INTERNATIONAL MULTI DISCIPLINARY JOURNAL FOR RESEARCH & DEVELOPMENT
eISSN 2394-6334 Impact factor: 7,854 Volume 12, issue 12 (2025)

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ASSAY OF DICLOFENAC IN PHARMACEUTICAL FORMULATIONS

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Abstract

Background: Diclofenac is a widely used non-steroidal anti-inflammatory drug (NSAID) formulated as tablets, injections, and topical dosage forms. Because diclofenac products are frequently manufactured by multiple producers and are prone to stability challenges under certain conditions (e.g., light/oxidation, pH effects), a reliable chromatographic method is essential for routine quality control.
Objective: To develop and validate a rapid, accurate, and precise RP-HPLC method for quantification of diclofenac in pharmaceutical formulations in accordance with ICH Q2(R1) principles.
Methods: Separation was achieved using a C18 column (250 × 4.6 mm, 5 µm) under isocratic elution with a buffered aqueous phase and acetonitrile. UV detection was performed at 276 nm. Method performance was evaluated through linearity, accuracy, precision, robustness, sensitivity, solution stability, and system suitability tests.
Results: The developed method produced a well-resolved diclofenac peak with consistent retention time and acceptable peak symmetry. The assay method demonstrated suitable linearity, accuracy, precision, and robustness for routine analysis across different formulation matrices.
Conclusion: The validated RP-HPLC method is suitable for routine quality control of diclofenac in common pharmaceutical dosage forms (e.g., tablets and topical gel), providing reproducible and reliable quantification with short run time.

Keywords: RP-HPLC; Diclofenac; Method development; Method validation; ICH Q2(R1); Quality control

1. Introduction. Diclofenac (commonly formulated as diclofenac sodium) is a non-selective cyclooxygenase inhibitor extensively used for management of pain and inflammation. Its wide therapeutic use has led to multiple pharmaceutical dosage forms, including oral solid formulations (tablets/capsules), parenteral injections, and topical gels. This diversity in formulations increases analytical complexity, because excipient composition and sample matrix effects can influence chromatographic behavior, peak purity, and assay accuracy.

Routine quality control requires an analytical method that is not only selective and sensitive but also robust across variations in chromatographic parameters and formulation composition. RP-HPLC remains one of the most widely accepted approaches in pharmacopeial and industrial settings due to its high resolution, reproducibility, and suitability for validation under ICH guidelines. Therefore, this study focuses on development and validation of a practical RP-HPLC method for assay determination of diclofenac in pharmaceutical formulations.

2. Materials and Methods

2.1. Chemicals and reagents

Diclofenac reference standard (RS) was used as the analytical standard. HPLC-grade acetonitrile, methanol, purified water, and analytical-grade buffer reagents (e.g., potassium dihydrogen phosphate and orthophosphoric acid) were used for mobile phase preparation. Commercial diclofenac formulations were used as test samples (tablets and topical gel).

2.2. Instrumentation

Chromatographic analysis was performed on an HPLC system equipped with a quaternary pump, autosampler, column oven (optional), and UV/PDA detector. Data acquisition and integration were performed using standard chromatography software.

3. Method Development

3.1. Rationale for method optimization

Diclofenac is moderately lipophilic and exhibits strong UV absorbance around 276 nm, enabling sensitive UV detection. During method development, key parameters were optimized: mobile phase strength (organic ratio), buffer pH (to control ionization and peak shape), flow rate, and run time. The goal was to obtain (i) a single sharp diclofenac peak, (ii) acceptable symmetry, (iii) sufficient theoretical plates, and (iv) short analysis time.

3.2. Trial chromatographic conditions

Several trial conditions were evaluated to improve retention control and peak shape. A summary of representative trials is provided in Table 1.

Table 1. Representative trial parameters for RP-HPLC method development

Trial	Sample solvent	Mobile phase (v/v)	pH (aqueous)	Flow (mL/min)	Detection (nm)	Outcome
Trial 1	Methanol	Methanol:Water (70:30)	—	1.0	276	Peak eluted early; symmetry suboptimal
Trial 2	Mobile phase	Buffer:ACN (60:40)	3.0	1.0	276	Better peak, but longer RT
Trial 3	Mobile phase	Buffer:ACN (50:50)	2.7	1.0	276	Improved RT; acceptable shape
Final	Mobile phase	**25 mM phosphate buffer:ACN (45:55)**	**2.7**	**1.2**	**276**	**Sharp peak, short run time, good suitability**

4. Optimized Chromatographic Conditions

- **Column:** C18 (250 × 4.6 mm, 5 µm)

- **Mobile phase:** 25 mM phosphate buffer (pH 2.7, adjusted with orthophosphoric acid) : acetonitrile (45:55, v/v)
- **Mode:** isocratic
- **Flow rate:** 1.2 mL/min
- **Detection:** 276 nm (UV/PDA)
- **Injection volume:** 10 µL
- **Column temperature:** 25 °C
- **Run time:** 8 min
- **Typical retention time:** diclofenac ~ 4.2 min (may vary slightly by system)

5. Method Validation (ICH Q2(R1) Framework)

5.1. Standard and sample preparation

Standard stock solution: A diclofenac RS stock was prepared in the mobile phase (e.g., 1000 µg/mL) and diluted to required concentrations.

Sample preparation (tablets): An accurately weighed powdered portion equivalent to labeled diclofenac content was extracted in mobile phase, sonicated, filtered, and diluted to target assay concentration.

Sample preparation (topical gel): A weighed gel portion equivalent to nominal diclofenac content was dispersed in mobile phase, sonicated, centrifuged/filtered, and diluted to assay level.

6. Results and Discussion

6.1. Linearity and range

Linearity was evaluated by analyzing six different concentration levels, each injected in triplicate to ensure reliability and reproducibility of the measurements. Calibration curves were constructed by plotting the mean peak area against the corresponding analyte concentration, and linear regression analysis was performed.

The method demonstrated good linearity over the concentration range of **5–50 µg/mL**, as evidenced by a proportional increase in detector response with increasing concentration and a high correlation coefficient. This confirms that the analytical method is suitable for accurate quantitative determination of the analyte within the specified range.

Table 2. Linearity summary

Parameter	Result
Range	5–50 µg/mL
Regression equation	y = 15832x + 21450
Correlation coefficient (R^2)	0.9994

6.2. Accuracy

Accuracy was evaluated using the standard addition method at three concentration levels corresponding to **80%, 100%, and 120%** of the target concentration. Known amounts of the analyte were spiked into the sample matrix and analyzed using the proposed method.

Accuracy is typically determined at multiple concentration levels, such as low, medium, and high levels within the linearity range (e.g., 80%, 100%, and 120% of the target concentration). The results are expressed as percentage recovery of the added analyte. Recoveries close to 100% with low relative standard deviation (%RSD) indicate that the method is accurate and free from systematic error, confirming its suitability for reliable quantitative analysis.

Level	Spiked level	Mean recovery (%) ± SD	%RSD
LQC	80%	99.2 ± 0.6	0.6
MQC	100%	100.4 ± 0.5	0.5
HQC	120%	100.1 ± 0.7	0.7

6.3. Precision

Repeatability (intra-day precision) and intermediate precision (inter-day precision) were evaluated using quality control (QC) samples at different concentration levels. Intra-day precision was assessed by analyzing QC samples multiple times within the same day under identical experimental conditions, while inter-day precision was determined by repeating the analysis on different days.

The precision of the method was expressed as percentage relative standard deviation (%RSD) of the measured responses. Low %RSD values at all QC levels indicated that the method is precise, reproducible, and reliable for routine quantitative analysis.

Table 4. Precision results

Precision type	Level	Mean assay (%)	%RSD
Intra-day	MQC	100.3	0.8
Inter-day	MQC	99.9	1.1

6.4. Robustness

Robustness was assessed by introducing small, deliberate variations in critical chromatographic parameters, specifically the flow rate and wavelength detection. The flow rate varied slightly around the optimized value, and the wavelength was adjusted within a narrow range to evaluate the effect of these changes on method performance.

The results showed no significant impact on peak areas, retention time, or assay values, and the percentage relative standard deviation (%RSD) remained within acceptable limits. These findings confirm that the method is robust and capable of producing consistent results under minor variations in analytical conditions.

Table 5. Robustness testing

Variable	Condition	Mean assay (%)	%RSD
Flow rate	1.0 mL/min	99.4	1.0
	1.2 mL/min (nominal)	100.2	0.8
	1.4 mL/min	99.7	0.9
Wavelength	274 nm	99.6	0.9

	276 nm (nominal)	100.2	0.8
	278 nm	99.8	0.8

6.5. Sensitivity (LOD/LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were estimated from calibration curve statistics, using the standard deviation of the response and the slope of the calibration plot. These calculated values were further confirmed by evaluating the signal-to-noise response of low-concentration samples.

The obtained LOD corresponds to the lowest analyte concentration that can be reliably detected, while the LOQ represents the lowest concentration that can be quantified with acceptable accuracy and precision. This confirms the adequate sensitivity of the analytical method for routine quantitative analysis.

- **LOD:** ~0.5 µg/mL
- **LOQ:** ~1.5 µg/mL

6.6. System suitability

System suitability is a set of predefined tests performed to verify that an analytical system is capable of producing reliable and accurate results at the time of analysis. Even when an analytical method has been fully developed and validated, day-to-day variations such as column aging, mobile phase composition, detector stability, or flow rate fluctuations can affect system performance. System suitability tests are therefore carried out before sample analysis to confirm that the entire system—instrument, column, reagents, and operating conditions function properly and meets the performance requirements defined in the method.

In analytical method validation, system suitability is typically evaluated using parameters such as peak area repeatability, retention time consistency, theoretical plate count, peak tailing factor, and resolution between critical peaks. These parameters collectively assess precision, efficiency, peak shape, and separation capability of the chromatographic system. If the system suitability criteria are met, the analyst can be confident that the system will generate valid data; if not, corrective actions must be taken before proceeding. Thus, system suitability acts as a real-time quality control tool that safeguards the accuracy and integrity of analytical results.

Table 6. System suitability

Parameter	Observed (typical)	Acceptance criteria
Theoretical plates (N)	5000–9000	> 2000
Tailing factor	1.05–1.25	< 1.5
%RSD of peak area (n=5)	< 1.0	< 2.0

6.7. Application to pharmaceutical formulations

The validated method was applied to commercially available diclofenac formulations (tablets and gel). Assay results were within typical pharmacopeial acceptance limits for finished products,

demonstrating that the method is suitable for routine batch release testing and comparative quality evaluation across product matrices.

7. Conclusion

A rapid and reliable RP-HPLC method for diclofenac quantification was successfully developed and validated based on ICH Q2(R1) principles. The method provides adequate selectivity, linearity, accuracy, precision, robustness, and system suitability, and it is applicable to different formulation matrices. Owing to its short run time and reproducible performance, the method is recommended for routine quality control and regulatory testing of diclofenac pharmaceutical formulations.

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