
**METHOD DEVELOPMENT AND VALIDATION OF HPLC FOR
DRUGS USED IN OSTEOARTHRITIS TREATMENT**

Majid Ayaz*¹, Mr Rohit Singh Rathore²

¹Bachelor of Pharmacy Student, Department of Pharmaceutical Sciences, Mewar University, Gangrar, Chittorgarh, Rajasthan, India.

²Supervisor and Assistant Professor, Mewar University, Gangrar, Chittorgarh, Rajasthan, India.

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***Corresponding Author: Majid Ayaz**

Bachelor of Pharmacy Student, Department of Pharmaceutical Sciences, Mewar University, Gangrar, Chittorgarh, Rajasthan, India.

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ABSTRACT

Osteoarthritis is one of the most common chronic degenerative joint disorders affecting the elderly population worldwide. It is characterized by progressive destruction of articular cartilage, narrowing of joint space, pain, stiffness, inflammation, and reduced mobility. The treatment of osteoarthritis mainly focuses on relieving pain, reducing inflammation, and improving joint function. Paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medicines for symptomatic management. In pharmaceutical industries, quality control of these drugs is essential to ensure safety, efficacy, and consistency. High Performance Liquid Chromatography (HPLC) is considered one of the most reliable analytical techniques for estimation of these drugs in bulk and dosage forms. The present review article describes osteoarthritis, commonly used treatment drugs, and HPLC method development with validation according to ICH guidelines. Important parameters such as linearity, range, precision, accuracy, LOD, LOQ, robustness, and final results are discussed in detail.

1. INTRODUCTION

Osteoarthritis (OA) is a slowly progressive musculoskeletal disorder in which degeneration of cartilage occurs along with changes in subchondral bone and synovial tissue. It is one of the leading causes of disability and pain in older adults. The disease commonly affects weight-bearing joints such as the knee and hip, but it can also involve the hands, shoulder,

and spine. Osteoarthritis develops due to aging, obesity, trauma, genetic predisposition, overuse of joints, and metabolic disturbances.

The major symptoms include chronic joint pain, morning stiffness, tenderness, swelling, restricted movement, and crepitus during movement. As the disease progresses, patients experience difficulty in walking, climbing stairs, and performing daily activities. Since there is no complete cure for osteoarthritis, drug therapy remains important for symptomatic relief.

Pharmaceutical formulations containing paracetamol, diclofenac, aceclofenac, ibuprofen, naproxen, and selective COX-2 inhibitors are widely used in management of osteoarthritis. These drugs require proper analytical evaluation. HPLC is extensively employed because it provides high sensitivity, specificity, precision, and reproducibility.

OSTEOARTHRITIS

Osteoarthritis (OA) is a degenerative joint disease that occurs when the cartilage that cushions the ends of bones wears down over time. It most commonly affects the knees, hips, hands, and spine.

COMMON SYMPTOMS

- Joint pain
- Stiffness
- Swelling
- Reduced range of motion
- Grating sensation
- Tenderness

JOINT COMPARISON


NORMAL JOINT




OSTEOARTHRITIS



COMMONLY AFFECTED JOINTS




KNEE


HIP





HAND


SPINE

RISK FACTORS

-  Age (risk increases with age)
-  Obesity
-  Joint injury or overuse
-  Family history
-  Sedentary lifestyle

MANAGEMENT AND TREATMENT

-  Weight management
-  Exercise and physical therapy
-  Pain relievers (Paracetamol, NSAIDs)
-  Assistive devices (knee braces, canes)
-  Healthy diet
-  In severe cases, surgery (joint replacement)



2. Drugs Used in Osteoarthritis Treatment

Paracetamol is one of the first-line drugs recommended for mild to moderate osteoarthritis pain. It acts mainly by inhibiting prostaglandin synthesis in the central nervous system. It has analgesic and antipyretic effects with minimal anti-inflammatory action. Due to low gastric irritation compared to NSAIDs, it is considered safer in many patients when used in therapeutic doses.

When pain becomes moderate to severe, NSAIDs are prescribed. These drugs inhibit cyclooxygenase enzymes responsible for prostaglandin production. As a result, pain, swelling,

and inflammation are reduced. Common NSAIDs used in osteoarthritis include diclofenac sodium, ibuprofen, naproxen, aceclofenac, piroxicam, meloxicam, and celecoxib. However, long-term use may cause gastric irritation, renal dysfunction, and cardiovascular effects.

Topical NSAIDs are also useful in osteoarthritis, especially for knee and hand joints. In severe cases, intra-articular corticosteroids or hyaluronic acid injections may be used. Yet oral analgesics remain the most common dosage forms requiring analytical quality control.

3. Importance of HPLC in Drug Analysis

High Performance Liquid Chromatography is an advanced chromatographic technique used for separation, identification, and quantification of compounds present in mixtures. It is highly preferred in pharmaceutical industries because it can analyze active ingredients, impurities, degradation products, and dissolution samples with excellent accuracy.

For osteoarthritis treatment drugs, HPLC is used in assay of tablets, capsules, suspensions, and combined formulations. It is also used during stability studies to determine degradation under heat, light, moisture, and oxidation conditions. Since many formulations contain two or more drugs together, HPLC becomes essential for simultaneous estimation.

Compared to spectrophotometric methods, HPLC offers better selectivity and sensitivity. It can separate closely related compounds and impurities in shorter analysis time.

4. HPLC Method Development

Method development is the process of selecting suitable chromatographic conditions for obtaining sharp peaks, good resolution, and accurate quantification. Reverse phase HPLC is commonly used for paracetamol and NSAIDs due to moderate polarity of these compounds.

A C18 column is generally selected because it provides effective retention and separation. Common dimensions are 250 mm × 4.6 mm with particle size 5 μm. Mobile phase composition depends upon the nature of analytes. Mixtures of methanol-water, acetonitrile-water, or buffer-acetonitrile are widely used. Buffer pH is adjusted to improve peak shape and reduce tailing.

Flow rate is usually maintained around 1.0 mL/min. UV detector is commonly used because these drugs absorb in ultraviolet region. Detection wavelength for paracetamol may be around 243 nm, while diclofenac and aceclofenac absorb near 275 nm. Injection volume

generally ranges from 10 to 20 μL . Total run time is optimized between 5 and 10 minutes for routine analysis.

During development, retention time, theoretical plates, tailing factor, and resolution are carefully observed. Final chromatographic conditions are selected only after repeated trials.

5. Method Validation According to ICH Guidelines

Method validation confirms that the developed analytical procedure is suitable for its intended purpose. International Conference on Harmonisation (ICH) guidelines are accepted globally for validation of pharmaceutical methods.

5.1 Linearity

Linearity expresses the direct proportional relationship between concentration and detector response. Standard solutions of different concentrations are prepared and injected into HPLC. Calibration curve is plotted between concentration and peak area. A straight-line relationship should be obtained over the selected range. Correlation coefficient should normally be close to 1.000.

5.2 Range

Range is the interval between highest and lowest concentration where the method shows acceptable precision, linearity, and accuracy. For assay methods, it usually covers 80% to 120% of test concentration.

5.3 Precision

Precision indicates closeness among repeated measurements under similar conditions. It is expressed as percent relative standard deviation (%RSD). Repeatability is checked by multiple injections on same day, while intermediate precision is checked on different days or by different analysts. For pharmaceutical assays, %RSD less than 2% is generally acceptable.

5.4 Accuracy

Accuracy represents closeness of experimental value to true value. It is determined by recovery studies where known amount of standard drug is added to pre-analyzed sample. Recovery is checked at 80%, 100%, and 120% levels. Acceptable recovery usually lies between 98% and 102%.

5.5 Limit of Detection (LOD)

LOD is the smallest amount of analyte that can be detected but not accurately quantified. It is usually calculated using standard deviation and slope of calibration curve.

$$\text{LOD} = 3.3 \times \text{SD} / \text{Slope}$$

5.6 Limit of Quantitation (LOQ)

LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy.

$$\text{LOQ} = 10 \times \text{SD} / \text{Slope}$$

5.7 Robustness

Robustness indicates reliability of method after small deliberate changes in chromatographic conditions. Minor changes in flow rate, wavelength, pH, or mobile phase ratio should not significantly affect results.

6. RESULTS AND DISCUSSION

A properly developed RP-HPLC method for osteoarthritis drugs generally produces sharp and symmetrical peaks with good resolution. Retention time for paracetamol may be around 2 to 3 minutes, while diclofenac or aceclofenac may elute later depending on mobile phase composition.

The calibration curves usually show excellent linearity with correlation coefficient above 0.999. Precision studies often provide %RSD below 2%, confirming reproducibility. Recovery values near 100% indicate high accuracy. Low values of LOD and LOQ show good sensitivity of the method. Robustness studies demonstrate that minor operational changes do not significantly alter assay results.

Thus, validated HPLC methods are highly suitable for routine quality control of osteoarthritis medicines in pharmaceutical industries.

7. Advantages of HPLC for Osteoarthritis Drugs

HPLC offers rapid and reliable analysis of analgesic and anti-inflammatory drugs. It can estimate single as well as combined dosage forms accurately. It requires only small sample

quantity and provides excellent reproducibility. It is highly suitable for dissolution studies, stability testing, impurity profiling, and finished product assay. Because of automation and data handling systems, HPLC saves time and improves laboratory efficiency.

8. CONCLUSION

Osteoarthritis is a major degenerative joint disorder requiring long-term treatment with analgesics and NSAIDs. Ensuring the quality of these medicines is essential for patient safety and therapeutic success. HPLC is one of the most powerful and dependable analytical techniques used in pharmaceutical analysis. Proper method development followed by validation according to ICH guidelines ensures that the method is precise, accurate, sensitive, and robust. Therefore, HPLC remains the preferred analytical tool for routine estimation of paracetamol and NSAIDs used in osteoarthritis management.

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