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Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 349–363
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Review

An overview of the recent developments in analytical methodologies for determination of COX-2 inhibitors in bulk drugs, pharmaceuticals and biological matrices

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Received 21 September 2004; received in revised form 16 March 2005; accepted 20 March 2005
Available online 11 July 2005

Abstract

An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the instrumental analytical methods which were developed and used for determination of COX-2 inhibitors in bulk drugs, formulations and biological fluids have been reviewed. This review covers the time period from 1995 to 2004 during which 138 analytical methods including all types of spectrophotmetric and chromatographic techniques were reported. HPLC with UV detection was found to be the technique of choice for many workers and more than 100 methods were based on LC and UV. A critical analysis of the reported data has been carried out and the present state-of-art of the analytical techniques for determination of celecoxib, rofecoxib, etoricoxib, etodolac, nimesulide and meloxicam has been discussed.

Keywords: NSAIDs; COX-2 inhibitors; Bulk drugs; Pharmaceuticals; Biological fluids; Spectrophotometry; Chromatography

Contents

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[1] IICT communication no.: 040822.

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doi:10.1016/j.jpba.2005.03.040

1. Introduction

Inflammation is one of the few conditions in which PGE_2 is produced as a major product of COX enzyme in several of the human tissues. It is induced by mechanical trauma, corrosive chemicals, burning irradiation, antigen-antibody reactions and modulates all aspects of inflammatory responses like vasodilation, oedema and pain [1]. The PGE_2 acts on different neurons and contributes to the systemic inflammations such as fever, fatigue and pain [2]. Both the enzymes of COX essentially carry out the same catalytic reactions, but differ in expression, function and structure. COX-1 is constitutively expressed in most of the tissues involved in the regulation of physiological "house keeping" functions such as platelet aggregation and homeostasis of the GI tract and kidney. COX-2 in contrast, is inducible in inflammatory cells in response to proinflammatory stimuli such as cytokines, growth factors, tumor-promoting agents and bacterial endotoxins [3,4]. Both are implicated in pathological processes such as Alzheimer's and Parkinson diseases as well as colorectal and breast cancers [5,6]. The enzymatic structures of COX-1 and COX-2 contain 576 and 587 amino acids and show a high degree of identity in their spatial arrangement [7,8]. Both are heme containing integral membrane proteins, located on the lumenal surface of the endoplasmic reticulum [9]. The three dimensional structure of COX-2 exists as homodimers, each monomer comprised of three distinctly folded units [10]. The active site is quite similar in both isozymes and consists of a long narrow hydrophobic channel extending from the membrane domain to the catalytic core [11,12]. Despite the similarity, the COX-2 active site is larger and has a slightly different form than that of COX-1. These differences open up a small hydrophilic side pocket off the main channel increasing the volume of the active site of COX-2. It could be seen from Fig. 1, that within the side pocket of the COX-2 there is an arginine residue in the place of His-513 in COX-1, which can interact with the polar moieties. Another essential amino acid difference between the two isoforms is that it does not alter the shape of the binding site but changes the chemical environment.

2. COX-2 inhibitors

Even before the introduction of aspirin in 1898, several plant extracts were used for the treatment of algesia. Since then a continuous development has been recorded and numerous anti-inflammatory agents such as indomethacin, aryl propionic acid derivatives, diclofenac and naproxen were introduced [13]. The first generation of these compounds came from animal models, before the notion of an inducible isoform was introduced. Nimesulide, meloxicam, and etodolac were the first NSAIDs possessing enhanced safety profile, which were later considered as preferential inhibitors. The main structural feature of these compounds is the absence of the carboxyl group. The most characteristic of classical NSAIDs, is the presence of a sulphone ($–SO_2$) or the sulphonamide moiety ($–SO_2NH_2–$), which can interact with Arg-513 in the hydrophilic side pocket of the COX-2. Despite the wide use of NSAIDs over the last century, their mechanism of action was not fully understood until Vane identified the molecular target as the COX enzyme [14]. In the early 1990s, the second isoform COX-2 was discovered [15]. The

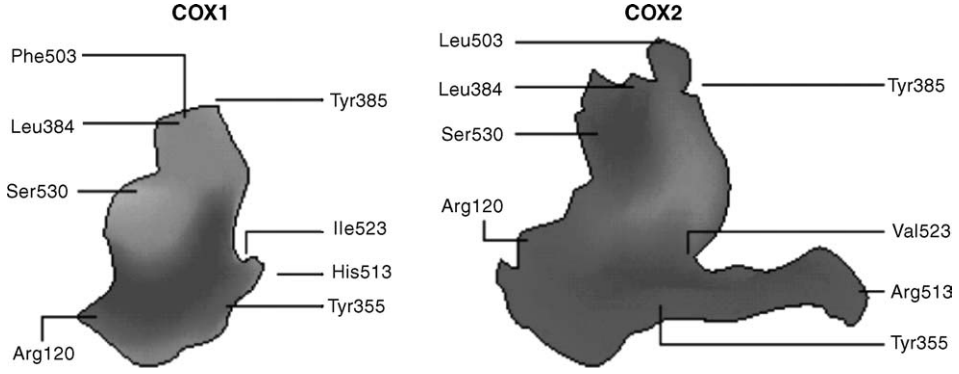


Fig. 1. Sequence of amino acids in COX-1 and COX-2. Reproduced from Ref. [13] with permission from Elsevier.

Table 1
Activity of COX-1 and COX-2 inhibitors (reproduced from Ref. [69] with permission from Elsevier)

Compound	COX-1 IC_{50} μM	COX-2 IC_{50} μM	COX-1/COX-2
Aspirin	1.7–4.5	13.9 to >100	<0.22
Celecoxib	1.2–6.7	0.1–1.0	8.3
Etodolac	9.0–19.6	2.2–3.7	4.8
Etoricoxib	116.0	1.1	105
Lumiracoxib	70.0	0.1	700
Meloxicam	1.4–5.7	0.25–2.1	3.1
Nimesulide	4–10.0	0.18–1.9	9.1
Rofecoxib	18.8–63.0	0.20–0.84	63
Valdecoxib	25.4	0.89	29

inhibitory activity and selectivity of the COX-2 inhibitors are presented in Table 1. The inhibitors of COX-2 were investigated for the treatment of several other inflammatory conditions such as rheumatoid arthiritis, osteoarthiritis, pain relief, cancer treatment and prevention and migraine [16,17].

2.1. Chemical classification

To date, there are only a few COX-2 inhibitors reported in the literature. Various suggestions were made for a new classification of these drugs [18]. Werner et al. have surveyed most of the available COX-2 inhibitors and proposed an extremely useful classification system based on selectivity, which has been widely adopted [19,20]. Sastry et al. have classified these compounds based on their chemical nature [21]. They have included NMD in miscellaneous but not under di-aryl molecules. Since NMD is made up of a di-aryl moiety, it would be appropriate to modify their approach not only to include the new drugs but also the NMD under di-aryl derivatives. The following classification as shown in Fig. 2 has been proposed for the currently known COX-2 inhibitors based on their active functional groups involved in the chemical structures.

COX-2 inhibitors are the recent development of NSAIDs and there is a great need to review the analytical work reported so far in the literature. Till today not even a single article of this nature has been appeared in the literature. Our objective is to compile all the published analytical methods with an emphasis to the spectrophotometric and chromatographic conditions of analysis dealing with formulated, unformulated drugs, biological samples including metabolites, enantiomers, stability and degradation studies. Efforts have been made to collect the literature from 1995 to till date and all the analytical procedures have been tabulated in the proceeding sections. The present review comprises of all analytical methods for the analysis of COX-2 inhibitors in bulk drugs, pharmaceuticals, therapeutic monitoring studies viz., bioavailability and pharmacokinetics published in the last 10 years.

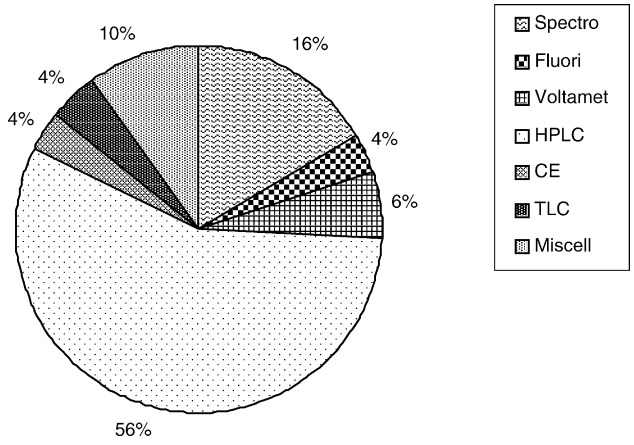


Fig. 3. Different techniques used for analysis of COX-2 inhibitors.

Techniques like spectrophotometric, fluorimetric, voltametric, thin layer chromatography [TLC], high-performance liquid chromatography [HPLC], capillary electrophoresis [CE] and others have been used for analysis. The percentage of their utility is shown in Fig. 3 from which it could be seen that HPLC followed by spectrophotometric methods have used extensively. Further analysis of this data has indicated that these techniques are applied mostly for analysis of bulk drugs, formulations, biological matrices and stability studies (Fig. 4).

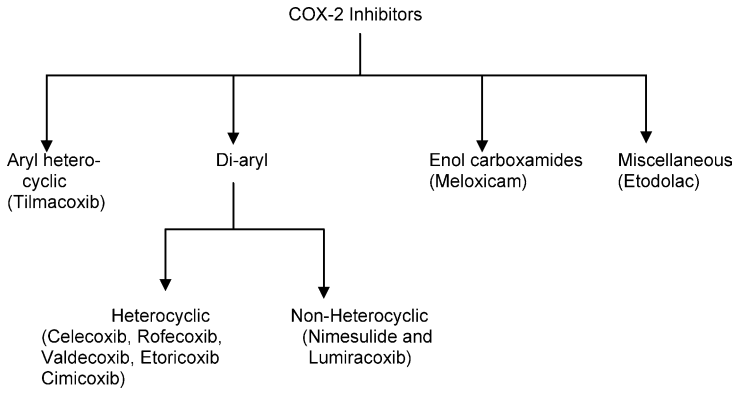


Fig. 2. Classification of COX-2 inhibitors.

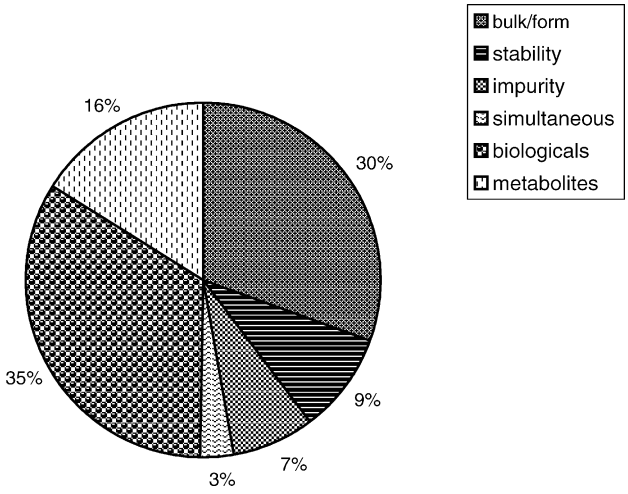
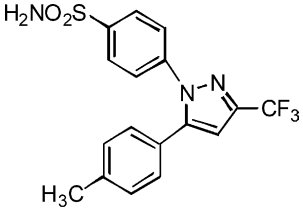


Fig. 4. Drugs analysed during the period of review.

3. Celecoxib (CXB)

4-[5-(4-Methylphenyl)-3-(trifluoromethyl)pyrazole-1yl] benzenesulphonamide, known as Celecoxib (CXB) is a selective inhibitor of COX-2 for the treatment of rheumatoid arthritis and osteoarthritis [22]. It was primarily designed to reduce the adverse effects associated with conventional NSAIDs. It showed an excellent selectivity for COX-2 over COX-1 in vitro studies. In contrast to other NSAIDs, it produces neither acute nor chronic gastro-intestinal toxicities. One of the latest studies showed that CXB could reduce the polyps formation in patients with familial adenomatus polyposis. It has a good bioavailability distribution as well as an established safety profile in preclinical models with $t_{1/2}$ and t_{max} of 2 and 12 h, respectively [23].



Celecoxib

A thorough literature search has revealed that a limited number of spectrophotometric, fluorimetric, voltametric and electrophoretic methods were reported. Chromatography was used extensively when compared to other techniques. The UV spectrophotometric determination was based on the absorption at 251 nm for the assay of CXB in bulk drugs and capsules [24]. Recently a spectrofluorimetric method based on the characteristic fluorescence of the ethanolic solution of the drug, which exhibited excitation at 272 nm and emission at 355 ± 5 nm for determination of CXB in capsules was reported [25]. Adsorptive stripping voltammetry involving the reduction of CN group of pyrazole ring at the Hg electrode surface in Britton-Robinson buffer at pH 7.0 showed a single well-defined peak at −1.54 V using Ag/AgCl electrode and its applicability for determination of CXB in human serum was discussed [26].

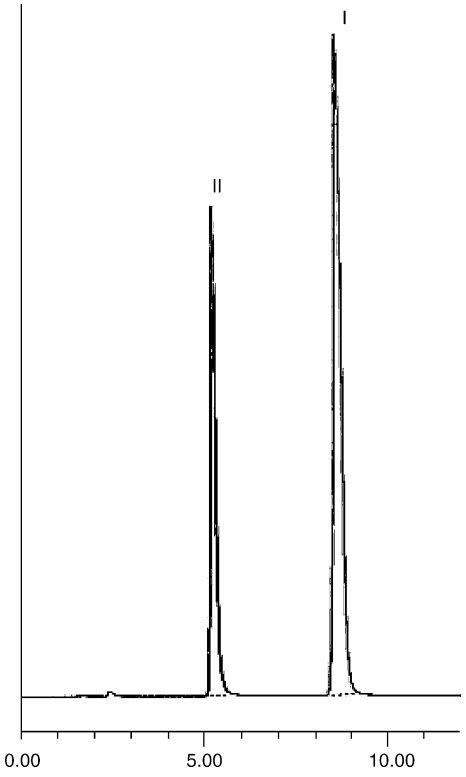


Fig. 5. HPLC chromatogram of a capsule formulation (I) celecoxib and (II) internal standard. Reproduced from Ref. [27] with permission from Elsevier.

3.1. Chromatographic methods

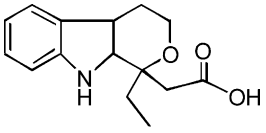
Many chromatographic methods for assay as well as for determination of impurities of CXB were reported. The purity of CXB in bulk drugs and formulations were evaluated by LC using 5-methyl 2-nitro-phenol as an internal standard (Fig. 5). Since the method suffers from resolution between *ortho* and *meta* isomers of CXB, later the same group of workers have proposed a modified LC method for separation of positional isomers using a chiral column [27,28]. Bebawy et al. have used LC, TLC, and HPTLC for development of stability indicating assay of CXB in the presence of 5-(4-methylphenyl)-3-(trifluoromethyl)1-phenyl pyrazole formed as a degradation product [29,30]. Micellar electrokinetic chromatography and HPTLC were applied for rapid quantification of CXB in pharmaceutical dosage forms [31,32]. Gradient RP-HPLC was carried out for the determination of not only CXB but also its hydroxy and carboxy metabolites simultaneously [33]. LC was used mostly for determination of CXB in human plasma and serum using UV, fluorimetric and APCI-MS/MS as detectors [34–39]. Liquid–liquid extraction followed by LC tandem MS to establish pharmacokinetic profiles of CXB and characterize the metabolites in

human and rabbit plasma was studied [40–42]. Solid-phase extraction of CXB from the plasma followed by LC determination was also reported [43,44]. Normal phase LC with column switching technique has been reported for its determination in plasma [45]. Table 3A records the chromatographic conditions including the details of detectors, mobile phases and sample matrices used by different workers.

4. Etodolac (ETD)

[(±)1,8-Diethyl-1,3,4,9-tetrahydro-pyrano[3,4-b]indole 1-aceticacid] known as Etodolac is a chiral non-steroidal anti-inflammatory, marketed as a racemic mixture of active *S*-(+) and inactive *R*-(−) enantiomers [46]. Sixteen methods were developed by different techniques and their percentage distribution is shown in Fig. 6.

El Kousy had proposed both spectrophotometric and fluorimetric methods for the analysis of ETD in bulk and dosage forms [47]. The UV method was based on the formation of a colored complex between the drug and *p*-DMB in presence of sulphuric acid and ferric chloride.



Etodolac

The absorbance was measured at 591.5 nm.The ethanolic solution of the ETD exhibits native fluorescence with excitation and emission wavelengths of 235 and 345 nm, respectively.

4.1. Chromatographic methods

The chiral nature of ETD emphasizes on the enantiomeric separation of racemates rather than the estimation of them in the bulk drugs. Cellulose tris(3,5-dichloro-phenyl)carbamate and 2-propanol were used as stationary and mobile phases respectively [48]. Sequential derivatization with ethylchloroformate and *l* (−)-α-phenylethylamine was found to be suitable for pharmacokinetic studies. The diastereoisomers thus formed were extracted and chromatographed on a normal-phase column, with a mobile phase consisting of hexane: ethyl acetate: 2-propanol (85:15:0.2, v/v/v) at a flow rate of 2 ml/min. The separation was accomplished based on the formation of diastereoisomers, either permanently or transiently with a resolution of 6.4 and detection at 280 nm [49]. Exhaustive methylation followed by GC–MS was used to detect ETD in urine for toxicological analysis. Human biological fluids were analysed by capillary GC and separated metabolites were identified by MS in a full screen mode [50,51]. Different LC procedures for separation and determination of ETD in plasma using UV detection were reported [52–54]. HPTLC determination of ETD in formulations and human plasma has been described [55]. On-line coupling of capillary electrochromatography with ESI-MS was used for the qualitative investigation of biotransformation of ETD. The coupling of this electro-driven separation technique with mass spectrometry demonstrated the presence of different metabolites of ETD extracted from human urine [56–60].

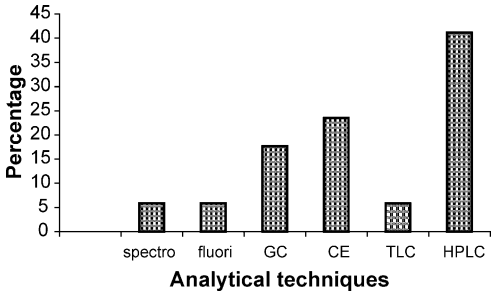
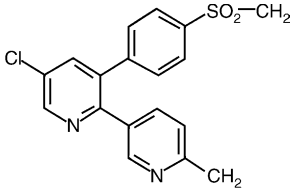


Fig. 6. Different analytical techniques used for analysis of ETD.

5. Etoricoxib (EXB)

EXB is {5-choloro-3-(4-methanesulphonyl-phenyl)-6′-methyl-[2,3′]bipyridinyl}, a second-generation COX-2 having higher in vitro selectivity compared to other drugs marketed currently. Merck, USA has developed this drug for the treatment of osteoarthritis [61–63].



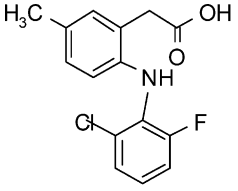
Etoricoxib

Being a latest molecule, very few HPLC methods were reported so far in the literature.

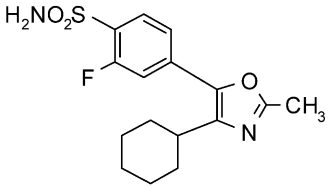
LC–MS/MS with atmospheric pressure chemical ionization [APCI] using a stable isotope of EXB as an internal standard was reported and validated over the concentration range of 0.5–250 ng/ml [64]. In another ESI-LC–MS/MS study, phenazone, a structurally unrelated moiety, was used as an internal standard and validated over the concentration range of 0.2–200 ng/ml [65]. The details are given in Table 3B. Matthews et al. have developed an analytical method for the determination of EXB in human plasma and urine using solid phase extraction (SPE) followed by HPLC with photochemical cyclization and fluorescence detection using one of the structural analogues as internal standard. The limit of quantification was found to be 5 ng/ml [66]. Abrahim et al. have developed a derivatization method coupled with RP-HPLC for determination of enolate and its intermediates, and a stability indicating method for the quantitative analysis of impurities [67,68].

6. Lumiracoxib (LXB)

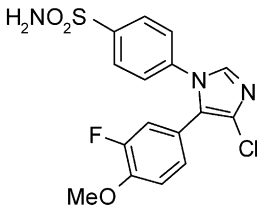
The in vitro studies of lumiracoxib have shown high selectivity for COX-2 over COX-1. Its activity was found to be between 100- and 1400-fold over diclofenac and naproxen. Unlike celecoxib and rofecoxib, LXB causes 100% inhibition of TXB2 production even at a dose of 300 μM. It absorbs rapidly with the t_{max} of 2–3 h, with the elimination half-life ranging from 3 to 6 h. This is the latest drug in the forthcoming COX-2 inhibitors while tilmacoxib (TXB) and cimicoxib (CMX) are undergoing clinical trial [69,70]. There are no analytical methods available for these three drugs in the literature.



Lumiracoxib



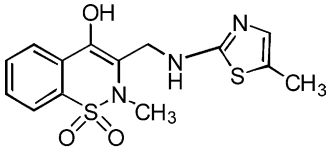
Tilmacoxib



Cimicoxib

7. Meloxicam (MXM)

MXM is a preferential selective COX-2 inhibitor related to piroxicam structurally [71]. Chemically, it is 4-hydroxy-2-methyl *N*-(5-methyl-2-thiazolyl)2H-1, 2-benzothiazine 3-carboxamide. On oral consumption, it is absorbed slowly but more or less completely with t_{max} of 5–6 h and elimination half-life of 20 h. Around thirty methods were available; 35% of them are spectrophotometric and 45% are LC as shown in Fig. 7.



Meloxicam

Different spectrophotometric methods including flow injection techniques for determination of MXM in pharmaceutical dosages were reported [72–74]. First derivative spectrophotometry for stability indicating assay of MXM in presence of 5-methyl 2-amino thiazole and benzothiazine carboxylic acid in 0.1N NaOH at 338 nm was used [75]. In addition to the differential spectrophotometric methods, Hassan has carried out the estimation of MXM by complexing the drug with safranin T in tablets and suppositories. The same solutions were diluted with chloroform for determination by fluorimetry [72]. The absorption and emission spectra of MXM are shown in Fig. 8. A stability indicating assay for detection and determination of degradation products viz., 5-methyl 2-aminothiazole and benzothiazine carboxylic acid at 450 nm was reported [76,77]. Different complexes of MXM with Fe(III) in methanol, basic methylene blue in phosphate buffer at pH 8.0, 2,3-dicholro 5,6-dicyano *p*-benzoquinone and with Folin–Ciocalteu reagent were developed for colorimetric estimations [78–80]. Ion pairing extractive dyes viz., bromocresol purple, bromothymol blue and bromophenol blue were also used for its determination in pharmaceutical preparations [81]. The details are given in Table 2A.

The electroanalytical methods play a significant role in the quantification of MXM in bulk drugs and formulations. The voltammetric behaviour of MXM was studied by DC, DPP and cyclic voltammetry [82]. Square wave cathodic adsorptive stripping voltammetric conditions were optimized for its determination in different dosages [83,84]. Second order single sweep oscillopolarography was carried out in ethano-

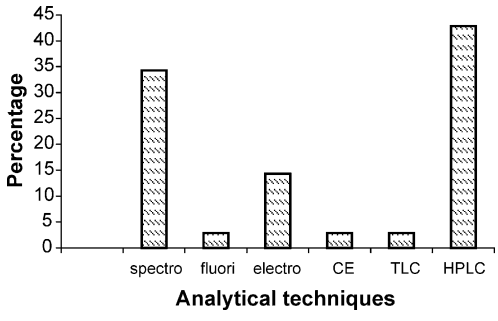


Fig. 7. Different analytical methods available for MXM.

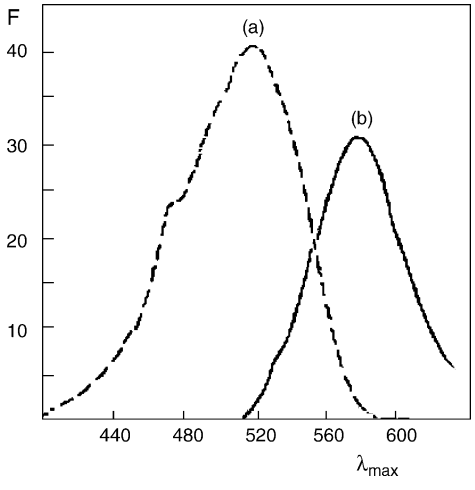


Fig. 8. Excitation and emission spectra of MXM. Reproduced from Ref. [72] with permission from Elsevier.

Table 2
Spectral data of meloxicam and nimesulide

Sample matrix	Solvent/reagent	Linearity range (μg/ml)	Detection, λ (nm)	Ref.
(A) Meloxicam				
Bulk/dosage forms	Ethanolic sodium hydroxide Ethanolic hydrochloric acid	1–10	UV 322–368	[72]
Bulk/dosage forms	Safranine-T	4–12	Vis 518	[72]
Tablets	Acidified acetonitrile		UV 341	[73]
Tablets and capsules	Dimethylformamide acidified water	1–14	UV 357	[74]
Pharmaceuticals	Ferric chloride in methanol	2–200	Vis 570	[77]
Pharmaceuticals	0.1 M sodium hydroxide	0.5–20	UV 362	[78]
Formulations	2,3-Dichloro5,6-cyano *p*-benzoquine	40–160	Vis 455	[79]
Formulations	Methylene blue	1–5	Vis 653	[79]
Formulations	Folin-Ciocaltau	1.5–22.5	Vis 700	[80]
Dosage forms	Bromocresol purple	1–27	UV 386	[81]
	Bromothymol blue	2–30	Vis 394	[81]
	Bromophenol blue	3–36	Vis 395	[81]
(B) Nimesulide				
Dosage forms	50% and 100% acetonitrile	10–50	UV 300	[102]
Tablets	Amm. sulphamate (0.5%) and NED (0.1%)	0–40	Vis 560	[103]
Tablets	DMPD and chloramine-T		Vis 540	[104]
Tablets	MBTH + $FeCl_3$		Vis 600	[104]
Tablets	Metol + pot. dichromate alc. imino dibenzyl/acid	10–50	Vis 600	[105]
Formulations	3-Aminophenol/acetic acid	10–50	Vis 470	[105]
Bulk drugs and formulations	Chloroform and ethanol	2–90	UV–vis 200–500	[106]

lic solution in acetate buffer at pH 4.7 [85]. The influence of pH, concentration of buffer, organic modifier, capillary temperature, applied voltage and injection volumes on the electrophoretic behavior of MXM were investigated [86–88].

7.1. Chromatographic methods

In addition to spectrophotometric and electroanalytical, LC methods were proposed for the estimation of MXM in pharmaceutical dosage forms [72,82]. Ion-pair HPLC determination of MXM and its related substances including intermediates and degradation products was developed using octane sulphonate as a reagent [76]. TLC on silica gel GF 254 in the presence of 5-methyl-2-amino thiazole and benzothiazine carboxylic acid, using ethyl acetate/methanol/NH_3 17:2:1 and detection at 365 nm for both identification and stability of MXM was used [75,89–91]. MXM was simultaneously determined in presence of NSAI oxicams using Technopak 10 column and detection at 360 nm [92]. LC methods for the quantitative estimation of MXM in human plasma including relative bioavailability in dispersible tablets at 355 nm were reported [93,94]. Pharmacokinetics of MXM was studied by RP-LC methods in which the solvent extraction avoided completely. The plasma proteins were precipitated using 1:1 mixture of acetonitrile and perchloric acid, the supernatant liquid was injected on a Lichrosphere C_{18} column directly with the mobile phase containing of acetonitrile and sodium acetate and detection at 355 nm. The detector response was linear over a range of 50–1500 ng/ml in human plasma. Few LC methods described the extraction of the drug from plasma and biological fluids followed by chromatography using a C_{18} column using acetonitrile/sodium acetate buffer pH 3.3 and methanol/phosphate buffer pH 3.2 as mobile phase and detection at 346 and 355 nm [95–97]. MXM was quantified in human plasma of healthy volunteers using RP-LC and tandem mass spectrometry. A triple quadrupole mass spectrometer in multiple reaction-monitoring modes using turbo ion spray in the positive ion mode was used [98]. The detailed chromatographic conditions are described in Table 3C.

8. Nimesulide (NMD)

NMD was introduced in 1985 and it is one of the most potent NSAIDs advocated for use in various inflammatory conditions. It is official in British Pharmacopoeia. Chemically it is [4-nitro-2-(phenoxy)methane sulphoanilide] and has a structure potentially capable of accessing the COX-2 side pocket when the two COX isoforms became organized. It was found to be 5–16-fold selective for COX-2. Oral absorption is nearly complete with the t_{max} between 1.2 and 3.2 h. NMD is entirely metabolized and has an elimination half-life between 1.6 and 5.0 h. Clinical studies have shown that NMD to be analgesic, anti-inflammatory and antipyretic in a wide range of conditions. However, symptomatic gastrointestinal tolerability is not superior to other NSAIDs [99–102]. Around 30 methods were reported during the period of review and their distribution is shown in Fig. 9.The nitro group of the compound is reduced to amino, diazotized and coupled with

Table 3
Chromatographic data of COX-2 inhibitors

Sample matrix	Column	Mobile phase (v/v)	Detector (nm)	Ref.
(A) Celecoxib				
Pharm dosage forms	Inertsil C_{18} (4.6 × 250 mm)	Water/acetonitrile (35:65)	UV 230	[35]
Bulk drug	Novapak C_{18} (3.9 × 300 mm)	Pot. dihydrogen phosphate (0.01 M)/acetonitrile (45:55)	UV 252	[38]
Bulk drug and formulations	ChiralpakAD (4.6 × 250 mm)	Cyclohexane/ethanol (94:06)	UV 255	[39]
Bulk drug and formulations	Hichrome C_{18} (4.6 × 250 mm)	Pot. dihydrogen phosphate (0.01 M)/acetonitrile (45:55)	UV 253	[40]
Formulations	RP C_{18}	Methanol/water (85:15)	MS	[42]
Human serum	Prontosil C_{18} (3.0 × 150 mm)	Acetonitrile/water (60:40)	Fluorescence λ_{Ex} 240, λ_{Em} 380	[46]
Human plasma	Hypersil C_{18} (4.6 × 250 mm)	Pot. dihydrogen phosphate(0.01 M)/acetonitrile/methanol (40:30:30)	UV 238	[47]
Human serum	Wakosil C_{18} (4.6 × 150 mm)	10 mm pot. dihydrogen phosphate (pH 3.2) acetonitrile (50:50)	UV 250	[49]
Biological fluid	Novapak C_{18} (3.9 × 150 mm)	Acetonitrile:amm. acetate (gradient flow)	MS	[51]
Human plasma	Novapak C_8 (3.8 × 150 mm)	Acetonitrile/tetrahydrofuran/sod. acetate buffer pH 5.0 (30:8:62)	UV 215	[52]
(B) Etoricoxib				
Bulk drug	Nucleodur C_{18}	Acetonitrile/water (9:1)	MS	[65]
(C) Meloxicam				
Tablets	Lichrosphere 100RP-C_{18} (4.0 × 125 mm)	Acetonitrile/50 mm tris buffer/1.0 %tetrabutyl ammonium phosphate (35:64:1)	UV 360	[73]
Bulk/formulations	Spherisorb ODS (4.6 × 250 mm)	Methanol:acetate buffer pH 4.3	UV 254	[81]
Degradation products in bulk drugs	AltimaODS (4.6 × 250 mm)	Acetonitrile/sod. dihydrogen phosphate (0.1 M)/0.25% octane sulphonate (13:7)	UV 355	[88]
Plasma	Lichrosphere C_{18}	Tris acetate/tetrabutylammonium reagent/tetrahydrofuran/acetonitrile	UV 360	[91]
Plasma	Hypersil ODS (4.6 × 250 mm)	Methanol/water/triethylamine/acetic acid (2500:1500:15:7)	UV 355	[92]
Plasma	Kromasil ODS	Water/acetonitrile/acetic acid	UV 355	[93]
Plasma	LichrosphereC_{18} (4.0 × 125 mm)	Sodium acetate buffer/acetonitrile (62:38)	UV 355	[94]
Plasma	RP-C_{18}	Methanol/phosphate buffer pH 3.2 (40:60)	UV 346	[95]
Plasma	Phenomenex Luna C_{18} (2.0 × 150 mm)	Acetonitrile/formic acid (0.2%) (65:35)	Tandem MS	[96]
Plasma	Lichrocart C_{18} (4.0 × 125 mm)	Methanol/acetonitrile/amm. phosphate (4:1:5)	UV 364	[98]
(D) Nimesulide				
Bulk drug and formulation	Lichrosphere (3.9 × 250 mm)	Acetonitrile/water (2:3)	UV 210	[112]
Bulk drugs and formulation	Chromosorb RP-C_{18} (4.6 × 250 mm)	Amm. acetate 0.1 M/acetonitrile/methanol pH 6.0 (3:5:2)	UV 210	[113]
Bulk drugs and formulations	Shimpak C_{18} (4.6 × 150 mm)	Methanol/water/acetic acid (67:32:1)	UV 230	[117]
Bulk drugs and formulation	SeparonSGX C_{18} (4.6 × 250 mm)	Acetonitrile/amm. phosphate 0.02 M pH 7.9 (35:65)	UV 245	[118]
Tablets	Bondapak C_{18}/μ porosil C_{18} (3.9 × 150 mm)	Phosphate buffer (pH 3)/methanol (3:2)	Electrochemical	[119]
Human plasma	Nucleosil C_{18} (4.0 × 50 mm)	Acetonitrile/methanol/pot. dihydrogen phosphate solution pH 7.3 (30:5:65)	UV 404	[120]
Human plasma	RP-C_{18}	Acetonitrile/methanol/phosphate buffer (pH 5.5) (50:20:30)	UV 230	[121]
Rat. blood	RP-C_{18}	Methanol/pot. dihydrogen phosphate (pH 5.0)	UV 290	[122]

Table 3 (*Continued*)

Sample matrix	Column	Mobile phase (v/v)	Detector (nm)	Ref.
Plasma	Hypersil BDS C_{18}	Methanol/citrate buffer (pH 3.0) (68:32)	UV 240	[123]
Urine	Supelcosil LC_{18} (4.6 × 250 mm)	Sod. dihydrogen phosphate (pH 3.0)/methanol (gradient flow)	UV 230	[124]
R plasma and CSF	C_{18} Symmetry (4.6 × 150 mm)	Acetonitrile/phosphate buffer (pH 3.0) (53:47)	UV 240	[126]
Human plasma (gradient condition)	Lichrosphere 100 RP	Methanol/phosphate buffer, methanol/phosphate buffer	UV 230	[127]
TLC				
Dosage forms	Silica gel60F_{254}	Chloroform/toluene 9:1	UV 300	[114]
Dosage forms	Silica gel160f_{254}	Methanol/toluene 1:4	UV 324	[115]
Dosage forms	Silica gel60F_{254}	Cyclohexane/ethyl acetate (3:2)	UV 295	[116]
Bulk/formulations	Silica gelG	Toluene/ethyl acetate (4:1)	UV	[117]
(E) Rofecoxib				
Bulk drugs and tablets	Symmetric C_{18} (4.6 × 250)	Acetonitrile/water (50:50)	UV 225	[139]
Bulk drugs	Hichrome RPb C_{18} (4.6 × 250 mm)	Acetonitrile/water(50:50)	UV 225	[140]
Bulk drugs	Symmetry C_8	0.1%phosphoric acid/acetonitrile (gradient flow)	UV 220	[141]
Plasma	Chromolith (4.6 × 50 mm)	Water/acetonitrile (65:35)	Fluorescence λ_{em} 400	[144]
Human plasma	Hypersil BDS C_{18} (4.6 × 100 mm)	Acetonitrile/water (65:34)	Fluorescence λ_{em} 375	[145]
Human plasma	Hypersil ODS	Acetonitrile/water (1:1)	UV 254	[146]
Human plasma	YMC ODS (3.0 × 100 mm)	Acetonitrile/water (1:1) MS	147	
Human plasma	Zorbax phenyl (4.6 × 250 mm)	Acetonitrile/water (60:40)	UV 261	[148]
Tablets	C_{18} (4.6 × 100 mm)	Acetonitrile/acetic acid/triethylamine water (77:23:0.1:0.03)	UV 272	[149]
Tablets	Symmetry C_{18}	Acetonitrile/water (65:35)	Fluorescence λ_{ex} 240, λ_{em} 400	[150]
Human plasma	C_{18}	Acetonitrile/water (65:35)	MS	[153]
Human plasma	Symmetry C_{18}	Acetonitrile/water (65:35)	UV 225	[153]
Human plasma	Spherisorb ODSI	Acetonirile/methanol/phosphate	UV 244	[154]
Human plasma	Prism RP (4.6 × 150 mm)	Acetonitrile/acetate buffer pH 4.0	Fluorescence λ_{ex} 260, λ_{em}375	[155]
(F) Valdecoxib				
Bulk drugs and formulation	Supelco diphenyl (4.2 × 250 mm)	Acetonitrile/phosphate buffer pH 3 (gradient flow)	PDA	[163]
Human urine	Prism RP-C_{18}	Acetonitrile/buffer pH.6 10 mm 4-methyl morpholine	MS	[164]
Human plasma	YMC ODS-AQ (4.6 mm × 250 mm)	Water/methanol (47:53)	UV 210	[165]

different reagents. For example, the formation of a charge transfer complex with *p*-methyl aminophenol sulphate in the presence of potassium dichromate and the intensity of the color was studied [102–104]. Nagaraja et al. have used an alcoholic imino dibenzyl or 3-aminophenol in acid medium as a coupling reagent to determine NMD by colorimetry [105].

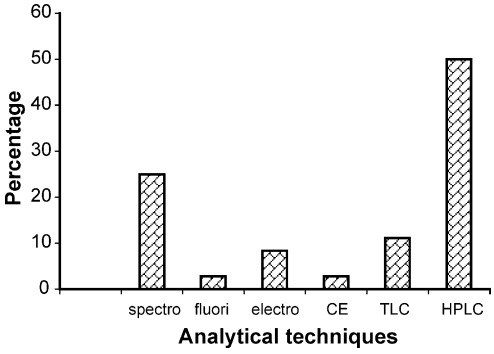
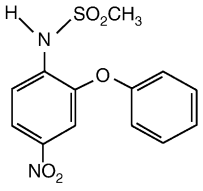


Fig. 9. Different instrumental methods used for analysis of NMD.



Nimesulide

Altinoz et al. have developed a second order derivative spectrophotometry and claimed that it could be applied to pharmaceutical preparations directly [106]. A simultaneous determination of NMD and chlorozoxazone in tablets has been reported [107]. The spectrophotometric conditions are presented in Table 2B. Fluorimetric and voltammetric studies were also carried out [108–111].

8.1. Chromatographic methods

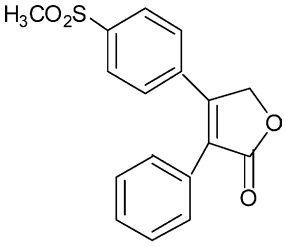
British Pharmacopoeia has described a RP-HPLC method for the assay and determination of relative substances in bulk drugs [100]. Simultaneous determinations of NMD and paracetamol or tizanidine in tablets were reported [112,113]. HPTLC methods for determination of NMD in pharmaceutical dosage forms were carried out (Table 3D) [114–116]. Stability studies were performed under different temperatures, humidity and UV conditions. The degradation products were analyzed by GC and RP-LC [117]. Kovarikova et al. have used both HPLC and TLC to detect the degraded products of 2-phenoxy 4-nitroanaline and methanesulphonic acid obtained after irradiating the drug with UV light at 254 nm [118].

HPLC was extensively used for estimation as well as pharmacokinetic studies of NMD in plasma and blood using electrochemical, spectrophotometric and tandem mass spectrometry [119–124]. Pre-treatment with acetonitrile for deproteination of aqueous humor followed by RP-LC determination at 300 nm was reported [125]. Simultaneous LC estimation of NMD and hydroxy NMD in rat plasma, CSF and brain was carried out [126]. Specific HPLC methods for simultaneous determination of NMD and its hydroxy metabolite in human plasma in healthy volunteers were developed [127]. Sarkar et al. have reported the detection of an unique amino metabolite (reduction of nitro group) in equine blood and urine for the first time by TLC [128]. HPTLC methods for separation and quantification of metabolites in urine were reported. The samples were subjected to liquid-liquid extraction and the metabolites were separated and characterized by TLC and electron-impact mass spectrometry respectively [129]. Different HPTLC methods were discussed for determination of NMD in human plasma and its application in human studies [130,131]. Table 3D indicates the details of the chromatographic conditions. Capillary zone electrophoretic method has been reported for the estimation of nimesulide in pharmaceutical preparations [132].

9. Rofecoxib (RXB)

RXB is 4[4-(methyl sulphonyl)phenyl]3-phenyl furan-2(5H) one, is a new COX-2 inhibitor developed by Merck in 1997. It is a highly selective time dependent COX-2 inhibitor, forms a tightly bound, slowly reversible enzyme inhibitor complex with 1:1 stoichiometry. On the other hand it is weak, time independent rapidly reversible inhibitor of COX-1 [133]. It has been approved for the treatment of osteoarthiritis and for the relief of acute pain. Recently its use has become more controversial due to cardiovascular side effects on prolonged use. Only 25 methods were found in the literature. It indicates the scope for development of newer analytical methods not only for forensic investigation but also quality control.

Spectrophotometric determinations of RXB in pharmaceutical preparations were reported [134–137]. RXB was converted to its photodegradate in a mixture of acetonitrile–water having excitation and emission wavelengths at 247 and 377 nm, respectively.



Rofecoxib

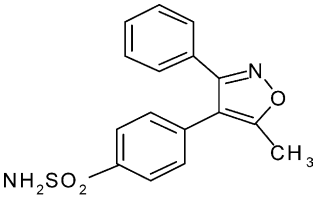
The drug and its photodegradation products in plasma were determined by first derivative spectrophotometry and fluorimetry [138].

9.1. Chromatographic methods

Radhakrishna et al. have developed an isocratic RP-LC method for determination and purity evaluation of RXB in bulk drugs and pharmaceutical formulations using photodiode array detection at 225 nm. This method was found to be capable of detecting main process related impurities present at trace levels in the finished products. The method was found to be useful for process monitoring during its production. Further they have extended their work to characterize the process related impurities 4-[4(methyl sulphony)phenyl]-3-phenyl, 5-hydroxy furan-2-one (1) and 4-[4(methyl sulphony)phenyl]-3-phenyl, 2,5-furandione using preparative HPLC, NMR and MS. Impurity profiles were generated. The data was validated and the reported percentage recoveries ranged from 98.2 to 102.6 [139,140]. The stability of RXB under different alkaline, photolytic stress conditions was studied. The main degradates were identified to be the cyclization products formed by photocyclization and dicarboxylate formed by ring opening. The mechanistic pathway for the formation of degradates was discussed. RP-LC, MEKC and chemometric methods were developed for the stability-indicating assay and validated [141–143]. Monolithic ODS columns and post column chemical derivatization techniques using spectrophotometric, fluorimetric and tandem mass spectrophotometric detectors for estimation of RXB in bulk drugs, formulations including the human plasma were developed [134,144–154]. The conditions are discussed in Table 3E. An automated on-line solid phase micro extraction of RXB from human plasma followed by fluorimetric detection was reported [155,156]. LC coupled with APCI-MS was used to study the pharmacokinetic profiles of RXB in healthy volunteers [157]. CXB was used as an internal standard for the quantitative determination of drug in human plasma [158]. LC tandem mass spectrometric evaluation and determination of stable isotope labeled analogs of RXB in the plasma for the oral bioavailability studies were carried out [159].

10. Valdecoxib (VXB)

In an attempt to overcome solubility restrictions of CXB, the researchers at Searle employed the prodrug approach, which resulted in the discovery of VXB. The prodrug, Parecoxib sodium converts rapidly to VXB, a potent, new anti-inflammatory drug that is highly selective for the inhibition of COX-2. US FDA has approved VXB from Pharmacia Corporation for the treatment of rheumatoid arthritis [160]. VXB is metabolised primarily by cytochrome p450, 2C9 and 3A4 to the pharmacologically active hydroxylated metabolite and carboxylic acid metabolite in humans[161,162]. To the best of our knowledge till now neither spectrophotometric nor voltametric methods were reported in the literature.



Valdecoxib

10.1. Chromatographic methods

Packed column supercritical fluid and liquid chromatographic procedures were reported in the literature. A Supelcosil LC-CN column with a mixture of methanol and isopropylamine as mobile phase at 3 ml/min flow, temperature gradient and detection at 240 nm was used. While the HPLC method used a Supelco diphenyl LC-DP column maintained at the gradient flow of different proportions of acetonitrile and phosphate buffer pH-3 and detected at 215 nm.

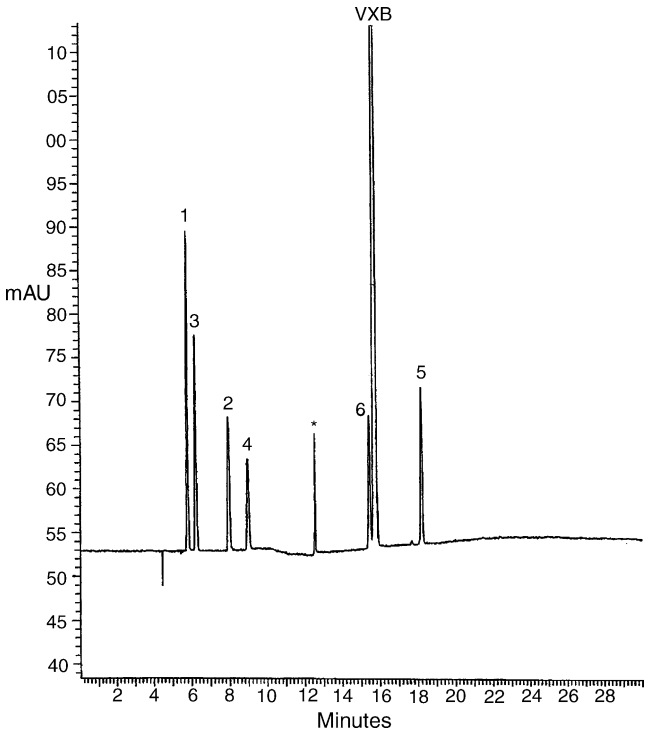


Fig. 11. Chromatogram generated with PCSFC using four columns. Reproduced from Ref. [163] with permission from Elsevier.

The process related impurities and a typical SFC chromatogram of VXB are shown in Figs. 10 and 11, respectively [163].

A sensitive analytical method was required for the determination of VXB and its metabolites in human urine in order to understand their renal excretion in clinical studies.

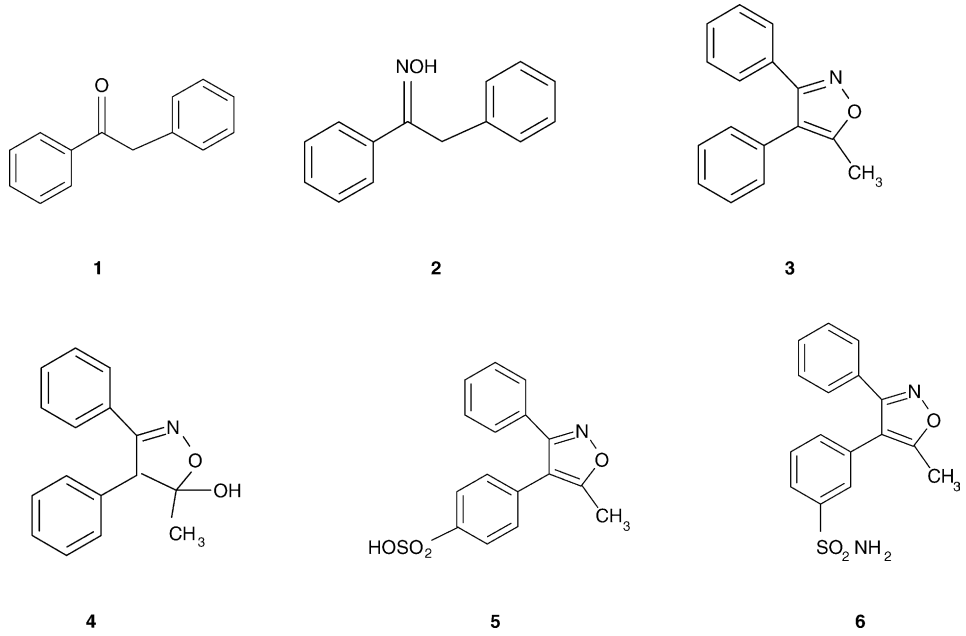


Fig. 10. Process related impurities of valdecoxib.

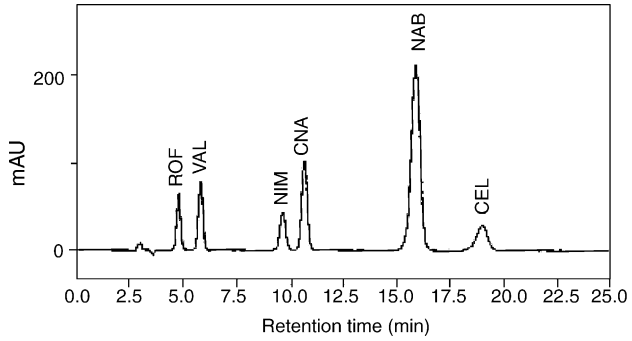


Fig. 12. Typical chromatogram of synthetic mixture containing COX-2 inhibitors and CNA (each 10 μg/ml) used as internal standard.

Zhang et al. have developed a LC method for determination of VXB, hydroxy metabolite and carboxy metabolite in human urine and plasma and validated [164]. The analytes were extracted on to a C_{18} solid phase extraction cartridge using a Zymark column with acetonitrile-water (50:50, v/v) containing 10 mM 4-methyl morpholine as a mobile phase at pH 6.0. The analytes were subjected to negative ion electrospray ionization mass spectrometry and detected by multiple reaction monitoring with a tandem mass spectrometer [165,166].

Liquid–liquid extraction followed by a LC method for quantification of VXB was reported [167,168]. Chromatographic details are presented in Table 3F.

11. Simultaneous determinations of COX-2 inhibitors

In addition to the analytical methods for single components, some of the simultaneous determinations are also included in this review. Simultaneous electrospray ionization tandem mass spectroscopic determination of 14-non steroidal drugs in human serum was described [169]. LC–MS of sulphur containing NSAIDs and its application to pharmaceutical products was reported [170]. The analytes viz., celecoxib and rofecoxib were determined by ion-trap mass spectrometer using APCI as an ionization process using different mobile phases.

Semipermeable surface or hydrophobic shielded columns were used for direct injection of some of the NSAIDs [171]. Enantioseparation of noval COX-2 inhibitors by capillary electrophoresis using sulphabutyl ether cyclodextrin systems as chiral selector using 50 mM sodium tetraborate buffer at pH 9.2 was reported [172]. A capillary GC–MS for the detection of COX-2 inhibitors in human plasma was discussed [173]. We have developed a simultaneous RP-LC method for determination of COX-2 inhibitors viz., nimesulide, celecoxib, rofecoxib and valdecoxib for its application to biological fluids recently (Fig. 12) [174].

12. Conclusions

An overview of the current state-of art analytical methods for determination of COX-2 inhibitors has been presented. The literature compilation has revealed that a variety of methods are available for the first generation of COX-2 inhibitors viz., NMD and MXM. For drugs like celecoxib, valdecoxib and etoricoxib only a limited number of methods were reported while no methods for lumiracoxib, tilmacoxib and cimicoxib. This is because of the fact that selective COX-2 inhibitors were introduced in late nineties. Our analysis of the published data revealed that the HPLC was extensively used for estimation of COX-2 inhibitors in biological fluids. Of the 138 methods published during the period of 1995–2004, methods based on HPLC were 80 showing that it is the technique of choice for analysis of COX-2 inhibitors. Most of the workers have used the reversed-phase mode with UV absorbance detection because this provided with best available reliability, repeatability, analysis time and sensitivity. LC coupled with mass detector (LC–ESI-MS) was used not only to detect most of the metabolites of COX-2 inhibitors in human urine and plasma but also the degradation products of bulk drugs and formulations. Other detectors such as fluorescence and electrochemical were also used in the evaluation and control of purity of COX-2 inhibitors. In this review we have discussed the present state-of the analytical methods for determination of not only preferential COX-2 inhibitors viz., NMD, MXM but also selective agents such as CXB, RXB and VXB. There is a great scope for development of newer analytical methods for latest drugs such as EXB, LXB and CMB. Since some of these drugs are recently introduced into the market, we have undertaken a comprehensive program to develop and validate new analytical methods using modern techniques such as HPLC, CE and LC–MS in drugs and pharmaceuticals as well as biological materials.

Acknowledgments

The authors wish to thank Dr. J.S. Yadav, Director and Dr. M. Vairamani, Head of the Analytical Division, Indian Institute of Chemical Technology for encouragement and permission to communicate the manuscript for publication.

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