro acetic acid (10%) was used as a protein precipitating agent and the percentage of recovery was calculated by adding known volume of dexibuprofen as internal standard. The HPLC separation was achieved on reversed-phase C18 column and the separations were detection by Photodiode Array (PDA) detector at 306 nm. Acetonitrile and 5 mM ammonium acetate (pH 3.5), in the ratio of 60:40% v/v was used as mobile phase at the isocratic flow rate of 1.0 ml/min. The method was validated according to FDA Guidance for Industry. The percentage of recovery of ketorolac was 93.7 \_ 0.12 (mean \_ SD; n ¼ 5). The linearity was achieved in this method range of 10.0e125.0 ng/ml with regression coefficient range of 0.999. Ketorolac bioanalytical method was developed and validated according to FDA Guidance for Industry. [10]

 Vijay Kumar. R et al. described Analytical method development and validation of Piroxicam by RP-HPLC. This paper describes the analytical method suitable for validation of Piroxicam by reversed Phase High Performance liquid chromatography (RP-HPLC) method. The method utilizedRP-HPLC (Water 2695 with PDA detector) model and a column, 150mm ´ 4.6 mm, 5m (Inertsil, ODS- 3V, 150mm ´ 4.6mm, 5m). The mobile phases were comprised of A, B of Methanol and Buffer pH 3.0 (55:45v/v). Validation experiments were performed todemonstrate System suitability, precision, linearity and Range, Accuracy study, stability of analytical solution and robustness. The method was linear over the concentration range of 5- 150 mg/ML-1. The method showed good recoveries (98.0 – 99.8%). [11]

 Alina-Diana Panainte et al. Described Fast HPLC Method for the Determination of Piroxicamand its Application toStability Study. A fast, robust RP-HPLC isocratic method was developed for determination of Piroxicam in bulk materials and pharmaceutical formulations. Optimum separation of piroxicam and stress induced degradation products were achived with a stationary phase SB-C18 Eclipse column(150x4.6; 5µm). The mobile phase was a mixture of water:acetonitrile (50:50) with a flow rate of 0.5mL/min. The UV detection was performedat 360nm. The method was validated in accordance with the current ICH guidelines in terms oflinearity, limit of detection, limit of quantification, precision, accuracy, recovery and system suitability.The retention time for piroxicam was 2.55 minute. The calibration graph was linear in the concentration range 5-90µg/mL. The assay proved to be sensitive, specific and reproducible.The method was applied for the determination of piroxicamin tablets. [12]

 Abdullah et al. describedThe Application of UV-VIS Spectrophotometry Method to Identify Phenylbutazone in Herbal Packages Circulating in Big Cities in Indonesia. The aim of this research is to find out how to analyze the chemical content of the drug phenylbutazone in herbal medicine using thin layer chromatography and UV-Vis’s spectrophotometers. The samples in this study were obtained from several drug stores and traditional markets that sell herbal medicine. The sampling technique used was non-probability sampling using purposive sampling. The conclusion drawn from the results of this research is as follows: There is one sample of tested herbal medicine that is suspected to contain BKO phenylbutazone, namely 0.3%. In analysis using TLC, there was one sample containing BKO phenylbutazone with an Rf value in three consecutive mobile phases of 0.5, 0.7, and 0.6. Meanwhile, in the analysis using UV-Vis’s spectrophotometry, data was obtained in the form of maximum wavelength, absorbance, calibration curve equation, concentration for calculating precision tests, accuracy, and sample levels. In the quantitative analysis of phenylbutazone, it was found that the limit of detection (LOD) was 0.09 μg⁄mL and the limit of quantitation (LOQ) was 0.3 μg⁄m. [13]

 M.I. Gonza´lez Mart´ın et al. described Determination by high-performance liquid chromatography of phenylbutazone in samples of plasma from fighting bulls. The purpose of this study was to investigate the possible presence of phenylbutazone in plasma samples from fighting bulls killed in 2nd and 3rd category bullrings in the province of Salamanca (Spain) in 1998, 1999 and 2000. For quantitative and qualitative determination, a high-performance liquid chromatograph was used, equipped with a photodiode-array detector and setting wavelengths at 240, 254 and 284 nm. The mobile phase optimized for the simultaneous detection of dexamethasone, betamethasone, flunixin and phenylbutazone, was 0.01 *M* acetic acid pH 3 in methanol (35:65 v/v) at a flow rate of 1 ml /min. Plasma samples were deproteinized with 400 ml of acetonitrile and 20 ml of the supernatant were injected directly into the chromatographic system equipped with a Lichrospher 60 RP select B column and guard column. For the quantitative analysis, standard calibration curves were made in a concentration range between 0.25 and 30 mg/ ml, using betamethasone as internal standard. The retention time of phenylbutazone was 8.760.2 min and recovery was 83%. The detection and quantification limits were 0.016 and 0.029, respectively for l5240 nm. The study results show that 17 of the 74 samples analyzed in 1998, 18 of those from 1999 and 10 of those from 2000 were positive for phenylbutazone. Ó 2002 Elsevier Science B.V. All rights reserved. [14]

 Hanan A. Merey et al. described Validated simultaneous determination of antipyrine and benzocaine HCl in the presence of benzocaine HCl degradation product. Two validated, sensitive and highly selective stability-indicating methods were adopted for the simultaneous quantitative determination of antipyrine (ANT) and benzocaine HCl (BEN) in the presence of the degradation product of benzocaine HCl [p-aminobenzoic acid (PABA)]. The first method was high performance liquid chromatography, where a mixture of antipyrine (ANT), benzocaine HCl (BEN) and degradation product of benzocaine HCl (PABA) is separated on a C8 ZORBAX analytical column (5 mm, 4.6 \_ 150 mm I.D.) using acetonitrile–phosphate buffer of pH 5.5 (25 : 75, v/v) as the mobile phase. The drugs were detected at 270 nm over a concentration range of 10–100 mg mL\_1 and 5–100 mg mL\_1, with mean percentage recoveries of 100.22% (S.D. 1.375) and 99.77% (S.D. 1.089) for antipyrine and benzocaine HCl, respectively. The second method was thin layer chromatography combined with the densitometric determination of the separated bands at 275 nm. Adequate separation was achieved using silica gel 60 TLC F254 plates and toluene–acetone–methanol–ammonia (8 : 3 : 3 : 0.1 by volume) as the mobile phase. The proposed methods were applied for the analysis of antipyrine and benzocaine HCl in their pharmaceutical formulation, and the results were statistically compared with the reported methods. [15]

 K.--T-. Ng, T. Snyderman Described Determinatlon Of Zomepirac In Plasma By High-Pressure Liquid Chromatography. A sensitive, specific and precise high-pressure liquid-chromatographic method for the determination of the analgesic agent, zomepirac, in plasma samples is described. The lowest concentration of zomepirac that can be measured accurately and precisely (coefficient of variation <20%) in a z-ml plasma sample is 10 ng/ml. The standard curve is linear in the concentration range of 10 to 5000 n\_g/ml. To date, this procedure has been employed successfully in analysin g over 10,000 clinical plasma samples. [16]

 Idris et al. described Simultaneous determination of methaqaulone, saccharin, paracetamol, and phenacetin in illicit drug samples by hplc. Saccharin, a low calorie artificial sweetener was found as a new diluent / adulterant present along with paracetamol and phenacetin in an illicit methaqualone sample. All these components were simultaneously analyzed by the proposed reverse phase high performance liquid chromatography method using C18 column using acetonitrile: water (90:10 v/v) as mobile phase with a flow rate of 1mL /min. The percentages of saccharin, phenacetin, paracetamol and methaqualone in illicit drug sample were found to be 15.0, 45.6, 25.1 and 12.0 respectively. The method was validated for limit of detection, limit of quantification, linearity, accuracy, precision and reproducibility with the help of the exhibit and simulated samples. The proposed method is simple, accurate and fast. It can be applied to the routine analysis of illicit methaqualone samples as well as for their impurity profiles for tracing the origin. [17]

 Lufeng Hu et al. described Rapid LC-APCI-MS-MS Method for Simultaneous Determination of Phenacetin and Its Metabolite Paracetamol in Rabbit Plasma. A highly sensitive liquid chromatographic-atmospheric pressure chemical ionization-tandem mass spectrometric method is developed to quantitate phenacetin and its metabolite paracetamol in rabbit plasma. The analytes and internal standard oxazepam are extracted from plasma by liquid–liquid extraction using ethyl acetate, and separated on a Zorbax SB-C18 column (2.1 mm 9 150 mm, 5 lm) using acetonitrile–0.1% formic acid in water (40:60 v/v) at a flow of 0.4 mL min-1. Detection is carried out by multiple reaction monitoring on a iontrap LC-MS-MS system with an atmospheric pressure chemical ionization interface. The assay is linear over the range 4–1,600 ng mL-1 for phenacetin and 3–2,000 ng mL-1 for paracetamol, with a lower limit of quantitation of 4 ng mL-1 for phenacetin and 3 ng mL-1 for paracetamol. Intra- and inter-day precision are less than 7.1% and the accuracy are in the range 97.3–103.5%. The validated method is successfully used to analyze the drug in samples of rabbit plasma for pharmacokinetic study. [18]

 Ms Fayeza Batool Et Al. Described An Assay Method For The Simultaneous Estimation Of Acetaminophen And Tramadol Using Rp-Hplc Technology. A simple and selective RP-HPLC method is described for the determination of Acetaminophen and Tramadol dosage forms. Chromatographic separation was achieved on a C18 column using mobile phase consisting of a mixture of mixed Phosphate buffer pH: 3.4 Acetonitrile (30:70v/v/v), with detection of 236nm and flow rate at 1.2mL/min. Linearity was observed in the range 100-300 μg /ml for Acetaminophen (r2 =0.99) & 10-30μg /ml for Tramadol (r2 =0.99) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods, sensitivity, precision and reproducibility. It can be used for routine analysis of pharmaceutical dosage form. [19]

 Ca˘linescu et al. described Determination of acetaminophen and its main impurities: 4-nitrophenol, 4’-chloroacetanilide, as well as 4-aminophenol and its degradation products, p-benzoquinone and hydroquinone has been developed and validated by a new high-performance liquid chromatography method. Chromatographic separation has been obtained on a Hypersil Duet C18/SCX column, using gradient elution, with a mixture of phosphate buffer (pH 5 4.88) and methanol as a mobile phase. Analysis time did not exceed 14.5 min and good resolutions, peak shapes and asymmetries have resulted. The linearity of the method has been tested in the range of 5.0–60 mg/ mL for acetaminophen and 0.5–6 mg/mL for the other compounds. The limits of detection and quantification have been also established to be lower than 0.1 mg/mL and 0.5 mg/mL, respectively. The method has been successfully applied for the analysis of commercial acetaminophen preparations. [20]

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 **REVIEW WORK**

**4. REVIEW WORK**

**ABSTRACT:**

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used pharmaceuticals in both human and animal medicines for the treatment of certain conditions such as inflammation, fever, and pain. The role of validated analytical methods has become highly important in the quantification of drug substances from their pharmaceuticals as precise product [quality control](https://www.sciencedirect.com/topics/chemistry/quality-control) is required. In the present review, we have summarized various [sample preparation methods](https://www.sciencedirect.com/topics/chemistry/sample-preparation-method) and analytical methods developed for the quantification of NSAIDs during the past decade (2012-till date). Furthermore, an in-depth description of numerous techniques including [chromatography](https://www.sciencedirect.com/topics/chemical-engineering/chromatography), spectrometry (UV), high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), thin-layer chromatography (TLC), High performance thin-layer chromatography (HPTLC), liquid chromatography-mass spectrometry (LCMS), and gas chromatography (GC) approaches used to determine and quantify NSAIDs are provided. Based on the matrix utilized, the following details were discussed: analytical conditions, detection limits, and solvent used in sample preparation. The present compilation provides valuable insights and crucial information on quantification methods for NSAIDs and would assist the scientific community to select the best and economical method for drug analysis in pharmaceuticals and biological samples. UV spectrometry and HPLC were the most commonly used.

**KEYWORDS:** Anti-inflammatory drugs, Analytical methods, validation parameters.

 **I. INTRODUCTION:**

Non-steroidal anti-inflammatory drugs (NSAIDs) constitute some of the highly recognized categories of pharmaceutical agents having a variety of advantages. These agents are generally employed as therapeutics in humans and animals due to their ability to function as antipyretic, analgesic, and anti-inflammatory agents. Drugs included within this category differ in terms of their chemical properties and almost all agents possess varying amounts of therapeutic efficacy.  NSAIDs are widely employed to alleviate several debilitating conditions including fever, migraines, [menstrual irregularities](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/menstrual-irregularity), [rheumatoid arthritis](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/rheumatoid-arthritis), [osteoarthritis](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/osteoarthritis), gout, and [postoperative complications](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/postoperative-complication). Several reports in the literature have suggested that NSAIDs have certain functions in reducing the risk of acquiring malignancies such as colorectal, breast, ovarian, hepatocellular, prostate, pancreatic, and [head and neck cancers](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/head-and-neck-cancer).

* 1. **SALICYATES:-**

**4.1.1 ASPIRIN**



**IUPAC name :** 2-Acetyloxybenzoic acid

**Molecular formula:** C9H8O4

**Molecular weight:** 180.16g/mol

**Physical properties Appearance:** colourless to white crystalline powder

**Solubility:** Sparingly soluble in water; soluble in alcohol and organic solvents

**Boiling point:** 140 °C

**Melting point:** 135 °C

**Pharmacological action :** Aspirin's mechanism of action involves the inhibition of cyclooxygenase (COX) enzymes, primarily COX-1 and COX-2. Inhibition of COX: By acetylating a serine residue in the COX enzymes, aspirin permanently inhibits their activity. This prevents the conversion of arachidonic acid into prostaglandins and thromboxanes. Reduced Prostaglandin Production: Prostaglandins are mediators of inflammation, pain, and fever. By reducing their synthesis, aspirin alleviates these symptoms. Antiplatelet Effect: In low doses, aspirin inhibits thromboxane A2 production in platelets, which reduces platelet aggregation and helps prevent blood clots. This combination of actions makes aspirin effective for pain relief, inflammation reduction, and cardiovascular protection.

**ANALYTICAL METHODS :**

**HPLC ASSAY METHOD VALIDATION ASPIRIN**

A sensitive, specific, precise and cost effective High Performance Liquid Chromatographic method of analysis for aspirin in presence of its degradation products is developed and validated. The method employed Hypersil BDS C18 (100 x 4.6 mm 5μ) column as stationary phase.The mobile phase consisted of sodium perchlorate buffer (pH 2.5): acetonitrile: isopropyl alcohol (85:14:1 % v/v). It is pumped through the chromatographic system at a flow rate of 1.5 ml min−1.The UV detector is operated at 275 nm. This system was found to give good resolution between aspirin and its degradation products. Method was validated as per ICH guidelines. [1]

**Table 2: Effect of pH**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **pH 2.3** | **pH 2.5** | **pH 2.7** |
| **Retention Time** | 5.2 | 4.6 | 4.7 |
| **Theoretical Plates** | 3686 | 2806 | 3461 |
| **Capacity Factors** | 6.42 | 4.8 | 4.2 |

**Table 3: Effect Of stationary Phases:**

|  |  |  |
| --- | --- | --- |
| **Parameters** | **BDS Hypersil C18 column****(25 cm × 4. 6mm i.e., 5μm)** | **BDS Hypersil C18 column****(5 cm × 4. 6mm i.e., 5μm)** |
| **Retention Time** | 9.4 | 3.8 |
| **Asymmetry Factors** | 1.2 | 1.0 |

**Table 4: Effect of Flow Rate**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **1.3 ml/min** | **1.5 ml/min** | **1.7 ml/min** |
| **Retention Time** | 5.3 | 4.6 | 4.1 |
| **Theoretical Plates** | NA | 2806 | 1246 |
| **Capacity Factors** | 5.6 | 4.8 | 4.8 |

**Table 5: validation Parameters:**

|  |  |
| --- | --- |
|  **Parameters** | **Obtained Results** |
| **r2 (Representive linearity)** | 0.997 |
| **Accuracy (% RSD)** | Recoverylevels | 50% | 97.8 % |
| 75% | 98.0 % |
|  |  | 100% | 99.8 % |
| 125% | 100.0 % |
|  | 150% | 100.0 % |
| Assay | 101.1 % w/w |
| **Precision (% RSD)** | System Precision | 0.6 % |
|  | IntermediatePrecision | Analyst 1 | 0.48 % |
| Analyst 2 | 0.32 % |
| Method Precision | 1.3 % |
| **Robustness (%****RSD)**  | Effect ofvariation in pH | pH 2.3 | 0.16 % |
| pH 2.5 | 0.10 % |
| pH 2.7 | 0.20 % |
| Effect ofvariation inflow rate | 1.3 ml/min | 0.52 % |
| 1.5 ml/min | 0.57 % |
| 1.7 ml/min | 0.65 % |
| **Ruggedness** | System tosystemvariability | System 1 | 0.10 % |
| System 2 | 0.63 % |

**Table 6: Filter Description:**

|  |  |
| --- | --- |
| **Filter Description** | **Filters** |
| **NYLON66** | **PVDF** |
| **Manufacturers Name** | Micro Devices | Milex |
| **Size** | 0.45μm | 0.45μm |

**Table 7: Filter Validation:**

|  |  |  |
| --- | --- | --- |
|  **Centrifuged** | **PVDF** |  **NYLON 66** |
|  **% Assay** | **% Assay** |  **% Assay** |
|  **1** | **2** |  **3** |  **1** |  **2** |  **3** |  **1** | **2** | **3** |
|  100.8 | 100.8 | 101 | 101 | 101.7 | 100.7 |  101.5 |  100 |  99.17 |

**Table 8: System Suitability Parameters:**

|  |  |
| --- | --- |
|  **Parameters** | **Aspirin** |
|  **USP plate Count** | 3254 |
|  **USP tailing** | 1.0 |
|  **% RSD of six Replicate Injection** | 0.6 |

* 1. **ANTHRANILLIC ACID:-**

**4.2.1 MEFENAMIC ACID**

****

**IUPAC name :** 2-(2,3-dimethylanilino)benzoic acid

 **Molecular formula:** C15H15NO2

**Molecular weight:** 241.28 g/mol

**Physical properties Appearance:** White to off-white, crystalline powder

**Solubility:** Slightly soluble in water; soluble in organic solvents like ethanol and acetone.

**Boiling point:** 364 °C

**Melting point:** 230-231 °C

**Pharmacological action :** Mefenamic acid is a non-steroidal anti-inflammatory drug (NSAID) that primarily works by inhibiting cyclooxygenase (COX) enzymes, leading to decreased synthesis of prostaglandins. This results in reduced pain, inflammation, and fever, making it effective for treating conditions like menstrual pain and arthritis.

**ANALYTICAL METHODS :**



 **Fig. Distribution of analytical method described in the literature for the determination of MFA**

**Thin layer chromatography (TLC)**

For the simultaneous determination of MFA (MFA) and its two toxic impurities, Martha M. Morcoss et al established and validated a process. The proposed TLC- densitometric system using a mobile phase consisted of of chloroform: acetone: acetic acid: ammonia (70:30:2:2, v/v/v/v) and TLC aluminum plates 60 F254 was used as a stationary phase and the separated bands were UV-scanned at 225 nm. Harrizul Rivai et al. studied and validated thin layer chromatography-densitometry method for analysis of MFA in tablet. The accuracy and reliability of the method was assessed by evaluation of linearity (50-300 µg/ml), precision intra-day and inter-day relative standard deviation values were always less than 2, accuracy (102.45 % ± 1.36% for Sample A and 100.28% ± 1.90% for Sample B) in accordance with ICH guidelines. The detailed information is depicted in Table 9. [2]

 **Table 9. TLC for analysis of MFA**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Title**  | **Mobile phase** | **Stationary phase** | **λmax** | **LOD** |
| Different chromatographic methods for simultaneous determination of MFA and two of its toxic impurities |  Chloroform:acetone: acetic acid:ammonia solution(70:30:2 :2)v/v/v/v | Aluminium plates 60 F254 | 225 nm  | 0.3-2 μg/band |
| Development and validation of thin layer chromatography-densitometry method for analysis of MFA in tablet | Chloroform :methanol (9.0:0.1,v:v) | Silica gel 60 F254 | 320 nm  | 50-300 μg/ml |

 *ª λmax-maximum wave length; ᵇ LOD –linearity*

**Fluorimetric Method**

A simple spectro fluorimetric procedure for assessment of MFA in pharmaceutical preparation and urine was reported by Ahad Bavili Tabriz. The process includes oxidizing MFA using cerium (IV) to create cerium (III) and the fluorescence of cerium (III) after stimulation at 255 nm was measured at 354 nm. A sensitive and simple spectro fluorimetric approach was developed using terbium sensitized fluorescence. The process is based on the conversion of radiative energy from anthranilates to terbium ions in alkaline methanolic solutions, with detection limits 1.4x 10-8 reported by Pinelopi C. Ioannou at el. Studies are summarized in Table 10.

 **Table 10. Fluorimetric method**

|  |
| --- |
| **Title Objective Evaluation**  |
| A Simple Spectrofluorimetric The process includes oxidizing The fluorescence of cerium (III) method for determination of MFA using cerium (IV) after stimulation at 255nmMFA in pharmaceutical to create cerium (III), was measured at 354nm.preparation and urine  |
| Spectrofluorimetric The process is based on the The detection limit of MFA determination of anthranilic conversion of radiative energy was1.4x10-8acid derivatives based on from anthranilates to terbiumterbium sensitized ions in alkaline methanolicfluorescence solutions.  |

* + 1. **FLUFENAMIC ACID**

****

**IUPAC name :** 2-[3-(trifluoromethyl)anilino]benzoic acid

**Molecular formula:** C14H10F3NO2

**Molecular weight:** 281.23 g/mol

**Physical properties Appearance:** White to off-white crystalline powder

**Solubility:** Sparingly soluble in water; soluble in organic solvents like ethanol and acetone

**Boiling point:** Not well-defined due to decomposition

**Melting point:** 133.5°c

**Pharmacological action :** Flufenamic acid works by inhibiting the cyclooxygenase (COX) enzymes, specifically COX-1 and COX-2. These enzymes are involved in the conversion of arachidonic acid into prostaglandins, which play a key role in inflammation, pain, and fever. By blocking this pathway, flufenamic acid reduces the production of prostaglandins, leading to decreased inflammation and pain relief.

**ANALYTICAL METHODS :**

**Spectrophotometric method**

A spectrophotometric method was developed for the determination of flufenamic in the pure form and in pharmaceutical dosage forms. The method depends on their complexation with copper(II) ammonium sulphate. The complex is extracted with chloroform and treated with diethyldithiocarbamate solution, whereupon another copper(II) complex (λmax 430 nm) is formed. Beer’s law is followed over the concentration ranges 6.0–60 lg mL-1 for flufenamic acid. [6]

**Visible spectrophotometric method**

A simple visible spectrophotometric method was described for the determination of enfenamic acid in bulk samples and pharmaceutical preparations. The method is based on the reaction of enfenamic acid with p-N,N-dimethylphenylenediamine in the presence of S2O82- or Cr(VI) whereby an intensely coloured product having maximum absorbance at 720 nm is developed. The reaction is sensitive enough to permit the determination of 0.125–2.0 lg mL-1. [6]

**Table 11. Visible spectrophotometric method**

|  |  |  |  |
| --- | --- | --- | --- |
| Name of Drug | Method | λmax (nm) | Linear range(lg mL\_1) |
| Flufenamic acid | Copper(II) ammine sulphate/DiethyldithiocarbamateSpectrofluorometry withconcentrated sulfuric acid | 430λem= 450λex =400  | 6.0–602.0–20 ng mL-1 |

* 1. **INDOLE ACETIC ACID:-**
		1. **INDOMETHACIN**



**IUPAC name :** 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid

**Molecular formula:** C19H16ClNO4

**Molecular weight:** 357.80 g/mol

**Physical properties Appearance:** Pale-yellow to yellow-tan, crystalline powder

**Solubility:** Slightly soluble in water; soluble in organic solvents.

**Boiling point:** Decomposes before boiling.

**Melting point:** 160-162 °C

**Pharmacological action :** Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that primarily inhibits cyclooxygenase (COX) enzymes, leading to decreased synthesis of prostaglandins. This action results in reduced pain, inflammation, and fever, making it effective for treating conditions such as arthritis, bursitis, and tendinitis.

**ANALYTICAL METHODS :**

**UV Spectroscopy:**

The method was validated through linearity, sensitivity, precision and accuracy, as shown in Table.10. The results were checked by UV-Vis. spectroscopy method measure the absorbance at (318.4 nm)of the solution IND diluting with(9:1) methanol: hydrochloric acid(0.1N) solvent[18] the results are in good agreement with this method. [3]

**Table.12: Optical characteristics, parameters of the method**

|  |  |  |
| --- | --- | --- |
| **No.** | **Parameters** | **Result** |
| 1 |  Absorption maxima(λmax) |  228 nm  |
|  |  Linear equation |  Y = 0.1263 x – 0.002  |
|  |  Regression co-efficient |  0.9947  |
|  |  Linearity |  1-10 mg/L  |
|  |  LOD (mg/L) |  0.602- 0.129  |
| 6 |  LOQ (mg/L) |  1.825 - 0.390 |

**UV-Spectrophotometer by mixed solvency concept**

The developed UV-spectrophotometric method was validated as per ICH guidelines in terms of linearity, and range, specificity, precision, sensitivity and accuracy. In order to determine linearity range of developed method, a series of solutions were prepared using Indomethacin stock solution at concentration range of 10-50µg/ml.The absorbances of the resultant solutions were measured at 320 nm against reagent blank. [4]

**Table 13: Developed UV method specification**

|  |  |
| --- | --- |
| Instrument and specification | UV-Spectrophotometer Shimadzu 1800  |
| Scanning Range | 200 nm to 400 nm  |
| Solvent Used | Hydrotropic solvent  |
| Strength of Solvent | 10% Sodium caprylate, 10% Sodium Benzoate and 10% Niacinamide  |
| Composition of Solvent | 10% Sodium caprylate, 10% Sodium Benzoate and 10% Niacinamide  |
| Wavelength Maxima of Indomethacin |  |

* 1. **ARYL PROPIONIC ACIDS:-**

**4.4.1 IBUPROFEN**



**IUPAC name :** 2-[4-(2-methylpropyl)phenyl]propanoic acid

**Molecular formula:** C13H18O2

**Molecular weight:** 206.28 g/ml

**Physical properties Appearance:** Colorless, crystalline stable solid

**Solubility:** Ibuprofen is slightly soluble in water, but soluble in organic solvents like ethanol and acetone.

**Boiling point:** 157ºC

**Melting point:** 75-77.5 ºC

**Pharmacological action :** Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) that works by: **Inhibition of COX Enzymes:** Like sulindac, ibuprofen inhibits cyclooxygenase (COX-1 and COX-2), reducing the production of prostaglandins. **Anti-inflammatory Effects:** This leads to decreased inflammation and pain relief. **Analgesic and Antipyretic Actions:** Ibuprofen is effective for relieving mild to moderate pain and reducing fever.

**ANALYTICAL METHODS :**

**Chromatographic and ultraviolet (UV) spectrophotometric methods**

In early ages, most of the analytical methods were done using thin-layer spectrophotometric, differential pulse polygraph, colorimetric, gas-liquid chromatography, paper chromatography, or direct liquid introduction mass spectrometry (MS). However, due to greater facilitate sample preparation, those early methods have largely been replaced by high-performance liquid chromatography (HPLC), gas chromatography (GC), and GC-MS, and a recent report describes the use of HPLC Table13. [5]

**Table 14: Chromatographic and UV spectrophotometric methods for various experiments**

****

* + 1. **NAPROXEN:-**

****

**IUPAC name :** (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoic acid

**Molecular formula:** C14H14O3

**Molecular weight:** 230.26 g/mol

**Physical properties Appearance:** White to off-white crystalline powder

**Solubility:** Slightly soluble in water; soluble in alcohol and chloroform

**Boiling point:** 250 °C

**Melting point:** 154 °C

**Pharmacological action :** Naproxen primarily works by inhibiting the cyclooxygenase (COX) enzymes, specifically COX-1 and COX-2. These enzymes are responsible for converting arachidonic acid into prostaglandins, which are lipid compounds that mediate inflammation, pain, and fever. By inhibiting both COX-1 and COX-2, naproxen reduces the synthesis of prostaglandins, resulting in decreased inflammation, alleviation of pain, and reduction of fever. This mechanism makes it effective in treating various inflammatory conditions and pain syndromes.

**ANALYTICAL METHODS :**

**Spectrophotometric method:**

A second-derivative spectrophotometric method for the determination of naproxen in the absence or presence of its 6- desmethyl metabolite in human plasma is described. The method consists of direct extraction of the non-ionized form of the drug with pure diethyl ether and determination of the naproxen by measuring the peak amplitude (mm) in the second-order derivative spectrum at a wavelength of 328.2 nm. The efficiency of the extraction procedure expressed by the absolute recovery was 94.6 }0.7% (mean } SD) for the concentration range tested, and the LOQ attained according to the IUPAC definition was 2.42 lg mL\_1. [6]

**Table 15. Spectrophotometric method**

|  |  |  |  |
| --- | --- | --- | --- |
| Name of the drug | Method | λmax (nm) | Linear range(lg mL\_1) |
| Naproxen | Second-derivative1-Naphthylarnine and sodium nitriteMBTH with Ce(VI) or Fe(III)2,6-Dichloro-p-benzoquinone-4-chlorimine (gibbs reagent)TCNE, DDQ, p-CHL | 328.2460–480  | 10–65 |

* 1. **HETERO ARYL ACETIC ACIDS:-**

**4.5.1 TOLMETIN**



**IUPAC name :** 2-[1-methyl-5-(4-methylbenzoyl)pyrrol-2-yl]acetic acid

**Molecular formula:** C15H15NO3

**Molecular weight:** 257.28 g/mol

**Physical properties Appearance:** White to off-white crystalline powder

**Solubility:** Practically insoluble in water, ethanol, and acetone; sparingly soluble in chloroform

**Boiling point:** Not specifically reported (decomposes before boiling)

**Melting point:** 156 °C

**Pharmacological action :** Tolmetin’s mechanism of action (MOA) primarily involves the inhibition of cyclooxygenase (COX) enzymes, specifically COX-1 and COX-2. By blocking these enzymes, Tolmetin reduces the synthesis of prostaglandins, which are mediators of inflammation, pain, and fever. This leads to its anti-inflammatory and analgesic effects, making it effective for conditions like arthritis and other inflammatory disorders. In analytical chemistry, the mechanism of action (MOA) of Tolmetin can be explored through various techniques that elucidate its interactions with biological targets. Enzyme assays can assess Tolmetin's inhibition of cyclooxygenase (COX) enzymes by measuring the production of prostaglandins, often using HPLC or mass spectrometry for quantification. Binding studies, such as Surface Plasmon Resonance (SPR), can evaluate the binding affinity of Tolmetin to COX enzymes, providing insights into its pharmacodynamics.

**ANALYTICAL METHODS :**

**Spectrophotometric Determination Of Tolmetin Sodium**

The accuracy and reproducibility of both procedures have been assessed by adopting the standard additions technique, where different added amounts (20-80%) of claimed tolmetin sodium in Tolectin® were tried. The concomitant added percent recoveries obtained were 99.60 ± 0.22 (n = 6) and 100.16 ± (126 for the zero order and first derivative methods respectively. In conclusion it can be stated that the two proposed spectrophotometric methods are rapid, simple, precise and accurate; and any of them is suitable for quality control of totmain sodium. However, the first derivative method has the advantage of eliminating irrelevant absorption due to possible interfering co-existing substances. [7]

**Table 16: Determination of Tolmetin Sodium by Zero-Order and First-Derivative Spectrophotometry.**

Zero-Order Method First-Derivative Method

|  |
| --- |
|  |

Pharmaceutical Nominal % Found % of Added % Found % of Added

Preparation concentration ± SD\* recovery ± SD\* ± SD\* recovery ± SD\*

 μg/ml

|  |
| --- |
|  |

Tolectin® 1-15 100.42 ± 0.33 99.60 ± 0.22 100.28±0.26 100.16±0.26

capsules (n=6) (n=6) (n=6) (n=6)

(200 mg)

|  |
| --- |
|  |

SD\* = Standard deviation.

**High-Performance Liquid Chromatography and Method Validation**

The intra- and interday (Table 17 ) standard deviations (SDs) of six replicate determinations for six consecutive days at the usual working concentrations of 5.0–100μM were between 0.087 and 0.518 with coefficients of variation (CVs) of between 0.20% and 1.77% for the former, and 0.129 to 0.293 with CVs of between 0.12% and 3.40% for the latter. The accuracies of the method, as referring to the recovery test at the 5 concentrations of 5, 10, 25, 50, and 100μM (expressed as the closeness of the observed mean to the true value), were determined to be 98.7%, 101.7%, 99.7%, 99.8%, and 100.0%, respectively. There was no significant difference in comparison with the results having 100% recovery (*p* > 0.05), which indicates good accuracy for the assay method. Clearly, the established assay method is reliable and applicable for stability assessment of TLM degraded under photoirradiated conditions. [8]

|  |
| --- |
| **Table 17**. Intra- and Interday (*n* = 6) Analytical Precisions for TLM |
| **Concentration** **(μM)** | **Intraday** |  **Interday** |
| **Mean Rel.** **(SD) CV (%) error (%)** |  **Mean Rel.** **(SD) CV (%) error(%)**  |
|  5 10 25 50 100 | 4.909 (0.087) 1.77 –1.8210.003 (0.157) 1.57 0.0025.219 (0.255) 1.01 0.8749.843 (0.518) 1.03 – 0.31100.023 (0.201) 0.20 0.02 |  4.937 (0.168) 3.40 –1.2610.173 (0.194) 1.91 1.7324.928 (0.293) 1.17 –0.2949.918 (0.273) 0.54 –0.16100.041 (0.129) 0.12 0.04 |

* + 1. **DICLOFENAC**

****

**IUPAC name :** 2-[2-(2,6-dichloroanilino)phenyl]acetic acid

**Molecular formula:** C14H11Cl2NO2

**Molecular weight:** 296.1 g/mol

**Physical properties Appearance:**  Usually appears as a white to pale yellow crystalline powder.

**Solubility:** Slightly soluble in water; more soluble in organic solvents like ethanol and dimethyl sulfoxide (DMSO).

**Boiling point:** 295 °C

**Melting point:** 283-285 °C

**Pharmacological action :** Diclofenac's mechanism of action (MOA) primarily involves the inhibition of cyclooxygenase (COX) enzymes, specifically COX-1 and COX-2. By blocking these enzymes, diclofenac reduces the synthesis of prostaglandins, which are mediators of inflammation, pain, and fever. This results in decreased inflammation and pain relief. Additionally, diclofenac may also have effects on other inflammatory pathways, contributing to its therapeutic effects.

**ANALYTICAL METHODS :**

 **Validation of Simple UV-Spectrophotometric Method**

A simple, selective, accurate, precise spectroscopic method for the estimation of diclofenac sodium in bulk and pharmaceutical tablet dosage form has been developed and validated. The linearity range of the diclofenac is 10-30 μg/ml and all parameters shown in Table 9. The LOD and LOQ were found to be 1.03 μg/ml and 3.12 μg/ml respectively. The amount of diclofenac was calculated as 99.42%. Further the precision of the method was confirmed by the repeatable analysis of solution. The % RSD was found to be 1.370 it indicated that the method has good precision. The percentage recovery was found to be in the range of 98.72-99.15%. The % recovery was calculated for 80%, 100% and 120%. RSD value indicated that there is no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed. All the procedure followed in the present study as per the ICH guidelines. [9]

 **Table 18: Results of Validation Parameters**

|  |  |  |
| --- | --- | --- |
|  **S. No.** | **Parameter** |  **Results** |
|  1 2 3 4 5 6 7 8 9 10 | Absorption maxima (nm)Beers range (μg/ml)Standard Regression EquationCorrelation Coefficient (r2)%AssayPrecision (%RSD)Intraday PrecisionInterday PrecisionAccuracyRobustness (%RSD)LODLOQ |  276.20 10-30 μg/ml Y= 0.0324x + 0.0021 0.9995 99.42 ± 0.0840.622 0.724 99.72 ± 0.080 0.9204 1.03 3.12 |

 **4.5.3** **KETOROLAC**

****

**IUPAC name :** 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid

**Molecular formula:** C15H13NO3

**Molecular weight:** 255.27 g/mol

**Physical properties Appearance:**  white to off-white crystalline powder.

**Solubility:**  Soluble in water, methanol, and ethanol; slightly soluble in chloroform.

**Boiling point:**  Not typically specified, but it decomposes before boiling.

**Melting point:** 162-165 °C

**Pharmacological action :**

Cyclooxygenase Inhibition: Ketorolac inhibits both COX-1 and COX-2 enzymes. COX-1 is primarily involved in the production of prostaglandins that protect the stomach lining and support kidney function, while COX-2 is induced during inflammation and is responsible for pain and swelling. By inhibiting COX-2, ketorolac effectively reduces the synthesis of pro-inflammatory prostaglandins, leading to decreased pain and inflammation. Analgesic Effect: Ketorolac is known for its strong analgesic properties, comparable to opioids but without the risk of addiction associated with narcotics. It is often used in postoperative settings for short-term pain management. Anti-Inflammatory Effect: The reduction of prostaglandins also contributes to ketorolac’s anti-inflammatory effects, making it useful in conditions involving inflammation.

**ANALYTICAL METHODS :**

 **Determination Of Ketorolac In Plasma By HPLC**

A simple and sensitive method for the determination of ketorolac in plasma by HPLC was developed and validated. Adequate specificity, precision and accuracy of the proposed method were demonstrated over the concentration range of 10.0e125.0 ng/ml. The method was accurate, reproducible, specific and applicable to the evaluation of pharmacokinetic study of ketorolac. [10]

**Table 19: Intraday and interday accuracy and precision of ketorolac**

|  |  |
| --- | --- |
| Standard concentration(ng/mL) | Average calculatedconcentration (ng/mL) Mean ± SD |
| Interday (n ₌ 3) 10 80 125Intraday (n ₌ 3) 10 80 125SD = Standard deviation | 9.87 ± 0.0779.47 ± 0.46124.54 ± 0.529.79 ± 0.0879.87 ± 0.71124.49 ± 0.60 |

* 1. **OXICAMS :-**

**4.6.1 PIROXICAM**

 

**IUPAC name :** 4-hydroxy-2-methyl-1,1-dioxo-*N*-pyridin-2-yl-1λ6,2-benzothiazine-3-carboxamide

**Molecular formula:** C15H13N3O4S

**Molecular weight:** 331.3 g/mol

**Physical properties Appearance:**  yellow to orange crystalline powder.

 **Solubility:** Not typically specified; tends to decompose before boiling.

**Boiling point:**  Not typically specified; tends to decompose before boiling.

**Melting point:** 198-200 °C

**Pharmacological action :**

COX Inhibition: It inhibits both COX-1 and COX-2 enzymes, leading to decreased synthesis of prostaglandins. This results in reduced inflammation, pain, and fever. Analgesic and Anti-inflammatory Effects: Piroxicam is effective in managing pain and inflammation in conditions such as arthritis and other inflammatory disorders. Long Half-Life: It has a relatively long half-life (around 50 hours), allowing for once-daily dosing.

**ANALYTICAL METHODS :**

**HPLC:**

HPLC is at present one of the most sophisticated tools of analysis. The estimation of Piroxicam is done by RP-HPLC. The mobile phase consists of buffer (volumes of phosphate buffer, 55 volumes of Methanol and 45 volumes of buffer. The ratio pH was found to be 3.0. Then finally filtered using 0.45μ nylon membrane filter and degassed in sonicator for 10 minutes). The detection is carried out using PDA detector set at 240nm. The solutions are chromatographer at the constant flow rate of 0.8 ml/min. The Retention time for Piroxicam was around 7.0 minutes. Linearity range for Piroxicam is 50 to 150μg/ml. The quantitative estimation was carried out on the tablet by RP-HPLC taking a concentration of 50μg/ml. the quantitative results obtained is subjected to the statistical validation. The values of RSD are less than 2.0% indicating the accuracy and precision of the method. The % recovery 98.0% to 99.80% for Piroxicam. The results obtained on the validation parameter met the requirements. It inferred that the method was found to be Simple, Specific, Precision, and Linearity, Proportional i.e. it follows Lambert-Beer’s law. The method was found to have a suitable application in routine laboratory analysis with a high degree of Accuracy and Precision. [11]

 **Table 20. Results of HPLC system suitability parameters for Piroxicam**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S.NO | Parameter | Calibration range (mg/ml)  | Theoretical plates  | Tailing factor  | Correlation Coefficient(r2)  | % Recovery  | System Suitability %RSD  | Method Repeatability %RSD  |
| Drug name  |
| 1. | Piroxicam | 5-150 | 8881.6 | 1.20 | 0.9991 | 98.0% - 99.8% | 0.4% | 1.9% |

**HPLC with UV detection:**

The present study describe the development and validation of a novel fast and accurate HPLC method with UV detection for the quantitative determination of Piroxicam in bulk and pharmaceutical formulations.In the study, the recovery percentage of piroxicam were higher than those obtained using othermethods.The newly developed method has a much faster analysis time.It is a simple method that eliminates the need for mass spectrometryand large volumes of organic solvents. The proposed method can be used to for the analysis of piroxicam in pharmaceuticals formulations used for the treatment of artrithis. These studies also provide information about the degradation pathways and degradation products that could form during storage. [12]

 **Table 21. Stress testing results of piroxicam**

|  |  |  |  |
| --- | --- | --- | --- |
| **Nature of stress** | **Storage conditions** | **Time (h)** | **Amount of piroxicam remaining (%)** |
| 2M HCl | 40°C | 5.00 | 95.56 |
| 2M NaOH | 40°C | 10.0 | 90.25 |
| 3% H2O2 | ambient | 0.30 | 80.25 |
| Photolytic | sunlight | 0.30 | 91.25 |
| UV  | UV lamp | 0.30 | 79.56 |
| Thermal | 105°C | 2.00 | 60.25 |

* 1. **PYRAZOLONE AND PYRAZOLIDINEDIONE :-**

**4.7.1 PHENYLBUTAZONE**

 ****

**IUPAC name :** 4-butyl-1,2-diphenylpyrazolidine-3,5-dione

**Molecular formula:** C19H20N2O2

**Molecular weight:** 308.4 g/mol

**Physical properties Appearance:** White to off-white crystalline powder.

**Solubility:** Slightly soluble in water; soluble in organic solvents like ethanol and acetone

**Boiling point:** 438 °C

**Melting point:** 105°C

**Pharmacological action :** Phenylbutazone acts as a nonsteroidal anti-inflammatory drug (NSAID). It reduces inflammation and pain by inhibiting cyclooxygenase (COX) enzymes, which play a key role in the production of prostaglandins—compounds involved in inflammation and pain signaling.

**ANALYTICAL METHODS :**

**Using TLC:**

In analysis using TLC, There is one sample of tested herbal medicine that is suspected to contain BKO phenylbutazone, namely 0.3%. There was one sample containing BKO phenylbutazone with an Rf value in three consecutive mobile phases of 0.5, 0.7, and 0.6. Meanwhile, in the analysis using UV-Vis’s spectrophotometry, data was obtained in the form of maximum wavelength, absorbance, calibration curve equation, concentration for calculating precision tests, accuracy, and sample levels. In the quantitative analysis of phenylbutazone, it was found that the limit of detection (LOD) was 0.09 μg⁄mL and the limit of quantitation (LOQ) was 0.3 μg⁄m. [13]

**Table 22: Phenylbutazone Mobile Phase Optimization Results.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|

|  |  |  |
| --- | --- | --- |
| Movement Phase  |  |  Rf Score  |
| Ethyl Acetate: N-hexane (1:4)  |  |  0.6  |
| Chloroform: Ethanol (9:1)  |  |  0.9  |
| N-hexane: Chloroform: Methanol (6:3:1)  |  |  0.8  |
| Ethyl Acetate: Chloroform (2:1)  |  |  0.87  |
| Ethyl Acetate: Methanol: Ammonia (7:2:1)  |  |  0.95  |

 |

 **HPLC**

The procedure described allows the quantitative and qualitative detection of phenylbutazone in plas- ma samples from fighting bulls. The sensitivity of the method, with a detection limit at 0.016 μg/ml and a quantification limit of 0.029 μg/ml (λ= 240 nm), is better than that of Salvadori et al. The presence of phenylbutazone was checked and quantified in unknown samples of plasma from bulls killed in 2nd and 3rd category bullrings in the province of Salamanca (Spain), and it is possible to use the method for the simultaneous analysis of dexamethasone, betamethasone, phenylbutazone and flunixin in plasma from fighting bulls. [14]

 **Table 23. Results of HPLC system suitability parameters for phenylbutazone**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S.NO |  ParameterDrug name | λ | Sensitivity (mg/ml) | Intra-assay C.V. (%) | Inter-assay C.V. (%) | Recovery (%) |
| Detection limit | Quantitation limit | Plasmaa | Methanolb | Plasmaa | Methanolb |
| 1. | Phenylbutazone | 240 nm | 0.016 | 0.029 | 2.04 | 1.73 | 1.72 | 1.83 | 83 |
| 254 nm  | 0.027 | 0.061 | 1.69 | 1.87 | 1.69 | 1.69 | 84 |

* + 1. **ANTIPYRINE**

 ****

**IUPAC name :** 1,5-dimethyl-2-phenylpyrazol-3-one

**Molecular formula:** C11H12N2O

**Molecular weight:** 188.23 g/mol

**Physical properties Appearance:** White to light yellow crystalline powder

**Solubility:** Soluble in alcohol and chloroform; slightly soluble in water

**Boiling point:** 319°C

**Melting point:** 114°C

**Pharmacological action :** Antipyrine is an analgesic (pain reliever) and antipyretic (fever reducer) agent. It is used to relieve pain and reduce fever, often in combination with other medications. Antipyrine has anti-inflammatory properties and can also be used as a diagnostic tool in ear examinations.

**ANALYTICAL METHODS :**

**TLC-densitometric method**

In this work, two stability-indicating methods were developed for the simultaneous determination of antipyrine in the presence of the reported BEN degradation product (PABA). The advantage of the TLC-densitometric method is that several samples can be run simultaneously using a small quantity of the mobile phase, thus lowering the analysis time and cost per analysis, as well as providing high sensitivity and selectivity. [15]

**Table 24. Results of TLC system suitability parameters for Antipyrine**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| S.NO |  ParametersDrug name | Mean | S.D | *n* | Variance | Student’s *t*-test | *F*-test |
| 1. | Antipyrine | 100.22 | 1.375 | 6 | 1.89 | 0.222 (2.262) | 3.88 (6.26) |

**HPLC**

In this work, two stability-indicating methods were developed for the simultaneous determination of antipyrine in the presence of the reported BEN degradation product (PABA). HPLC has the advantage of being highly selective compared with other published HPLC methods, which cannot separate ANT and BEN in the presence of PABA. [15]

**Table 25. Results of HPLC system suitability parameters for Antipyrine**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| S.NO |  ParametersDrug name | Mean | S.D | *n* | Variance | Student’s *t*-test | *F*-test |
| 1. | Antipyrine | 99.93 | 0.985 | 7 | 0.985 | 0.271 (2.228) | 1.99 (6.16) |

* 1. **PYRROLE-ACETIC ACID :-**

**4.8.1 ZOMEPIRAC**

****

**IUPAC name :** 2-[5-(4-chlorobenzoyl)-1,4-dimethylpyrrol-2-yl]acetic acid

**Molecular formula:** C15H14ClNO3

**Molecular weight:** 291.73 g/mol

**Physical properties Appearance:** White to off-white crystalline powder

**Solubility:** Soluble in water and organic solvents

**Boiling point:** Not readily available, as it's typically not reported

**Melting point:** 178.5°C

**Pharmacological action :** Zomepirac acts primarily as a nonsteroidal anti-inflammatory drug (NSAID). Its mechanism of action involves: **Inhibition of Cyclooxygenase (COX) Enzymes:** Zomepirac inhibits COX-1 and COX-2 enzymes, which play a key role in the conversion of arachidonic acid to prostaglandins. Prostaglandins are mediators of inflammation, pain, and fever. **Reduction of Prostaglandin Synthesis:** By inhibiting COX enzymes, zomepirac decreases the synthesis of prostaglandins, leading to reduced inflammation, pain relief, and a decrease in fever. Overall, its analgesic and anti-inflammatory effects stem from the reduction of prostaglandin levels in the body.

**ANALYTICAL METHODS :**

**HPLC**

The lowest concentration of zomepirac that can be measured accurately and precisely (coefficient of variation <20%) in a z-ml plasma sample is 10 ng/ml. The standard curve is linear in the concentration range of 10 to 5000 n\_g/ml. To date, this procedure has been employed successfully in analysin g over 10,000 clinical plasma samples. [16]

**Table 26. Results of HPLC system suitability parameters for Zomepirac**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| S.NO |  ParametersDrug name | Plasma conc.ng/ml | Mean peak height ratio | Standard deviation  | Co-efficient of variation (%) |
| 1. | Zomepirac | 10-5000 | 0.10-57.96 | 0.01-3.25 | 9.7-5.6 |

* 1. **PARA-AMINO PHENOL:-**

**4.9.1 PHENACITIN**

****

**IUPAC name :** *N*-(4-ethoxyphenyl)acetamide

**Molecular formula:** C10H13NO2

**Molecular weight:** 179.22 g/mol

**Physical properties Appearance:** White crystalline powder

**Solubility:** Soluble in alcohol, ether, and chloroform; slightly soluble in water

**Boiling point:** 242-245 °C

**Melting point:** 134-135 °C

**Pharmacological action :** Phenacetin is an analgesic and antipyretic agent, primarily used for pain relief and to reduce fever. It works by inhibiting the synthesis of prostaglandins, similar to other NSAIDs. However, phenacetin has been largely withdrawn from the market in many countries due to safety concerns, including potential links to kidney damage and cancer.

**ANALYTICAL METHODS :**

**HPLC**

The proposed method is simple, accurate, reproducible and fast. It can determine the phenacetin, the adulterants/ diluents including saccharin simultaneously. The present method can be routinely used for the analysis of these components in illicit phenacetin samples and it will be a valuable method for drug profiling. [18]

 **Table 27. Results of HPLC system suitability parameters for Phenacetin**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| S.NO |  ParametersDrug name | Limit ofdetection(LOD) | Limit ofquantification(LOQ) | Linearityrange | %Recovery | Intraday recovery ± S.D | Interday recovery ± S.D |
| 1. | Phenacetin | 10 ng | 33 ng | 200ng – 2000ng | 101.53 | 1.47 | 1.92 |

* + 1. **ACETAMINOPHEN**

****

**IUPAC name :** *N*-(4-hydroxyphenyl)acetamide

**Molecular formula:** C8H9NO2

**Molecular weight:** 151.16 g/mol

**Physical properties Appearance:** White crystalline powder

**Solubility:** Soluble in water, alcohol, and ether

**Boiling point:** >500 °C

**Melting point:** 169-170.5 °C

**Pharmacological action :** Acetaminophen is an analgesic and antipyretic medication used to relieve pain and reduce fever. **Inhibition of Prostaglandin Synthesis:** Acetaminophen primarily inhibits the cyclooxygenase (COX) enzymes, particularly in the brain, leading to decreased production of prostaglandins, which are mediators of pain and fever. **Central Nervous System Action:** It is thought to have a central analgesic effect, altering the perception of pain. Acetaminophen is widely used for its effectiveness and safety when taken at recommended doses.

**ANALYTICAL METHODS :**

**RP-HPLC**

The developed method was validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for the simultaneous estimation of Acetaminophen in tablet dosage form, different chromatographic conditions for its validation were applied & the results observed are presented (Shown in table). The results of Correlation coefficient (r) LOD, LOQ, Accuracy, Precision, Robustness and Ruggedness Shown in table. [19]

**Table 28: Results from Analysis and Calibration Curves.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S.NO |  ParametersDrug name | Correlation coefficient (r) |  LOD (μg/mL) | LOQ (μg/mL) |  Accuracy (%) ± % RSD |  Precision ( % RSD) | Robustness | Ruggedness |
| 1. | Acetaminophen |  0.99  | 0.05 |  0.12  | 99.96%  | 1.33  |  0.62  | 0.81% |

**HPLC**

A new HPLC method for the determination of acetaminophen was developed using a mixed-mode Hypersil Duet C18/SCX stationary phase. The separation was achieved using a mixture of phosphate buffer (pH ¼ 4.88)–methanol (80:20 v/v) as mobile phase. Both elution and flow rate gradients contribute to the total time of analysis lower than 15 minutes. The selectivity and the efficiency of the separation are very good. The chromatographic method was validated in the laboratory. A fluorescence program for detection in HPLC was also developed, with the aim of analyzing samples with low concentrations of acetaminophen impurities. The influence of the temperature on the separation of the analyte was also studied; the linearity of the van’t Hoff plots indicates that the retention mechanism of each compound does not change when temperature increases. The method was applied with good results on commercially available acetaminophen tablets. [20]

 **Table 29. Results of HPLC system suitability parameters for acetaminophen**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.NO |  ParametersDrug name |  Concentration | Recovery [%+s.d.] |   Precission (% R.S.D.) |
| Intra-day | Inter-day |
| 1. | Acetaminophen | 40 mg/mL | 100.7+ 0.42 | 1.29 | 1.45 |

**CONCLUSION**

 **5. CONCLUSION**

In this literature study, the most popular Spectrophotometric and Chromatographic analytical procedures has been listed. The development of these methods involved the use of analytical equipment such as UV-Visible Spectrophotometers, High-Performance Liquid Chromatography, Reverse-Phase High-Performance Liquid Chromatography and Thin Layer Chromatography. It was developed to describe particular anti-inflammatory drugs. Salicylates, anthranillic acids, indole acetic acids, aryl propionic acids, oxicams in their pure form, human plasma, and other biological fluids. Validation parameters mainly addressed on flow rate, wavelength, linearity, LOD, LOQ, accuracy and precision.

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