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Review article

Analytical methods for quantification of non-steroidal anti-inflammatory drugs in pharmaceutical and biological samples: An overview of developments in the last decade

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used pharmaceuticals in both human and animal medicines for the treatment of certain conditions such as inflammation, fever, and pain. The role of validated analytical methods has become highly important in the quantification of drug substances from their pharmaceuticals as precise product quality control is required. In the present review, we have summarized various sample preparation methods and analytical methods developed for the quantification of NSAIDs during the past decade (2012-till date). Furthermore, an in-depth description of numerous techniques including chromatography (89), UV spectrophotometry (5), spectroflurometry (4), IR spectroscopy (3), electrophoresis (4), and

Abbreviations: μLPME, Microfluidic-based liquid-phase microextraction; μSPE, Micro solid phase extraction; AA, Arachidonic acid; BR, Britton Robinson; CE, Capillary electrophoresis; CMS, Capillary microsample; CMD, Cerebral micro dialysis; COX, Cyclooxygenase; CPE-MWCNT, Carbonate paste-multiwalled carbon nanotubes electrode; CPE, Cloud point extraction; CV, Cyclic voltammetry; CZE, Capillary zone electrophoresis; DAD/PDA, Photodiode array; DBS, Dried blood spot; DPS, Dried plasma spot; DSS, Dried saliva spot; DUS, Dried urine spot; DES, Deep eutectic solvent; DLLME, Dispersive liquid–liquid microextraction; DMSPE, Dispersive magnetic solid phase extraction; DPV, Differential pulse voltammetry; EME, Electro membrane extraction; FTIR, Fourier transform infrared; FD, Fluorescence detection; $Fe_3O_4/SiO_2/TiO_2$, Magnetite/silica/titania composite; FID, Flame ionization detector; FL, Spectro fluorimetry; GC, Gas chromatography; GC-QqQ-MS/MS, Gas chromatography-tandem mass spectrometry with the triple quadrupole; GC-FID, Gas chromatography-flame ionization detection; GC-EI-MS, Gas chromatograph–electron impact-mass spectrometer; GPC, Gel permeation chromatography; HCl, Hydrochloric acid; HDTMA-ZSM5/Fe_2O_3, zeolite-based composite decorated with iron oxide magnetic nanoparticles and modified with hexadecyltrimethylammonium bromide surfactant; HF-LPME, Hollow fiber liquid-phase microextraction; HILIC, Hydrophilic interaction liquid chromatography; HPLC, High-performance liquid chromatography; HRMS, High-resolution mass spectroscopy; HPLC-FD, high-performance liquid chromatography-florescence detector; HPLC-MS, High-performance liquid chromatography-mass spectrometry; HPLC-PDA, High-performance liquid chromatography-photo diode array; HPLC-MS/MS, High-performance liquid chromatography-tandem mass spectrometry; HRMS, High-resolution mass spectrometry; IR, Infrared; LC, Liquid chromatography; LLE, Liquid-liquid extraction; LLME, Liquid-liquid microextraction; LPME, liquid-phase microextraction; LOD, Limit of detection; LOQ, Limit of quantitation; LC-HRMS, Liquid chromatography-high resolution mass spectrometry; MCM-41, Mesoporous silica material; MDSPE, Magnetic dispersive solid-phase extraction; MEKC, Micellar electrokinetic capillary chromatography; MEPS, Microextraction by packed sorbent; MIP, Molecularly imprinted polymer; MP A, Moblie phase A; MP B, Moblie phase B; MS/MS, tandem mass spectrometry; MS, Mass spectrometry; MSPD, Matrix solid-phase dispersion method; MSPE, Magnetic solid phase extraction; NaOH, Sodium hydroxide; RDSE, Rotating disk sorptive extraction; RP, Reversed phase; SEME-SFOD, Surfactant-enhanced emulsification microextraction method based on solidification of floating organic drop; SFC, Supercritical fluid chromatography; SPE, Solid-phase extraction; SPME, Solid phase microextraction; SPMTE, Solid phase membrane tip extraction; TAALLM, Tandem air agitated liquid–liquid microextraction; TFME, Thin film microextraction; TX, Thromboxane; UA-Dμ-SPE-S-UA-LLME-SFO, Ultrasound assisted dispersive micro solid-phase extraction coupled with salting-out ultrasound-assisted liquid–liquid microextraction based on solidification of a floating organic droplet; UHPLC, Ultra-high performance liquid chromatography; UPLC, Ultra-performance liquid chromatography; UV, Ultraviolet.

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electrochemical (9) approaches used to determine and quantify NSAIDs are provided. Based on the matrix utilized, the following details were discussed: analytical conditions, detection limits, and solvent used in sample preparation. The present compilation provides valuable insights and crucial information on quantification methods for NSAIDs and would assist the scientific community to select the best and economical method for drug analysis in pharmaceuticals and biological samples.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) constitute some of the highly recognized categories of pharmaceutical agents having a variety of advantages. These agents are generally employed as therapeutics in humans and animals due to their ability to function as antipyretic, analgesic, and anti-inflammatory agents (I. Olives et al., 2012; Izadi et al., 2020). Drugs included within this category differ in terms of their chemical properties and almost all agents possess varying amounts of therapeutic efficacy (Kress et al., 2016; Modi et al., 2012; Smith, 2014). NSAIDs are widely employed to alleviate several debilitating conditions including fever, migraines, menstrual irregularities, rheumatoid arthritis, osteoarthritis, gout, and postoperative complications (Katturajan and Sabina, 2021; McCarberg and Gibofsky, 2012). Several reports in the literature have suggested that NSAIDs have certain functions in reducing the risk of acquiring malignancies such as colorectal, breast, ovarian, hepatocellular, prostate, pancreatic, and head and neck cancers (Amici et al., 2006; Bindu et al., 2020; Ruder et al., 2011). These agents act on cyclooxygenase enzymes (COX-1 and COX-2) that are involved in the conversion of arachidonic acid (AA) into prostaglandins (PGs) and also generate thromboxane's (TX), which is ultimately involved in the modulation of the inflammatory response (Omran, 2013; Shishov et al., 2019; Smith et al., 1998). NSAIDs can be further subdivided into COX-1 selective, COX-1 non-selective, and COX-2 selective based on their mechanism of action (Haag et al., 2008). The physicochemical properties of selected NSAIDs are outlined in Table 1.

Sample preparation is considered as one of the crucial steps in an analytical process, as it plays a significant role in mitigating the impact of interfering factors on the analytical performance of various techniques. Moreover, appropriate sample preparation can be useful in several analytical methods (Li et al., 2020). Techniques such as homogenization, solid-phase extraction (SPE), liquid–liquid extraction (LLE), and centrifugation are commonly employed in sample preparations to improve the sensitivity, specificity, and accuracy of the analytical results (Yilmaz et al., 2020). This review provides an overview of different analytical techniques used in the detection and quantification of NSAIDs in pharmaceutical preparations and biological samples. Moreover, it gives preliminary data on developed analytical methods (chromatography, spectrophotometry, spectroscopy, electrophoresis, and electrochemical techniques) that have been used in the determination of NSAIDs containing pharmaceuticals and biological samples.

Table 1
Classification and physicochemical properties of selected NSAIDs.

Class	Drugs	Mol. weight (g/mol)	Log P	pKa	Water solubility (mg/mL)
Nonselective COX inhibitors					
Salicylic acid derivatives	Aspirin	180.16	1.18	3.50	10.0
Pyrazole derivatives	Phenylbutazone	308.37	3.16	4.50	47.5
Anthranilic acid derivatives	Mefenamic acid	241.28	5.12	4.20	20.0
	Flufenamic acid	281.23	5.25	3.88	9.1
Propionic acid derivatives	Ibuprofen	206.29	3.97	5.30	21.0
	Naproxen	230.26	3.18	4.15	15.9
	Ketoprofen	254.28	3.12	4.45	51.0
Acetic acid derivatives	Diclofenac	296.15	4.51	4.15	2.37
	Ketorolac	255.27	2.10	3.84	0.86
Indole derivatives	Indomethacin	357.79	4.27	4.50	0.93
Enolic acid derivatives	Piroxicam	331.35	3.06	6.30	23.0
	Meloxicam	351.40	1.16	4.47	22.0
	Tenoxicam	337.38	1.19	2.21	14.1
Selective COX-2 inhibitors					
Coxibs	Celecoxib	381.37	3.53	11.10	0.000503
	Etoricoxib	358.84	2.79	16.19	0.00328

2. Pharmacokinetics and metabolism

Drugs included in the NSAID category possess both hydrophilic groups, such as carboxylic or enolic groups, and lipophilic groups, such as aromatic rings or halogen atoms (Starek and Krzek, 2009). The gastrointestinal tract serves as the primary route of absorption for most of these drugs. These medications typically have high bioavailability making them highly effective agents. However, certain NSAIDs, such as diclofenac, are subjected to hepatic first-pass metabolism, which reduces their bioavailability. Conversely, prodrugs, such as sulindac and parecoxib, require hepatic metabolism to convert to their active metabolites, sulindac sulphide, and valdecoxib, through which they can exert their action. A majority of NSAIDs that exist in the plasma remain in highly ionized forms. The strong binding capacity (>97 %) of NSAIDs to plasma proteins is a result of their favorable amphiphilic properties, which displace other drugs from protein binding leading to increased persistence in the body and a high duration of action (Starek and Krzek, 2009). The volume of distribution is generally low for NSAIDs ranging from 0.1 to 0.3 L/kg of body weight since they are lipophilic in nature, and are not metabolized or excreted by the liver or kidneys. The half-life of NSAIDs varies to a great extent depending on their physicochemical properties. For example, the half-life of aspirin is 0.25–0.3 h, while for piroxicam it is 45–50 h (Awtry and Loscalzo, 2000). These pharmacokinetic parameters are influenced by the patient's age, protein binding, and the distribution of the drug (Wongrakpanich et al., 2018).

3. Sample preparation

A brief summary of pre-treatment methods that are utilized for the analysis of NSAIDs in biological specimens is provided in this section. Recent developments in analytical instrumentation have resulted in improvements in sensitivity, efficiency, and selectivity. Nevertheless, there are a few challenges in directly analyzing low or trace amounts of analytes in complicated samples. These difficulties include the existence of matrix effects, interferences, and analytical instrument incompatibilities. In order to overcome these difficulties, scientists frequently isolate and purify the target analyte before analysis using sample preparation procedures like extraction, separation, or purification (Ocaña-González et al., 2016; Yan and Wang, 2013).

Sample preparation is an essential step in the analytical process, where different issues that may come up when analyzing complex samples are carefully considered. Once the barriers have been identified, particular actions can be considered, like eliminating substances that interfere, boosting the analyte concentration, and converting the analytes into a form that is appropriate for separation and detection. These actions aid in guaranteeing the accuracy and dependability of the analytical results (Ansari and Karimi, 2017). Pre-treatment procedures are essential for the analysis of NSAIDs in biological samples differ significantly from those needed for the analysis of NSAIDs in

pharmaceutical products because the matrix of biological samples containing NSAIDs can vary widely in terms of concentration levels, interferences present, and whether the sample is in a liquid or solid state. The objective of using analytical techniques for pharmaceutical products and bulk drugs is to evaluate the quality of these substances and identify any impurities or degradation products that might be present. The efficacy, safety, and regulatory compliance of drugs are guaranteed by this procedure. Utilizing analytical techniques for medications such as NSAIDs also has the benefit of allowing for the long-term monitoring of the drug's stability and the detection of any alterations in the drug's quality that might happen during transportation or storage. (I. Olives et al., 2012).

In biological matrices such as blood, serum, plasma, saliva, urine, sweat or tissues, the estimation of NSAID is essential for pharmacokinetic and pharmacodynamic studies (Al-Khateeb and Dahas, 2021). During analysis of therapeutic drugs in different biological sample matrices, selecting a suitable sample preparation method is considered to be the most important step in the analytical process. This is a result of the intricacy of such matrices, which include a range of endogenous substances that can impede the analysis, including proteins, salts, and particulate matter. Furthermore, the presence of drugs in biological matrices at very low concentrations often complicates the analysis even more. As a result, selecting a reliable and safe sampling technique is crucial (Saito and Nakagami, 2020; Vasconcelos and Fernandes, 2017). One example of a bioanalytical analysis technique is the protein precipitation method, which typically involves centrifuging the sample first and adding an organic solvent, such as methanol or acetonitrile (Lakshmana and K. Suriyaprakash, 2012). For sample preparation in complicated matrices, the traditional procedures of LLE, protein precipitation with subsequent centrifugation, and SPE have been utilized most frequently (Carasek and Merib, 2015; Płotka-Wasylka et al., 2016). Recently, there has been a shift towards miniaturization and automation in sample preparation techniques in order to address the issues of time-consuming, labour-intensive, and costly procedures, as well as the negative environmental impact of the use and disposal of large amounts of organic solvents (Magiera et al., 2013). Furthermore, different microextraction techniques have been developed for sample preparation in bioanalytical applications. These techniques include solid-phase microextraction (SPME), micro extraction by packed sorbent (MEPS), stir bar sorptive extraction (SBSE), thin-film microextraction (TFME), liquid-phase microextraction (LPME), dispersive liquid–liquid microextraction (DLLME), single-drop microextraction (SDME), electromembrane extraction (EME), solvent bar microextraction (SBME), *etc.* These techniques have become more acceptable due to their advantages in terms of their simple and rapid operation, accuracy, and sensitivity, during clinical investigations. Therefore, continuous improvement of novel sample preparation and microfluidics-based techniques is necessary to accelerate bioanalytical research (Alexovič et al., 2018; Boyaci et al., 2015; Buszewski and Szultka, 2012; Daryanavard et al., 2021; Kabir et al., 2017; Vas and Vékey, 2004).

Microsampling is an another approach of sample preparation which require small sample volumes (<50 μL) of biological fluids. It has gained significant attention at ICH level and reflected in ICH M10, SA3, and S11 guidelines. This technique assists remote sampling, easy shipment, storage and analysis during clinical trials. Example of microsampling techniques linclude cloud point extraction (CPE), microdialysis, cerebral microdialysis (CMD), dried blood spot (DBS), dried plasma spot (DPS), dried saliva spot (DSS), dried urine spot (DUS), volumetric absorptive microsample (VAMS), capillary microsample (CMS), spin column extraction (SCE) (Ingle et al., 2022; Londhe and Rajadhyaksha, 2020).

4. Analytical techniques

Although reviews for the quantification of NSAIDs have been published in the past, none of these reviews include all the reported analytical techniques for the analysis of NSAIDs in pharmaceuticals and biological samples. Additionally, a lot of fresh analytical methods have been reported in the recent past. With the development of novel sample preparation procedures as well as sophisticated chromatographic and spectrometric techniques during the past ten years, drug analysis has experienced enormous expansion. Therefore, an updated and comprehensive review that can briefly explain various analytical methodologies for NSAIDs is required. Furthermore, a concise tabular explanation of each analytical technique along with its procedure and specifics, such as analytical conditions, matrices, and limit of detection (LOD) is mentioned. A graphical representation of different analytical techniques used for quantification NSAIDs in pharmaceuticals and biological samples is shown in Fig. 1.

4.1. Spectrometric and spectroscopic techniques

Spectrometric and spectroscopic methods have several advantages over other analytical methods such as being an easy, uncomplicated procedure as well as low reagent consumption. Thus, they are considered cost-effective and time-saving methods as compared to most of the other methods. Furthermore, these methods can be used for the determination of drugs in laboratories where modern and expensive equipment such as gas chromatography (GC) or high-performance liquid chromatography (HPLC) are not available.

4.1.1. UV spectrophotometric technique

Several studies have reported UV spectrophotometric techniques for the determination of NSAIDs in pharmaceuticals and biological samples. A summary of these studies is presented in Table 2 and Table 3. Liposome-encapsulated diclofenac sodium was prepared by **Goh *et al.*** and analyzed using a simple, economical, and reliable UV spectrophotometric technique employing dimethyl sulfoxide as a solvent. The detection wavelength was 295 nm with a linearity range of 5–35 μg/mL and a 0.9978 regression coefficient. The LOD and limit of quantitation (LOQ) were 1.19 and 3.62 μg/mL, respectively (Goh et al., 2014). **Zaazaa *et al.*** carried out an analysis of ibuprofen with famotidine in a combined form wherein they analyzed ibuprofen in the form of tablet matrices through ratio difference UV spectrophotometric analysis with methanol and 0.1 N NaOH as solvents. The wavelength applied for detection was 262.5–271.7 nm. The LOD and LOQ values were found to be 13.68 and 41.47 μg/mL, respectively. This ratio difference method was simpler than the Q-absorption and absorption correction methods as it involves fewer data processing steps (Lotfy et al., 2015; Zaazaa et al., 2015). For the simultaneous estimation of naproxen, imatinib, and nalbuphine in a quaternary mixture and human urine, **Belal *et al.*** used the spectrophotometric technique. This work used a univariate method (extended derivative ratio) as well as the multivariate method (partial least squares in original and derivative mode). They asserted that these methods work in laboratories without liquid chromatographic equipment and can be used for quality control analysis without the need for any preliminary separation steps (Belal et al., 2018). **Mabrouk *et al.*** analyzed ketorolac in biological samples of human plasma with 0.1 N HCl and phosphate buffer as solvents to simulate human conditions. The wavelength used was 223 nm. The LOD value was found to be 0.08 μg/mL with a linearity range of 2–20 μg/mL. This method differentiated and measured ketorolac after the removal of potential interfering substances in the blank biological matrix without using large volumes of organic solvents, such as methanol, acetonitrile, and *n*-hexane, sophisticated instruments (Mabrouk et al., 2020). The described spectrophotometric methods showed several advantages such as simplicity, low cost, and speed of analysis. However, disadvantages such as the inability to analyze related substances and less sensitivity often make them unattractive in analysis.

4.1.2. Spectrofluorimetric technique

Table S1 and Table S2 summarize the studies utilizing spectrofluorimetric techniques for the estimation of NSAIDs in pharmaceuticals

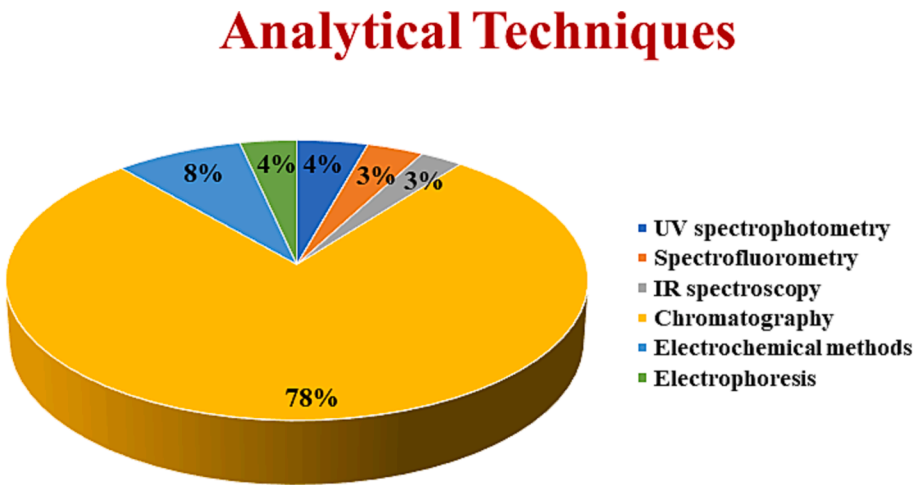

Fig. 1. Graphical representation of different analytical techniques used for NSAIDs determination in pharmaceuticals and biological samples.

Table 2
UV spectrophotometric techniques for NSAIDs determination in pharmaceuticals.

Analyte	Formulation	Solvent(s)	Detection (nm)	Linearity (μg/mL)	LOD (μg/mL)	Ref.
Diclofenac sodium	Liposome encapsulated formulation	Dimethyl sulfoxide	295.0	5–35	1.19	(Goh et al., 2014)
Ibuprofen	Tablet	Methanol and 0.01 N Sodium hydroxide	262.5–271.7	50–600	13.68	(Zaazaa et al., 2015)
Naproxen	Tablet	Methanol	239.0	1–6	0.09	(Belal et al., 2018)
Mefenamic acid	Tablet	0.1 N Sodium hydroxide	285	2–10	–	(Pabla et al., 2018)

Table 3
UV spectrophotometric techniques for NSAIDs determination in biological samples.

Analyte	Matrix	Sample Preparation	Sorbents/Solvents used in Sample Preparation	Detection (nm)	Linearity (μg/mL)	LOD (μg/mL)	Ref.
Naproxen	Human urine	Weighing, dilution, filtration	Methanol	239.0	1.0–6.0	0.09	(Belal et al., 2018)
Ketorolac	Human plasma	SPE	Ion exchange MIP (Chitosan-based)	223.0	2–20	0.08	(Mabrouk et al., 2020)

and biological samples. **Lian *et al.*** used a complex imprinted membrane as the recognition material to develop a simple and effective solid-surface fluorescence method for determining naproxen in a capsule dosage form. The extraction, concentration, and detection steps were combined into one step by a complex imprinted membrane, which might have improved the effectiveness of the analytical process. A satisfactory linearity was attained between 0.50 and 20 mg/L concentrations, and LOD was 0.11 mg/L at emission and excitation wavelengths of 352 and 284 nm, respectively (Lian et al., 2013).

Amjadi *et al.* performed the biological analysis of celecoxib in human serum using ratiometric fluorescent nanosensor at emissive and excitatory wavelengths of 550–440 and 360 nm, respectively. They have developed a new sensor for selective identification and quantitative determination of target analyte, by integrating the exceptional selectivity of the molecularly imprinted polymer (MIP), the effective characteristics of the ratiometric approach, and the sensitivity of mesoporous silica. The LOD and linearity range was found to be 57 μM and 0.08–0.90 μM, respectively (Amjadi and Jalili, 2018). **Attala *et al.*** used an improved first derivative synchronous spectrofluorimetric technique to identify amlodipine and celecoxib in a combined dosage form and human plasma. Compared to traditional native fluorescence techniques, synchronous spectrofluorimetric simplified emission spectra, narrows spectral bands, and constricts spectral range. To increase the fluorescence intensity, a complex was formed between the drugs and sodium dodecyl sulphate. Linearity, LOD, LOQ, precision, and accuracy of the validation parameters were within acceptable limits (Attala et al., 2020). Only being able to analyze fluorescent compounds or requiring a derivatization step that increases complexity and analysis time, and typically prevents the analysis of related substances, are its limitations, which restrict the use of this technique in quality control applications.

4.1.3. IR spectroscopic techniques

Haskell *et al.* analyzed ibuprofen in tablet formulation and urine sample using transmission Fourier transform infrared (FTIR) method in which LOD values were found to be 0.77 μg/mL in both samples and the linearity range was found to be 10–100 μg/mL. The main outcomes of this method were analytical simplicity, improved rapidity, improved accuracy, and improved sensitivity for ibuprofen quantification. The range from 1807 to 1461 cm^{-1} was used to create a partial least square calibration model as shown in **Table S3 and Table S4** (Khaskheli et al., 2013). For the determination of four NSAIDs, etodolac, tolfenamic acid, bumadizone, and diacerein, either alone or in the presence of their degradation products, **Hassib *et al.*** used ATR-FTIR methods like direct measurement, first derivative, and second derivative with minimal sample preparation. To get over the spectrum overlap and enable simultaneous assessment of drugs and their degradation product, derivative spectroscopy was utilized for data processing. From the pharmaceutical preparation, active constituents were extracted in

chloroform, and then directly measured in liquid form. The LOD of etodolac, bumadizone, and tolfenamic acid were observed to be 1.523, 2.773, and 1.193 μg/mL, respectively. The reported techniques could serve as a substitute for methods that utilize the separation processes. IR spectroscopic technique has the potential to decrease both chemical waste and costly laboratory testing (Hassib et al., 2017). Nevertheless, this technique does not allow analyte separation.

4.2. Chromatographic techniques

Several chromatographic techniques were used in the analysis of NSAID drugs such as high-performance liquid chromatography-UV (HPLC-UV), high-performance liquid chromatography-floroscence detector (HPLC-FD), high-performance liquid chromatography-mass spectrometry (HPLC-MS), high-performance liquid chromatography-photo diode array (HPLC-PDA), high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), ultra-high performance liquid chromatography-tandem mass spectrophotometry (UHPLC-MS/MS), supercritical fluid chromatography (SFC), gas chromatography- flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC–MS), gas chromatography-tandem mass spectrometry (GC–MS/MS), and liquid chromatography-high resolution mass spectrometry (LC-HRMS), and hydrophilic liquid chromatography (HILIC). These methods are listed in **Table S5 and Table S6.** The quantitation of NSAIDs in pharmaceutical formulations (tablet, capsule, caplet, eye drops, and suspension) and biological samples (human plasma, urine, blood, breast milk, rat plasma, rabbit tissue, swine muscle, *etc.*) using chromatographic techniques with various types of detectors, including UV, fluorescence, PDA, MS, and MS/MS are represented in Fig. 2**.**

The determination of mefenamic acid in urine and pharmaceutical samples was carried out by **Rezaei Kahkha *et al.*** using HPLC-UV following pipette-tip solid phase microextraction using carbon nanotubes modified with zinc sulphide. The method was observed to have good linearity in the range of 0.7–100 μg/L. The LOD was found to be 0.075 μg/L. Additionally, a quick analysis time of 9 min was attained (Rezaei Kahkha et al., 2016). For the biological sample analysis of naproxen, diclofenac, and mefenamic acid, the HPLC-UV analytical technique was employed by **Aqda *et al.*** which was analyzed in the plasma sample. The mobile phase of methanol and water in an 80:20 proportion was pumped at a flow rate of 1.0 mL/min with the C_{18} column as a stationary phase. LOD values were found to be 1.80 μg/mL, 2.40 μg/mL, and 2.00 μg/mL for naproxen, diclofenac, and mefenamic acid, respectively (Golzari Aqda et al., 2018). In 2023 **Han *et al.*** coupled the Fe_3O_4@magnetic ionic liquid hypercrosslinked polymer composite based magnetic solid-phase extraction (MSPE) with HPLC-DAD to detect specific NSAIDs in urine and water samples with sensitivity. Using Friedel-Crafts alkylation, a magnetic ionic liquid hyper crosslinked polymer composite was produced as an MSPE adsorbent. The developed composite was able to extract NSAIDs from real samples with a wide linear range, low LODs, and satisfactory recoveries (Han et al., 2023). Using a simple cysteine-triggered in situ growth strategy, **Ji *et al.*** created amino-bearing metal–organic frameworks modified on cotton fibers and used as an in-situ SPME adsorbent combined with HPLC-UV for the extraction and quantitation of three NSAIDs *i.e.* diclofenac sodium, ketoprofen, and flurbiprofen in human plasma samples. The experiment yielded good linear range, good reproducibility, good recoveries (66.5 % – 98.9 % with less than 6.62 % relative standard deviations), and satisfactory sensitivity. For the quantitative analysis and pretreatment of NSAIDs in complex samples, the suggested method showed potential (Ji et al., 2023).

For the detection and quantification of NSAIDs such as acetylsalicylic acid, ketoprofen, diclofenac, naproxen, and ibuprofen in human urine, **Magiera *et al.*** reported a novel method based on MEPS and an RP-UHPLC-UV. The analytes were separated using a binary mobile phase (aqueous 0.1 % trifluoroacetic acid:Acetonitrile) in the gradient elution mode on a core–shell C_{18} column (100 × 3.0 mm × 2.7 μm). For the target drugs, good linearity (1.07–16.2 ng/mL) and LOD were reported (Magiera et al., 2013). For the simultaneous separation and determination of NSAIDs in human urine and plasma, **Ferrone *et al.*** developed graphene/Fe_3O_4-based dispersive magnetic solid phase extraction coupled with UHPLC-PDA. It was found that using an isocratic elution mode with acetonitrile and 10 mM potassium dihydrogen phosphate (pH 2) in water (50:50, v/v) pumped at a flow rate of 0.55 mL/min resulted in higher separation efficiency, better peak shape, and short run time (Ferrone et al., 2018). The chiral HPLC is used to distinguish between the enantiomers of naproxen, etodolac, and ibuprofen. **Hewala *et al.*** performed the analysis of the etodolac drug which existed in their *R*- and *S*- enantiomers in the form of tablet pharmaceutical preparation and human plasma through HPLC-DAD. The mobile phase was hexane, isopropanol, and trifluoracetic acid in a 90:10:0.1 proportion. The stationary phase was the Kromasil Cellucoat Chiral column (Hewala et al., 2014). In order to quickly determine *S*-ibuprofen and *R*-ibuprofen at low concentration levels typically present in human breast milk, **León-**

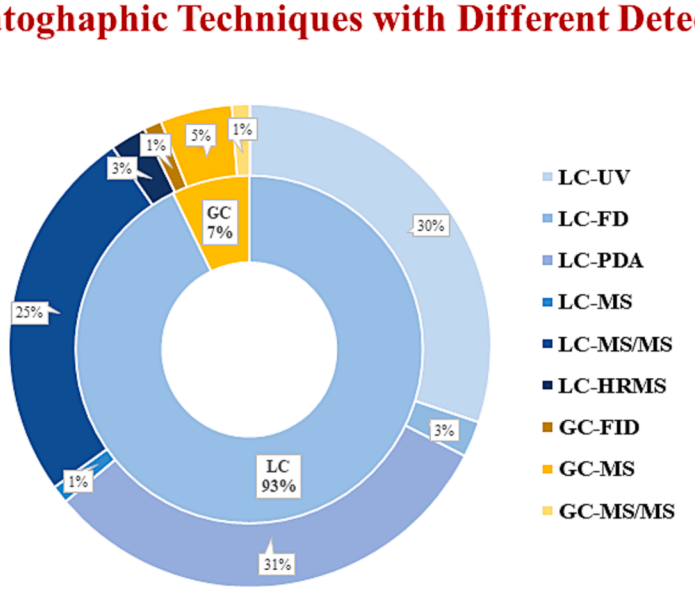

Fig. 2. Summary of various chromatographic techniques along with different detectors used for NSAIDs determination in pharmaceuticals and biological samples.

González *et al.* combined the optimized vortex-assisted matrix solid-phase dispersion method (MSPD) procedure with direct chiral LC-UV detection. Upon analysis, LOD was found to be 0.042 μg/g for *R*-ibuprofen and 0.045 μg/g for *S*-ibuprofen (León-González and Rosales-Conrado, 2017). LC-MS and LC-MS/MS are widely used to determine NSAID residues in complex biological matrices such as human plasma, urine, beef liver, whole blood, and animal tissues. LC coupled to MS or MS/MS is preferred over other detectors due to its high analytical selectivity and sensitivity. The accurate identification of poisons or drugs is the primary objective of toxicology investigations. Since substances with chemically similar structures may potentially cause interference, it is important to accurately discriminate drugs from all potential sources of interference. This can be done by using LC-MS/MS techniques. In order to simultaneously quantify sixty drugs that are frequently found in postmortem blood, **Al-Asmari** has developed an LC-MS/MS technique. The method could be used for NSAIDs like acetaminophen, ibuprofen, mefenamic acid, and diclofenac as well as a wide range of other compounds and their metabolites in forensic toxicology cases. Gradient elution was carried out using biphenyl columns (50 × 3.0 mm, 2.7 μm) at a flow rate of 0.30 mL/min. This method was found to quickly and accurately identify multiple drugs. The author has claimed that this technique is for the multianalyte screening of sixty drugs and their metabolites that are commonly encountered in postmortem toxicology and can be applied to the routine analysis of autopsy blood samples (Al-Asmari, 2020). **Sun *et al.*** developed a UPLC-MS/MS method for the detection of NSAIDs in swine kidneys, fat, muscle, and liver. Phosphorylated acetonitrile was used to extract the swine tissue samples, which were then purified using a hydrophile-lipophile balance (HLB)-SPE column and separated using an Acquity^{TM} UPLC BEH shield RP_{18} column in gradient elution mode, and 0.1 % formic acid in acetonitrile and water as a mobile phase. The method was able to separate and detect ten different types of NSAIDs in 10 min with good recovery and repeatability. The suggested technique would be helpful for regulatory monitoring of NSAID residues in swine fat, liver, kidneys, and muscle (Sun et al., 2023). There are some drawbacks to LC techniques, including sample pretreatment or extractions, derivatization, lengthy analysis times, significant waste disposal, and high instrument and maintenance costs.

The fact that GC requires a derivatization step before analysis, which adds to the experimental effort, makes it a less popular method than LC, but it was still used to determine NSAIDs in a number of different matrices. The extraction of salicylic acid (hydrolysis product of aspirin), diclofenac, and ibuprofen, in human urine is done before their detection by GC-FID, **Barfi *et al.*** compared two dispersive-based LLME methods which include USE-AALLME and LDS-DLLME. The findings demonstrated that USE-AALLME combined with GC-FID was significantly more efficient method, offering high analyte recoveries without the need for a derivatization step and detection limits of 0.1–1.0 μg/L (Barfi et al., 2015). Ibuprofen and its four likely metabolites were simultaneously determined in equine urine samples using a novel assay developed by **Waraksa *et al.*** using GC-EI-MS. In the sample preparation step, methyl-derivatization was carried out by adding 100 mL of ethyl acetate, 50 mL of methyl iodide, and 50 mg of anhydrous potassium carbonate. An Agilent HP-1 MS column (17 m × 200 μm, 0.11 μm) was used for the separation. As a carrier gas, helium was used with a constant flow rate of 1.8 mL/min. The LOD for ibuprofen, 1-hydroxy ibuprofen, 2-hydroxy ibuprofen, 3-hydroxy ibuprofen, and carboxy ibuprofen was found to be 0.58, 0.20, 1.37, 0.19, and 1.33 μg/mL, respectively, and also produced a satisfactory linear concentration range (Waraksa et al., 2018). **Szpot et al.** developed an ultra-sensitive GC-QqQ-MS/MS with an electron impact ionization source for direct detection of diclofenac in whole blood samples. The multiple-reaction monitoring mode was used to determine the analytes. Helium served as the carrier gas and the SH-RXI-5MS column (30.0 m × 0.25 mm, 0.25 μm) served as the stationary phase. The column temperature was initially maintained at 60 °C for 2 min before being raised to 320 °C and maintained for another 2 min. The method had a regression coefficient of 0.999 and was linear from 0.1 to 200 ng/mL. Moreover, the LOD and LOQ attained were 0.05 ng/mL and 0.1 ng/mL, respectively, both of which were relatively low (Szpot et al., 2021). GC has been widely used for NSAIDs analysis, however, further research is needed to address a few drawbacks such as the need for a derivatization step for compounds that exhibit low volatility and have poor thermal stability.

4.3. Electrochemical techniques

Recently, a number of electrochemical techniques have drawn a lot of interest because they have been found successful in quantifying various pharmaceutical compounds. In most scenarios, sample pretreatment is unnecessary, non-destructive, and takes less time. Its main advantage over conventional methods is that they enable the low-cost simultaneous analysis of multiple analytes at once. For the analysis of NSAIDs, both potentiometric and voltammetric techniques were reported; however, voltammetry is the technique that is most frequently employed due to its high sensitivity.

4.3.1. Potentiometry techniques

Potentiometric methods are an intriguing alternative to traditional drug testing because they offer straightforward procedures, inexpensive electrodes, and good sensitivity. Potentiometric techniques used in the analysis of NSAIDs are listed in **Table S7**. A new potentiometric sensor based on doped polypyrrole films for the detection of diclofenac was prepared and characterized in work reports by **Oliveira *et al.*** Diclofenac anion is incorporated into polypyrrole film during polymer electrochemical synthesis to create a membrane with a selective potentiometric response for the dopant ion. The pharmaceutical analysis of diclofenac existed in the form of tablet preparation and the solvent used was 10 mL of hydroalcoholic solution (20 %). With an electric charge of 35 mC and an electric current density of 0.045 mA/cm^2, it was observed that the sensor displayed a linear dynamic response in the concentration range from 3.1 × 10^{-4} to 1.1 × 10^{-2} mol/L (Oliveira et al., 2014).

4.3.2. Voltammetric techniques

Electroanalytical approaches, particularly voltammetric techniques are also used in the analysis of pharmaceuticals in recent years due to their precise ability to quantify analytes, less expensive equipment, and sensitivity. Various voltammetric methods have been highlighted in **Table S8 and Table S9.** The differential pulse voltammetry (DPV) method is primarily employed to determine NSAIDs in pharmaceuticals and biological samples. **Aguilar-Lira *et al.*** carried out a DPV analysis of diclofenac analyte which was present in the form of tablet pharmaceutical preparation. The solvent used was Britton-Robinson buffer (BR buffer) having pH 8. The electrodes used for the analysis were carbonate paste-multiwalled carbon nanotubes electrodes (CPE-MWCNT). The analyte concentration was found to be 2.49–10 μmol/L and the LOD value was 0.74 μmol/L (Aguilar-Lira et al., 2017). **Hendawy *et al.*** performed an analysis of naproxen in human plasma biological samples in a DPV technique. The electrode employed was a nanomaterial-based carbon paste electrode in which the concentration range of the analyte was found to be 4.35–65.5 μM and the LOD value was 6.255 μM and a quick run time of 66 s (Nigović et al., 2018). Although electrochemical techniques possess many benefits such as selectivity and accuracy, drawbacks such as extended analysis time and the necessity of complicated sample preparation steps reduce the enthusiasm for their use.

4.4. Electrophoresis techniques

Electrophoresis methods (**Table S10 and Table S11**) like capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC) have many benefits, including high separation efficiency, short run times, easy-to-use instruments, low operating costs, and compatibility with relatively small volumes. It has been

demonstrated to be among the most effective methods for analyzing biological samples. **Zhang *et al.*** performed an analytical estimation of naproxen in biological samples of human urine in which a borate buffer of pH 10.0 was used as a solvent. Capillary electrophoresis with chemiluminescence was used for analysis. The voltage was adjusted to 16 kV due to its high separation efficiency and selectivity of chemiluminescence system towards analytes. This method could determine naproxen in relatively complicated matrices. The linear concentration range was found to be 10.0–2000 μg/L and LOD and LOQ values were 2.7 μg/L and 2.8 μg/L, respectively (Zhang et al., 2018). **Dal *et al.*** carried out an analysis of piroxicam in tablet formulation in which borate buffer having pH 9.0 along with 10 % v/v methanol was used as a solvent. The analysis was conducted using capillary zone electrophoresis. The LOD and LOQ values are 0.07 μg/mL and 0.19 μg/mL, respectively (Dal et al., 2014). To analyze five pharmaceutical formulations containing three NSAIDs, **El-Kommos *et al.*** created a quick and precise MEKC method. All mixtures were separated using a 20 mM borate buffer of pH 9, 100 mM sodium dodecyl sulphate, and methanol (15 % v/v), with a voltage of 15 kV. The detection was carried out at 214 nm. The technique could be used in quality control labs not only for the binary mixtures under investigation but also for potential future binary mixtures with the same components (El-Kommos et al., 2013).

5. Conclusion

Due to the fact that NSAIDs are frequently prescribed for a wide range of indications, their analysis becomes an important aspect to ensure proper quality control. The present review summarizes various analytical techniques and sample preparation methods of NSAIDs both in pharmaceutical preparations and biological samples reported in the previous decade (2012-present). Appropriate analysis of NSAIDs not only ensures proper quality control but can also be employed in their pharmacokinetic and pharmacodynamic studies in order to examine drug toxicity, stability, and interaction studies, which provide valuable insights into the drug profile of NSAIDs. The commonly used pretreatment techniques include LLE, SPE, and the environmentally friendly novel techniques including LLME (DLLME, HF-LPME). Upon evaluation, we observed that the most widely utilized separation and analytical techniques are LC using UV, FD, MS, and tandem MS/MS techniques for their estimation. LC methods have several advantages over other methods in terms of simple to perform and cost-effective, which is why they are widely used in the clinical monitoring. However, other chromatographic methods such as GC are not commonly employed in the estimation of NSAIDs. Another example of a method that has come to light is spectrophotometric analysis in UV along with fluorimetry. A drawback of this technique is that in spite of widely available pieces of equipment, their use is limited, especially with a complex matrix. In recent times, electrochemical (voltammetric) methods have gained attention for the estimation of pharmaceutical products as they possess the advantages of being cheap, widely available, rapid to perform, simple to perform, and provide results that are more sensitive than HPLC and spectrometric methods of analysis. To conclude, it would be helpful for researchers to gain insights into the various methods employed for the estimation of NSAIDs and select the method most convenient for their research.

Author contributions

Bhavarth Dave, Jyoti Solanki, Kunal Maheshwari: Gathered information from the mentioned references and finalized the layout of the article along with the addition of content and prepared Figures, diagrams, and tables. All four authors also contributed to the subsequent manuscript draft review, editing, and subsequent changes during the manuscript preparation.

Palak Parikh, Ketan Ranch, Anuradha Gajjar, Bharaneeswar Renukuntla: Design of content and skeleton, Manuscript draft review and editing, Figures and diagram conception, overall monitoring and guidance throughout the study duration.

Nisha Parikh, Sai HS Boddu: Topic conception, preparation of the article, and formatting it according to the specified requirements.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2023.105446.

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