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A REVIEW ON UV, HPLC AND HPTLC ASSESSMENT TECHNIQUES AVAILABLE ON PAIN RELEIVING AND ANTI INFLAMMATRY DRUGS

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ABSTRACT

The evaluation of pain-relieving and anti-inflammatory drugs, including NSAIDs and opioids, is crucial for ensuring therapeutic efficacy and safety. Analytical techniques such as UV spectrophotometry, High-Performance Liquid Chromatography (HPLC), and High-Performance Thin Layer Chromatography (HPTLC) are widely utilized for this purpose. UV spectrophotometry offers a rapid and cost-effective means of quantifying drug compounds based on UV light absorption, though it lacks specificity compared to chromatographic methods. HPLC provides high sensitivity and resolution, enabling precise separation, identification, and quantification of drug components in complex matrices, making it a gold standard for drug analysis. HPTLC is a versatile and efficient method that allows simultaneous analysis of multiple samples with minimal solvent use, but it generally has lower sensitivity compared to HPLC. This review outlines the principles, advantages, and limitations of these techniques, highlighting their roles in drug quality control and regulatory compliance.

KEYWORDS: Pain-relieving drugs, Anti-inflammatory drugs, NSAIDs, Opioids, UV spectrophotometry, High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC), Drug analysis, Analytical techniques, Drug quality control, Therapeutic efficacy, Regulatory compliance.

INTRODUCTION

Anti inflammatory[1] and pain relieving drugs[2]

Anti-inflammatory and pain-relieving drugs are medications designed to reduce inflammation and alleviate pain. Anti-inflammatory drugs, such as NSAIDs (e.g., ibuprofen and aspirin), work by inhibiting enzymes involved in the inflammatory process, thereby reducing swelling and pain. Pain-relieving drugs, including opioids (e.g., morphine and oxycodone), target the central nervous system to block pain signals. Both types of drugs play critical roles in managing conditions like arthritis, injuries, and chronic pain. While anti-inflammatory drugs focus on reducing inflammation, pain-relievers aim to diminish pain sensation, contributing to overall patient comfort and recovery.

Codeine

Codeine[3] is an opioid analgesic used to treat mild to moderate pain and to alleviate cough. It is derived from the opium poppy, making it a natural opioid, but it is less potent than morphine and other stronger opioids.[4] Codeine works by binding to opioid receptors in the brain and spinal cord, which helps block pain signals and reduce coughing. It is often prescribed in combination with other medications, such as acetaminophen or aspirin, to enhance its pain-relieving effects.[5] Despite its effectiveness, codeine can cause side effects such as drowsiness, constipation, and nausea.[6] It also carries a risk of dependence and abuse, especially with prolonged use.[7] In some countries, codeine is available over-the-counter in lower doses, but higher doses require a prescription due to its potential for addiction and misuse.[8]

- **IUPAC Name:** (5α,6α)-7,8-Didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol[9]
- **Molecular Formula**: $C_{18}H_{21}NO_3$[10]
- **Molecular Weight**: 299.37 g/mol[11]
- **CAS Number**: 76-57-3[12]

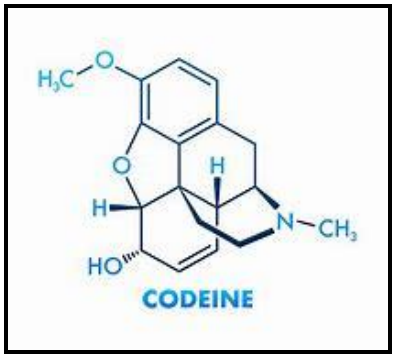

Fig: 1 Structure of Codeine.

Diclofenac

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) used to relieve pain, reduce inflammation, and lower fever.[13] It works by inhibiting enzymes involved in prostaglandin production, which helps decrease pain and swelling. Commonly prescribed for conditions like arthritis, musculoskeletal injuries, and menstrual pain, diclofenac is available in oral, topical, and suppository forms. While effective, it can cause side effects such as gastrointestinal discomfort, ulcers, and increased cardiovascular risk. Due to these potential issues, it is generally recommended for short-term use at the lowest effective dose, with regular monitoring by a healthcare provider.[14]

- I**UPAC Name**: 2-[(2,6-Dichlorophenyl) amino] benzeneacetic acid[15]
- **Molecular Formula**: $C_{14}H_{11}Cl_2NO_2$[16]
- **Molecular Weight**: 296.15 g/mol[17]
- **CAS Number**: 15307-86-5[17]

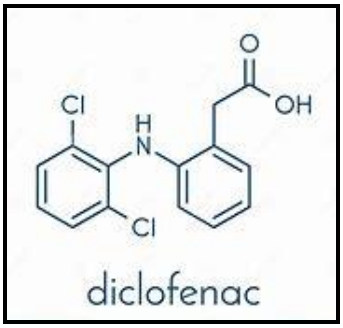

Fig. 2: Structure of Diclofenac.

Combined Use

Combining diclofenac and codeine can enhance pain relief by leveraging their different mechanisms. Diclofenac, an NSAID, reduces inflammation and pain by inhibiting prostaglandin production, while codeine, an opioid, alleviates pain by acting on the central nervous system. This combination is often used for moderate to severe pain when a single medication is insufficient. However, it requires careful management due to potential side effects such as gastrointestinal issues from diclofenac and sedation or dependence from codeine. Always use this combination under the guidance of a healthcare provider to balance efficacy and safety.[18,19,20]

Pharmacokinetic Properties of both Cofeine and diclofenac

CODEINE[21]

1. Absorption

- **Bioavailability:** Well absorbed orally, with 50-60% bioavailability due to first-pass metabolism.
- **Peak Plasma Concentration:** Reached within 1 hour after oral intake.
- **Effect of Food:** Food may delay absorption slightly but does not significantly affect it.

2. Distribution

- **Plasma Protein Binding:** Moderately bound to plasma proteins (25-30%).
- **Volume of Distribution:** Approximately 3-6 L/kg.
- **Tissue Distribution:** Distributed throughout the body, including the CNS, for analgesic effects.

3. Metabolism

- **Primary Metabolism:** Metabolized in the liver by CYP2D6, converting 5-10% to morphine.
- **First Pass Effect:** Reduces unchanged codeine in systemic circulation.

4. Excretion

- **Elimination Half-life:** About 2.5-3 hours.
- **Routes of Excretion:** Primarily excreted in urine, with 90% eliminated within 24 hours.
- **Renal Clearance:** High, with most excreted as metabolites.

DICLOFENAC[22]

1. Absorption

- **Bioavailability:** Oral bioavailability is 50-60% due to extensive first-pass metabolism.
- **Peak Plasma Concentration:** Achieved within 1-2 hours after administration.
- **Effect of Food:** Food may delay absorption and reduce peak concentrations but does not affect the total absorption.

2. Distribution

- **Plasma Protein Binding:** Highly bound to plasma proteins (99.7%), primarily albumin.
- **Volume of Distribution:** Approximately 0.12-0.17 L/kg.
- **Tissue Distribution:** Widely distributed, including in synovial fluid, for anti-inflammatory effects.

3. Metabolism

- **Primary Metabolism:** Metabolized in the liver by CYP2C9 into largely inactive hydroxylated metabolites.
- **First Pass Effect:** Reduces bioavailability.

4. Excretion

- **Elimination Half-life:** About 1-2 hours.
- **Routes of Excretion:** Primarily excreted in urine (65%), with some in bile and feces.

- **Renal Clearance:** Low for unchanged diclofenac due to extensive metabolism.

Pharmacodynamic Properties[23,24,25]

Pharmacodynamic properties describe the effects of a drug on the body, focusing on how the drug interacts with its target (e.g., receptors, enzymes) to produce therapeutic and side effects.

Codeine

1. Mechanism of Action

Codeine, an opioid analgesic, binds to mu-opioid receptors in the central nervous system, inhibiting pain pathways and altering pain perception. About 5-10% is metabolized into morphine, which has a higher affinity for opioid receptors and contributes significantly to pain relief.

2. Effects

- **Analgesia:** Provides relief for mild to moderate pain.
- **Cough Suppression:** Acts on the cough center in the medulla to suppress coughing.
- **Sedation:** Can cause drowsiness due to CNS depressant effects.
- **Respiratory Depression:** Higher doses may depress respiratory function, posing risks.

3. Side Effects

Common side effects include constipation, nausea, dizziness, and drowsiness. Higher doses may lead to respiratory depression, hypotension, and potential addiction or abuse.

Diclofenac[26,27,28]

1. Mechanism of Action

Diclofenac, an NSAID, inhibits cyclooxygenase (COX) enzymes (COX-1 and COX-2), reducing prostaglandin synthesis. This leads to decreased inflammation, pain, and fever.

2. Effects

- **Anti-inflammatory:** Reduces inflammation in conditions like arthritis.
- **Analgesic:** Alleviates mild to moderate pain in osteoarthritis, rheumatoid arthritis, and musculoskeletal injuries.
- **Antipyretic:** Lowers fever, though this is not its primary use.

3. Side Effects

Common side effects include gastrointestinal issues (nausea, dyspepsia, abdominal pain), risk of ulcers and bleeding, headaches, dizziness, and liver enzyme elevations. Long-term use can increase cardiovascular risk.

Combination of Codeine with Other Drugs[29,30]

- **Codeine and Acetaminophen:** Enhances pain relief by combining codeine's opioid effects with acetaminophen's analgesic properties.
- **Codeine and NSAIDs (e.g., Ibuprofen or Diclofenac):** Provides combined central and peripheral pain relief, addressing both inflammation and pain.
- **Codeine and Muscle Relaxants:** Offers comprehensive relief from pain and muscle spasms.
- **Codeine and Cough Suppressants:** Codeine's cough-suppressing effects can be boosted by other cough medications for severe cough control.

Analytical methods involved in both Codeine and diclofenac

1. UV Spectrophotometry[31]

UV spectrophotometry measures how a substance absorbs ultraviolet or visible light (200-800 nm). This technique is based on the absorption of light by molecules, which excites electrons to higher energy levels. In a UV spectrometer, light passes through a sample, and absorbance is recorded, creating a spectrum of absorbance versus wavelength. It is widely used for identifying and quantifying organic compounds, offering advantages in pharmaceutical analysis, environmental testing, and quality control due to its simplicity and non-destructive nature. However, it requires the sample to be appropriately prepared and may struggle with complex mixtures due to overlapping absorption bands.

2. High-Performance Liquid Chromatography (HPLC)[32]

HPLC is an analytical technique used to separate, identify, and quantify components in a mixture. It involves passing a liquid sample through a column packed with a stationary phase while a mobile phase (solvent) flows through. Components interact differently with the stationary phase, leading to separation. Separated components are detected using UV/VIS detectors, or other methods like fluorescence or mass spectrometry. HPLC offers high resolution and sensitivity, making it ideal for analyzing complex mixtures in pharmaceuticals, environmental samples, and biochemical research. Despite its accuracy and reproducibility, HPLC requires careful method development and equipment maintenance.

3. High-Performance Thin-Layer Chromatography (HPTLC)[33]

HPLC separates, identifies, and quantifies components in a mixture by passing a liquid sample through a column with a stationary phase, while a mobile phase flows through. Components interact differently with the stationary phase, causing separation. Detectors like UV/Vis, fluorescence, or mass spectrometry then identify the separated components. HPLC is highly sensitive and provides high resolution, making it suitable for pharmaceuticals, environmental samples, and biochemical research. It requires careful method

development and maintenance despite its accuracy and reproducibility.

Table 1: Different methods involved in UV Spectrophotometry.

Drug	Solvent	Wavelength	Methods	Methods description	Reference
Codeine	Methanol, Water	260 nm	Direct UV Absorption	Measures the absorbance of codeine directly in solution.	[34]
Codeine	Varies (e.g., Acetonitrile)	Specific to derivatized form	Derivatization Techniques	Codeine is derivatized to enhance UV absorbance or differentiate from other substances.	*[35]*
Codeine	Water, Methanol, or Acetonitrile	Variable (e.g., 260 nm)	Difference Spectroscopy	Compares UV absorbance before and after specific reactions to identify and quantify codeine.	*[36]*
Codeine	Water, Methanol, or Acetonitrile	260 nm	Spectrophotometric Method with Solvent Extraction	Codeine is extracted from a sample using a solvent, and its concentration is determined by UV measurement.	*[37]*
Codeine	Methanol, Water	Variable (e.g., 260 nm)	Spectrophotometric Method with Solvent Extraction	Utilizes derivative or ratio spectra methods to analyze mixtures containing codeine and other substances.	*[38]*

Table 2: Different methods involved in HPLC.

Method	Drug Column	Mobile Phase	RF Value	Reverse Phase	Injection Volume	Reference
Method 1	C18 column (150 mm × 4.6 mm, 5 µm)	Methanol: Phosphate Buffer (70:30 v/v, pH 3.5)	6.2 min	Yes	20 µL	*[39]*
Method 2	Hypersil BDS C18 (250 mm × 4.6 mm, 5 µm)	Acetonitrile: Water (60:40 v/v) with 0.1% trifluoroacetic acid	5.1 min	Yes	10 µL	*[40]*
Method 3	Symmetry C18 (100 mm × 4.6 mm, 3.5 µm)	Methanol: Acetonitrile: Phosphate Buffer (50:25:25 v/v, pH 2.8)	4.0 min	Yes	25 µL	*[41]*
Method 4	Zorbax Eclipse XDB-C18 (150 mm × 4.6 mm, 3.5 µm)	Water: Methanol (50:50 v/v)	5.7 min	Yes	15 µL	*[42]*
Method 5	Phenomenex Luna C18 (250 mm × 4.6 mm, 5 µm)	Water: Acetonitrile (65:35 v/v, pH 3.0)	6.5 min	Yes	20 µL	*[43]*

Table 3: Different methods involved in HPTLC.

Method	Stationary Phase	Mobile Phase	RF Value	Linearity	Reference
HPTLC Method 1	Silica gel 60 F254	Ethyl acetate: Methanol: Ammonia (80:10:10 v/v/v)	0.32	100 - 600 ng/spot (r^2 = 0.997)	*[44]]*
HPTLC Method 2	Silica gel 60 F254	Chloroform: Methanol (90:10 v/v)	0.44	200 - 1000 ng/spot (r^2 = 0.999)L	*[45]*
HPTLC Method 3	Silica gel 60 F254	Toluene: Methanol: Acetic acid (80:10:10 v/v/v)	0.29	50 - 500 ng/spot (r^2 = 0.998)	*[46]*
HPTLC Method 4	Silica gel 60 F254	Methanol: Ethyl acetate: Ammonia (70:25:5 v/v/v)	0.40	100 - 500 ng/spot (r^2 = 0.996)	*[47]*
HPTLC Method 5	Silica gel 60 F254	Methanol: Dichloromethane (80:20 v/v)	0.36	150 - 750 ng/spot (r^2 = 0.995)	*[48]*

Analytical methods used for the detection and quantification of diclofenac

Table 1: UV-Visible Spectrophotometry.

Method	Solvent	Wavelength (nm)	Method Description	Reference
Direct UV Spectrophotometric Method	Methanol	276 nm	Simple method where Diclofenac is dissolved in methanol and absorbance measured at 276 nm.	[49]
Derivative Spectrophotometry (1st Derivative)	Methanol, water	276 nm	First derivative spectra improve peak resolution by reducing baseline drift.	[50]
UV Method with Fe(III) Complexation	Water	425 nm	Diclofenac forms a colored complex with Fe(III), which shifts the absorbance to 425 nm.	[51]
Simultaneous Estimation in Combination Drugs	Methanol	2765nm	Used for formulations containing Diclofenac and Paracetamol, applying multicomponent analysis.	[52]
Area Under Curve (AUC) Method	Methanol, Water	270-280 nm	Measures absorbance over a wavelength range to improve accuracy.	[53]

Table 2: High-Performance Liquid Chromatography (HPLC).

Method	Drug Column	Mobile Phase	RF Value	Reverse Phase	Injection Volume	Reference
HPLC Method 1	C18 (e.g., Waters XTerra)	Methanol/Phosphate Buffer (70:30, v/v)	Not applicable	Yes	20 µL	[54]
HPLC Method 2	C18 (e.g., Phenomenex Luna)	Acetonitrile/Water (60:40, v/v)	Not applicable	Yes	10-20 µL	[56]
HPLC Method 3	C18 (e.g., Agilent Zorbax)	Methanol/Water (50:50, v/v)	Not applicable	Yes	20 µL	[57]
HPLC Method 4	C18 (e.g., Shim-pack CLC-ODS)	Phosphate Buffer/Methanol (75:25, v/v)	Not applicable	Yes	10 µL	[58]
HPLC Method 5	C18 (e.g., Supelco LC-18)	Acetonitrile/Phosphate Buffer (60:40, v/v)	Not applicable	Yes	20 µL	[59]

Table 3: High-Performance Thin-Layer Chromatography (HPTLC).

Method	Stationary Phase	Mobile Phase	RF Value	Linearity	Reference
HPTLC Method 1	Silica Gel 60 F254	Ethyl Acetate/Hexane (4:6, v/v)	~0.40	1-25 µg/mL	[60]
HPTLC Method 2	Silica Gel 60 F254	Toluene/Ethyl Acetate/Glacial Acetic Acid (5:4:1, v/v/v)	~0.45	0.5-20 µg/mL	[61]
HPTLC Method 3	Aluminum Oxide 60 F254	Chloroform/Methanol (9:1, v/v)	~0.42	1-15 µg/mL	[62]
HPTLC Method 4	Silica Gel 60 F254	Methanol/Chloroform (1:9, v/v)	~0.38	0.5-25 µg/mL	[63]
HPTLC Method 5	Silica Gel 60 F254	Ethanol/Water (7:3, v/v)	~0.47	0.1-20 µg/mL	[64]

Combination of diclofenac with other drug[65,66,67,68]

Diclofenac is frequently combined with other drugs to enhance treatment efficacy or reduce side effects. For example, diclofenac is often paired with misoprostol to protect the stomach lining and reduce gastrointestinal issues associated with NSAIDs. Another common combination is with opioids like codeine or tramadol, which helps manage severe pain while allowing for lower opioid doses and reducing the risk of dependency. Additionally, diclofenac can be combined with muscle relaxants, such as cyclobenzaprine, to address both inflammation and muscle spasms in conditions like acute musculoskeletal pain. Each combination is carefully selected to balance efficacy and safety.

CONCLUSION

The assessment techniques for pain-relieving and anti-inflammatory drugs such as UV spectrophotometry, High-Performance Liquid Chromatography (HPLC), and High-Performance Thin Layer Chromatography (HPTLC) each offer distinct advantages and limitations. UV spectrophotometry, while cost-effective and simple,

lacks the specificity required for complex drug formulations. HPLC, on the other hand, is the gold standard in analytical chemistry due to its high sensitivity, precision, and ability to separate, identify, and quantify drug components even in complex matrices. HPTLC, though less sensitive than HPLC, offers efficiency in simultaneously analyzing multiple samples with minimal solvent use, making it suitable for high-throughput drug screening.

For the analysis of drugs like codeine (an opioid) and diclofenac (an NSAID), these methods play a crucial role in ensuring quality control, therapeutic efficacy, and regulatory compliance. The combination of these drugs, while potentially enhancing pain relief through their complementary mechanisms, requires careful analysis to manage risks such as gastrointestinal issues from diclofenac and dependence from codeine. Overall, UV, HPLC, and HPTLC techniques contribute to the rigorous evaluation of drug safety and efficacy, ensuring that medications meet regulatory standards and offer the intended therapeutic benefits to patients.

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