Talanta 85 (2011) 8–27

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Review

Review of the applications of different analytical techniques for coxibs research

Małgorzata Starek

Jagiellonian University, Collegium Medicum, Faculty of Pharmacy, Department of Inorganic and Analytical Chemistry, 9 Medyczna Str., 30-688 Cracow, Poland

ARTICLE INFO

Article history:
Received 13 October 2010
Received in revised form 15 April 2011
Accepted 19 April 2011
Available online 28 April 2011

Keywords:
NSAIDs
COX-2 inhibitors
Analytical methods
Pharmaceuticals
Biological samples
Validation

ABSTRACT

An extensive survey of the literature published in analytical and pharmaceutical chemistry journals has been conducted and analytical methods which were developed and used for the determination of some of the COX-2 inhibitors, a subclass of non-steroidal anti-inflammatory drugs (NSAIDs) in bulk drugs, formulations, and biological fluids have been reviewed. This review covers the time period from 1999 to present, during which over 140 analytical procedures including chromatographic, spectrometric, electrophoretic and voltammetric techniques were reported. Presented applications concern analysis of coxibs from pharmaceutical formulations and biological samples.

Contents

Abbreviations: ACN, acetonitrile; AI, active ingredient; APCI, atmospheric pressure chemical ionization; BCG, bromocresol green; BCP, bromocresol purple; CD, cyclodextrin; CE, capillary electrophoresis; CMS, chlorophenylmethylsulfone; COX, cyclooxygenase; CZE, capillary zone electrophoresis; ^{1}D, first derivative spectrophotometry; DAD, diode array detector; EtOH, ethanol; FDA, Food and Drug Administration; HMDE, hanging mercury drop electrode; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin layer chromatography; I.S., internal standard; ICP, inductively coupled plasma; IR, infrared; λ, wavelength; LC, liquid chromatography; LOD, detection limit; LOQ, quantitation limit; M, concentration [mol L^{-1}]; MEKC, micellar electrokinetic capillary chromatography; MeOH, methanol; MS, mass spectrometry; NMR, nuclear magnetic resonance; NSAIDs, non-steroidal anti-inflammatory drugs; OSA, octane sulfonic acid; PDA, photo diode array; PG, prostaglandin; PPAC, 4-n-pentyl-phenylacetic acid; RP-HPLC, reversed-phase high-performance liquid chromatography; RRLC, rapid resolution liquid chromatography; RSD, relative standard deviation; SDS, sodium dodecyl sulfate; TBA, tetrabutylammonium; TBAHS, tetrabutylammonium hydrogen sulfate; TCAA, trichloroacetic acid; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; UPLC, ultra performance liquid chromatography; UV, ultraviolet.

E-mail address: mstarek@cm-uj.krakow.pl

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.04.052

1. Introduction

Rheumatoid arthritis is an autoimmune disease that causes inflammation in the lining of the joints, which results in pain, stiffness, swelling, joint damage and loss of function in the joints. Osteoarthritis is the result of wear and tear of the material that cushions joints, usually in weight-bearing joints. Osteoarthritis is often accompanied by some inflammation but not to the degree seen in rheumatoid arthritis. Presently millions of people live with the pain of arthritis, which may be mild or debilitating. There are many medications available to relieve arthritis pain and slow the progression of the disease.

Non-steroidal anti-inflammatory drugs (NSAIDs) are readily available and effective and thus are extensively used by patients. The growing demand for these agents stimulates a search for new, even more effective drugs, but also calls for higher levels of quality control of these therapeutic substances and preparations, so that they are in the highest possible degree free from any impurities that may come from the production process, as well as from decomposition products of active or auxiliary substances. Therefore, it seems appropriate to develop new analytical methods regarding their analysis.

The progress of analytical chemistry in the scope of instrumentalisation of the methods of chemical analysis is reflected in the use thereof in pharmacopeia monographs as well as in the standards adopted by manufacturers. A constant place is occupied by chromatographic (especially liquid chromatography (LC)) and spectrophotometric methods. Electromigrational and voltamperometric methods are also used for the determination of NSAIDs.

2. NSAIDs—COX-1 and COX-2 inhibitors

Form a historical viewpoint, the first NSAID with therapeutic benefits was aspirin, which has now been used for more than 100 years. In the 1970s, a scientific breakthrough occurred with the elucidation of the molecular mechanism of aspirin and other NSAIDs. Vane, Samuelson and Bergstrom succeeded in showing that these anti-inflammatory substances block the biosynthesis of prostaglandins (PGs) which contribute to a variety of physiological and pathophysiological functions. PGs are produced by most cells and are also present in tissues, which explains their broad spectrum of biological responses. PGs mediate a number of characteristic features of the body's response to tissue injury or inflammation. The outstanding effects of the PGs include their cytoprotective properties in the gastrointestinal tract and control of renal functions in the kidney. PGE_2 is the most important PG which mediates the typical symptoms of inflammation: rubor, calor, tumor, dolor and function laesa. Dilatation of small blood vessels initiates the development of redness and heat; the increase in vascular permeability causes the characteristic swelling of tissues. Moreover, PGs sensitize peripheral nerve endings and nociceptors to transmit pain signals to the brain and the spinal cord.

Prostaglandins, formed by cyclooxygenases (COX) are important mediators for a number of physiological processes and pathophysiological conditions, including inflammation and pain. COX catalyzes the conversion of arachidonic acid (or other 20 carbon fatty acids) to PGG_2 and PGH_2, which are subsequently converted to a variety of eicosanoids that include PGE_2, PGD_2, $PGF_{2,a}$, PG_{T2} and thromboxane. The prostacyclins, prostaglandins, and thromboxanes act as important mediators of physiological and inflammatory responses. Two main isoforms of cyclooxygenase have been identified. The discovery of two COX, the constitutive form COX-1 and the inducible form COX-2, brought forth a new generation of NSAIDs, COX-2 inhibitors [1]. The COX-1/COX-2 model did not explain the properties of paracetamol (acetaminophen). Although its antipyretic and analgesic effects might be explained by inhibition of COX-2, it was not anti-inflammatory. In 2002, Dan Simmons' group reported the discovery of a new COX isoenzyme that was putatively the specific target of acetaminophen. They have named COX-3 [2].

Generally, the NSAIDs inhibit both COX-1 and COX-2. Most NSAIDs are mainly COX-1 selective (e.g., aspirin, ketoprofen, indomethacin, piroxicam). Others are considered slightly selective for COX-1 (e.g., ibuprofen, naproxen) and others may be considered slightly selective for COX-2 (e.g., nabumetone, meloxicam). The mechanism of action of coxibs (e.g., celecoxib) is primarily selective inhibition of COX-2. At therapeutic concentrations, the COX-1 isoenzyme is not inhibited thus gastrointestinal toxicity may be decreased. COX-1 inhibitors inactivate platelet cyclooxygenase irreversibly and at high dosages the COX-1 inhibition is generalized and more damage to the gastrointestinal tract results, so that COX-1 is mainly associated with homeostasis. On the contrary, inducible COX-2 would be the mayor isoenzyme responsible for the production of proinflammatory mediators, and for these reasons COX-2 inhibitors had no effect on platelet aggregation, and lower rates of gastrointestinal, pulmonary and renal side effects would be expected. The main advantage of selective COX-2 inhibitors is that they cause fewer gastrointestinal complications than conventional NSAIDs.

The term NSAID is an abbreviation of a class of drugs as non-steroidal anti-inflammatory drugs. This nomenclature was given to the class to differentiate it from steroids, the other major anti-inflammatory class of drugs. In addition to their anti-inflammatory effects, agents belonging to the NSAID class possess both analgesic and antipyretic activities. Hence, NSAIDs are sometimes referred to as non-narcotic analgesics or as aspirin-like drugs (aspirin was the first member of the class to be discovered). The class of NSAID drugs illustrates the close relationship between the chemical structure of drugs on one side and their biological effects and kinetic properties on the other side. Recently, some NSAIDs have emerged as part of a new class of cancer chemotherapeutic and chemopreventive agents. NSAIDs are agents that reduce inflammation by inhibiting the COX enzymes. However, in spite of their beneficial effects, NSAIDs have a tendency to interfere with the body's ability to protect the stomach lining as well as protect platelet function. Therefore, manifestations of toxicity may be unacceptable in many patients. This paved the way for the discovery and development of newer agents called COX-2-specific inhibitors (coxibs), which are like NSAIDs in that they inhibit the inflammatory conditions while they preserve homeostatic functions such as the integrity of the stomach lining or platelet control. The new class of agents has efficacy comparable to NSAIDs, but with a much improved safety profile such that their use in both the treatment of acute and chronic pain, with or without inflammatory conditions, has been widely accepted. In less than a two decades after the discovery of COX-2, clinical trials have demonstrated that treatment with highly selective COX-2 inhibitors (especially more selective coxibs) causes significantly fewer serious gastrointestinal adverse events than does treatment with classical NSAIDs [3].

Coxibs are appropriate second-line agents when a patient has specific factors that preclude NSAID use. The choice of appropriate NSAID or coxib should be based on patient risk factors, adverse effects and cost. Today, there is an abundant availability of NSAIDs and coxibs that patients may be initially treated with, and perhaps switched during the therapy. Therefore, development of an assay that has a generic application for quantitative determinations of a number of NSAIDs and coxibs has significant utility.

NSAIDs are widely prescribed drugs in clinical practice for the treatment of osteoarthritis, rheumatoid arthritis and other painful conditions. All available NSAIDs can inhibit both COX-1 and COX-2 enzymes at a given dose. NSAIDs side effects are mostly caused by COX-1 inhibition in the stomach, kidney, uterus and platelets

leading to many clinically undesirable side effects. The selectivity of a given NSAID can be expressed by the ration of the concentration of drug required to reduce enzyme activity of COX-2 and COX-1. Various risk factors (i.e. advanced age, ulcers, higher doses of NSAIDs, infections with *Helicobacter pylori*, alcohol) that have been identified in analysis of NSAIDs were a studies of gastroduodenal ulcer or gastrointestinal bleeding. In patients with current ulcers or a history of ulcers who were using NSAIDs to combined treatment with, e.g. omeprazole, infection with *H. pylori* increased the risk of gastroduodenal mucosal injury associated with NSAIDs use minimally [4]. Long-term studies of osteoarthritis patients have reported a significantly lower 12-month cumulative incidence of perforations, ulcers and upper gastrointestinal tract bleeding with COX-2 inhibitors than with other NSAIDS. The selective COX-2 inhibitors have recently been marketed as an alternative to conventional NSAIDs for the treatment of osteoarthritis and rheumatoid arthritis on the basis of the lower risk of adverse gastrointestinal effects. Moreover, overexpression of COX-2 enzyme is not only limited to the inflammatory process, but also has been observed in various types of cancer. The potential use of COX-2 selective NSAIDs in the prevention of colon cancer is suggested from the distribution of COX-2 enzyme in adenomatous polyps and colon cancer [5].

By far the greatest amount of research in the COX-2 area has been performed in the preparation and evaluation of this class of compounds. The compounds are characterized by a central carbocyclic or heterocyclic ring system bearing two vicinal aryl moieties. During the last few years a large number of compounds have been developed as potential candidates as COX-2, specific inhibitors. Occasionally this structure is defined in the literature as a "tricyclic compound" which is not really appropriate according to chemical nomenclature. The chemical structures of COX-2 inhibitors are heterogenic so that a further classification of this group will be made in the following chapter. Contrary to the classic NSAIDs, this new class of enzyme inhibitors is lacking a carboxylic group, thus effecting COX-2 affinity by a different orientation within the enzyme without formation of a salt bridge in the hydrophobic channel of the enzyme. Selective inhibitors belong to different structural classes: 1, diaryl- or aryl-heteroaryl-ethers and thioethers (sulfonanilide inhibitors): nimesulide, flosulide; 2, carbocycles and heterocycles with vicinal aryl substitution (coxibs): celecoxib, rofecoxib, parecoxib sodium, valdecoxib; 3, structurally modified known NSAIDs to improve COX-2 selectivity: meloxicam, etodolac; 4, antioxidative compounds (ibuprofen, diclofenac); 5, 1,2-diarylethylene derivatives (indomethacin).

The structure activity relationship studies have shown that the substituted sulfonyl group present in the structure of coxibs, is considered one of the pharmacophoric moieties responsible for the selective recognition with the key amino acid residues at COX-2 active site pocket. Also, it has been reported that compounds having aryl methylsufone or sulfonamide moieties display a propensity for COX-2 selectivity. It is now known that under basal conditions the constitutive enzyme COX-1 is expressed in nearly all tissues including the colon, kidney, spleen, stomach, liver, lung, heart and brain. In both the kidney and the stomach, for example, prostanoids synthesized by COX-1 act as vasodilatators. In the kidney these prostanoids help to maintain renal plasma flow and glomerular filtration during periods of systemic vasoconstriction. Similarly, in the gastric antrum, local vasodilatation appears to be critical in maintaining mucosal defenses. COX-1 in platelets on the other hand generates thromboxane which plays a key role in mediating platelet aggregation [6]. In contrast, COX-2 expression is largely undetectable unless induced by inflammatory stimuli in cells such as synovicytes, macrophages and endothelial cells. Such stimuli are proinflammatory cytokines, lipopolysaccharides, mitogenes and oncogenes, growth factors, hormones and disorders of water – electrolyte hemostasis [7]. Indeed, the functions of COX-1 and COX-2 are more complex. Findings, largely from animal studies, have suggested a broader spectrum of biological activity of COX-2. Apart from its induction in inflammatory cells, COX-2 is known to be induced in the kidney in response to sodium depletion or in hyperfiltration states, in postsynaptic excitatory neurons in the brain after electroconvulsive stimulation and in colon adenoma and carcinoma cells; this opens a new spectrum for therapy with COX-2 inhibitors.

However, emerging evidence suggests that adverse reactions such as gastrointestinal irradiation or ulceration and renal liabilities are associated with prolonged use of COX-2 selective inhibitors. They have not been approved for use in children younger than 18 years old. In adults, some factors for the use of COX-2 inhibitors have been suggested, such as age over 60 years, history of gastrointestinal bleeding, toxicity, cardiovascular disease, concomitant use of glucocorticoids and combinations of various NSAIDs. Recently, clinical investigations have demonstrated a link between the use of the sulfone COX-2 inhibitor, rofecoxib, and increased risk for atherothrombotic events. This increased risk was not observed for a sulfonamide COX-2 inhibitor (celecoxib), indicating a potential non-enzymatic mechanism for rofecoxib. Two coxibs – celecoxib and valdecoxib – have been approved for use by the Food and Drug Administration (FDA); a fourth, etoricoxib, has been approved by the European regulatory authority, and it and a fifth, lumiracoxib, are currently under consideration for FDA approval. Although it is evident that high COX-2/COX-1 ratios correlate high risk of gastrointestinal side-effects, the clinical efficacy and tolerability of selective COX-2 inhibition cannot yet be assessed since none of the NSAIDs in clinical use have COX-2 selectivity. Clinical trials have demonstrated that treatment with highly selective COX-2 inhibitors causes significantly fewer serious gastrointestinal adverse events than does treatment with non-selective NSAIDs. Additional information on the pharmacology of the coxibs, changes in blood pressure, elucidation of the cardiovascular and renal effects of those drugs and their interactions with potential adjuvant therapies is imperative [8].

3. Analytical techniques in assay of coxibs

The analysis of pharmaceuticals is an integral and increasingly important part of an overall drug development process. Technological and scientific progress has led to the development of numerous synthetic drugs. It is therefore imperative to develop analytical methods to determine these drugs both in the quality control manufacturing phase of the pharmaceutical formulations and their determination in the human body. Several techniques like atomic absorption spectrometry, capillary electrophoresis, spectrofluorimetry, liquid chromatography, mass spectrometry, luminescence, voltammetry and polarography have been used for the analysis of pharmaceutical compounds. Chromatographic methods have been extensively used and recommended. However these methods generally require complex and expensive equipment, provision for use and disposal of solvents, labour-intensive sample preparation procedures and personnel skilled in chromatographic techniques.

Coxibs are the recent development of NSAIDs and there is a great need to review the analytical work reported so far in the literature. Several years ago, some of analytical methods used in the analysis of COX-2 inhibitors (celecoxib, etoricoxib, rofecoxib, nimesulide, meloxicam) were summarized in a review paper by Rao et al. [9]. Our objective was to compile the published analytical methods dealing with formulated, unformulated drugs, and biological samples including metabolites and degradants. Techniques like spectrophotometry, fluorimetry, chromatography, electrophoresis, voltammetry and others have been used for analysis. The percentage of their utility is shown in Fig. 1, from which it can be seen that

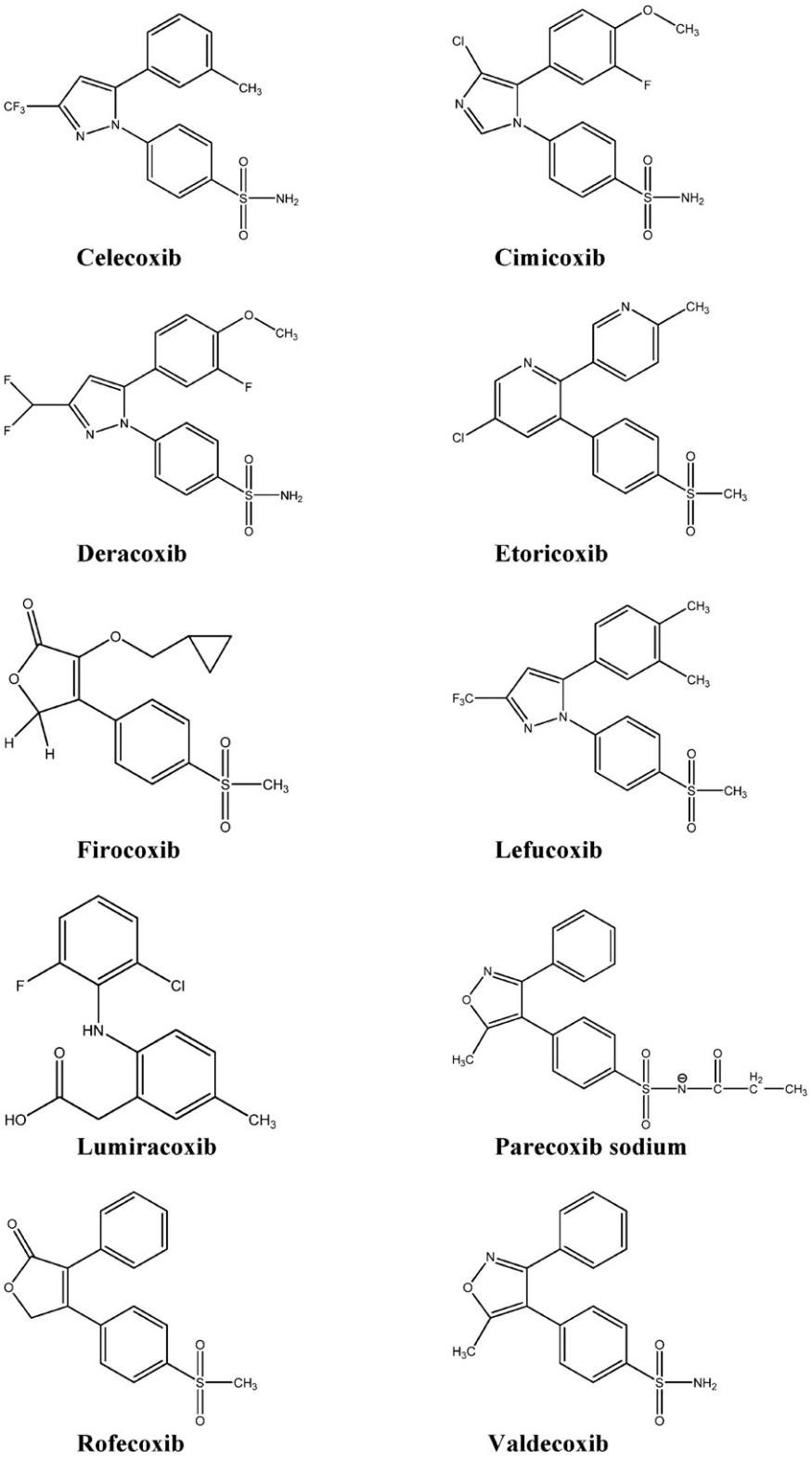

Fig. 1. Chemical structures of coxibs.

high-performance liquid chromatography (HPLC) and spectrophotometric methods have been most extensively used.

The purpose of this paper is to present the material about the assay of coxibs by different analytical methods published between 1999 and 2010 in order to complete available information. The structures of the investigated drugs are illustrated in Fig. 2. This paper is intended to help in selecting the analytical procedure for the assay of coxibs in biological material or pharmaceuticals, in certain concentrations ranges, and with great accuracy and precision.

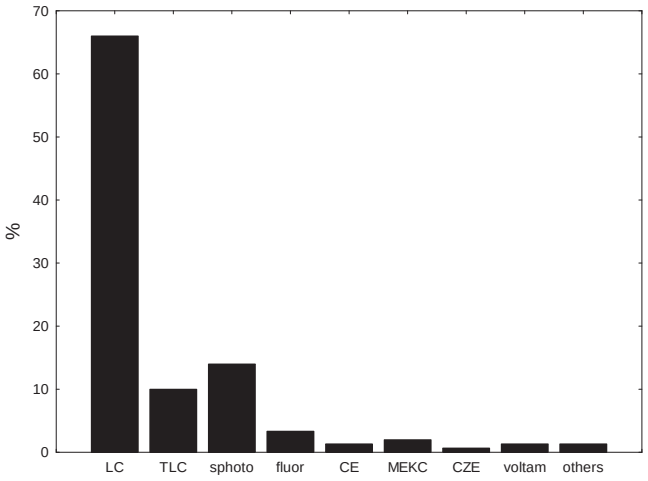

Fig. 2. Different analytical techniques used for analysis of coxibs.

3.1. Rofecoxib

The historical discovery of COX-2 enzyme played an important role in studies of inflammation. The COX-2 program began at Merck Frosst in July, 1992. The result was Vioxx®, approved in the U.S. in May 1999 and in Canada in November 1999, making it the second fastest drug development success in the history of Merck & Co. Inc. Vioxx (active substance – rofecoxib) is a prescription COX-2 selective, NSAID that was approved by FDA in May 1999 for the relief of the signs and symptoms of osteoarthritis, for the management of acute pain in adults, and for the treatment of menstrual symptoms. It is also approved for the relief of the signs and symptoms of rheumatoid arthritis in adults and children.

Rofecoxib, chemically 3-(4-methylsulfonylphenyl)-4-phenyl-2H-furan-2-one (IUPAC), is a white to light yellow powder. It is sparingly soluble in acetone, slightly soluble in methanol and isopropyl acetate, very slightly soluble in ethanol, practically insoluble in octanol, and insoluble in water. The empirical formula for rofecoxib is $C_{17}H_{14}O_4S$, and the molecular weight is 314.357 g mol^{-1}. Its bioavailability is 93% with protein binding of 87%. Rofecoxib has a plasma high-life of ca. 17 h.

Results of the VIGOR (Vioxx Gastrointestinal Outcomes Research) study, released in March 2000, demonstrated that the risk of gastrointestinal toxicity with Vioxx was less than with naproxen, but indicated an increased risk of cardiovascular events versus naproxen. On September 27, 2004, Merck & Co. Inc. was informed that the Data Safety Monitoring Board for an ongoing long-term study of Vioxx had recommended that the study be stopped early for safety reasons. The study was being conducted in patients at risk for developing recurrent colon polyps. The study showed an increased risk of cardiovascular events (including heart attack and stroke) in patients on Vioxx compared to placebo, particularly those who had been taking the drug for longer than 18 months. Based on this new safety information, Merck and FDA officials were informed that Merck was voluntarily withdrawing Vioxx from the market place.

Among chromatographic methods HPLC is the most popular technique, with normal or reversed-phases [10–18]. It has been used with spectrophotometry [19,20] or mass spectrometry (MS) [21,22] detection. The LC-chromatographic conditions are presented in Table 1. Human plasma extracts with rofecoxib also were analyzed via HPLC with post-column photochemical excitation and fluorescence detection [23]. Matthews et al. presented a HPLC method with fluorescence detection (excitation wavelength of 250 nm, emission wavelength of 400 nm) for the determination of rofecoxib in human plasma using sample preparation via 96-well solid-phase extraction [24]. The objective of the next work was to develop and validate a HPLC method for the determination of rofecoxib in rat and human plasma using liquid–liquid extraction [25]. A HPLC method for measuring rofecoxib in human plasma and breast milk was also presented. That method could be used in the study of rofecoxib distribution into human milk [26].

A RP-HPLC method with photo diode array (PDA) detection was developed to separate impurities and degradation products from the rofecoxib drug substance in order to monitor its quality and establish a stability profile. Stress testing of the drug substance, which was carried out under extreme thermal, humid, oxidative, acidic, alkaline and photolytic conditions, helped to determine the intrinsic stability of the molecule [27]. Two unknown impurities in rofecoxib bulk drug at levels below 0.1% were detected by a isocratic RP-HPLC method. These impurities were isolated from crude samples of rofecoxib using reversed-phase preparative HPLC [28]. A fully automated on-line solid-phase extraction method combined with HPLC for the determination of rofecoxib in human plasma has been described. Under postcolumn ultraviolet-irradiation conditions, the analytes were photochemically converted to highly fluorescent products and then monitored by a fluorescence detector [29].

Chavez-Eng et al. described the proper choice of mobile phase and the type of ionization (ESI or APCI) and mode (positive or negative) which were found to be critical to the successful development of an assay for rofecoxib by HPLC with tandem mass spectrometric detection [30].

Turbulent flow was applied to the measurement of rofecoxib in rat plasma by HPLC-MS/MS method, to determine the feasibility of this technique to minimize sample manipulation in laboratory. This analytical methodology was achieved during the loading of the biological sample onto the extraction column, with the advantage of accelerating the clean-up of the sample while maintaining high extraction efficiency. In order to generate turbulent flow, the extraction column was used a small internal diameter (<1 mm) packed with restricted access material (RAM) particles of large diameter (40–63 μm) [31]. The details of a HPLC-MS/MS method for the simultaneous determination of rofecoxib and [^{13}C]–labeled rofecoxib in human plasma in support of oral bioavailability study were presented. The isotopic integrity of the labeled compounds and the assessment of the isotopic content of rofecoxib and [^{13}C]–labeled rofecoxib from MS and MS/MS responses, the development of a simultaneous assay for those compounds required demonstration of the absence of cross-talk effects, a careful assessment of assay selectivity and the demonstration of the utility of the method for supporting the human bioavailability study [32].

Vallano et al. described an HPLC method for the determination of rofecoxib and an experimental COX-2 inhibitor, 3-isopropoxy-4-(4-methanesulfonylphenyl)-5,5′-dimethyl-5H-furan-2-one (DFP), in human plasma. The assay for rofecoxib utilizes fluorescence detection, while the assay for DFP used detection based on the native fluorescence of this compound [33]. The aim of the work also was to develop methods for simultaneous determination of rofecoxib in the presence of both its photodegradate and alkaline degradation products. This was achieved by developing an HPLC method and two chemometric methods. In the chemometric methods, a quantitative, multivariate, spectral analysis by classical least squares (CLS) requires that the concentrations of all spectrally active constituents to be known and included in the calibration samples before a stable prediction model can be developed [34].

Apart from HPLC, thin-layer chromatographic (TLC) methods were used for the determination of rofecoxib. Kaul et al. presented two methods for simultaneous determination of tizanidine and rofecoxib in tablets using HPTLC-densitometry and reversed-phase HPLC. A HPTLC method based on the separation of drugs

Table 1
LC-chromatographic conditions using for the analysis of coxibs.

Compound	Sample matrix	I.S.	Chromatographic conditions					Ref.
			Column	Mobile phase (v/v)	Flow-rate ($mL\,min^{-1}$)	Detection	Retention time (min)	
Celecoxib	Human serum	–	C18 Hypersil ODS (150 × 4.1 mm; 5 μm)	Sol.A: 0.1% TFAA; sol.B: ACN; gradient 0–3′ 15% B, 3–3.1′ to 40% B, 3.1–8′ 40% B, 8–8.1′ to 60% B, 8.1–15′ 60% B, 15–15.1′ to 15% B	1.0	UV 254 nm	13.6	[13]
	Human plasma	PPAC	Zorbax SB-CN (5 μm)	ACN + water containing 0.1 mol L^{--1} KH_2PO_4 buffer (pH 2.4 with 85% H_3PO_4) (42 + 58)	1	UV 254 nm	–	[14]
	Pharm. prep., biological fluids	Chloro-2-nitroaniline	Intersil C18 ODS (250 × 4.6 mm; 5 μm)	MeOH + 0.05% glacial acetic acid (68 + 32)	1.0	UV 230 nm	18.99	[16]
	Tablets, capsules	–	Shim-Pack	ACN + 1% acetic acid (4 + 1)	1.0	MS *m/z* 382	3.47	[18]
	Capsules	–	Partisil C-18 (250 × 4.6 mm; 5 μm)	ACN + water (60 + 40)	1.0	UV 254 nm	6.35	[45]
	Human plasma	Flutamide	Nucleosil 100-5 CN (250 × 4.6 mm)	Water + ACN (60 + 40)	0.9	UV 260 nm	7.02	[46]
	Capsules	–	HiQSil RP C-18 (250 × 4.6 mm; 5 μm)	MeOH + water (85 + 15)	0.8	UV	4.96	[47]
	Human serum	Tolbutamide	C18 Wakosil	10 mM KH_2PO_4 (pH 3.2) + ACN (50 + 50)	1.0	UV 250 nm	9.6	[48]
	Bulk drug, microemulsion	–	516 C18 DB RP (250 × 4.6 mm; 5 μm)	MeOH + water (75 + 25)	1.25	UV 250 nm	4.8	[49]
	Human plasma, breast milk	–	Aqua C18 (75 × 4.6 mm; 5 μm)	ACN + 0.01 M phosphate buffer pH 3.5 (50 + 50) containing 0.1% TEA	1.0	UV 254 nm	8.2	[50]
	Human plasma	Mefenamic acid	Chromolith performance RP-18e (100 × 4.6 mm)	ACN + MeOH + water (45 + 10 + 45) containing 0.2% acetic acid (pH 3.5)	2.0	UV 254 nm	3.6	[51]
	Human plasma	I.S.	Nucleosil-NO_2 (10 × 3.2 mm;5 μm) + Nucleosil-NO_2 (150 × 4.6 mm; 5 μm)	Hexane + methylene chloride + isopropyl alcohol (70 + 25 + 5)	1.4	UV 260 nm	7.3	[52]
	Human plasma	DRF-4367	Kromasil KR100-5C18 (250 × 4.6 mm; 5 μm)	phase A: 0.05 mol L^{-1} formic acid pH 3.0; phase B: water + ACN (9 + 95); phase C: water + MeOH (10 + 90) gradient program	1.0	UV 235 nm	32.22	[53]
	Capsules	I.S.	Prontosil C18 AQ (150 × 3 mm; 3 μm)	Water + ACN (40 + 60)	0.35	λ_{exc} 240 nm, λ_{em} 380 nm	11.7	[54]
	Rat plasma	Ibuprofen	RP Phenomenex C18 (100 × 4.6 mm; 5 μm)	ACN + water + acetic acid + TEA (47 + 53 + 0.1 + 0.03)	1.0	UV 254 nm	11.0	[55]
	Bulk drugs	–	RP Intersil ODS-3 (250 × 4.6 mm; 5 μm)	ACN + water (55 + 45)	1.0	UV 242 nm	16.5	[56]
	Human plasma	I.S.	Nucleosil C18 (30 × 2 mm; 5 μm)	ACN + water + NH_3 aq (85 + 15 + 0.1)	0.2	MS *m/z* 380→316	2.7	[57]
	Human plasma	Rofecoxib	Nucleosil C8 120-5 (11 × 2 mm)	MeOH + water (50 + 50) + 1% acetic acid	200 $\mu L\,min^{-1}$	MS *m/z* 382.4→362	2.3	[58]
	Human plasma	Sulindac	Shim Pack GLC-CN C18 (150 × 6 mm; 5 μm)	ACN + 1% acetic acid (4 + 1)	1.0	MS *m/z* 382	3.47	[59]
	Human plasma	SC-236	Nova Pak C8 (150 × 3.9 mm; 5 μm)	ACN + THF + 0.02 M sodium acetate buffer pH 5.0 (30 + 8 + 62)	1.5	UV 215 nm	~18	[60]
	Bulk drug, pharm. prep.	5-Methyl-2-nitrophenol	Novapak C18 (300 × 3.9 mm; 4 μm)	aqueous KH_2PO_4 (0.01 M, pH 4.8) + ACN (45 + 55)	1.0	UV 252 nm	9.1	[61]

Table 1(*Continued*).

Compound	Sample matrix	I.S.	Chromatographic conditions					Ref.
			Column	Mobile phase (v/v)	Flow-rate ($mL\,min^{-1}$)	Detection	Retention time (min)	
	Rabbit urine, plasma, feces	–	Waters NovaPak C18 (150 × 3.9 mm; 4 μm)	sol.A: ACN + 0.025 M ammonium acetate (20 + 80, pH 4.5); sol.B: ACN + 0.025 M ammonium acetate (60 + 40, pH 4.5); gradient 0-5′ A, 5.1-20′ to 100% B, 20.1-25′ to 100% A	1.0	MS *m/z* 380	19.6	[62]
	Human milk	Rofecoxib	Zorbax SB-C18 (150 × 3 mm; 3.5 μm)	ACN + water with 1% TFAA (pH 2.2) (60 + 40)	0.5	UV 254 nm	–	[63]
	Rat serum	I.S.	octadecyl bonder phase	several phases containing ACN + phosphate buffer	–	UV	–	[64]
	Human plasma	Phenacetin	Phenomenex Luna C18 (150 × 4.6 mm; 5 μm)	sol.A: 10% ACN + 90% 0.01 M NaH_2PO_4 buffer (pH 5.4); sol.B: 80% ACN + 20% 0.01 M NaH_2PO_4 buffer (pH 5.4); gradient 0–10′ 10% B, 10.1–47′ to 38% B, 47.1-63′ to 73% B, 63.1-68′ 73% B, at 68.1′ reduced to 10% B	1.5	UV 254 nm	62	[65]
	Bulk drug, pharm. prep.	–	Chiralpak AD (250 × 4.6 mm; 10 μm)	hexane + ethanol (94 + 6)	1.0	UV 255 nm	26–37	[66]
	Pharm. prep.	I.S.	RP C18	buffer + ACN (40 + 60)	–	UV 254 nm	–	[67]
	Drug	–	Hichrom C18 (250 × 4.6 mm; 5 μm)	0.01 M KH_2PO_4 in water + ACN (45 + 55)	1.0	UV 252 nm	–	[68]
	Drug	–	Hyperprep-C18 (250 × 10 mm; 4.6 μm)	ACN + water (50 + 50)	2.0	UV 252 nm	–	[68]
	Drug	–	Zorbax C18 (250 × 4.6 mm)	0.01 M CH_3COONH_4 + ACN (40 + 60)	0.4	MS *m/z* 381	–	[68]
	Serum, synovial fluids	–	Acquity C18 BEH (150 × 2.1 mm; 1.7 μm)	solA: water 0.1% formic acid, sol.B: MeOH 0.1% formic acid; gradient start at 10% B changing to 100% B over 7′	0.3	MS	6.18	[69]
	Pure, solid dosage forms	–	Inertsil® C8 (250 × 4.6 mm; 5 μm)	ACN + water (65 + 35)	1.25	UV 230 nm	8.05	[70]
Etoricoxib	Human plasma	DRF-4367	Kromasil KR100-5C18 (250 × 4.6 mm; 5 μm)	phase A: 0.05 M formic acid pH 3.0; phase B: water + ACN (9 + 95); phase C: water + MeOH (10 + 90) gradient program	1.0	UV 235 nm	15.63	[53]
	Tablets	Rofecoxib	Kromasil 100 C-18 (250 × 4.6 mm; 5 μm)	ACN + MeOH + 10 mM KH_2PO_4 (pH 3.0 with 1% H_3PO_4) (35 + 35 + 30)	1.0	UV 234 nm	–	[82]
	Pharm. prep.	Amlodipine	Hypersil pack ODS C18 (250 × 4.6 mm; 5 μm)	ACN + 10 mM TBAHS (25 + 75)	0.8	UV 237 nm	3.2	[83]
	Human plasma	Rofecoxib	BDS-Hypersil-C18 (250 × 4.6 mm; 5 μm)	aqueous buffer pH 2.55 (consist of 0.3 mL TEA + 0.4 mL H_3PO_4 per liter) + ACN (62 + 38)	1.25	UV 284 nm	5.11	[84]
	Human plasma	Valdecoxib	BDS Hypersil C18 (150 × 4.6 mm; 5 μm)	10 mM ammonium acetate buffer + ACN (65 + 35)	1.0	UV 235 nm	5.975	[85]
	Human plasma	–	Waters Symmetry® C18 (250 × 4.6 mm; 5 μm)	water + ACN (58 + 42)	1.2	UV 284 nm	7.8	[86]
	Pharm. prep.	Piroxicam	RP Phenomenex Synergi Fusion C18 (150 × 4.6 mm; 4 μm)	0.01 M phosphoric acid (pH 3.0 with 3 M NaOH) + ACN (62 + 38)	1.0	UV 234 nm	4.68	[87]
	Pharm. prep.	Piroxicam	RP Phenomenex Luna C18 (50 × 3 mm; 3 μm)	ACN + water (95 + 5) + 0.1% acetic acid (90 + 10)	0.4	MS *m/z* 359.3→280	0.79	[87]
	Human plasma, pharm. prep.	Piroxicam	C18 (50 × 3 mm)	ACN + water (95 + 5)/0.1% acetic acid (90 + 10)	–	MS	< 2	[88]

	Human plasma	Phenazone	Nucleodur C18 (30 × 2 mm; 5 μm)	ACN + water (90 + 10)	0.3	MS *m/z* 359→280	~0.5	[89]
	Human plasma	Antipyrin	Phenomenex Luna C18 (50 × 3 mm; 3 μm)	ACN + water (95 + 5) + ammonium acetate (pH 4.0; 10 mM)	0.6	MS *m/z* 359.6→280	–	[90]
	Human plasma	Valdecoxib	Nucleosil C8 120-5 (8 × 3 mm)	MeOH + water (50 + 50) + 1% acetic acid	300 μL min^{-1}	MS *m/z* 359.2→280	1.05	[91]
	Human plasma	I.S.	BDS Hypersil C18 (50 × 3 mm; 3 μm)	ACN + 10 mM ammonium acetate (pH 4.0) (35 + 65)	0.6	MS *m/z* 359→280	2.1	[92]
	Substance	–	Hypersil HyPURITY®-C18 (50 × 3 mm;5 μm)	ACN + 10 mM ammonium acetate buffer (pH 4)	0.6	UV 254 nm MS *m/z* 357	2.6	[93]
	Substance	–	Thermo Hypersil Hypurity C 18 (150 × 2 mm; 5 μm)	sol.A: 10/90 CD_3CN/10 mM ND_4COOCD_3 pD ~ 4.93, sol.B: 90/10 CD_3CN/D_2O; gradient 0-3′ 40% B, 3-28′ 40-90% B, 28.1-32′ 90% B, 32.1′ 40% B	0.3	ID ^1H NMR	–	[93]
	Substance	–	UPLC™ BEH C18 (100 × 21 mm; 1.7 μm)	acetate buffer pH 5.0 + ACN (60 + 40)	0.3	UV 235 nm	2.66	[96]
	Tablets	–	BDS Hypersil C-8 (150 × 4.6 mm; 5 μm)	water + ACN + MeOH (50 + 25 + 25)	1.25	UV 284 nm	4.8	[100]
Firocoxib	Horse, dog plasma	–	Intersil™ ODS-3 (150 × 4.6 mm; 5 μm)	ACN + water + TFAA (45 + 55 + 0.025)	1.0–1.2	UV 290 nm	9.2	[135]
	Horse, dog urine, plasma	–	Phenomenex Luna (100 × 3 mm; 3 μm)	ACN + aqueous buffer pH 4.0 (30% ammonium hydroxide + 2 mM ammonium formate with 0.1% formic acid) (45 + 55)	–	MS *m/z* 337→283	4.4	[137]
	Bovine milk	–	Agilent Eclipse Plus C18 (50 × 2.1 mm; 1.8 μm)	sol.A: water + ACN (90 + 10) + 0.001 M acetic acid; sol.B: ACN; gradient 0-0.4′ 90% A, 1′ 85% A, 3.1′ 80% A, 4.7′ 90% A	0.75	MS *m/z* 337.2→283	2.57	[138]
Lefucoxib	Rat plasma, urine	Celecoxib	Kromasil C18 (250 × 4.6 mm)	sol.A: MeOH, sol.B: water; for plasma: A + B (80 + 20); for urine: gradient 0-6′ 60% A 40% B, 6-8′ 60% A 40%B→80% A 20% B, 8-16′ 80% A 20% B, 16-17′ 80% A 20% B→100% A, 17-25′ 100% A	1.0	λ_{exc} 254 nm, λ_{em} 430 nm MS *m/z* 394	for plasma: 8.1; for urine: 15.4	[139]
	Rat plasma, urine, fecal samples	–	Kromasil C18 (250 × 4.6 mm)	sol.A: MeOH; sol.B: water; gradient 0-6′ 60% A 40% B, 6-8′ to 80% A, 8-16′ 80% A, 20% B, 16-17′ to 100% A, 17-25′ 100% A, 25-27′ 60% A 40% B	1.0	λ_{exc} 254 nm λ_{em} 430 nm MS *m/z* 395→375→296	~16	[140]
Lumiracoxib	Tablets	–	Phenomenex Synergi Fusion C18 (150 × 4.6 mm; 4 μm)	Phosphoric acid (pH 3.0, 25 mM) + ACN (40 + 60)	1.0	UV 272 nm MS *m/z* 294→276, 143	5.8	[141]
	Human plasma	Niflumic acid	CC 124/4 Nucleosil 120-3 C8	ACN + 0.05% TCAA (35 + 65)	1.0	UV 270 nm	16.9	[142]
Rofecoxib	Tablets	Nimesulide	Waters Spherisorb ODS (150 × 4.6 mm; 0.5 μm)	1% TEA + ACN (55 + 45)	0.8	UV 303 nm	3.2	[6]
	Tablets	Valdecoxib	Luna C18	MeOH + phosphate buffer pH 3.5 (55 + 45)	1.0	UV 240 nm	4.53	[7]

Table 1(*Continued*).

Compound	Sample matrix	I.S.	Chromatographic conditions					Ref.
			Column	Mobile phase (v/v)	Flow-rate ($mL\,min^{-1}$)	Detection	Retention time (min)	
	Bulk drug, tablets, human plasma	Etodolac	Spherisorb ODSI	ACN + MeOH + 0.067 M KH_2PO_4 (27 + 20 + 53) (pH 6.95 with 3 M NaOH)	–	UV 244 nm	7.5	[8]
	Nimesulide	RPC-18 (250 × 4.6 mm; 5 μm)	MeOH + water (consisting of 0.04% TEA + 0.15% glacial acetic acid) (50 + 50)	1.0	UV 272 nm	7.95	[9]	
	Bulk drug, pharm. prep.	CMS	Symmetry C18 (150 × 4.6 mm; 5 μm)	ACN + water (50 + 50)	1.0	UV 225 nm	7.5	[12]
	Human serum	–	C18 Hypersil ODS (150 × 4.1 mm; 5 μm)	sol.A: 0.1% TFAA, sol.B: ACN; gradient 0-3′ 15% B, 3-3.1′ to 40% B, 3.1-8′ 40% B, 8-8.1′ to 60% B, 8.1-15′ 60% B, 15-15.1′ to 15% B	1.0	UV 263 nm	9.6	[13]
	Human plasma	PPAC	Zorbax SB-CN (5 μm)	ACN + water containing 0.1 M KH_2PO_4 buffer (pH 2.4 with 85% H_3PO_4) (42 + 58)	1.0	UV 254 nm	–	[14]
	Human serum	Diazepam	Novapak-C18 (150 × 4.6 mm; 5 μm)	ACN + water (37.5 + 62.5)	1.0	UV 254 nm	3.15	[15]
	Human plasma	Celecoxib	Nucleosil C8 120-5 (11 × 2 mm)	MeOH + water (50 + 50) + 1% acetic acid	200 $\mu g\,min^{-1}$	MS *m/z* 315.4→297	1.2	[17]
	Tablets, capsules	–	Shim-Pack	ACN + 20 mM ammonium acetate buffer (4 + 1)	–	MS *m/z* 313.2	–	[18]
	Human plasma	I.S.	BDS-hypersil C18 (100 × 4.6 mm; 5 μm)	ACN + water (35 + 65)	1.2	λ_{exc} 250 nm λ_{em} 375 nm	5.8	[19]
	Plasma	I.S.	Waters Symmetry C18 (50 × 4.6 mm; 3.5 μm)	ACN + water (35 + 65)	1.2	λ_{exc} 250 nm λ_{em} 400 nm	4.9	[20]
	Rat, human plasma	Racemic ketoprofen	C_{18} (100 × 4.6 mm; 5 μm)	water + ACN + acetic acid + TEA (77 + 23 + 0.1 + 0.03)	1.0	UV 272 nm	13	[21]
	Human plasma, breast milk	Valdecoxib	Aqua C18 (75 × 4.6 mm; 5 μm)	MeOH + water (50 + 50)	1.0	UV 272 nm	4.1	[22]
	Substance	–	Waters Symmetry C8 (250 × 4.6 mm; 5 μm)	Sol.A: 0.1% H_3PO_4, sol.B: ACN; gradient 70 + 30% to 40 + 60% A+B over 15′, to 15 + 85% A+B over 10′	1.0	UV 220 nm	13.4	[23]
	Substance	–	Hichrom RPB (250 × 4.6 mm)	Water + ACN (50 + 50)	1.0	UV 225 nm	7.5	[24]
	Substance	–	Hyperprep-HS-C18 (250 × 10 mm; 10 μm)	Water + ACN (65 + 35)	3.0	UV 225 nm	20-23	[24]
	Human plasma	I.S.	YMC ODS AQ (20 × 3 mm) + YMC ODS AQ (10 × ×3 mm; 3 μm)	ACN + water (50 + 50)	0.4	MS *m/z* 313→257	5.0	[26]
	Rat plasma	L-752	Chromolit Speed ROD RP-18 (50 × 4.6 mm)	water + ACN (95 + 5)	5.0	MS *m/z* 315→297	2.44	[27]
	Human plasma	I.S.	YMC ODS AQ (20 × 2 mm) + YMC ODS AQ (100 × 3 mm; 3 μm)	ACN + water (50 + 50)	0.5	MS *m/z* 313→257	3.0	[28]
	Human plasma	I.S.	Chromolith Speed Rod RP-18e (50 × 4.6 mm) + Keystone Javelin Beta Basic C18 (10 × 4 mm; 5 μm)	water + ACN (65 + 35)	3.0	λ_{exc} 250 nm λ_{em} 400 nm	1.24	[29]
	Tablets	–	Shimpak VP-ODS C18 (150 × 4.5 mm; 5 μm)	ACN + 0.05% H_3PO_4 (pH 2.6) (35 + 65)	1.0	UV 220 nm	13.92	[30]

	Tablets	–	Kromasil C18 (250 × 4.6 mm; 5 μm) + Finepak SIL-5 C18 (250 × 4.6 mm; 5 μm)	phosphate buffer (pH 5.5) + MeOH (45 + 55)	1.0	UV 235 nm	7.109	[31]
	Tablets	Celecoxib	Kromasil 100-5C18 RP	ACN + water (65 + 35)	1.0	UV 225 nm	4.0	[35]
	Pharm. prep.	Atorvastatin	RP-Phenomenex Partisil 5ODS(3) (250 × 4.6 mm; 5 μm)	ACN + water (50 + 50)	1.0	UV 225 nm	3.3	[37]
Valdecoxib	Human plasma	DRF-4367	Kromasil KR100-5C18 (250 × 4.6 mm; 5 μm)	Phase A: 0.05 M formic acid pH 3.0; phase B: water + ACN (9 + 95); phase C: water + MeOH (10 + 90) gradient program	1.0	UV 235 nm	21.66	[53]
	Human plasma	Etoricoxib	Nucleosil C8 120-5 (8 × 3 mm)	MeOH + water (50 + 50) + 1% acetic acid	300 μL min^{-1}	MS *m/z* 315.1→235	1.08	[91]
	Tablets	–	Hypersil C-18 (250 × 4.6 mm; 5 μm)	0.1 M ammonium acetate buffer (pH 6.0) + + MeOH + ACN (50 + 30 + 20)	1.0	UV 232 nm	4.5	[108]
	Tablets	–	Spherisorb ODS (150 × 0.4 mm; 0.5 μm)	water + ACN (50 + 50)	1.2	UV 237 nm	2.4	[109]
	Pharm. prep.	–	RP	0.01 M ammonium acetate in water + ACN (50 + 50)	1.5	UV 243 nm	–	[110]
	Human plasma	Rofecoxib	Cosmosil C18 (150 × 4.6 mm; 5 μm)	ammonium acetate buffer + ACN (60 + 40) containing 0.1% TEA (pH 6.5 with glacial acetic acid)	1.0	UV 239 nm	8.8	[111]
	Human serum	Celecoxib	C18	ACN + water, acidified with H_3PO_4 (pH 3.2) (60 + 40)	1.0	UV 240 nm	–	[112]
	Tablets	Nimesulide	Phenomenex LUNA C18 (250 × 4.6 mm; 5 μm)	ACN + 0.5% TEA (pH 3.0 with H_3PO_4) (50 + 50)	1.0	UV 240 nm	8.95	[113]
	Tablets	Bromohexine	Phenomenex Gemini C18 (250 × 4.6 mm; 5 μm)	ACN + 20 mM OSA (pH 3.0 with H_3PO_4) (50 + 50)	1.0	UV 250 nm	8.05	[114]
	Tablets	–	RP	MeOH + water (55 + 45)	–	UV 243 nm	–	[115]
	Tablets	–	Phenomenex Synergi Fusion C18 (150 × 4.6 mm; 4 μm)	water pH 7.0 with 0.1 M NaOH + ACN (52 + 48)	1.0	UV 210 nm	5.51	[116]
	Human plasma	Rofecoxib	YMC ODS-AQ (250 × 4.6 mm; 5 μm)	water + MeOH (47 + 53)	1.0	UV 210 nm	11.9	[117]
	Bulk drug	–	Agilent Zorbax SB-CN (250 × 4.6 mm; 2 μm)	sol.A: (80 + 20) 0.01 M KH_2PO_4, 0.01 M 1-octane sulfonic acid sodium salt (pH 3.0 with H_3PO_4) + ACN; sol.B: (30 + 70) water + ACN; gradient % B was 0/20, 5/20, 30/75, 35/75, 40/20, 45/20	1.0	UV 240 nm	14.5	[118]
	Substance	–	XTerra™ RP18 (150 × 4.6 mm; 5 μm)	MeOH + 1% TEA water solution (52 + 48) (pH 7.35 with 85% H_3PO_4)	1.0	UV 220 nm	–	[119]
	Tablets	Esmolol	Waters chromolith performance RP-18e (100 × 4.6 mm; 2 μm)	1% aqueous TEA solution (pH 7.4) + MeOH (64 + 36)	3.5	UV 220 nm	6.01	[120]
	Human plasma	I.S.	Zorbax XDB-C8	ACN + water (50 + 50) containing 10 mM ammonium acetate	100 μL min^{-1}	MS *m/z* 313→118	3.2	[121]
	Human urine	I.S.	Keystone Prism RP (50 × 2 mm;5 μm)	ACN + water (50 + 50) containing 10 mM 4-methylmorpholine (pH 6.0)	100 μL min^{-1}	MS *m/z* 313→118	4.0	[122]
	Substance	–	Novapak C-8 (150 × 3.9 mm; 4 μm)	sol.A: ACN + MeOH + 25 mM ammonium acetate buffer (pH 4.0) (1 + 2 + 27); sol.B: ACN + MeOH + 25 mM ammonium acetate buffer (pH 4.0) (1 + 2 + 3); gradient 100% A to 100% B in 25′, followed by isocratic conditions of 100% B for 5′	1.0	MS *m/z* 345→196	–	[123]

between silica gel aluminium 60 F254 plates as a stationary phase and mixture: toluene + methanol + acetone (7.5 + 2.5 + 1, v/v/v) as a mobile phase, followed by densitometric measurements of spots at 311 nm. The retention factor (R_F) value for rofecoxib was 0.65 [35]. A HPTLC method has been developed for the simultaneous estimation of tizanidine and rofecoxib in tablet formulation. The silica gel G 60 F254 was used as a stationary phase. The mobile phase used was n-butyl acetate + formic acid + chloroform (6 + 4 + 2, v/v/v). Spots were scanning at 315 nm. The R_F value was 0.69 for rofecoxib [36]. Pawar et al. reported a HPTLC method for the assay of two drugs viz., rofecoxib and tizanidine in combined dosage forms. The experiments were performed on silica gel 60 F254 HPTLC plates using mobile phase comprised of toluene + ethyl acetate + methanol + triethylamine (6 + 3 + 0.5 + 0.1, v/v/v/v). The plates were pre-washed with methanol and activated in an oven at 110 °C for 1 h before use. Rosiglitazone was used as internal standard (I.S.). Spots were scanned at 235 nm. The R_F value for rofecoxib was 0.68 [37]. A TLC-densitometric method for simultaneous determination of rofecoxib and its degradation products in tablets and solutions was developed. The stability of the drug was investigated, including an effect of solution pH, temperature and incubation time. Assay was performed on silica gel F254 TLC plates as stationary phase and chloroform + acetone + toluene + glacial acetic acid (12 + 5 + 2 + 0.1, v/v/v/v) as mobile phase. Densitometric scanning was acquired at 256 nm. The R_F value for rofecoxib was 0.69 [38].

Rofecoxib content was determined by different spectrophotometric methods. Duran et al. presented two methods for the quantitative determination of rofecoxib in commercial tablets. One of these methods was based on UV spectrophotometric detection at 220.2 nm and 285.3 nm, while the second one was HPLC [39]. A different spectrophotometric method was developed for the estimation of rofecoxib in bulk drug and in pharmaceutical formulations. Different spectrum obtained by keeping rofecoxib in 0.1 M NaOH in the reference cell and rofecoxib in 0.1 M HNO_3 in the sample cell showed two characteristic peaks at 219.2 nm and 260.8 nm with negative and positive absorbance, respectively. The difference in absorbance between these two maxima was calculated to find out the amplitude, which was plotted against concentration [40].

Two different UV spectrophotometric methods were developed for the determination of rofecoxib in bulk form and in pharmaceutical formulations. The first method was based on the linear relationship between the rofecoxib concentration and the λ_{max} amplitude at 279 nm. The second one, the first derivative ^{1}D, was based on the linear relationship between the rofecoxib concentration and the first derivative amplitude at 228, 256 and 308 nm. The results obtained were compared with a HPLC method as a reference method [41]. The aim of the work also was to develop sensitive stability indicating derivative spectrophotometric and spectrofluorimetric methods for the determination of rofecoxib in the presence of its photodegradation product. The amplitude values for determination of rofecoxib were measured at 316.3 nm for ^{1}D, at 284 nm for ^{1}DD and fluorimetrically at excitation λ = 247 nm and emission λ = 377 nm. The developed methods were applied to the analysis of rofecoxib in some pharmaceuticals and in human plasma. A kinetic study of the photodegradation reaction was also carried out [42].

Two spectrofluorophotometric methods have been developed for the determination of rofecoxib and mosapride citrate in their individual dosage forms. Spectra of rofecoxib in 0.1 M sulfuric acid and mosapride in methanol showed excitation wavelengths of 282 nm and 331 nm, and emission wavelengths of 406 nm and 360 nm, respectively [43]. Shehata et al. reported stability – indicating spectrophotometric and spectrofluorimetric methods for the determination of rofecoxib in the presence of its photodegradation product. The developed methods were applied to the analysis of rofecoxib in some pharmaceuticals and in human plasma. A kinetic study of the photodegradation reaction was also carried out. Spectrofluorimetric method was described to determine rofecoxib at very low concentrations, where it was converted to its photodegradate product, which possesses a native fluorescence, that could be measured [42].

A micellar electrokinetic capillary chromatographic (MEKC) method for determination of rofecoxib in the presence of its photodegradation product in pharmaceutical preparations with nifedipine as an I.S. and detection at 225 nm has been developed and validated. A hanging mercury-drop electrode was the working electrode. Ag/AgCl and Pt were reference and counter electrodes, respectively. The reduction current for rofecoxib was measured in Britton-Robinson buffer pH 9.0 at a peak potential of −1.55 V. Under these conditions rofecoxib eluted in 6.93 min [44]. Capillary electrophoresis (CE) with UV detection at 200 nm was described for the simultaneous determination of celecoxib, meloxicam and rofecoxib. The analysis was performed in Tris buffer (10 mM; pH 11) with sodium octane sulfonate (60 mM) and 20% acetonitrile as an anionic surfactant and organic modifier, respectively. Diclofenac was used as I.S. Migration times were 3.62 min for rofecoxib, 5.06 min for celecoxib, 6.26 min for meloxicam and 6.86 min for diclofenac [45].

Adsorption and reduction of rofecoxib were investigated by cyclic and square-wave voltammetry on a hanging mercury drop electrode (HMDE) incorporating an Ag/AgCl reference electrode and a platinum wire auxiliary electrode in electrolytes of various pH values. The reduction process on HMDE gave rise to a single peak within the entire pH range (2.0–11.5). In alkaline solutions, rofecoxib gave a sensitive adsorptive reductive peak; approximately 10 times larger than those obtained by applying a square-wave scan without prior accumulation [46].

3.2. Celecoxib

There are several other COX-2 inhibitors similar to rofecoxib (Vioxx) on the market including: celecoxib (Celebrex) approved in 1999, and second generation coxibs with improved COX-2 selectivity including valdecoxib (Bextra, approved in 2001), etoricoxib (Arcoxia), lumiracoxib (Prexige), parecoxib (Dynastat), deracoxib (Dermaxx; for veterinary use), firocoxib (Previcox, Equioxx; for veterinary use), tiracoxib and cimicoxib. Several others coxibs are currently undergoing clinical development.

Celecoxib was approved by the FDA for the treatment of osteoarthritis and chronical polyarthritis in 1998. It exhibits in vitro and in vivo selectivity for COX-2 over COX-1. The substance shows potent and selective in vitro activity and marked anti-inflammatory activity in the rat adjuvant-induced arthritis assay. Studies showed that the analgesic and antiphlogistic efficacy of 400 mg celecoxib daily is comparable to a daily dosage of 1000 mg naproxen or 150 mg diclofenac. Celecoxib is contraindicated during pregnancy and interestingly in patients with GI ulcers. At this point the role of COX-2 for wound healing becomes evident. Once an ulcerative injury is present, COX-2 expression is elevated in response to this disease and the COX-2 enzyme seems to be essential for wound healing in the stomach by enhancing gastric blood flow, reducing gastric acid secretion and allowing epithelial cell proliferation and granulation tissue contraction. Consequently, selective COX-2 inhibitors such as celecoxib lead to delayed wound healing and can aggravate the injury [47].

Celecoxib chemically is a diaryl substituted pyrazole designated as 4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide (IUPAC). Its formula is $C_{17}H_{14}F_3N_3O_7S$ and molecular weight is 381.373 g mol^{-1}. The mechanism of action is believed to be due to inhibition of prostaglandin synthesis by blocking COX-2. At therapeutic concentrations in humans, celecoxib does not inhibit the COX-1 isoenzyme.

Celecoxib is a COX inhibitor that possesses anti-inflammatory, analgesic and antipyretic activities. The drug has similar efficacy as conventional NSAIDs in improving the symptoms of osteoarthritis and rheumatoid arthritis, but it is associated with a lower incidence of gastrointestinal ulceration and complications. This promising gastrointestinal safety profile, together with sustained symptomatic pain relief, places celecoxib as an alternative to conventional NSAIDs in the treatment of rheumatoid diseases, particularly in patients at high risk of developing gastrointestinal problems. In practice, its primary indication is in patients who need regular and long term pain relief: there is probably no advantage to using celecoxib for short term or acute pain relief over conventional NSAIDs. In addition, the pain relief offered by celecoxib is similar to that offered by paracetamol.

Celecoxib was the first selective COX-2 inhibitor developed to reduce the serious side-effects of NSAIDs associated with the inhibition of COX-1 seen with non-selective COX inhibitors. It was introduced to clinical practice in 1999 and it is marketed under the brand name Celebrex. Its bioavailability is 40% following oral administration with protein binding of 97% (mainly to serum albumin). Its metabolism is hepatic, and the drug has a plasma half-life of ca. 11 h [48].

For the determination of celecoxib in pharmaceutical formulations and biological material, HPLC methods were the most widely used [17,18,49–53]. They have been used with UV [20,54–57] and fluorimetric [58] detection. Guirguis et al. reported an HPLC technique using solid–liquid extraction for the determination of celecoxib and delineated the pharmacokinetics of the drug in the rat in the presence and absence of inflammation [59]. A UV-RP-HPLC method for the separation and determination of process-related impurities of celecoxib in bulk drugs and pharmaceuticals was developed [60]. A HPLC method with MS detection for the determination of celecoxib in human plasma. The assay allows a faster analysis of plasma samples and delivers an improved sensitivity for the determination of celecoxib in microdialysis samples [61]. A HPLC-MS assay of five sulfur-containing NSAIDs, namely, celecoxib, piroxicam, rofecoxib, sulindac and tenoxicam, in available tablets and capsules [22]. A HPLC method coupled to atmospheric pressure chemical ionization (APCI) mass spectrometry after liquid–liquid extraction was reported. Presented method was used to assay plasma specimens taken from healthy volunteers after oral administration of normal daily dose of celecoxib and pharmacokinetic profile was described [62]. Next work reports on the application of APCI-LC-MS technique, using single-ion monitoring for the quantitation of celecoxib in human plasma [63]. Sherry Chow et al. presented a RP-HPLC procedure for determination of celecoxib in human plasma. This method was intended for monitoring adherence to the study medication and examining the patient-to-patient variations in plasma celecoxib levels [64].

A LC method was developed for the purity evaluation and the quantitative determination of celecoxib in bulk drug and in pharmaceutical dosages. The stability studies were performed for celecoxib solution placed on laboratory bench and in refrigerator for one hundred days [65]. The paper describes the application of LC-MS/MS to determine the metabolites of celecoxib in female rabbits. The metabolic profiles of [^{14}C] celecoxib in plasma, urine and feces and the percentages of dose excreted in urine and feces were determined. After separation by on-line chromatography, the crude urine samples and plasma and fecal extracts were analyzed with turbo-ionspray ionization in negative ion mode [66]. In the study authors described the transfer of celecoxib into human milk in two different groups of breastfeeding mothers. In one group, three breastfeeding mothers were taking celecoxib once daily for many weeks and were at steady state and breastfeeding their infants. In a second group, intravenous lines were placed in two patients who were then given a single oral dose of celecoxib and both maternal plasma and milk concentrations of celecoxib were determined by HPLC method, over an 8-h period until the milk levels dropped below the quantitation limit (LOQ) [67]. A simplified solid phase extraction method of serum samples on a poly(divinylbenzene-co-N-vinylpyrrolidone) sorbent, eliminating a preliminary protein precipitation step has been developed for the determination of celecoxib in rat plasma [68]. Authors developed and validated a method for the simultaneous determination of celecoxib, hydroxycelecoxib and carboxycelecoxib in human plasma using solid-phase extraction combined with gradient elution RP-HPLC with UV detection [69]. A HPLC method for the separation of positional isomers of celecoxib in bulk and formulation samples was also developed [70]. A RP-HPLC method to determine assay and known impurity profiles of celecoxib was reported [71]. Satyanarayana et al. described the identification of the five impurities present in Crude celecoxib drug. Compounds were characterized by LC-MS and were isolated by a reversed phase preparative HPLC system [72]. The specific detection of the two sulfur-containing drug substances, etoricoxib and celecoxib, by inductively coupled plasma mass spectroscopy (ICP-MS) in serum and synovial fluid samples was performed. Chromatographic separation was performed by ultra performance liquid chromatography (UPLC) [73]. The LC-chromatographic conditions are presented in Table 1.

Two analytical methods for the estimation of celecoxib in pure form and in its solid dosage forms were developed. One paper describes a UV spectrophotometric method at 251 nm and a RP-HPLC method at 230 nm. The methods were also assessed for their suitability as stability indicating assays [74]. Bebawy et al. reported spectrophotometric and densitometric stability-indicating methods for the analysis of doxazosin mesylate and celecoxib in the presence of their degradation products. TLC separation was provided on silica gel G F254 plates as stationary phase with a solvent mixture of: cyclohexane + dichloromethane + diethylamine (50 + 40 + 10, v/v/v) as mobile phase. Spots were scanned at 253 nm. The R_F value for celecoxib was 0.23. The first derivative technique was used to resolve the spectral over lapping and show zero-crossing point for the compounds at 256, 269 for celecoxib [75].

A HPTLC method for the determination of celecoxib in capsules has been described. The method uses silica gel 60 F254 as a stationary phase and n-hexane + ethyl acetate (60 + 40, v/v) as mobile phase and loratadine as I.S. Detection was performed at 262 nm. The linearity range was from 200 to 2000 ng [76]. Celecoxib was identified by Primo et al., by its melting range (161.3–162.2 °C), ultraviolet (λ_{max} = 252 nm) and infrared (band frequency: 1150–1350, 1550–1600 and 3300–3500 cm^{-1}) spectrophotometry, thin layer chromatography (silica gel 60 F254 plates as stationary phase, mobile phase as chloroform + ethyl acetate + ether (10 + 5 + 1, v/v/v) and detection under UV light at 254 nm and 365 nm; R_F value 0.45) and nuclear magnetic resonance (characteristic peaks in the range of 2.0–4.0 ppm) [77]. Determination of celecoxib, etoricoxib and valdecoxib in pharmaceutical preparations by TLC with densitometric detection has been described. Chromatography was performed on silica gel 60 F254 plates with chloroform + acetone + toluene (12 + 5 + 2, v/v/v) as mobile phase. Chromatograms were scanned at 254 nm for celecoxib and valdecoxib, and at 290 nm for etoricoxib. The R_F values were 0.65, 0.32 and 0.57 for celecoxib, etoricoxib and valdecoxib, respectively [78].

Apart from chromatographic methods, spectrophotometry was used for the assay of celecoxib. Two spectrophotometric methods for the estimation of celecoxib from capsule formulation were presented. The first method was a UV spectrophotometric method using methanol as a solvent; the drug showed an absorption maximum at 253.2 nm. The second was a visible spectrophotometric method based on formation of a red coloured complex of drug with 1,10-phenantroline and ferric chloride. The complex showed an

absorbance maximum at 509.2 nm [79]. In the next presented study the host-guest complexation of celecoxib with β-cyclodextrin (β-CD) was investigated by using fluorescence spectroscopy. The stochiometry and association constant of the β-CD-celecoxib complex were studied and its thermodynamic parameters (ΔH°, ΔS°, ΔG°) were obtained. Based on the inclusion reaction, a sensitive spectrofluorimetric method for the determination of celecoxib was developed and compared with the results obtained in the absence of β-CD. The relative fluorescence intensity of the methanolic solutions was measured at 390 nm with excitation at 270 nm. The presented method was applied to the assay of celecoxib in capsules [80]. A spectrofluorimetric method for the quantitation of celecoxib in capsules was also designed and validated. Authors used ethanol or acetonitrile to dissolve celecoxib in capsules and measured of direct fluorescence emission at 355 nm (exciting at 272 nm) [81].

Celecoxib content was also determined by electrophoretic and voltammetric techniques. CE with UV detection was described for the determination of celecoxib beside meloxicam and rofecoxib. The analysis was performed in Tris buffer with sodium octane sulfonate/acetonitrile, and with diclofenac as I.S. The migration time for celecoxib was 5.06 min [45]. A MEKC was also described using sodium dodecyl sulfate (SDS) as the surfactant and 2-nitroaniline as an I.S. for the determination of celecoxib in pharmaceutical formulations. The detection wavelength was set at 252 nm. A migration time for celecoxib was 6.3 min [82]. A square-wave adsorptive cathodic stripping voltammetric procedure was optimized for the determination of celecoxib drug in pharmaceutical formulation and human serum. The analytical procedure was based on the reduction of the C-N of the pyrazole ring of the drug molecule at HMDE in Britton–Robinson buffer of pH 7.0. The voltammogram of celecoxib showed a single well-defined peak at −1.54 V (vs. Ag/AgCl/KCl) using an accumulation potential of −0.70 V, frequency of 120 Hz, scan increment of 10 mV and pulse amplitude of 25 mV [83].

If the chemical structure of the active ingredient (AI) contains at least one specific heteroatom (i.e. Li, B, F, S, Cl, Br, etc.), the quantification of the AI in commercial solid drugs become possible via the quantification of the heteroatom of interest by using appropriate ion beam elemental analysis like the PIXE and the PIGE techniques. In the present paper authors demonstrate the reliability of the TT-PIXE (thick target PIXE) and the TT-PIGE techniques via the quantification of sulfur and fluorine, respectively, for rapid quantification of celecoxib AI molecule in various solid drugs, with a pure celecoxib sample as an external standard. Both experiments were carried out by using a 3 MeV proton beam delivered by the NEC 1.7 MV 5-SDH tandem accelerator. The emitted gamma rays were detected [84].

3.3. Etoricoxib

Etoricoxib (chemically 5-chloro-2-(6-methylpyridin-3-yl)-3-(4-(methylsulfonyl-phenyl]pyridine; IUPAC) represents a second-generation COX-2 inhibitor that has been developed for the treatment of inflammatory diseases such as rheumatoid arthritis, osteoarthritis, pain relief, and acute gout, and causes fewer gastrointestinal complications than conventional NSAIDs. Etoricoxib, empirical formula $C_{18}H_{15}ClN_2O_2S$, molecular weight 358.842 g mol^{-1}, is a NSAID available as a white to off-white powder, relatively insoluble in water, soluble in methanol and acetone and freely soluble in alkaline aqueous solutions.

Etoricoxib has been launched in 38 countries worldwide in Europe, Latin America and the Asia Pacific region. Recently Merck & Co. Inc., has submitted a new drug application for Arcoxia (etoricoxib) to the U.S. FDA for the treatment of osteoarthritis, rheumatoid arthritis, chronic low back pain, acute pain, dysmenorrhea, acute gouty arthritis and ankylosing spondylitis. Etoricoxib, commercially available under the brand names Arcoxia, Nurcoxia, Tauxib and Algix, is a more recent COX-2 selective inhibitor introduced for the treatment of osteoarthritis. Current therapeutic indications for etoricoxib in addition to osteoarthritis include treatment of rheumatoid arthritis, ankylosing spondylitis, chronic low back pain, acute pain and gout. As a second-generation COX-2 inhibitor it has higher in vitro selectivity compared to other drugs marketed currently. Its bioavailability is close to 100% following oral administration with a protein binding of 92%. The biotransformation of etoricoxib is hepatic, and the drug has a plasma half-life of ca. 22 h [85]. Etoricoxib may be an effective drug and has minimal side effects compared to other NSAIDs, but the patient should inform his doctor if he/she has a dysfunctional or impaired heart, kidneys or liver so that his/her health will be closely monitored while he/she is on the medication. If a patient has a medical history of decreased liver function, decreased kidney function, high blood pressure, dehydration, liver cirrhosis, heart disease, or heart failure, he/she would inform the doctor immediately so that his/her medication can be managed and monitored properly.

Etoricoxib content was determined especially by HPLC method [86,87]. It has been used with spectrophotometric [57,88–90] or MS [91–93] detection. The LC-chromatographic conditions are presented in Table 1. An automated high though put on-line SPE-LC-MS/MS method for the determination of etoricoxib in human plasma was presented [94]. Werner et al. developed a LC-MS/MS method for the determination of two COX-2 inhibitors, etoricoxib and valdecoxib, in human plasma. They used APCI detection after a liquid-liquid extraction. Mass analysis was performed in the positive ion mode [95]. A HPLC-MS/MS method for the simultaneous determination of etoricoxib and its carbon-13 analogue ($^{13}C_6$-etoricoxib) from human plasma was also developed. During the study, the bioavailability of etoricoxib was determined based on the coadministration of multiple oral doses of etoricoxib together with a single intravenous dose of ^{13}C-labeled drug [96]. To elucidate and confirm the formation of photolysis products of etoricoxib, a series of photolysis experiments was conducted, the products of photolysis were isolated and their chemical structures were confirmed using HPLC-UV, HPLC-NMR and HPLC-MS/MS techniques [97].

Stability-indicating HPLC methods for both the impurity and quantitative analysis of etoricoxib exist. Method development incorporated the optimization of stationary phase, pH, temperature and mobile phase composition for the resolution of thirteen process impurities and three major degradation products. The identities of etoricoxib decomposition products were confirmed by UV, LC-MS and NMR spectra [98]. A LC method has been developed to monitor the formation of an enolate intermediate in a synthetic route to etoricoxib. The presented method requires the derivatization of the enolate with methyl iodide to form a stable methylketosulfone derivative followed by RP-HPLC analysis [99]. Vora et al. reported a UPLC method for separation of etoricoxib and its degradation products, which are formed by forced degradation under acidic (1 M HCl), alkaline (1 M NaOH), strong oxidizing (50% H_2O_2), thermal (105 °C) and photolytic (light 254 nm) conditions [100].

Conditions for quantitation of celecoxib, etoricoxib and valdecoxib in pharmaceuticals by TLC with UV densitometry have been described. Chromatography was performed on silica gel 60 F254 plates with chloroform + acetone + toluene (12 + 5 + 2, v/v/v) as the mobile phase. Chromatograms were scanned at 254 nm for determination of celecoxib and valdecoxib, and at 290 nm for etoricoxib. The R_F values were 0.32 for celecoxib [78]. Shah et al. reported a HPTLC method for the determination of etoricoxib in bulk drug and tablet formulations. Separation was performed on silica gel 60 F254 TLC plates as stationary phase and chloroform + methanol + toluene (4 + 2 + 4, v/v/v) as the mobile phase. Scanning was done at 289 nm. The R_F value for etoricoxib was 0.58 [101]. The objective of the next paper was to develop and validate an HPTLC method for the rapid quantitation of etoricoxib and to develop a stability-indicating

technique which could be used for routine quantification of low levels of etoricoxib in the presence of degradation products and related impurities for assessment of the purity of the bulk drug and the stability of its bulk dosage forms. The drug undergoes degradation when subjected to neutral (H_2O), acidic (0.1 M HCl) or basic (0.1 M NaOH) hydrolysis, oxidation (3% H_2O_2) or dry heat treatment (80 °C). Chromatography was performed on silica gel 60 F254 plates as the stationary phase and toluene + 1,4-dioxane + methanol (8.5 + 1 + 0.5, v/v/v) as the mobile phase with rofecoxib as a I.S. Densitometric scanning was performed at 235 nm. The R_F values were 0.24 for etoricoxib and 0.38 for I.S. [102]. A HPTLC determination of etoricoxib and thiocolchicoside in combined tablet dosage form has also been developed and validated. Separation was performed on silica gel 60 F254 plates, prewashed with methanol and activated at 110 °C for 5 minutes using ethyl acetate + methanol (8 + 2, v/v) as a mobile phase. Densitometric scanning was performed at 290 nm. The R_F value for etoricoxib was 0.70 [103].

Shakya et al. reported two analytical methods for the estimation of etoricoxib in tablets. The first method was HPLC with UV detection. The second was UV spectroscopy at 284 nm [104]. Another UV-spectrophotometric method (280 nm) for the estimation of etoricoxib in bulk drug, dosage form, and human plasma has also been described [105]. Gowri Sankar et al. have developed two UV spectrophotometric methods at 235 nm and 230 nm for the estimation of etoricoxib and ezetimibe in pure and in pharmaceutical dosage forms. Molar absorptivity for etoricoxib was 2.789×10^4 L mol^{-1} cm^{-1} [106]. UV and ^{1}D spectrophotometric methods have been developed for the determination of etoricoxib in bulk drug and its pharmaceutical formulations. The UV spectrum of etoricoxib in 0.1 M sodium hydroxide exhibits an absorption maximum at 284 nm where as in first derivative spectrum it shows a maximum at 301 nm and minimum at 266.8 nm [107]. Two spectrophotometric methods have been developed for the estimation of etoricoxib in tablet formulations. During the course of the study, it was observed that acidic solutions of the drug formed coloured ion-association complexes with bromocresol green (BCG) and bromocresol purple (BCP), which were soluble in chloroform. The complex of etoricoxib with BCG and BCP showed λ_{max} at 416 nm and 408 nm, and the molar absorptivities were 1.9331×10^4 and 1.6642×10^4 L mol^{-1} cm^{-1}, respectively [108].

A capillary zone electrophoresis (CZE) method was also developed and validated for the determination of etoricoxib in solid pharmaceutical dosage forms. All experiments were carried out on a fused-silica capillary, thermostatized at 35 °C and detected at 234 nm using a PDA detector. The buffer consisted of 25 mM trisphosphate solution at pH 2.5. Prilocaine was used as an I.S.; the migration time for etoricoxib was about 4.9 min [109].

3.4. Valdecoxib and parecoxib

Valdecoxib is the newest addition to the group of NSAIDs known as selective COX-2 inhibitors. It is classified as an NSAID, so it should not be taken by anyone who is allergic to these types of drugs.

Valdecoxib is chemically designated as 4-(5-methyl-3-phenyl-1,2-oxazol-4-yl)benzenesulfonamide, and is a diaryl substituted isoxazole. The empirical formula is $C_{16}H_{14}N_2O_3S$, and the molecular weight is 314.364 g mol^{-1}. It is a white crystalline powder that is relatively insoluble in water (10 μg mL^{-1}) at 25 °C and pH 7.0, soluble in methanol and ethanol, and freely soluble in organic solvents and alkaline (pH 12) aqueous solutions.

Valdecoxib is currently in clinical evaluation for the management of pain and inflammation with high selectivity. It potently inhibits recombinant COX-2, with an IC_{50} of 0.005 μM. This compares with IC_{50} values of 0.05 μM for celecoxib, 0.5 μM for rofecoxib and 5 μM for etoricoxib. Unique binding interactions of valdecoxib with COX-2 translate into a fast rate of inactivation of COX-2 [110]. Valdecoxib showed inhibitory activity towards COX-1, screened on human recombinant enzymes. These in vitro data were confirmed in in vivo models, i.e. rat carrageenan foot edema for acute inflammation assay, the rat adjuvant arthritis model for chronic anti-inflammatory activity and the rat carrageenan air pouch model for blocking PG synthesis at the inflammatory site [111]. Its bioavailability is 83% with a protein binding of 98%. The drug has a plasma half-life of 8–11 h. Valdecoxib is given orally in the treatment of osteoarthritis, rheumatoid arthritis and for pain of dysmenorrhoea. It has been granted a marketing authorization license as a single-ingredient preparation in the form of film-coated tablets, Bextra.

Parecoxib sodium is a water-soluble prodrug ([4-(5-methyl-3-phenyl-1,2-oxazol-4-yl)phenyl]sulfonylpropanoylazanide;with formula $C_{19}H_{18}N_2O_4S$; molecular weight 370.422 g mol^{-1}) of a novel, second-generation COX-2-specific inhibitor and the first such agent to be developed for injectable use. It is a parenterally administered inactive ester amide prodrug that undergoes rapid hepatic enzymatic hydrolysis in vivo to the pharmacologically active COX-2-specific compound valdecoxib. Valdecoxib undergoes conversion, involving both cytochrome P-450-mediated and non-cytochrome P-450-mediated metabolic pathways, to a hydroxylated metabolite, which is also a COX-2-specific inhibitor. Its bioavailability is 100% with a protein binding of ca. 98%. The metabolism of parecoxib is hepatic, to valdecoxib and propionic acid and it has a plasma half-life of ca. 22 min. Preclinical and healthy volunteer clinical studies have shown that parecoxib sodium, i.e. valdecoxib provides potent anti-inflammatory activity and possesses a markedly improved gastrointestinal safety profile relative to conventional NSAIDs [112].

Parecoxib sodium is a highly selective non-steroidal COX-2 inhibitor undergoing clinical development, with intended use perioperatively as an analgesic agent. It may be administered perioperatively or postoperatively for its analgesic and anti-inflammatory effects. Both parenteral parecoxib and oral valdecoxib have shown effeciacy in pain relief that is similar to the currently available parenteral non-selective COX inhibitor, ketorolac. Moreover, parecoxib (valdecoxib) has significantly fewer side effects (gastrointestinal, platelet aggregation) than ketorolac. Substances such as celecoxib, rofecoxib or valdecoxib possess a low aqueous solubility which prevents their use for parenteral administration. The development of parecoxib sodium, which is a prodrug of valdecoxib, as an injectable COX-2 inhibitor broadens the spectrum for the treatment of severe acute pain and particularly postoperative pain.

In 2005, the U.S. FDA issued a letter of non-approval for parecoxib in the United States. Study noted increased occurrences of heart attacks following cardiac bypass surgery compared to placebo when high doses of parecoxib were used to control pain after surgery. It is also important to remember that rare but severe allergic reactions have been described with valdecoxib, the molecule to which parecoxib is converted. The drug is not approved for use after cardiac surgery in Europe.

Among analytical techniques, HPLC is the most popular method for determination of valdecoxib [20,113–120]. The aim of the work was to develop and validate a RP-HPLC method to be applied to the analysis and dissolution rate studies of valdecoxib in tablets, contributing therefore to the quality control and safety of this type of pharmaceutical preparation [121]. A UV-HPLC method for simultaneous determination of etoricoxib, salicylic acid, valdecoxib, ketoprofen, nimesulide and celecoxib in human plasma was developed and validated [57]. A UV-HPLC method to determine the plasma concentration of valdecoxib using liquid/liquid extraction was reported. The method provides information about the stability of valdecoxib in plasma and during sample processing [122]. A gradient RP-HPLC assay was developed for the quantitative deter-

mination of valdecoxib in bulk drug. This paper reports on the forced degradation of valdecoxib under a variety of stress conditions such as acid (2 M HCl) and base hydrolysis (2 M NaOH), oxidation (6% H_2O_2), heat (60 °C) and UV light (at 254 nm) [123]. A RP-HPLC method has also been developed for the separation of valdecoxib and impurity SC-77852 [124]. The principal subject of the paper was to set up the optimal chromatographic conditions for the separation of valdecoxib and a degradation product consisting of α and β-N-lactosyl sulfonamide, i.e. α and β anomers, in dosage forms, using a HPLC technique with UV detection [125].

A LC-MS/MS assay was developed to quantitate valdecoxib and its hydroxylated metabolite in human plasma [126]. Zhang et al. reported the development and validation of a LC-MS/MS method for the simultaneous quantitation of valdecoxib and its metabolites in human urine. The assay was used to support clinical studies for valdecoxib and its parenteral drug parecoxib [127]. Other scientists developed LC-MS/MS method for the determination of etoricoxib and valdecoxib in human plasma. APCI detection after a liquid–liquid extraction was used [95]. Metabolism studies of valdecoxib by LC-MS/MS were reported. Authors presented two-step rearrangement related to an isoxazole ring in the collision-induced dissociation spectra of the valdecoxib metabolites. They proposed that the first step consists of an intramolecular reaction with a five-membered ring rearrangement to form an intermediate. The second step involves a four-membered ring intramolecular rearrangement followed by a cleavage of the N—O bond on the isoxazole ring to form a unique fragment ion at *m/z* 196. Accurate mass measurement and stable isotope-labeled analogues were used to assist the elucidation of the fragmentation mechanism in their mass spectra. The unique rearrangement has been observed for a group of structurally related metabolites of valdecoxib that contain 5-hydroxymethyl or 5-carboxylic acid moieties [128].

Thin-layer chromatography methods also were used for determination of valdecoxib. A HPTLC on silica gel with toluene + ethyl acetate (1 + 1, v/v) as mobile phase with detection by densitometry at 262 nm was provided. Linearity range was 800–1000 ng per zone. Recovery was 98.9% [129]. Gandhimathi et al. described a HPTLC method for the estimation of paracetamol and valdecoxib from combined dosage form. Ethanolic extract from tablets was developed on silica gel 60 GF254 TLC plates as a stationary phase, using chloroform + isopropyl alcohol + glacial acetic acid (9.5 + 1 + 0.2, v/v/v) as a mobile phase. Detection was at 250 nm. R_F value for valdecoxib was 0.51 [130]. Authors described a conditions for determination of celecoxib, etoricoxib and valdecoxib in pharmaceutical preparations by TLC-densitometry. Chromatography was performed on silica gel 60 F254 plates with chloroform + acetone + toluene (12 + 5 + 2, v/v/v) as mobile phase. Chromatograms were scanned at 254 nm for assay of valdecoxib. The R_F values were 0.57 for valdecoxib, 0.65 for celecoxib and 0.32 for etoricoxib [78]. A stability-indicating method for the determination of valdecoxib in the presence of its degradation products and related impurities was conducted using HPTLC technique with UV detection at 236 nm. Separation was provided on silica gel 60 F254 plates, using toluene + acetone + 5% ammonia (7 + 5 + 1, v/v/v) as mobile phase. R_F value for valdecoxib was 0.56. Valdecoxib was degraded by exposure to alkali (0.1 M NaOH), acid (0.1 M HCl), oxidation (6% H_2O_2) and UV light [131].

For the determination of valdecoxib in pure and pharmaceutical dosage forms a spectrophotometric method has been developed. The method was based on the reaction of valdecoxib with potassium permanganate to form a bluish green coloured chromogen with an absorption maximum at 610 nm. Molar absorptivity was 7143.72 L mol^{-1} cm^{-1} [132]. UV and third derivative spectrophotometric methods have been developed for the determination of valdecoxib in bulk drug and in tablets. UV spectrum of valdecoxib in 0.1 M sodium hydroxide showed absorption maximum at 243 nm, where as in third derivative spectrum it showed maximum at 221.2 nm and a minimum at 213.6 nm [133]. A method for simultaneous estimation of valdecoxib and paracetamol in two-component tablet formulation has been developed. The method of analysis was derivative spectroscopy to eliminate spectral interference by measuring absorbance at two wavelengths 284 nm and 301 nm. The zero-order absorption spectra were derivatized and derivative spectra from first to fourth order were recorded. Considering all these derivative spectra, the second-order derivative spectrum for valdecoxib and first-order derivative spectrum for paracetamol were selected [134]. Nagulwar et al. reported a method for the simultaneous estimation of valdecoxib and paracetamol in combined dosage form by Vierodf's UV spectrophotometry. The λ_{max} values of valdecoxib and paracetamol in 0.1 M NaOH were 244 nm and 257 nm, respectively. Molar absorptivity was 1.6399×10^4 L mol^{-1} cm^{-1} [135]. Vierodot's and Q-analysis UV spectrophotometric method has been developed for the simultaneous determination of valdecoxib and tizanidine in combined tablet dosage form. The λ_{max} of valdecoxib was found to be 237 nm. In Q-analysis, the isoabsorptive point for both the drugs was found at 289.5 nm [136]. Two spectrophotometric methods have been developed for the estimation of valdecoxib and tizanidine HCl in the mixture. Valdecoxib has an absorbance maximum at 243 nm in methanol + 0.1 M HCl (1 + 1, v/v) mixture. The first method was based on the simultaneous equations at 243 nm and 228 nm, and the second method was based on *Q* absorbance ratio measured at 280.4 nm and 243 nm [137]. Two additional spectrophotometric methods for simultaneous estimation of valdecoxib and tizanidine in combined dosage form have been described. The first method involved formation of a *Q*-absorbance equation at 239.6 nm and 241 nm, while the second method involved formation of simultaneous equation at 241 nm and 229 nm using methanol as solvent [138].

A MEKC method for the simultaneous determination of nimesulide and valdecoxib in solid pharmaceutical dosage forms, with celecoxib as I.S. was developed and validated. Experiments were carried out in a fused silica capillary, thermostatted at 35 °C and detection at 234 nm using a PDA detector. Background electrolyte consisted of a mixture of 35 mM aqueous borate buffer and 35 mM anionic detergent SDS (pH 9.75) with acetonitrile (95 + 5, v/v). Migration times for valdecoxib were 6.88 min [139].

3.5. Firocoxib

Firocoxib belongs to the coxib class of non-narcotic NSAIDs. It is a white crystalline compound described chemically 3-(cyclopropylmethoxy)-5,5-dimethyl-4-(4-(methylsulfonylphenyl)furan-2-one. The empirical formula is $C_{17}H_{20}O_5S$, and the molecular weight is 336.402 g mol^{-1}.

Firocoxib was the first COX-2 inhibitor approved by the U.S. FDA for animals only. It is administered orally once daily for the control of pain and inflammation associated with osteoarthritis in dogs and horses. Dosages of firocoxib are species dependent, with the recommended dosage for dogs being 5 mg kg^{-1} every 24 h and for horses 0.1 mg kg^{-1} every 24 h. Firocoxib belongs to an important class of NSAIDs known as coxibs that are selective for COX-2 and sparing for COX-1. Development of this new phase of NSAIDs began in the 1990s after the discovery of a mitogen-inducible form of COX enzyme (also known as PG G/H synthase) [140]. Firocoxib's mode of action is similar to that of other NSAIDs, and is through inhibition of the arachidonic acid enzyme cascade that synthesizes various prostanoids, such as prostaglandins, thromboxanes and lipoxins, from arachidonic acid via the COX isozymes, and it specifically interrups the biosynthetic pathway of prostaglandin formation, an inflammation mediator, by inhibition of COX-2. The bioavailabil-

ity of Firocoxib is approximately 38% (with 96% a plasma protein binding) when administered as a 5 mg kg^{-1} oral dose to fasted adult dogs. It is rapidly cleared from the blood via hepatic metabolism and fecal excretion.

Firocoxib is known by the brand names of Equioxx and Previcox. It is used to control pain and inflammation associated with osteoarthritis. The use of firocoxib reduces lameness, pain, swelling and improves range of motion. Because firocoxib only targets the COX-2 enzyme, the COX-1 enzyme is available to execute its safeguarding role within the gastrointestinal tract, stomach and kidneys. Firocoxib is labeled for use for osteoarthritis in dogs. It is designed to relieve canine pain associated with arthritis in dogs, which are having difficulty running, jumping, rising or displaying symptoms of stiffness. The most common side effects are anorexia and vomiting. Firocoxib should not be used in animals (horses, dogs) with known hypersensitivity or allergy to the drug. It should be used with caution in animals that are dehydrated or those with kidney disease, heart disease or liver disease. Firocoxib should not be use in horses intended for human consumption.

Kvaternick et al. presented a reversde-phase HPLC method for the determination and quantitation of firocoxib in horse and dog plasma [141]. Quantitative analysis of firocoxib in urine and plasma from horse and dog using isocratic RP-HPLC with MS detection was reported [142]. A rapid resolution liquid chromatography (RRLC method) with MS detection was developed and validated for the analysis of firocoxib in bovine milk [143].

3.6. Deracoxib

Deracoxib (4-[3-(difluoromethyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazole-1-yl]benzenesulfonamide; IUPAC) is a NSAID of the coxibs class. Its formula is $C_{17}H_{14}F_3N_3O_3S$ and molecular weight is 397.38 g mol^{-1}. Deracoxib is use for dogs for the treatment of pain and inflammation associated with osteoarthritis, including hip dysplasia. It is also be used in the treatment of certain cancers. It is not recommended for use of pain control in cats. Deracoxib is known by the brand name of Deramaxx.

A RP-HPLC method with UV detection for determination of Deracoxib in feline plasma was developed and validated [144].

3.7. Lefucoxib

Lefucoxib (5-(3,4-dimethyl-phenyl)-1-methanesulfonyl-3-trifluoromethol-pyrazole; IUPAC) was one of new selective inhibitors of COX-2 and it showed potent anti-inflammatory and analgesic activity. The incidence of gastrointestinal complications caused by lefucoxib was significantly lower than that with the non-selective NSAIDs.

A reproducible and sensitive HPLC fluorescence method to quantify lefucoxib in a large number of low-volume biological matrices generated in pharmacokinetic studies was developed. The method was applied to the pharmacokinetic studies of lefucoxib in rats [145]. The in vivo and in vitro biotransformation study of lefucoxib was investigated. Through HPLC coupled with fluorescence detection and LC-MS analysis, hydroxylation was found to be the primary metabolism pathway of lefucoxib in rats [146].

3.8. Lumiracoxib

Selective COX-2 inhibitors currently used in the clinic are the sulfonamides celecoxib and valdecoxib, as well as the methylsulfones rofecoxib and etoricoxib. Furthermore, the phenylacetic acid derivative lumiracoxib has gained permission recently to be marketed in Europe.

Lumiracoxib (2-[2-(2-chloro-6-fluoroanilino)-5-methylphenyl]acetic acid; IUPAC) is a COX-2 selective inhibitor NSAID, manufactured by Novartis and still sold in countries including Mexico, Ecuador and the Dominican Republic, under the trade name Prexige. Its formula is $C_{15}H_{13}ClFNO_2$ and molecular weight is 293.72 g mol^{-1}. Its structure is different from that of other coxibs. Lumiracoxib is an analogue of diclofenac (one chlorine substituted by fluorine, the phenylacetic acid has another methyl group in meta position), making it a member of the arylalkanoic acid class of NSAIDs. It binds to a differ site on the COX-2 receptor than do other COX-2 inhibitors. It is the only acidic coxib. It displays extremely high selectivity. Its bioavailability is 74% following oral administration with protein binding of ca. 98%. The metabolism of lumiracoxib is hepatic by oxidation and hydroxylation and the drug has a plasma high-life of 4 h. It is eliminated predominantly via hepatic metabolism.

Since its original approval, lumiracoxib has been withdrawn from the market in several countries, mostly due to hepatotoxicity concerns (it may cause liver failure). In November 2006, Prexige received marketing approval for all European Union countries. However, in 2007 it was withdrawn from the market in Australia, Canada and several European Union countries.

A stability – indicating RP-HPLC method was developed and validated for the determination of lumiracoxib in pharmaceutical formulations [147]. Cheremina et al. described a HPLC method with UV detection for the determination of lumiracoxib in human plasma [148].

3.9. Cimicoxib

Cimicoxib, a new imidazole derivative, is a highly selective COX-2 inhibitor that exhibits promising anti-inflammatory and analgesic activity. Chemically it is 4-[4-chloro-5-(3-fluoro-4-methoxxyphenyl)imidazol-1-yl]benzenesulfonamide. Its formula is $C_{16}H_{13}ClFN_3O_3S$ and molecular weight 381.809 g mol^{-1}. The compound showed good oral activity when tested in experimental models of acute and chronic inflammation and pain, comparable to celecoxib and rofecoxib. Cimicoxib exhibited a good pharmacokinetic profile in rats and dogs and a high safety margin with regards to both the cardiorespiratory and central nervous system. Cimicoxib is a selective COX-2 inhibitor being developed by Affectis Pharmaceuticals as a treatment for depression and schizophrenia. In subjects with moderate to serve postoperative dental pain, cimicoxib (as Cimicoxith) produced significant analgesia. A prodrug for parenteral use has recently been identified and is now in preclinical testing. Cimicoxib continues to undergo clinical development as anti-inflammatory and analgesic agent.

4. Validation of the method

Introduction of new methods, enabling carrying out determination with maximum accuracy, contributes to increased interest in analytical methods as such. They should enable to simultaneously determine the individual components in multi-component preparations and in biological material. A range of guidelines, standardizing requirements concerning the quality of drugs, have been issued. Fulfilment of the quality standards confirms the product to the appropriate quality. These are numerical parameters that validate reliability of the results and enable comparing efficiency of the methods used. The process that is used to determine the above parameters is the so-called method validation [149]. Typical parameters that characterize each analytical method include: selectivity, specificity, range, linearity, accuracy (recovery), detection limit (LOD), quantitation limit (LOQ), precision (repeatability, reproducibility), robustness, ruggedness. Validation parameters of

Table 2
Validation data for analyzed drugs.

Compound	Method	Linear range	LOD	LOQ	Precision RSD [%]		Recovery [%] (RSD %)	Ref.
					Intra-day	Inter-day		
Celecoxib	LC	1.8–100.0 mg L^{-1}	0.6 mg L^{-1}	1.8 mg L^{-1}	8.0–13.8	8.9–13.1	73.3–103.0	[13]
		20–2000 μg L^{-1}	–	20 μg L^{-1}	–		–	[14]
		1–20 μg mL^{-1}	1.040 μg mL^{-1}	3.153 μg mL^{-1}	–		98.63–100.14 (0.24–1.93)	[16]
		5–100 μg mL^{-1}	1 μg mL^{-1}	3 μg mL^{-1}	1.29–2.18	1.69–1.81	> 95	[42]
		1–40 μg mL^{-1}	–	–	0.26–0.81	0.51–0.99	99.8–100.3	[45]
		10–1000 ng mL^{-1}	4 ng mL^{-1}	10 ng mL^{-1}	0.25–3.56	0.33–2.43	86.31–92.18	[46]
		2–50 μg mL^{-1}	–	–	0.675	1.35	97.42–100.40	[47]
		10–1000 ng mL^{-1}	–	–	< 10		70–80	[48]
		0.27–80.00 μg mL^{-1}	0.086 μg mL^{-1}	0.263 μg mL^{-1}	0.13–1.55	0.48–1.88	99.84–101.01 (0.49–1.59)	[49]
		10–2000 μg L^{-1}	–	10 μg L^{-1}	< 5	< 10	96.0–98.3	[50]
		10–800 ng mL^{-1}	–	10 ng mL^{-1}	3.8–7.2	3.7–7.9	98.1–102.0	[51]
		25–2000 ng mL^{-1}	–	25 ng mL^{-1}	3.5–5.7	–	85.7–96.1	[52]
		0.1–50.0 μg mL^{-1}	–	0.1 μg mL^{-1}	0.91–9.86	2.98–7.30	82–108	[53]
		12.5–1500.0 ng mL^{-1}	–	12.5 ng mL^{-1}	4.2–7.7	8.3–10.8	73.2–80.8	[54]
		20–1000 ng mL^{-1}	–	20 ng mL^{-1}	0.8–9.2	0.9–5.3	91.1–101.6	[55]
		1–100 μg mL^{-1}						
		0.25–1.00 μg mL^{-1}	S/N ratio: 2–3	S/N ratio: 9.5–10.4	0.15–1.20	–	89.94–106.94 (0.29–1.70)	[56]
		0.25–250.00 ng mL^{-1}	–	0.25 ng mL^{-1}	4.0–10.3	–	90	[57]
		5–2000 μg L^{-1}	–	5 μg L^{-1}	4.8–10.7	4.6–11.2	73.2–75.8	[58]
		5–1000 ng mL^{-1}	20 ng mL^{-1}	50 ng mL^{-1}	1.58–2.56	3.03–4.00	102.4–103.3	[59]
		40–4000 ng mL^{-1}	–	40 ng mL^{-1-1}	1.9–4.9	1.1–11.4	89–96	[60]
		0.05–0.15 mg mL^{-1}	–	–	0.3–1.5	0.4–1.9	98.4–101.2	[61]
		25–500 μg L^{-1}	10 μg L^{-1}	25 μg L^{-1}	< 12		–	[63]
		0.01–2.00 mg L^{-1}	0.005 mg L^{-1}	0.002 mg L^{-1}	4.6	5.0	94.8–95.3	[64]
		10–500 ng mL^{-1}	0.5 ng mL^{-1}	10 ng mL^{-1}	6.3–12.6	10.9–14.2	66–84 (8.9–11.0)	[65]
		0.25–0.75 mg mL^{-1}	38 ng	116 ng	–		98.2–101.9 (0.6–1.3)	[66]
		0.48–10.00 μg mL^{-1}	0.55 ng mL^{-1}	–	2.8	–	95–105	[69]
		100–1000 ng mL^{-1}	25 ng mL^{-1}	75 ng mL^{-1}	0.43	–	97.44–99.86	[70]
	TLC	1–4 μg per spot	–	–	–		98.44–100.39 (1.21–1.37)	[71]
		40–600 μg mL^{-1}	0.0017 μg per band	0.005 μg per band	1.42	1.31	99.38 (1.46)	[74]
	Sphoto	1–20 μg mL^{-1}	0.26 μg mL^{-1}	0.88 μg mL^{-1}	0.1	–	95.6–100.82	[70]
		1–20 μg mL^{-1}	–	–	–		100.35–100.62 (0.75–0.89)	[71]
		5–15 μg mL^{-1}	–	–	–		100.4–101.3	[75]
		50–400 μg mL^{-1-1}	–	–	–		99.15–99.22	[75]
		0.1–4.0 μg mL^{-1}	7.29–16.92 ng mL^{-1}	24.32–56.39 ng mL^{-1}	–		98.46–101.32	[76]
		EtOH: 0.13–20.00 mg L^{-1}	0.04 mg L^{-1}	0.13 mg L^{-1}	0.5–1.2	0.33–0.96	(0.98–1.01)	[77]
		ACN: 0.19–2.32 mg L^{-1}	0.06 mg L^{-1}	0.19 mg L^{-1}	0.09–1.00	0.10–1.02	(0.98–1.03)	
	MEKC	0.2–0.6 mg mL^{-1}	–	–	0.6–0.7	1.44–1.58	93.0–98.4 (0.7–1.2)	[78]
	Voltam	1×10^{-9}–5×10^{-7} mol L^{-1}	1×10^{-9} mol L^{-1}	4.7×10^{-9} mol L^{-1}	–		99.8–100.9	[79]
Etoricoxib	LC	0.1–50.0 μg mL^{-1}	–	0.1 μg mL^{-1}	1.05–10.15	2.57–7.32	87–112	[53]
		0.48–10.00 μg mL^{-1}	0.55 ng mL^{-1}	–	3.4	–	94–105	[69]
		25–400 ng, 20 μL^{-1}	–	–	0.28–1.26	0.35–1.39	99.52–100.59	[82]
		15–3200 ng mL^{-1}	–	15 ng mL^{-1}	2.2–5.4	0.8–6.7	76.5–80.5	[84]
		20–500 ng mL^{-1}	10 ng mL^{-1}	20 ng mL^{-1}	0.38–1.38	0.34–7.94	79.53–85.70	[85]
		5–2500 ng mL^{-1}	–	5 ng mL^{-1}	1.1–2.4	4.1–5.1	75.6–76.6	[86]
		0.02–150.00 μg mL^{-1}	2.0 ng mL^{-1}	6.9 ng mL^{-1}	0.17–0.34	0.09–0.32	100.2−−100.45 (0.10–0.17)	[87]
		1–5000 ng mL^{-1}	0.1 ng mL^{-1}	1.0 ng mL^{-1}	1.41–2.68	1.23–2.37	100.67–102.67	[87]
		1–5000 ng mL^{-1}	–	–	–		96.09	[88]
		0.2–200.0 ng mL^{-1}	–	0.2 ng mL^{-1}	2–12	–	95.1–105.3	[89]
		1–5000 ng mL^{-1}	–	1 ng mL^{-1}	4.15–5.87	2.89–3.97	93.72–96.18 (3.94–5.26)	[90]
		10–2500 μg L^{-1}	–	10 μg L^{-1}	3.1–4.4	5.4–5.6	75.9–88.5	[91]
		5–2500 ng mL^{-1}	–	–	1.1–7.8	2.1–10.8	71.4–85.3	[92]
		0.05–120.00 μg mL^{-1}	0.02 μg mL^{-1}	0.05 μg mL^{-1}	0.008	0.01	–	[96]
		5–50 μg mL^{-1}	0.25 μg mL^{-1}	10.00 μg mL^{-1}	1.09	1.11	100.04–101.18	[100]
	TLC	20–600 μg mL^{-1}	0.0122 μg per band	0.0369 μg per band	1.06	0.95	99.16 (1.79)	[74]
		100–600 ng per spot	50 ng per spot	100 ng per spot	0.25		–	[97]
		100–1500 ng per spot	30 ng per spot	100 ng per spot	0.89	0.62	98.77–100.47	[98]
	Sphoto	5–35 μg mL^{-1}	0.5 μg mL^{-1}	1.0 μg mL^{-1}	0.93	0.53	99.72–101.20	[100]
		Drug: 3–60 μg mL^{-1}	0.0194 μg mL^{-1}	–	–		–	[101]
			Plasma: 0.1–20 μg mL^{-1}	0.1750 μg mL^{-1}				
		2–10 μg mL^{-1}	–	–	–		99.8–100.3	[102]
		BCG: 2–18 μg mL^{-1}	–	–	–		99.73−−99.91	[104]
			BCF: 4–20 μg mL^{-1}				100.04–100.08	

Table 2 (*Continued*)

Compound	Method	Linear range	LOD	LOQ	Precision RSD [%]		Recovery [%] (RSD %)	Ref.
					Intra-day	Inter-day		
	CZE	2–150 μg mL^{-1}	0.58 μg mL^{-1}	1.94 μg mL^{-1}	0.60–0.63	0.40–0.45	98.60–101.97	[105]
Firocoxib	LC	25–5000 ng mL^{-1}	10 ng mL^{-1}	25 ng mL^{-1}	1–6	1–6	89–100	[135]
		plasma: 1––2500 ng mL^{-1}	0.25 ng mL^{-1}	1 ng mL^{-1}	1.6–9.3	3.0–12.2	68.5–94.0	[137]
		urine: 5–2500 ng mL^{-1}	0.25 ng mL^{-1}	5 ng mL^{-1}	2.4–––8.0	3.0–10.0	81.7––98.4	
		0–20 ng mL^{-1}	1.18 ng mL^{-1}	2.02 ng mL^{-1}	2.9–8.9	2.5–7.1	96.3–105.2	[138]
Lefucoxib	LC	5–1000 ng mL^{-1}	–	5 ng mL^{-1}	1.4–5.5	3.0–14.2	97.9–108.1	[139]
Lumiracoxib	LC	10–100 μg mL^{-1}	0.24 μg mL^{-1}	0.80 μg mL^{-1}	0.32–0.79	0.51–0.60	99.78–101.24 (<0.30)	[141]
		10–10000 ng mL^{-1}	–	10 ng mL^{-1}	1.3–7.5	4.5–8.3	82.4–93.2	[142]
Rofecoxib	LC	1.2–6.0 μg mL^{-1}	1 ng mL^{-1}	12 ng mL^{-1}	0.52		(0.0146)	[6]
		7.5–17.5 μg mL^{-1}	–	–	–		99.7	[7]
		0.005–30.000 μg mL^{-1}	0.00143 μg mL^{-1} 0.01–10.00 μg mL^{-1}	– 0.00301 μg mL^{-1}	–		–	[8]
		10–400 ng per 0.5 mL of plasma	–	5 ng per 0.5 mL of plasma	< 2	<2.3	95.66	[9]
		50–450 ng mL^{-1}	–	50 ng mL^{-1}	–		93.95–99.58	[10]
		125–500 μg mL^{-1}	–	–	0.86–1.54	0.32–0.49	99.5–101.3	[12]
		2.5–100.0 mg L^{-1}	0.8 mg L^{-1}	2.5 mg L^{-1}	7.7	9.0–11.9	76.4–96.6	[13]
		10–500 μg L^{-1}	–	10 μg L^{-1}	–		–	[14]
		10–500 ng mL^{-1}	2 ng mL^{-1}	–	< 8.4		89.61–93.55	[15]
		1–20 μg mL^{-1}	0.392 μg mL^{-1}	1.189 μg mL^{-1}	–		97.55–99.74 (0.09–1.12)	[16]
		1–500 μg L^{-1}	–	1 μg L^{-1}	2.6–6.8	8.0–9.5	95.6–96.3	[17]
		0.5–100.0 ng mL^{-1}	–	0.5 ng mL^{-1}	0.8–6.9	1.16–4.62	94.7–98.2 (0.1–4.3)	[19]
		0.5–80.0 ng mL^{-1}	–	0.5 ng mL^{-1}	0.8–7.5	4.8–6.7	98.1–102.7	[20]
		10–3000 ng mL^{-1}	–	10 ng mL^{-1}	2.2–7.2	0.98–10.3	96–107	[21]
		10–2000 μg L^{-1}	–	10 μg L^{-1}	1.6–6.1	4.5–9.5	84.7–87.6	[22]
		0.1–100.0 ng mL^{-1}	–	–	4.4–11.9 1.8–9.1	–	96.5–106.0 98.0–108.0	[26]
		0.3–30.0 μg mL^{-1}	–	40 ng mL^{-1}	–	3.8–10.2	75.8–86.3 (1.0–6.7)	[27]
		0.1–100.0 ng mL^{-1}	–	–	1.1–8.7	–	96.0–104.0	[28]
		5–400 ng mL^{-1}	–	–	2.5–4.9	–	96.7–107.1	[29]
		2–36 μg mL^{-1}	–	–	0.53	0.48	99.19–99.23	[30]
		625–1875 μg mL^{-1}	0.1 μg mL^{-1}	0.15 μg mL^{-1}	1.45	1.98	100.94	[31]
		5–50 μg mL^{-1}	–	–	–		101.51 (0.8753)	[35]
		0.05–35.00 μg mL^{-1}	0.09 μg mL^{-1}	0.64 μg mL^{-1}	0.35–0.58	–	99.9–101.0	[37]
		5–100 μg mL^{-1}	2 μg mL^{-1}	5 μg mL^{-1}	0.53–2.21	0.68–1.77	>95	[42]
	TLC	375–1000 ng per spot	20 ng per spot	40 ng per spot	1.21	1.34	100.48	[31]
		16–40 mg per spot	20 ng per spot	0.4 mg per spot	0.07	0.05	98.8–99.1 (0.9–1.5)	[32]
		3.75–11.25 μg per spot	45 ng per spot	135 ng per spot	–		99.97–100.43	[33]
		60–400 μg mL^{-1}	0.35 μg mL^{-1}	1.05 μg mL^{-1}	1.47	–	98.62 (2.52)	[34]
	Sphoto	2–30 μg mL^{-1}	–	–	–		101.12–101.81 (0.3031–0.9681)	[35]
		2.5–30.0 μg mL^{-1}	0.5 μg mL^{-1}	0.92 μg mL^{-1}	0.65–0.89	–	98.3–99.7	[37]
		1.5–35.0 μg mL^{-1}	0.32 μg mL^{-1}	0.79 μg mL^{-1}	0.48–1.43	–	97.8–99.8	[37]
		5.82–26.2 μg mL^{-1}	–	–	0.125–1.121	0.367–0.938	100.06–100.07	[38]
		25–540 ng mL^{-1}	–	–	1.102	1.02	101.48	[38]
		10–50 μg mL^{-1}	1.71 ng mL^{-1}	5.69 ng mL^{-1}	–		–	[39]
		5.8–26.2 μg mL^{-1}	–	–	0.125–1.121	0.376–0.938	97.5–104.0	[40]
		25–540 ng mL^{-1}	–	–	1.102	1.102	99.94 (1.389)	[40]
	MEKC	2.5–125.0 μg mL^{-1}	0.83 μg mL^{-1}	2.5 μg mL^{-1}	1.09–1.49	0.75–1.25	100.69 (<2.19)	[41]
	Voltam	5×10^{-9}–5×10^{-8} mol L^{-1}	1×10^{-9} mol L^{-1}	–	2.8	–	(1.9–2.9)	[43]
Valdecoxib	LC	1–20 μg mL^{-1}	0.357 μg mL^{-1}	1.081 μg mL^{-1}	–		97.40–99.72 (0.01–1.85) –	[16]
		0.1–50.0 μg mL^{-1}	–	0.1 μg mL^{-1}	0.69–8.67	2.29–6.51	102–111	[53]
		5 -1000 μg L^{-1}	–	5 μg L^{-1}	3.3–6.0	2.7–8.4	80.1–82.2	[91]
		1–100 μg mL^{-1}	0.1 μg mL^{-1}	0.4 μg mL^{-1}	–		100.1	[108]
		0.2–5.0 μg mL^{-1}	–	–	–		99.6–100.2	[109]
		5–150 μg mL^{-1}	5 μg mL^{-1}	–	< 1		–	[110]
		5–400 ng mL^{-1}	3 ng mL^{-1}	5 ng mL^{-1}	3.23–4.99	2.41–6.83	96.57–107.67	[111]
		20 ng mL^{-1}–200 μg mL^{-1}	≤10 ng mL^{-1}	–	< 18		∼90	[112]
		0.05–150.00 μg mL^{-1}	10 ng mL^{-1}	50 ng mL^{-1}	0.06	0.19–0.22	99.81–100.75	[116]
		10–500 ng mL^{-1}	–	10 ng mL^{-1}	0.79–6.12	1.27–7.45	–	[117]
		25–150 μg mL^{-1}	–	–	–	0.7–3.2	99.2–100.6	[118]
		1–3 mg mL^{-1}	0.2 μg mL^{-1}	0.4 μg mL^{-1}	0.8–1.2	0.8–1.6	98.39–102.35	[120]
		0.5–200.0 ng mL^{-1}	–	–	3.66–10.20	1.97–13.3	95.2–115.0	[121]
		1–200 ng mL^{-1}	–	1 ng mL^{-1}	2.04–9.01	–	94.7–105.0	[122]

Table 2 (*Continued*)

Compound	Method	Linear range	LOD	LOQ	Precision RSD [%]		Recovery [%] (RSD %)	Ref.
					Intra-day	Inter-day		
	TLC	40–500 μg mL^{-1}	0.028 μg per band	0.0848 μg per band	1.89	0.61	100.48 (1.94)	[74]
		50–250 ng per band	10.993 ng per band	33.314 ng per band	0.549	1.221	100.17–101.10 (0.844–1.273)	[99]
		0.1–0.5 μg per spot	10 ng	100 ng	0.24	0.109	98.6	[125]
		200–1000 ng μL^{-1}	50 ng μL^{-1}	200 ng μL^{-1}	0.145–0.308	0.128–0.201	97.52–99.37	[126]
	Sphoto	5–25 mg mL^{-1}	–	–	–		99.4–99.9	[127]
		0–25 mg mL^{-1}	–	–	–		98.8–101.5	[128]
		1–6 μg mL^{-1}	0.5 μg mL^{-1}	1.0 μg mL^{-1}	–		100.00–100.01	[129]
		5–30 μg mL^{-1}	–	–	–		98.5–102.0	[132]
		1°: 2.5–15 μg mL^{-1}	–	–	0.472		98.5	[133]
		2°: 2.5–15 μg mL^{-1}			0.467		99.5	
	MEKC	5–150 μg mL^{-1}	0.86 μg mL^{-1}	2.88 μg mL^{-1}	0.82–0.86	0.03–0.38	98.51–99.50 (0.22–0.62)	[134]

the methods used for the determination of coxibs are presented in Table 2. The date obtained make it possible to choose the proper analytical procedure, adapted to the kind of sample (pharmaceutical preparations, biological matrices), method of the determination or detection. Comparing validation parameters of researched methods, it can be concluded which of them are more sensitive (low LOD, LOQ values), accurate (precision, recovery) and allows marking in a needed linearity scope.

5. Conclusions

This review presents analytical methods applied to the determination of coxibs between 1999 and 2010. A great number of studies on rofecoxib, celecoxib, etoricoxib and valdecoxib can be noted, whereas for others coxibs there are only a few. Among all of the published methods, liquid chromatography with spectrophotometry or mass spectrometry detection is the most popular technique, which is used both for the analysis of pharmaceutical preparations and biological material. It is applied not only for the determination of active components, purity and stability studies, but also for pharmacokinetic analyse. Spectophotometric methods (classical and derivative mode) are also quite common, being most frequently used for quantification or confirmation of substance identity. Despite wide availability of the equipment, their use is however still limited, especially with a complicated matrix. The automation of some stages in the analytical procedure and the combination of different methods increase the potential of analysis and detection of components and lead to the development of new methods. The ultimate goal is to obtain results with precision and accuracy and at increasingly lower concentration levels of determined substances.

References

[1] G. Dannhardt, W. Kiefer, Eur. J. Med. Clin. 36 (1998) 109.
[2] J.A. Bela Kis, D.W. Snipes, Busija, J. Pharmacol. Exp. Ther. 315 (2005) 1.
[3] A.J.J. Wood, N. Engl. J. Med. 345 (2001) 433.
[4] M.M. Wolfe, D.R. Lichtenstein, G. Singh, N. Engl. J. Med. 340 (1999) 1888.
[5] D.B. Fournier, G.B. Gordon, J. Cell. Biochem. Suppl. 34 (2000) 97.
[6] L. Crofford, J. Rheumatol. 24 (1997) 15.
[7] Y.S. Bakhle, R.M. Botting, Mediators Inflamm. 5 (1996) 305.
[8] D.A. Al-Turki, L.A. Abou-Zeid, I.A. Shehata, M.A. Al-Omar, Int. J. Pharmacol. 6 (2010) 813.
[9] R.N. Rao, S. Meena, A.R. Rao, J. Pharm. Biomed. Anal. 39 (2005) 349.
[10] M. Gandhimathi, T.K. Ravi, S.J. Varghese, J. Pharm. Biomed. Anal. 37 (2005) 183.
[11] G. Subramanian, S. Pandey, N. Udupa, Indian J. Pharm. Sci. 66 (2004) 699.
[12] A. Savaşer, Y. Özkan, C.K. Özkan, Ç. Taş, S.A. Özkan, Anal. Lett. 37 (2004) 81.
[13] Y.S.R. Krishnaiah, G. Srinivasa Rao, P. Bhaskar, S.S. Shyale, Asian J. Chem. 15 (2003) 945.
[14] U. Mandal, M. Ganesan, M. Jayakumar, T.K. Pal, T.K. Chattaraj, K. Ray, S.N. Banerjee, J. Indian. Med. Assoc. 101 (2003) 486.
[15] M.K. Aravind, R. Prescilla, J.P. Ofenstein, J. Chromatogr. Sci. 40 (2002) 26.
[16] T. Radhakrishna, D. Sreenivas Rao, G. Om Reddy, J. Pharm. Biomed. Anal. 26 (2001) 617.
[17] N. Navas, R. Ureña, L.-F. Capitan-Vallvey, Chromatographia 67 (2008) 55.
[18] A.K. Hamama, J. Ray, R.O. Day, J.A. Brien, J. Chromatogr. Sci. 43 (2005) 351.
[19] M. Amini, M. PirAli Hamedani, M. Vosooghi, M. Nabavi, A. Shafiee, Anal. Bioanal. Chem. 382 (2005) 1265.
[20] R.N. Rao, S. Meena, D. Nagaraju, A. Raghu Ram Rao, Biomed. Chromatogr. 19 (2005) 362.
[21] U. Werner, D. Werner, R. Mundkowski, M. Gillich, K. Brune, J. Chromatogr. B 760 (2001) 83.
[22] M.E. Abdel-Hamid, J. Liq. Chromatogr. Rel. Technol. 23 (2000) 3095.
[23] E. Woolf, I. Fu, B. Matuszewski, J. Chromatogr. B 730 (1999) 221.
[24] C.Z. Matthews, E.J. Woolf, B.K. Matuszewski, J. Chromatogr. A 949 (2002) 83.
[25] S. Sattari, F. Jamali, J. Pharm. Pharmaceut. Sci. 3 (2000) 312.
[26] M. Zhang, G.A. Moore, S.J. Gardiner, E.J. Begg, J. Chromatogr. B 807 (2004) 217.
[27] B. Mao, A. Abrahim, Z. Ge, D.K. Ellison, R. Hartman, S.V. Prabhu, R.A. Reamer, J. Wyvratt, J. Pharm. Biomed. Anal. 28 (2002) 1101.
[28] K.V.S.R. Krishna Reddy, J. Moses Babu, P.K. Dubey, B. Chandra Sekhar, G. Om Reddy, K. Vyas, J. Pharm. Biomed. Anal. 29 (2002) 355.
[29] J.Y.-K. Hsieh, L. Lin, B.K. Matuszewski, J. Liq. Chromatogr. Rel. Techol. 24 (2001) 799.
[30] C.M. Chavez-Eng, M.L. Constanzer, B.K. Matuszewski, J. Chromatogr. B 748 (2000) 31.
[31] A. Vintiloiu, W.M. Mullett, R. Papp, D. Lubda, E. Kwong, J. Chromatogr. A 1082 (2005) 150.
[32] C.M. Chavez-Eng, M.L. Constanzer, B.K. Matuszewski, J. Chromatogr. B 767 (2002) 117.
[33] P.T. Vallano, R.S. Mazenko, E.J. Woolf, B.K. Matuszewski, J. Chromatogr. B 779 (2002) 249.
[34] M.A. Shehata, A. Ashour, N.Y. Hassan, A.S. Fayed, B.A. El-Zeany, Anal. Chim. Acta 519 (2004) 23.
[35] N. Kaul, S.R. Dhaneshwar, H. Agrawal, A. Kakad, B. Patil, J. Pharm. Biomed. Anal. 37 (2005) 27.
[36] T.K. Ravi, M. Gandhimathi, K.R. Sireesha, S. Jacob, Indian J. Pharm. Sci. 68 (2006) 234.
[37] U.D. Pawar, A.V. Sulebhavikar, A.V. Naik, S.G. Pingale, K.V. Mangaonkar, E.-J. Chem. 6 (2009) 95.
[38] M. Starek, J. Krzek, J. Dechnik, J. Anal. Chem. 64 (2009) 623.
[39] A. Duran, B. Bekçe, H.N. Doğan, Pharmazie 58 (2004) 71.
[40] S.J. Rajput, M.G. Sankalia, Indian J. Pharm. Sci. 65 (2003) 418.
[41] N. Erk, T.G. Altuntas, Pharmazie 59 (2004) 453.
[42] M.A. Shehata, N.Y. Hassan, A.S. Fayed, B.A. El-Zeany, Il Farmaco 59 (2004) 139.
[43] S.J. Rajput, M.G. Sankalia, F.T. Patel, Indian J. Pharm. Sci. 67 (2005) 582.
[44] E. Nemutlu, N. Özaltin, S. Altinöz, Anal. Bioanal. Chem. 378 (2004) 504.
[45] Y.-H. Hsieh, S.-J. Lin, S.-H. Chen, J. Sep. Sci. 29 (2006) 1009.
[46] A. Radi, Microchem. J. 72 (2002) 35.
[47] P.C. Konturek, T. Brzozowski, S. Konturek, A. Taut, Z. Sekiwowski, J. Stachura, E.G. Hahn, Eur. J. Pharmacol. 342 (1998) 55.
[48] S.K. Paulson, M.B. Vaughn, S.M. Jessen, Y. Lawal, C.J. Gresk, B. Yan, T.J. Maziasz, C.S. Cook, A. Karim, J. Pharmacol. Exp. Ther. 297 (2001) 638.
[49] D. Gowri Ankar, K. Devi Priya, M. Vamsi Krishna, P.V.M. Latha, Asian J. Chem. 18 (2006) 803.
[50] H. Jalalizadeh, M. Amini, V. Ziaee, A. Safa, H. Farsam, A. Shafiee, J. Pharm. Biomed. Anal. 35 (2004) 665.
[51] P.M. Dhabu, K.G. Akamanchi, Drug Dev. Ind. Pharm. 28 (2002) 815.
[52] G. Jayasagar, M.K. Kumar, K. Chandrasekhar, P.S. Prasad, Y.M. Rao, Pharmazie 57 (2002) 619.
[53] S. Baboota, S. Faiyaz, A. Ahuja, J. Ali, S. Shafiq, S. Ahmad, Acta Chromatogr. 18 (2007) 116.
[54] M. Zhang, G.A. Moore, S.J. Gardiner, E.J. Begg, J. Chromatogr. B 830 (2006) 245.
[55] A. Zarghi, A. Shafaati, S.M. Foroutan, A. Khoddam, J. Chromatogr. B 835 (2006) 100.

[56] M.J. Rose, E.J. Woolf, B.K. Matuszewski, J. Chromatogr. B 738 (2000) 377.
[57] V.V. Pavan Kumar, M.C.A. Vinu, A.V. Ramani, R. Mullangi, N.R. Srinivas, Biomed. Chromatogr. 20 (2006) 125.
[58] F. Schönberger, G. Heinkele, T.E. Mürdter, S. Brenner, U. Klotz, U. Hofmann, J. Chromatogr. B 768 (2002) 255.
[59] M.S. Guirguis, S. Sattari, F. Jamali, J. Pharm. Pharmaceut. Sci. 4 (2001) 1.
[60] R.N. Rao, S. Meena, D. Nagaraju, A.R. Rao, S. Ravikanth, Anal. Sci. 22 (2006) 1257.
[61] L. Bräutigam, G. Vetter, I. Tegeder, G. Heinkele, G. Geisslinger, J. Chromatogr. B 761 (2001) 203.
[62] U. Werner, D. Werner, A. Pahl, R. Mundkowski, M. Gillich, K. Brune, Biomed. Chromatogr. 16 (2002) 56.
[63] M. Abdel-Hamid, L. Novotny, H. Hamza, J. Chromatogr. B 753 (2001) 401.
[64] H.-H. Sherry Chow, N. Anavy, D. Salazar, D.H. Frank, D.S. Alberts, J. Pharm. Biomed. Anal. 34 (2004) 167.
[65] M.K. Srinivasu, Ch. Lakshimi Narayana, D. Sreenivas Rao, G. Om Reddy, J. Pharm. Biomed. Anal. 22 (2000) 949.
[66] J.Y. Zhang, Y.F. Wang, C. Dudkowski, D.-Ch. Yang, M. Chang, J. Yuan, S.K. Paulson, A.P. Breau, J. Mass Spectrom. 35 (2000) 1259.
[67] T.W. Hale, R. McDonald, J. Boger, J. Hum. Lact. 20 (2004) 397.
[68] M.H. Guermouche, A. Gharbi, Chromatographia 60 (2004) 341.
[69] E. Stömer, S. Bauer, J. Kirchheiner, J. Brockmöller, I. Roots, J. Chromatogr. B 783 (2003) 207.
[70] D. Sreenivas Rao, M.K. Srinivasu, Ch. Lakshimi Narayana, G. Om Reddy, J. Pharm. Biomed. Anal. 25 (2001) 21.
[71] A.S. Jadhav, M.S. Shingare, Drug Dev. Ind. Pharm. 31 (2005) 779.
[72] U. Satyanarayana, D. Sreenivas Rao, Y. Ravindra Kumar, J. Moses Babu, P. Rajender Kumar, J. Tirupathi Reddy, J. Pharm. Biomed. Anal. 35 (2004) 951.
[73] H.G. Gika, A. Theodoridou, F. Michopoulos, G. Theodoridis, E. Diza, L. Settas, P. Nikolaidis, Ch. Smith, I.D. Wilson, J. Pharm. Biomed. Anal. 49 (2009) 579.
[74] R.N. Saha, C. Sajeev, P.R. Jadhav, S.P. Patil, N. Srinivasan, J. Pharm. Biomed. Anal. 28 (2002) 741.
[75] L.I. Bebawy, A.A. Moustafa, N.F. Abo-Talib, J. Pharm. Biomed. Anal. 27 (2002) 779.
[76] R.T. Sane, S. Pandit, S. Khedkar, J. Planar Chromatogr. – Modern TLC 17 (2004) 61.
[77] F.T. Primo, P.E. Fröehlich, Acta Farm. Bonaerense 24 (2005) 421.
[78] M. Starek, M. Rejdych, J. Planar Chromatogr. 22 (2009) 399.
[79] S. Pillai, I. Singhvi, Asian J. Chem. 18 (2006) 1560.
[80] J.L. Manzoori, H. Abdolmohammad-Zadeh, M. Amjadi, Il Farmaco 60 (2005) 575.
[81] P. Damiani, M. Bearzotti, M.A. Cabezõn, Anal. Bioanal. Chem. 376 (2003) 1141.
[82] M.K. Srinivasu, D. Sreenivas Rao, G. Om Reddy, J. Pharm. Biomed. Anal. 28 (2002) 493.
[83] M.M. Ghoneim, A.M. Beltagi, Talanta 60 (2003) 911.
[84] B. Nsouli, K. Zahraman, A. Bejjani, S. Assi, F. El-Yazbi, M. Roumié, Nucl. Instr. Meth. Phys. Res. B 249 (2006) 692.
[85] N.G.B. Agrawal, C.Z. Matthews, R.S. Mazenko, E.J. Kline, E.J. Woolf, A.G. Porras, L.A. Geer, P.H. Wong, M. Cho, J. Cote, T.C. Marbury, J.W. Moncrief, H. Alcorn, S. Swan, M.R. Sack, R.A. Robson, K.J. Petty, J.I. Schwartz, K.M. Gottesdiener, J. Clin. Pharmacol. 44 (2004) 48.
[86] H.M. Patel, B.N. Suhagia, S.A. Shah, I.S. Rathod, Indian J. Pharm. Sci. 69 (2007) 703.
[87] K. Srinivasa Rao, K. Srinivas, The Indian Pharmacist 8 (2009) 81.
[88] A.K. Shakya, N.A. Khalaf, Asian J. Chem. 19 (2007) 5241.
[89] U. Mandal, D. Senthil Rajan, A. Bose, K.V. Gowda, A. Ghosh, T.K. Pal, Indian J. Pharm. Sci. 68 (2006) 485.
[90] N.V.S. Ramakrishna, K.N. Vishwottam, S. Wishu, M. Koteshwara, J. Chromatogr. B 816 (2005) 215.
[91] L. Brum Jr., M. Fronza, D.C. Ceni, T. Barth, S.L. Dalmora, J. AOAC Int. 89 (2006) 1268.
[92] L. Brum Jr., D.C. Ceni, M. Fronza, P.R. De Oliveira, S.L. Dalmora, J. Liq. Chromatogr. Rel. Technol. 29 (2006) 123.
[93] L. Bräutigam, J.U. Nefflen, G. Geisslinger, J. Chromatogr. B 788 (2003) 309.
[94] S.L. Dalmora, L. Brum Jr., R.M. Ferretto, P.R. De Oliveira, T. Barth, M. Da Silva Sangoi, Quim. Nova 31 (2008) 574.
[95] U. Werner, D. Werner, B. Hinz, Ch. Lambrecht, K. Brune, Biomed. Chromatogr. 19 (2005) 113.
[96] M.J. Rose, N. Agrawal, E.J. Woolf, B.K. Matuszewski, J. Pharm. Sci. 91 (2002) 405.
[97] C.Z. Matthews, R. Subramanian, E.J. Woolf, N. Foster, B.K. Matuszewski, Pharmazie 59 (2004) 913.
[98] R. Hartman, A. Abrahim, A. Clausen, B. Mao, L.S. Crocker, Z. Ge, J. Liq. Chromatogr. Rel. Technol. 26 (2003) 2551.
[99] A. Abraham, R. Hartman, Z. Ge, B. Mao, J. Marcoux, J. Liq. Chromatogr. Rel. Technol. 25 (2002) 1049.
[100] D.N. Vora, A.A. Kadav, Eurasian J. Anal. Chem. 2 (2007) 142.
[101] N.J. Shah, S.J. Shah, D.M. Patel, N.M. Patel, Indian J. Pharm. Sci. 68 (2006) 788.
[102] G. Maheshwari, G. Sundararajan Subramanian, A. Karthik, A. Ranjithkumar, P. Musmade, K. Ginjupalli, N. Udupa, J. Planar Chromatogr. 20 (2007) 335.
[103] V.S. Rajmane, S.V. Gandhi, U.P. Path, M.R. Sengar, J. AOAC Int. 93 (2010) 783.
[104] A.K. Shakya, N.A. Khalaf, Asian J. Chem. 19 (2007) 2059.
[105] G. Vadnerkar, S.K. Jain, D. Jain, Asian J. Chem. 18 (2006) 2895.
[106] D. Gowri Sankar, D.V.S.P. Kumar, M. Vamsi Krishna, P.V.M. Latha, Asian J. Chem. 17 (2005) 2812.
[107] B.N. Suhagia, H.M. Patel, S.A. Shah, I.S. Rathod, B.P. Marolia, Indian J. Pharm. Sci. 67 (2005) 634.
[108] K. Shah, A. Gupta, P. Mishra, E.-J.Chem. 6 (2009) 207.
[109] S.L. Dalmora, M. Da Silva Sangoi, L.M. Da Silva, R.O. Macedo, T. Barth, J. Sep. Sci. 31 (2008) 169.
[110] J.K. Gierse, Y. Zhang, W.F. Hood, M.C. Walker, J.S. Trigg, T.J. Maziasz, C.M. Koboldt, J.L. Muhammad, B.S. Zweifel, J.L. Masferrer, P.C. Isakson, K. Seibert, J. Pharmacol. Exp. Ther. 312 (2005) 1206.
[111] J.J. Talley, D.L. Brown, J.S. Carter, M.J. Graneto, C.M. Koboldt, J.L. Masferrer, W.E. Perkins, R.S. Rogers, A.F. Shaffer, Y.Y. Ahang, B.S. Zweifel, K. Seibert, J. Med. Chem. 43 (2000) 775.
[112] J.J. Talley, S.R. Bertenshaw, D.L. Brown, J.S. Carter, M.J. Graneto, M.S. Kellogg, C.M. Koboldt, J. Yuan, Y.Y. Zhang, K. Seibert, J. Med. Chem. 43 (2000) 661.
[113] C.S. Ramaa, D.K. Deshpande, A.R. Shiride, V.V. Wamorkar, V.V. Kakad, V.J. Kadam, Indian J. Pharm. Sci. 68 (2006) 514.
[114] A. Suganthi, H.B. Sivakumar, S. Shrikumar, M. Gandhimathi, M. Gopala Rao, T.K. Ravi, Indian J. Pharm. Sci. 67 (2005) 757.
[115] M.S. Srinivas, L.D. Srinivas, B.S. Sastry, Asian J. Chem. 16 (2004) 1119.
[116] R.T. Sane, S. Menon, A.Y. Deshpande, A. Jain, Chromatographia 61 (2005) 137.
[117] S. Keshetty, R.K. Venisetty, V. Molmoori, V. Ciddi, Pharmazie 61 (2006) 245.
[118] P. Senthamil Selvan, R. Gopinath, V.S. Saravanan, N. Gopal, Asian J. Chem. 18 (2006) 2505.
[119] P. Senthamil Selvan, R. Gopinath, V.S. Saravanan, K. Periyasamy, Asian J. Chem. 19 (2007) 1011.
[120] K. Raja Rajeswari, G.G. Sankar, A.L. Rao, D.B. Raju, J.V.L.N. Seshagiri Rao, Asian J. Chem. 18 (2006) 3117.
[121] M. Fronza, L. Brum Jr., M. Wrasse, T. Barth, S.L. Dalmora, Acta Farm. Bonaerense 25 (2006) 117.
[122] N.V.S. Ramakrishna, K.N. Vishwottam, S. Wishu, M. Koteshwara, J. Chromatogr. B 802 (2004) 271.
[123] T. Satyanarayana Raju, K.S.V. Raghavachary, A. Raghupathi Reddy, M. Satish Varma, M. Ravikumar, P. Yadagiri Swamy, Chromatographia 69 (2009) 507.
[124] M. Zečević, G. Savić, Lj. Živanović, Anal. Lett. 39 (2006) 1875.
[125] G. Savić, M. Zečević, B. Jocić, Lj. Živanović, Chromatographia 66 (2007) 29.
[126] J.Y. Zhang, D.M. Fast, A.P. Breau, J. Pharm. Biomed. Anal. 33 (2003) 61.
[127] J.Y. Zhang, D.M. Fast, A.P. Breau, J. Chromatogr. B 785 (2003) 123.
[128] J.Y. Zhang, F. Xu, A.P. Breau, J. Mass Spectrom. 39 (2004) 295.
[129] D.T. Baviskar, S.C. Jagdale, N.O. Girase, A.Y. Deshpande, D.K. Jain, Indian Drugs 44 (2007) 734.
[130] M. Gandhimathi, T.K. Ravi, N. Shukla, G. Sowmiya, Indian J. Pharm. Sci. 69 (2007) 145.
[131] T.K. Ravi, M. Gandhimathi, A. Suganthi, S. Sarovar, J. Sep. Sci. 29 (2006) 1647.
[132] A. Suganthi, H.B. Sivakumar, S.C. Vijayakumar, P. Ravimathi, T.K. Ravi, Indian J. Pharm. Sci. 68 (2006) 373.
[133] V.B. Sutariya, R. Mashru, M.G. Sankalia, P. Parikh, Indian J. Pharm. Sci. 66 (2004) 360.
[134] N. Aditya, R.K. Arora, M. Tiwari, Indian J. Pharm. Sci. 68 (2006) 370.
[135] V. Nagulwar, Y.R. Dhurvey, S. Deshpande, K. Upadhye, S. Bakhle, R. Wadetwar, Indian J. Pharm. Sci. 68 (2006) 639.
[136] V. Nagulwar, M.R. Tajne, K. Upadhye, S. Bakhle, S. Deshpande, R. Wadetwar, Indian J. Pharm. Sci. 67 (2005) 624.
[137] A.S.K. Sankar, K. Anandakumar, D. Nagavalli, M. Senthil Palaniappan, T. Vetrichelvan, K. Nithyanandham, Indian J. Pharm. Sci. 69 (2007) 132.
[138] L. Devarajan, Sivasubramanian, Indian J. Pharm. Sci. 68 (2006) 240.
[139] S.L. Dalmora, M. Fronza, D.R. Nogueira, R.B. Souto, R.M. Bernardi, J. Liq. Chromatogr. Rel. Technol. 30 (2007) 2863.
[140] W. Xie, D.L. Robertson, D.L. Simmons, Drug Dev. Res. 25 (1992) 249.
[141] V. Kvaternick, T. Malinski, J. Wortmann, J. Fischer, J. Chromatogr. B 854 (2007) 313.
[142] L. Letendre, V. Kvaternick, B. Tecle, J. Fischer, J. Chromatogr. B 853 (2007) 333.
[143] G. Dowling, P. Gallo, L. Regan, J. Chromatogr. B 877 (2009) 541.
[144] S.K. Cox, J. Roark, A. Gassel, K. Tobias, J. Chromatogr. B 819 (2005) 181.
[145] X. Bi, Z. Meng, G. Dou, J. Chromatogr. B 850 (2007) 199.
[146] X. Bi, Z. Meng, H. Chen, X. Zhu, G. Dou, J. Pharm. Biomed. Anal. 48 (2008) 134.
[147] P.R. Oliveira, L.S. Beruardi, C. Mendes, M.S. Sangoi, M.A.S. Silva, J. Pharm. Biomed. Anal. 51 (2010) 728.
[148] O. Cheremina, K. Brune, B. Hinz, Biomed. Chromatogr. 20 (2006) 1033.
[149] ICH Guidelines, International Conference on Harmonization of Technical requirements for Registration of Pharmaceuticals for Human Use, July 2010, Geneva, Switzerland.