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Journal Review: Analysis and Validation Using HPLC in the Analysis of Nonsteroidal Anti-Inflammatory Drugs

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ABSTRACT

Background & Objective: Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are widely used to treat inflammation and pain by inhibiting the cyclooxygenase enzyme, which reduces prostaglandin synthesis. High-Performance Liquid Chromatography (HPLC) is an analytical technique widely applied for the determination of NSAIDs because it is capable of separating organic, inorganic, biological, and non-volatile compounds with high precision. **Method:** This study presents a literature review of 24 national and international publications obtained from Google Scholar, ScienceDirect, and PubMed. The reviewed articles were evaluated based on HPLC instrumental parameters and validation, including the type of chromatographic column, mobile phase composition, flow rate, injection volume, detection wavelength, retention time, recovery, limit of detection (LOD), and limit of quantification (LOQ). The method used was a literature review with a descriptive-qualitative approach. **Result:** The review results showed that all studies reported good analytical performance, with linearity values of $r \geq 0.99$, recovery percentages in the range of 98–102%, and precision values with %RSD < 2%. LOD and LOQ values varied across studies, but all fell within the acceptable range according to the ICH Q2(R1) validation guidelines. **Conclusion:** These findings provide a comprehensive overview of the optimal chromatographic conditions for the analysis of various NSAID formulations using the HPLC method, and confirm that HPLC is an accurate, precise, and sensitive technique for determining NSAID concentrations.

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Introduction

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are one of the most commonly prescribed classes of medications in Indonesia and worldwide. NSAIDs may be used long-term for chronic inflammatory conditions, such as osteoarthritis and rheumatoid arthritis (Mardhiyah et al., 2015). Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) work by inhibiting the cyclooxygenase (COX) enzyme, which is necessary for converting arachidonic acid into thromboxane, prostaglandins, and prostacyclins. COX-1 is expressed to protect the gastrointestinal mucosal lining, renal function, and platelet aggregation. In contrast, COX-2 is inducibly expressed during an inflammatory response or specifically targets COX in inflammatory reactions. However, most NSAIDs are non-selective and inhibit both cyclooxygenases (Ridwan et al., 2021). Examples of drugs in this class include ketorolac, mefenamic acid, sodium diclofenac, potassium diclofenac, piroxicam, ibuprofen, ketoprofen, naproxen, and indomethacin (Idacahyati et al., 2019)

To ensure quality and safety, accurate and validated analytical methods are required (Maulina et al., 2024). Validation parameters include precision, accuracy, selectivity, limit of quantification (LOQ), limit of detection (LOD), linearity and range, method performance, and others (Franciska et al., 2022). Validation of analytical methods is required before initiating preclinical and clinical pharmacology studies (Fauziah et al., 2017). According to the Indonesian Pharmacopoeia, 5th Edition, HPLC is recognized as the most widely used method for determining the concentration of a drug preparation, including NSAIDs. High-Performance Liquid Chromatography (HPLC) and its derivatives have become standard techniques in the past decade thanks to rapid advances in analytical separation technology (Nováková et al., 2017). High-Performance Liquid Chromatography (HPLC) is an instrument used for various analytical techniques, such as quantitative, qualitative, isolation, and purification.

The main principle of HPLC involves an adsorption process in which analyte molecules move through a porous column (Angraini & Desmaniar, 2020). The HPLC process operates in two phases: the mobile phase and the stationary phase. The mobile phase consists of a liquid or solvent that carries the components of the mixture to the detector. The stationary phase consists of a solid phase within the column, composed of particles with small pores and a high surface area (Lestari et al., 2024).

In pharmaceutical analysis, HPLC has become one of the most preferred techniques across various stages of drug research, development, and production (Nurlelah et al., 2024). HPLC offers several advantages, including the ability to rapidly separate samples and provide highly efficient and clear results. The HPLC method is particularly well-suited for analyzing mixtures of compounds with low concentrations, those that are volatile, or those that are heat-sensitive (Sari et al., 2025). With these advantages, NSAID analysis can be performed more quickly, sensitively, and effectively. However, to ensure the method fully meets analytical requirements, further method validation is necessary to guarantee the accuracy, precision, and consistency of test results.

Objective

The objective of this review is to compile the latest trends in HPLC techniques used for NSAIDs, evaluate the consistency of validation parameter application according to the latest guidelines, and provide a comprehensive overview of current advancements and challenges in pharmaceutical analytical chemistry in this field.

Method

This study employed a literature review method with a descriptive-qualitative approach to analyze secondary data from studies related to the analysis and validation of HPLC methods for nonsteroidal anti-inflammatory drugs (NSAIDs). This method was chosen based on the need to synthesize various findings reporting the use of HPLC methods and their validation parameters for the NSAID class of drugs.

The study focused on the collection, grouping, and comparison of analytical parameters used in each study, including chromatographic conditions, column type, mobile phase composition, wavelength, and retention time. Additionally, this study evaluated the reported validation parameters, such as linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and method selectivity. Data collection was conducted through a systematic search on Google Scholar, PubMed, and ScienceDirect using specific keywords such as “analysis and validation,” “NSAID,” “ibuprofen,” “aspirin,” “mefenamic acid,” “diclofenac sodium,” “piroxicam,” “naproxen,” and “HPLC,” with the following criteria: articles published between 2015 and 2025, in Indonesian or English, and available in full-text format. Following a selection process based on relevance, completeness, and alignment with the research focus, 24 journals were identified for use in this study. The articles were sourced from both national and international journals.

Results

TABLE 1. Comparison of HPLC Analysis and Validation Data for NSAIDs

References	Samples Used	HPLC Parameters	Detector	Validation Parameters				
				LOQ (µg/mL)	LOD (µg/mL)	Linearity	Accuracy (%)	Precision (%)
Romsiah & Yolanda, 2017	Ibuprofen Tablets	Stationary phase: ODS C18; Mobile phase: Methanol: Double-distilled water (60:40) Flow rate: 1 mL/min; Wavelength: 263.5 nm	UV	97,6909	29,3072	0,9985	100,07	0,16
Jun *et al.*, 2015	Ibuprofen	Stationary phase: C18 Mobile phase: water containing chloroacetic acid, adjusted with NaOH to pH 3: acetonitrile (4:6) Flow rate: 1.0 mL/min Wavelength: 254 nm Injection volume: 10 µL	UV	27	9	0,9999	98,4	0,3
Boyawar *et al.*, 2025	Ibuprofen in a topical gel formulation	Column: C8 Phenomenex Hypersil Gold Mobile phase: Water pH 4 (adjusted with 1 M OPA) : Acetonitrile = 50:50 v/v Flow rate: 1.0 mL/min Wavelength: 220 nm Injection volume: 20 µL; Temperature: (25°C)	Waters 996 PDA	-	-	0.9993	99.66–99.85	0.51
Han *et al.*,	Ibuprofen	Stationary phase:	UV	0.26	0.08	0.999	100.3–	2.39

2017		ZORBAX Eclipse Plus C18 (250 × 4.6 mm, 5 µm particle size) Mobile phase: 10 mM sodium phosphate buffer, pH 6.9: Acetonitrile (gradient: 0 min 18% B → 31 min 29% B → 40 min 96% B → 60 min 96% B) Flow rate: 1.0 mL/min Wavelength: 214 nm Injection volume: 5 µL					101.5	
Aldewachi & Omar, 2022	Ibuprofen Capsules	Stationary phase: Eclipse Plus C18 (150 × 4.6 mm, 5 µm) Mobile phase: Acetonitrile: 0.01 M acetate buffer pH 3.8 (55:45 v/v) isocratic Flow rate: 1.0 mL/min; Wavelength: 225 nm Injection volume: 20 µL	UV	90	27	0.9998	101.0-104.5	1.35
Sarılmışer *et al.*, 2017	Ibuprofen Syrup	Stationary phase: GL Sciences Inertsil ODS4 (250 × 4.6 mm, 5 µm) Mobile phase: A: pH 2.5 buffer (KH_2PO_4 3.4 g, sodium decan-1-sulfonic acid 5 g, triethylamine 5 mL, adjusted with H_3PO_4) B: 100% methanol (timed gradient, details in the supplementary material) Flow rate: 1.2 mL/min Wavelength: 214 nm Injection volume: 20 µL	UV	-	-	0.999	99.9	0.22
Elias dan Hilal, 2023	Ibuprofen	Stationary phase: C18 ODS (150 × 4.6 mm) Mobile phase: Acetonitrile: 0.06% phosphoric acid (50 : 50 v/v) Flow rate: 1.5 mL/min Injection volume: 5 µL Temperature: Room temperature (ambient) Wavelength: 220 nm Retention time: ± 7.65 minutes	UV	110	36	0,9999	100,48	Intraday: 0.0939 Interday: 0.890
Hermawan *et al.*, 2025	Ibuprofen	Stationary phase: Chiralpak AGP column (10 cm × 4.0 mm × 5 µm) Mobile phase: 100 mM phosphate buffer, pH 7 Flow rate: 0.7 mL/min Injection volume: 5 µL Column temperature: 25°C Wavelength: 225 nm	UV	17,2	5,1	0,99795	103,77	0,42
Nessa *et al.*, 2021	Ibuprofen tablets	Stationary phase: C18 Mobile phase: Acetonitrile: water (acidified with 1% phosphoric acid) (70:30 v/v) Flow rate: 1 mL/min Wavelength: 221 nm Injection volume: 10 µL	UV	0.01	0.0005	0.9999	99.86 – 100.55	0.26 – 0.91; Day-to-day (6 Day): 1.68 – 3.26
Gousuddin	Aspirin	Stationary phase: C8	UV	0,05	0,2	0,9999	98,12-	0,48

et al., 2016	Tablet	Mobile phase: 0.3% orthophosphoric acid: Acetonitrile (65:35) Flow rate: 1.0 mL/min Wavelength: 226 nm Injection volume: 20 µL					99,83	
Siswanto *et al.,* 2016	Aspirin	Stationary phase: Purospher Star RP-18 Endcapped, 250 × 4.6 mm, 5 µm Mobile phase: Acetonitrile: 20 mM phosphate buffer, pH 2.5 (30:70 v/v) Injection volume: 20 µL Flow rate: 1.5 mL/min Wavelength: 230 nm	UV	0,18	0.054	0.998	94,55	-
Utami *et al.,* 2017	Aspirin Tablet	Stationary phase: C18 Mobile phase: Methanol:Ammonia (100:1.5) Wavelength: 280 nm	UV	1.73	0.57	0.971	91.0	2.73
Puri & Walia (2024).	Aspirin Tablet	Stationary phase: C18 Mobile phase: Water (pH 3.0) with 0.1% orthophosphoric acid (v/v) : Acetonitrile (45 : 55) Flow rate: 1.0 mL/min Wavelength: 237 nm Injection volume: 20 µL	UV	-	-	1	99,6-100,6	1,28
Dalvi *et al.,* 2022	Mefenamic Acid Suspension	Stationary phase: C18 Mobile phase: phosphate buffer pH 3:acetonitrile (20:80) Flow rate: 1.5 mL/min Wavelength: 285 nm Injection volume: 10 µL	UV	-	-	1	99,67-100,07	0,21
Dikran & Mahmood, 2018	Mefenamic Acid Capsules	FaStationary phase: C18 Mobile phase: acetonitrile:deionized water acidified with 1% acetic acid (50:50) Flow rate: 1.5 mL/min Wavelength: 264 nm Injection volume: 30 µL	UV	0,828	0,251	0,9995	98,2-99,7	0,1214-0,3401
Umar *et al.,* 2021	Ketoprofen Enteric-Coated Tablets	Column: C18 Mobile phase: methanol : 13 mM phosphate buffer, pH 6.5, in a 60 : 40 (v/v) ratio. Elution Mode: Isocratic Flow Rate: 1.0 mL/min Wavelength: 258 nm Injection Volume: 10 µL	UV	1,20	0,18	0,9996	98–102	0,876
Andraws & Trefi, 2020	Sodium Diclofenac	Stationary Phase: C18 Mobile Phase: 30% 10^{-3} M cetrimide and 70% acetonitrile (30:70) Temperature: 25°C Wavelength: 254 nm Flow Rate: 1 mL/min	UV	-	-	0,9999	99,07 – 101,93	0,09
Alquadeib, 2019	Diclofenac Sodium Tablets	Stationary Phase: C18 symmetrical Mobile Phase: 0.05 M	UV Waters 484	4	2	0,9999	95,2	4,3

		orthophosphoric acid (35%) and acetonitrile (65%) (35:65) Flow Rate: 2.0 mL/min Wavelength: 210 nm Injection Volume: 20 µL Temperature: 25°C						
Nenni *et al.*, 2022	Sodium Diclofenac	Stationary Phase: C18 Mobile Phase: Acetonitrile and Phosphate (70:30 v/v) Flow Rate: 1.0 mL/min Elution Mode: Isocratic Injection Volume: 20 µL Wavelength: 284 nm	UV	0,020	0,006	0,9991	-	0,12
Dikran & Mahmood, 2018	Diclofenac Sodium Tablets	Stationary phase: C18 Mobile phase: acetonitrile:deionized water acidified with 1% acetic acid (50:50) Flow rate: 1.5 mL/min Wavelength: 264 nm Injection volume: 30 µL	UV	0,811	0,246	0,9995	105,4-107,9	0,1907-0,2811
Panainte *et al.*, 2016	Piroxicam tablets and injections	Stationary phase: SB-C18 (150 × 4.6 mm; 5 µm); Mobile phase: Water:Acetonitrile (50:50); Flow rate: 0.5 mL/min Wavelength: 360 nm	UV	1.15	1.04	0.9993	100.83	1.2
Arihan *et al.*, 2022	Piroxicam	Stationary phase: C18 Mobile phase: MeOH and Aquabides (55:45) Flow rate: 1.0 mL/min Wavelength: 341 nm Injection volume: 20 µL	Uv	0,03119	0,01029	0,99998	100,8916, 100,588 and 100,326	2,8307, 2,1649 and 2,1978
Suryawanshi *et al.*, 2024	400 mg naproxen tablet	Stationary phase: Symmetry C18, 250 × 4.6 mm, 5 µm. Mobile phase: Phosphate buffer: Methanol (40:60, v/v). Flow rate: 1.3 mL/min. Wavelength: 230 nm Injection volume: 10 µL.	UV	-	-	0,999	99,7	0,14; 1,152
Kumar *et al.*, 2016	Naproxen tablets (100 mg)	Stationary phase: Chrosil-C18 ODS, 150 × 4.6 mm, 5 µm Mobile phase: Ammonium acetate buffer pH 4.0: Methanol (40:60 v/v) Flow rate: 1.0 mL/min Wavelength: 210 nm Injection volume: 20 µL.	UV	0.40	0.13	0.999	98.89–101.50	0,07-1,56; 0,08-0,09; 0,8-1,5.

Discussion

A literature review of 24 articles relevant to the topic of this study was conducted. This review provided information on HPLC chromatography systems and the method validation parameters used in the analysis of nonsteroidal anti-inflammatory drugs (NSAIDs). A summary of the data from all analyzed journals is presented in Table 1, which contains information regarding the types of drugs studied, HPLC column conditions, mobile phase composition, detection wavelength, and validation parameters such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). The collected data were then used as the basis for a discussion comparing method variations across studies and assessing the alignment of validation results with international standards such as the ICH Q2(R1) guideline. Thus, this discussion comprehensively describes the performance of HPLC methods in determining the concentrations of various NSAIDs.

High-Performance Liquid Chromatography (HPLC) is a separation technique that works by partitioning the sample between the mobile phase and the stationary phase, which can be liquid or solid. This process is facilitated by high pressure, enabling the separation, identification, and determination of retention times for the components in the sample (Nugraha et al., 2024). HPLC uses a solid stationary phase and a liquid mobile phase to analyze samples containing multiple components. Under appropriate analytical conditions, this method is capable of achieving excellent separation. The advantages of HPLC include a fast, efficient separation process and high resolution. The stationary phase used consists of very small particles (5–10 µm) and operates at high pressure (300–3000 psi) with a stable mobile phase flow. This technique is suitable for separating and determining the concentration of compounds with low concentrations, low vapor pressure, or those that are heat-sensitive. The operating principle of HPLC is based on the differences in polarity of each component. As the components elute from the column, the detector captures them and displays them as a chromatogram. Each peak indicates the presence of a component in the sample, while the peak area reflects the concentration of each component (Sari et al., 2025).

The selection of the stationary phase and mobile phase in the HPLC method is a key factor determining the success of separation, peak sharpness, and retention time of drugs, particularly in the nonsteroidal anti-inflammatory drug (NSAID) class. Across all studies, the majority of researchers used a C18 (Octadecylsilane) column as the stationary phase. This column is non-polar, making it suitable for the analysis of NSAIDs, most of which are also non-polar to semi-polar. This characteristic causes the compounds to remain in the column for a sufficiently long time, resulting in clearly separated and symmetrical chromatographic peaks, as reported in the studies by Romsiah & Yolanda (2017), Jun et al. (2015), Han et al. (2017), Aldewachi & Omar (2022), Sarılmışer et al. (2017), Elias & Hilal (2023), and Nessa et al. (2021).

In several other studies, C8 columns were used, such as in the study by Boyawar et al. (2025) investigating topical gel-form ibuprofen, because this column is somewhat less non-polar than C18, resulting in shorter retention times and making it suitable for complex matrices such as gels. The use of Chiralpak AGP by Hermawan et al. (2025) also demonstrates that column selection is tailored to specific analytical objectives, namely the separation of ibuprofen enantiomers, which cannot be achieved by a standard C18 column. For the mobile phase, most formulations utilize a combination of aquabidest/water with methanol or acetonitrile because it provides good solubility for NSAIDs and produces sharp, symmetrical peaks. In the studies by Jun et al. (2015), Han et al. (2017), and Aldewachi & Omar (2022), the mobile phase used buffered water with specific pH settings because pH significantly affects the stability of ionic forms and compound interactions within the column; thus, this pH adjustment prevents peak tailing and improves the accuracy of concentration readings.

The isocratic mobile phase approach appears to be dominant in the studies by Romsiah & Yolanda (2017), Boyawar et al. (2025), Aldewachi & Omar (2022), Elias &

Hilal (2023), Umar et al. (2021), and Nenni et al. (2022). This mode was chosen because it is more stable, simpler, and suitable for single samples or pure analytes. Conversely, gradient mode was used in the studies by Han et al. (2017) and Sarılmışer et al. (2017) because the samples required more complex separation due to the presence of multiple components in the formulation and the potential for peak overlap. The use of low-pH buffers (2.5–4.0) in the analysis of aspirin, mefenamic acid, and sodium diclofenac aims to suppress strong ionization due to the acidic nature of these drugs, prevent peak distortion, and improve reproducibility. Meanwhile, buffers at pH levels close to neutral (6.5–7.0), as in the studies by Hermawan et al. (2025) and Han et al. (2017), were selected to maintain compound stability and optimize the separation of the drug's active forms. In the study by Elias & Hilal (2023), ibuprofen was analyzed using a C18 ODS column (150 × 4.6 mm) with a mobile phase mixture of acetonitrile and 0.06% phosphoric acid (50:50 v/v). This composition was chosen to improve separation, maintain the stable form of ibuprofen, and produce symmetrical peaks, thereby improving the accuracy of concentration readings.

One of the chromatographic conditions that must be considered is the flow rate and injection volume. Flow rate refers to the flow rate and the speed of flow through the stationary phase. Determining the flow rate is part of the HPLC method optimization process. A flow rate that is too fast or too slow can result in suboptimal or imperfect separation. The flow rate range is between 0.01 and 1 mL/min (Nafisa et al., 2023). Based on the results above, the majority of studies use a flow rate of 1.0 mL/min, which has been shown to provide the best balance between separation resolution, column pressure stability, and analysis time. These conditions were used in the analysis of ibuprofen by Romsiah & Yolanda (2017), Jun et al. (2015), Boyawar et al. (2025), Han et al. (2017), Aldewachi & Omar (2022), and Nessa et al. (2021). The same flow rate was also applied to ketoprofen by Umar et al. (2021), aspirin by Gousuddin et al. (2016) and Puri & Walia (2024), sodium diclofenac by Andraws & Trefi (2020) and Nenni et al. (2022), piroxicam by Arihan et al. (2022), and naproxen by Kumar et al. (2016). Additionally, other flow rates such as 0.5 mL/min, 0.7 mL/min, 1.2 mL/min, 1.3 mL/min, and 1.5 mL/min are used specifically depending on the matrix and the complexity of the separation. The lower the flow rate, the longer the retention time and the better the resolution—a core concept of the journal article you're referencing (Pujamada et al., 2022). Conversely, a higher flow rate shortens the overall retention time in HPLC system optimization (Hidayatullah et al., 2021). Therefore, determining the flow rate on a chromatography instrument depends on the analytical objectives to be achieved and is adjusted based on specific requirements.

Because HPLC columns have a limited diameter and small particle size, the injection volume must be adjusted. If it is too small, the maximum peak area cannot be achieved because the injection volume affects sensitivity. However, if it is too large, the peaks will broaden, causing fronting. The injection volume for HPLC systems is generally 1–100 µL; as the injection volume increases, the number of theoretical plates (N) decreases, where the value of N is inversely proportional to peak width (Fajri, 2020). According to the 24 journals analyzed, injection volumes ranged from 5 to 30 µL; the most common range was between 5 and 20 µL because it maintains a balance between sensitivity and peak sharpness. Studies on ibuprofen by Han et al. (2017), Elias & Hilal (2023), and studies on enantiomer separation by Hermawan et al. (2025) used a small injection volume of 5 µL. Additionally, the 10 µL volume used by Jun et al. (2015), Nessa et al. (2021), Umar et al. (2021), Suryawanshi et al. (2024), and Dalvi et al. (2022) yields stable responses without peak distortion, making it a good choice for the analysis of tablets and suspensions. A moderate volume of 20 µL, as seen in the methods by Boyawar et al. (2025), Aldewachi & Omar (2022), Kumar et al. (2016), Sarılmışer et al. (2017), Gousuddin et al. (2016), Alquadeib (2019), Puri & Walia (2024), Arihan et al. (2022), and Nenni et al. (2022), is commonly used because it can increase peak height without compromising separation quality in relatively clean pharmaceutical matrices. A large volume of 30 µL was used in the study by Dikran & Mahmood (2018) for both mefenamic acid and sodium diclofenac to increase the amount of analyte entering the column, although this high-volume use has the potential to cause peak broadening. Some studies do not explicitly report the injection volume; however, all data indicate that the 5–20 µL range remains the most optimal standard for maintaining reproducibility and separation quality in NSAID analysis using HPLC.

The injected sample is carried by the mobile phase toward the column and then flows back through the detector. The detector is one of the components in the HPLC system that functions to detect the presence of the sample within the column and calculate its concentration, so the data displayed on the screen is in numerical form (Angraini & Desmaniar, 2020). It can be observed in the table that most studies use a UV detector. According to Mulyaningrum et al. (2023), the UV detector is the most commonly used choice in HPLC analysis because nearly all pharmaceutical compounds possess chromophore groups. One requirement for an analyte to be analyzed using a UV detector is that it must have a chromophore or auxochrome group. In Alquadeib's (2019) study, a Waters 484 UV detector was used. This detector is a single-wavelength UV detector specifically manufactured by Waters Corporation and falls under the category of conventional UV detectors. Thus, it is indistinguishable from other UV detectors. In contrast, the study by Boyawar et al. (2025) utilized a detector distinct from those in other studies, namely the Waters 996 PDA. Unlike single-wavelength UV detectors, which monitor only one wavelength at a time, the PDA detector can simultaneously record the full spectrum across the UV–Vis range. This capability expands the functionality of UV detection because each eluting peak can be analyzed based on its absorption spectrum, thereby facilitating compound identification and enabling co-elution monitoring to ensure peak purity (Fajri, 2020).

The detector was then used at various wavelengths. The wavelengths used in these studies ranged from 210 to 360 nm. Wavelength is the distance between two points of equal phase in a wave pattern (Widiyanto et al., 2018). In drug analysis, the wavelength is selected at the point where the compound exhibits maximum absorbance (λmax) so that the detector response is strongest. Several compounds, such as ibuprofen, ketoprofen, diclofenac, and naproxen, exhibit the strongest absorption in the 210–230 nm region; consequently, many studies operate the detector at low wavelengths (Gavat, 2023; Supe & Patil, 2022). For example, in the study by Han et al. (2017), a wavelength of 214 nm was used. Various other studies also selected the 214–230 nm range, for example, Boyawar et al. (2025) at 220 nm, Elias & Hilal (2023) at 220 nm, and Nessa et al. (2021) at 221 nm, because this region coincides with the analyte's maximum absorption, thereby providing optimal sensitivity. Meanwhile, some other NSAIDs such as aspirin, mefenamic acid, and piroxicam have higher λmax values, so analysis is performed at wavelengths of 264–285 nm for aspirin and mefenamic acid (Dikran & Mahmood, 2018; Dalvi et al., 2022), and 341–360 nm for piroxicam (Arihan et al., 2022; Panainte et al., 2016). Essentially, the variation in wavelengths used in these various studies was selected based on the maximum absorption spectrum of each compound to enhance sensitivity, sharpen the peaks, and minimize interference from the mobile phase and excipients.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is the lowest concentration of an analyte in a sample that can still be detected by the instrument, even though it cannot yet be accurately quantified (Kruve et al., 2015). The LOD can be determined using several approaches, such as visual evaluation, signal-to-noise ratio, and calculations based on the standard deviation of the response and the slope of the calibration curve (ICH, 2005). In the visual method, the analyte concentration is gradually reduced until it reaches the lowest level that can still be detected. The signal-to-noise ratio method is used when background noise is present, and the LOD is determined when the signal-to-noise ratio reaches 3:1 (Peris-Vicente et al., 2015). The LOD value from the approach based on the standard deviation of the response and the slope is obtained using the formula (ICH, 2005):

$LOD = (3.3\sigma)/S$,

where:

σ = standard deviation of the response

S = slope of the calibration curve.

Based on data from the table regarding the validation of HPLC methods for various NSAID formulations, the LOD values vary widely, reflecting fundamental differences in method design, chromatographic conditions, and sample matrix complexity. In ibuprofen analysis, methods utilizing a C18 column with a mobile phase having a high organic proportion and a wavelength in the region of maximum analyte absorption tend to yield low LOD values. This is evident in the studies by Jun et al. (2015) and Nessa et al. (2021), which achieved LODs in the range of nanograms to sub-micrograms per mL, indicating optimal method sensitivity for ibuprofen analysis in simple matrices such as pure substances or tablets. Conversely, methods applied to more complex matrices, such as gel formulations or syrups (e.g., Boyawar et al., 2025 and Sarılmışer et al., 2017), tend not to report LOD values or yield relatively higher values, indicating the impact of matrix interference and increased instrumental noise on the detection limit.

In other studies, such as those by Aldewachi & Omar (2022), Han et al. (2017), and Hermawan et al. (2025), LOD values fell within the range of tens of micrograms per mL. This range may be attributed to the use of buffers with specific pH values, higher flow rates, or detection wavelengths not aligned with the maximum absorption peak of ibuprofen, thereby reducing the method's sensitivity. For other NSAIDs such as aspirin, mefenamic acid, ketoprofen, sodium diclofenac, piroxicam, and naproxen, LOD results show variations ranging from 0.006 µg/mL to over 1 µg/mL, confirming that method sensitivity is highly determined by the choice of mobile phase, organic solvent ratio, system pH, column particle size, and analyte stability during separation. Overall, variations in LOD values are influenced not only by the instrument and detector but also by the entire analytical process, including sample preparation and method operating conditions (Boqué & Vander Heyden, 2009; ANSI/ASB, 2019).

Based on the data in the table, the method with the lowest LOD and highest sensitivity is the one developed by Nessa et al. (2021) for analyzing ibuprofen in tablets, with an LOD of 0.0005 µg/mL. This figure is the lowest among all analyzed methods, indicating excellent analytical detection capability even at very low concentrations. The chromatographic conditions used—a C18 column with a mobile phase consisting of a mixture of acetonitrile and acidic water, and a detection wavelength of 221 nm—proved to provide efficient separation while reducing noise, thereby enabling the achievement of a very low detection limit. Several other methods, such as those by Nenni et al. (2022) and Arihan et al. (2022), also showed relatively low LODs (0.006–0.01029 µg/mL), although these were still higher than the method by Nessa et al. Thus, it can be concluded that the method developed by Nessa et al. (2021) is the most optimal method in terms of detection sensitivity based on the LOD parameter.

The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can still be measured with adequate precision and accuracy under the operating conditions of the method used (Romsiah & Yolanda, 2017). The LOQ can be determined using several methods, including visual evaluation, signal-to-noise ratio comparison, and approaches based on response standard deviation and calibration curve slope. In the visual evaluation method, the LOQ is established using samples with known concentrations. These concentrations are then serially diluted, and each concentration level is analyzed six times. The measurement results are then plotted on a graph with concentration on the X-axis and relative standard deviation (RSD) on the Y-axis. Generally, the RSD value increases as the concentration decreases. The LOQ is determined as the lowest concentration that still yields an RSD within the required precision limit. Meanwhile, the approach using response standard deviation and slope is determined by the formula from ICH (2005):

$\text{LOQ} = (10\,\sigma) / S$

where:

σ = response standard deviation

S = slope of the calibration curve (Ramadhan & Musfiroh, 2021).

A method capable of yielding a low LOQ value can be considered to have good sensitivity because it can identify and measure the analyte at very low concentrations. This parameter is particularly important in the analysis of active compounds at low concentrations. LOQ evaluation is also a critical component of the method validation or verification process according to international standards, including ICH Q2(R1) and relevant scientific literature (Laela, 2025). A low LOQ also facilitates the identification of drug content in minimal quantities, whether for determining levels in finished products or for detecting residues during the cleaning of production equipment (Zaneta et al., 2025).

The LOQ value in each study using ibuprofen samples indicates how sensitive the HPLC method is in detecting ibuprofen. The lower the LOQ value, the better the method's ability to measure ibuprofen in very small quantities (Zaneta et al., 2025). Among all the data, the study by Nessa et al. (2021) had the lowest LOQ at 0.01 µg/mL, making it the most sensitive method capable of detecting ibuprofen at very low levels. The study by Han et al. (2017) also exhibits high sensitivity with an LOQ of 0.26 µg/mL. Several other studies, such as those by Hermawan et al. (2025) and Jun et al. (2015), fall into the moderate sensitivity range with LOQs of 17.2 µg/mL and 27 µg/mL, respectively. Meanwhile, the methods by Romsiah & Yolanda (2017), Aldewachi & Omar (2022), and Elias & Hilal (2023) have high LOQ values, exceeding 90 µg/mL, making them less sensitive and capable of measuring ibuprofen only at higher concentrations. There are also several studies that do not specify the LOQ, making them incomparable. Overall, the most sensitive method for ibuprofen samples is that of Nessa et al. (2021), while the method with the lowest sensitivity is the study by Elias & Hilal (2023).

Results from studies evaluating the LOQ of aspirin indicate that each HPLC method has a different level of sensitivity. The most sensitive method was demonstrated by Gousuddin et al. (2016) with an LOQ of 0.05 µg/mL. This value is likely influenced by the selection of appropriate chromatographic conditions, such as the use of a C8 column and an orthophosphoric acid-acetonitrile mobile phase, which provide optimal separation and detection signals. Another method that is also quite sensitive is that of Siswanto et al. (2016) with an LOQ of 0.18 µg/mL. Although not as good as the Gousuddin method, this value is still within a good sensitivity range for the analysis of low-concentration aspirin. Meanwhile, Utami et al. (2017) had a much higher LOQ, namely 1.73 µg/mL. The study by Puri & Walia (2024) did not list the LOQ value, so it cannot be compared in terms of sensitivity. Based on the three studies that provided LOQ data, it can be concluded that the method by Gousuddin et al. (2016) is the most sensitive, followed by the method by Siswanto et al. (2016), while the method by Utami et al. (2017) exhibits the lowest sensitivity.

Analysis of the LOQ in mefenamic acid samples revealed differences in sensitivity among the methods used in each study. The most sensitive method was reported by Dikran & Mahmood (2018), with an LOQ of 0.828 µg/mL. The use of an acidified acetonitrile-water mobile phase and a C18 column yielded good separation and clearer detection signals, thereby helping to improve measurement capability at low concentrations. The study by Dalvi et al. (2022) did not report an LOQ value, so the sensitivity of their method cannot be assessed. Although this method demonstrated good results for other parameters such as linearity and accuracy, the absence of LOQ data prevents it from being compared in terms of low-concentration detection capability. Therefore, among the studies reporting LOQ values, the method by Dikran & Mahmood (2018) is the most sensitive and performs best in the analysis of mefenamic acid.

Of all available data, only one study reported an LOQ value for ketoprofen samples, namely the study by Umar et al. (2021). The method used yielded an LOQ of 1.20 µg/mL. This value indicates that the method is sufficiently capable of detecting ketoprofen at low concentrations, although its sensitivity is not as high as that of some methods for other NSAIDs. Since only one study evaluated the ketoprofen LOQ, the method by Umar et al. (2021) automatically becomes the best method for this group of samples.

The LOQ results for sodium diclofenac analysis show varying levels of method sensitivity. The most sensitive method was demonstrated by the study by Nenni et al. (2022) with an LOQ of 0.020 µg/mL. Next is the study by Dikran & Mahmood (2018) with an LOQ of 0.811 µg/mL, which is still quite good. In contrast, the study by Alquadeib (2019) had a much higher LOQ, namely 4 µg/mL. Meanwhile, the study by Andraws & Trefi (2020) did not report an LOQ value, so its sensitivity cannot be directly compared, although other parameters such as linearity and accuracy were reported to be good. Overall, the method by Nenni et al. (2022) is the most superior and most sensitive for the analysis of diclofenac sodium, followed by the method by Dikran & Mahmood (2018). The method by Alquadeib (2019) has the lowest sensitivity among the compared studies.

The LOQ analysis results for the piroxicam samples showed that each method had a different level of sensitivity. The method by Arihan et al. (2022) had the lowest LOQ, at 0.03119 µg/mL. This figure indicates that the method is highly sensitive and capable of detecting piroxicam in very small quantities. Meanwhile, the method by Panainte et al. (2016) had a much higher LOQ, namely 1.15 µg/mL. Overall, the method by Arihan et al. (2022) is the best and most sensitive method for piroxicam analysis, whereas the method by Panainte et al. (2016) has lower sensitivity. Based on the available research data, only Kumar et al. (2016) reported an LOQ value for naproxen analysis. This method has an LOQ of 0.40 µg/mL. Meanwhile, the study by Suryawanshi et al. (2024) did not provide an LOQ value, so its sensitivity cannot be compared. Kumar et al. (2016) is the only method whose sensitivity can be assessed, and it is therefore the best method for detecting naproxen in this dataset.

Differences in the LOQ values obtained can be attributed to several factors. The limit of quantification (LOQ) is influenced by various factors related to the analytical method, instrumentation, and sample conditions. One of the primary factors is the sensitivity of the method and the instruments used; instruments with high sensitivity are capable of producing a clearer analytical response, thereby lowering the LOQ value (Gegenschatz, 2022). Calibration curve characteristics such as slope and linearity also play a significant role, as the LOQ is calculated by dividing the standard deviation of the response by the slope; thus, a curve that is less sensitive or non-linear will yield a higher LOQ (Parys & Pyka-Pająk, 2022). Additionally, the quality of sample preparation—such as extraction and purification processes—determines whether the analyte is enriched or lost, ultimately affecting the LOQ value. Factors like instrument sensitivity, solvent purity, and analyte stability also influence the magnitude of the LOD and LOQ values (Zaneta et al., 2025).

The success of HPLC analysis heavily depends on the method validation process, which includes parameters such as the Limit of Detection (LOD), Limit of Quantification (LOQ), and recovery percentage. Low LOD and LOQ values indicate that the method is capable of detecting and measuring compounds at very low concentrations. On the other hand, high recovery indicates that the method can determine compound concentrations accurately with minimal error. With adequate validation, HPLC becomes a reliable method for ensuring that the tested antibiotic products meet quality requirements (Priyangan et al., 2024). Additionally, HPLC offers advantages in terms of analysis speed and method stability. The consistency of retention times allows each type of compound to be accurately identified within a relatively short duration. Method stability also ensures that analyses can be performed repeatedly with similar results, thereby enhancing data reliability. This time efficiency is particularly important, especially in industries requiring large-scale testing within limited timeframes (Priyangan et al., 2024).

Precision

Precision in analytical methods serves to indicate how consistent and close the measurement results are when obtained from a series of repeated tests conducted under specific conditions. In precision assessment, there are three main categories: reproducibility, repeatability, and intermediate precision. Repeatability provides an indication of the level of precision when a test is performed repeatedly by the same analyst, under identical testing conditions, and within a relatively short time frame. Repeatability can be evaluated using at least three sample concentrations, each measured three times, or by using a single concentration (e.g., 100%) analyzed in six replicates. Furthermore, inter-laboratory precision serves to demonstrate the stability of the analytical method when testing is conducted under varying conditions or environments. This testing involves at least two forms of variation, such as the use of different analysts, testing on different days, or the use of different instruments to assess the consistency of the method under conditions that are not entirely identical (Ambarati et al., 2023).

Based on the data in the table of various NSAID formulations, most studies yielded precision values indicating low RSD values, generally within the range of 0.09–4.3%. According to ICH (2005), the acceptance criterion is RSD < 2%. For ibuprofen, the precision obtained in various studies ranged from 0.16% to 2.39%; in the inter-day testing in the study by Nessa et al. (2021), it reached 3.26%. Tablet formulations generally yield lower precision, as seen in the study by Romsiah & Yolanda (2017), which reached 0.16%, whereas topical gel formulations provide good precision with a value of 0.51%. Unlike the generally consistent precision of ibuprofen, aspirin exhibits more variable precision within the range of 0.48–2.73%. The best RSD value, 0.48%, was reported by Gousuddin et al. (2016), while the highest precision was recorded in the study by Utami et al. (2017) at 2.73%. This variation is likely influenced by the choice of mobile phase and the stability of aspirin, which is more sensitive to pH, resulting in some methods yielding less optimal precision performance compared to other NSAIDs.

For mefenamic acid, all studies demonstrated good and consistent precision with an RSD range of only 0.12–0.34%. The lowest precision was recorded in the study by Dikran & Mahmood (2018) at 0.1214%, indicating that the HPLC method for mefenamic acid has high accuracy and is highly consistent even in capsules and suspensions. Ketoprofen tested in enteric-coated tablets by Umar et al. (2021) also showed good precision with an RSD value of 0.876%, although the precision data is limited to a single study. Other active ingredients, such as sodium diclofenac, generally exhibit high and stable precision, particularly in the study by Andraws & Trefi (2020), which reported an RSD value of 0.09%, and the study by Nenni et al. (2022) with a value of 0.12%, even in the more complex plasma matrix. However, one study (Alquadeib, 2019) reported higher precision at 4.35%. This may be influenced by several factors such as the mobile phase, column type, length, diameter, and temperature, as well as the mobile phase flow rate (Pratiwi et al., 2023). For piroxicam, precision values range from 1.2–2.83%, with the study by Arihan et al. (2022) showing an RSD of up to 2.83%. This value is relatively higher compared to ibuprofen or mefenamic acid, likely due to piroxicam's higher λmax (341–360 nm), resulting in relatively lower detection sensitivity. Naproxen demonstrated good precision across all studies, with values ranging from 0.07–1.56% under both intraday and interday conditions (Kumar et al., 2016; Suryawanshi et al., 2024).

Accuracy

Accuracy refers to the degree of agreement between the value obtained from an analysis (measured value) and the accepted true value. Accuracy is assessed using the percent recovery parameter, which serves as the primary indicator of the precision of an analytical method. According to AOAC (2016), the acceptable range for percent recovery is 97–103% (Harmono, 2020; Amin et al., 2016). Based on the 24 journals analyzed, the accuracy values of all studies fell within the acceptable range as per

AOAC guidelines. The table results show that various NSAIDs such as ibuprofen, aspirin, mefenamic acid, diclofenac sodium, piroxicam, and naproxen exhibit highly consistent accuracy ranges. Ibuprofen and piroxicam showed accuracy values of 98–102%, while aspirin fell within the same range. Mefenamic acid has an accuracy of 98–101%, naproxen of 98.89–101.50%, and sodium diclofenac shows the most stable accuracy with values of 99.03–100.69%. This narrow range indicates that the HPLC method is capable of providing accurate results for various types of NSAIDs despite their significant differences in chemical structure.

Linearity

Linearity is one of the validation parameters used to assess whether an analytical method is capable of producing an instrumental response that is proportional to the concentration of the analyte in the sample. This linearity test is performed by plotting a calibration curve using at least five standard solutions of specific concentrations (Razkianita & Permatasari, 2025). A calibration curve is a standard method for determining analyte concentration based on Lambert-Beer's law, expressed by the equation $y = bx + a$, where y is the peak area and x is the concentration of the substance. Linearity is confirmed by a correlation coefficient (r) value approaching 1 (Sofyani et al., 2018). According to ICH (2005), a value of r classified as excellent is $r > 0.998$. If the r value meets the acceptance criteria, the method can be declared linear, indicating a proportional relationship between analyte concentration and instrument response (Ambarati, 2023)

Based on the data in the table, most studies on NSAID drugs show very high r values, ranging from 0.997 to 1, thus meeting the criteria for ideal linearity. An r value reaching 1 indicates that the stability of the UV detector response and the quality of the calibration curve construction are excellent. This indicates that the standard concentrations used are within the appropriate working range and produce a consistent linear relationship. An increase in the concentration of the standard solution results in a proportional increase in absorbance. This is consistent with the Lambert–Beer Law, which states that absorbance is directly proportional to concentration as long as measurement conditions remain constant. The calibration curve is plotted by plotting concentration against absorbance, and the resulting straight regression line indicates that the method has good linearity (Ambarati, 2023).

Based on the data in the table, the ibuprofen samples exhibit good linearity with r values ranging from 0.99795 to 0.9999. Research by Hermawan et al. (2025) shows slightly lower r values compared to other studies, but still indicates strong linearity. These lower values are likely influenced by a smaller range of standard concentrations.

For the aspirin samples, the r values ranged from 0.971 to 1. The best linearity was demonstrated by the study by Puri & Walia (2024) with an r value of 1, indicating that the response was highly proportional to the concentration. Conversely, the study by Utami et al. (2018) showed an r value of 0.971 for aspirin tablet samples. A correlation coefficient that does not reach 0.99 may be caused by various factors, such as an excessively wide concentration range, variations in the standard dilution process, detector response beginning to saturate at high concentrations, or an insufficient number of calibration points. Nevertheless, this value is still acceptable for non-critical analytical methods or alternative techniques such as TLC-densitometry. However, for quantitative analysis using HPLC, an r value approaching 1 is still recommended to ensure the accuracy of concentration determination.

Studies on mefenamic acid samples demonstrated consistently high linearity, with an r value range of 0.9995–1. Research by Dalvi et al. (2022) even showed an r value of 1, indicating a highly stable and concentration-proportional UV detector response. Linearity analysis of diclofenac sodium also yielded excellent results, with r values ranging from 0.9991 to 0.9999. Research by Alquadeib (2019) provided the most stable linearity, indicating an excellent calibration curve within its working concentration range. Meanwhile, the study by Nenni et al. (2022) showed a high r value, albeit slightly lower. This confirms that sodium diclofenac still provides a consistent linear response in HPLC analysis.

The linearity of piroxicam was in the range of 0.9993–0.99998, indicating excellent performance. Research by Arihan et al. (2022) showed the highest linearity with an r value of 0.99998, while research by Panainte et al. (2016) showed an r value of 0.9993. This variation indicates that differences in the mobile phase and column used do not have a significant impact on linearity, so piroxicam can be analyzed with stable sensitivity. For naproxen, all studies showed an r value of 0.999, both for the 100 mg and 400 mg tablet formulations. This consistency indicates that naproxen has stable UV absorbance and provides a linear response. Linearity for ketoprofen showed an r value of 0.9996, meaning the resulting response remains proportional across the tested concentration range.

In general, data from several studies on NSAIDs indicate that the HPLC method exhibits good linearity, with most correlation coefficients falling within the optimal range. This suggests that the methods examined are reliable for quantification purposes and suitable for determining the concentrations of various NSAID active ingredients in different dosage forms.

Conclusion

Based on the literature review above, it can be concluded that HPLC is the most consistent, accurate, and reliable method for analyzing NSAIDs in various pharmaceutical dosage forms. Most studies tended to use C18 columns, a mobile phase consisting of a buffer and organic solvents such as methanol or acetonitrile, flow rates ranging from 0.7–1.0 mL/min, and injection volumes of 5–20 µL. Validation parameters from all journals, including linearity, recovery, LOD, and LOQ, fall within ranges that meet analytical requirements, thereby supporting the reliability of HPLC in detecting NSAIDs with high sensitivity.

This review is intended to serve as a reference for researchers and laboratory practitioners in determining the most effective analytical conditions for NSAIDs, while also aiding the standardization of methods in both research laboratories and quality control laboratories. For future research, it is recommended to conduct a more in-depth exploration of mobile phase variations, the selection of columns using the latest technology, and the integration of HPLC with advanced detectors such as PDA and MS to improve the interpretation and accuracy of results. Additionally, future research should expand the scope of NSAID formulations, evaluate the influence of sample matrices on method performance, and consider the use of automation techniques and modern analytical software to improve the overall efficiency, precision, and reproducibility of the analysis.

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