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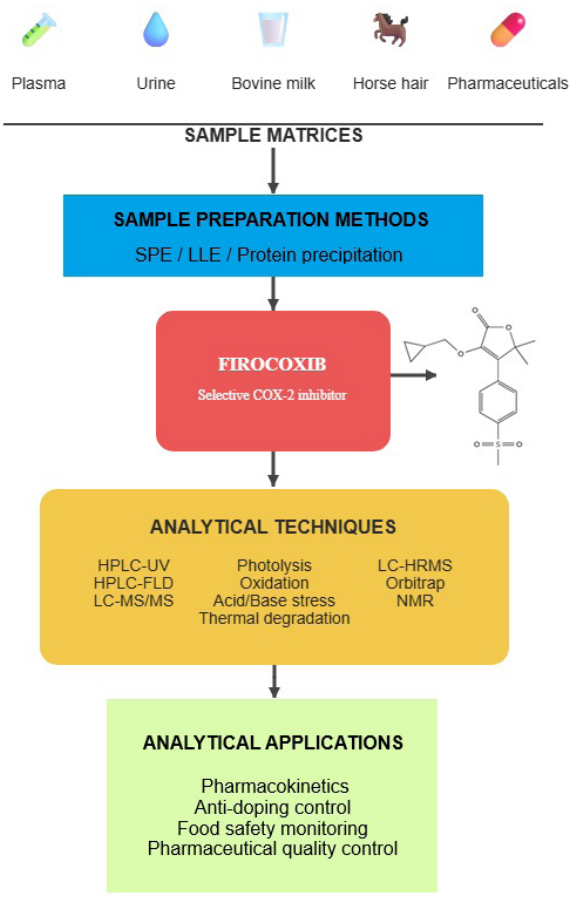
REVIEW

Analytical Methodologies for the Determination of the Drug Firocoxib in Various Matrices: A Review

Patrícia Espinosa dos Santos Brito*, **Najla Mohamad Kassab**

Laboratório de Tecnologia Farmacêutica, Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil



Firocoxib (FX) is a second-generation nonsteroidal anti-inflammatory drug characterized by its high selectivity for the cyclooxygenase-2 (COX-2) enzyme, primarily indicated for the treatment of pain and inflammation associated with osteoarthritis in horses and dogs, as well as for postoperative pain in orthopedic and soft tissue surgeries in dogs. Due to its significant clinical use and the stringent regulations in competitive equestrian sports, reliable quantification of FX in biological and pharmaceutical matrices is essential for pharmacokinetic studies, therapeutic drug monitoring, anti-doping control, and industrial quality control. This review presents analytical methods developed for the determination of FX, covering various matrices such as plasma, urine, bovine milk, horse hair, and pharmaceutical products. The reviewed methods include high-performance liquid chromatography (HPLC) coupled with ultraviolet or fluorescence detection, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), and high-resolution techniques such as Orbitrap mass spectrometry. This review also presents the degradation profile of FX under various stress conditions, the structural elucidation of its major degradation products using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) and nuclear magnetic resonance (NMR), and data from analyses performed on different species (horses, dogs, camels, and goats) to assist researchers and professionals in choosing the most appropriate analytical techniques for FX quantification to meet clinical, regulatory, and pharmaceutical demands.

Keywords: anti-inflammatory, firocoxib, analytical methods, chromatography, validation

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INTRODUCTION

The pharmaceutical sector is highly complex and lucrative, where the pursuit of innovation is constant to maintain competitiveness and customer loyalty. In this scenario, the implementation of quality control protocols, based on good manufacturing practices, becomes the fundamental pillar of production. The guarantee of safety and therapeutic efficacy depends directly on the application of analytical methods that demonstrate proven selectivity, accuracy, and robustness.[1]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely marketed and used drugs in the world, being extensively employed in the treatment of pain and fever in humans and animals.[2] The widespread use of this class is associated with a high incidence of degenerative diseases, such as osteoarthritis. However, the prolonged use of conventional NSAIDs can be limited by the occurrence of adverse effects, mainly in the gastrointestinal and renal systems.[3,4]

Side effects are related to the non-selective inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. While COX-1 is responsible for maintaining tissue homeostasis and protecting the gastric mucosa, COX-2 acts as the main pathway for mediating inflammation.[4,5] This functional distinction has driven the development of "coxibs," a class of second-generation drugs developed to act selectively on COX-2, reducing systemic toxicity.[3,6]

Currently, the coxib class includes drugs of great clinical importance, such as celecoxib, rofecoxib, and etoricoxib, which are widely used in human medicine.[2,7] In veterinary medicine, the available coxibs include deracoxib, firocoxib, robenacoxib, mavacoxib, cimicoxib, and, more recently, enflicoxib, each adapted to the metabolic needs of different domestic species.[2,8-10]

Firocoxib (FX) stands out in this class for its selectivity, being the first coxib approved by the FDA for horses and the second for dogs.[11,12] Its clinical efficacy is widely documented in the control of chronic pain associated with osteoarthritis and in postoperative analgesia of complex orthopedic surgeries, such as tibial plateau leveling osteotomy (TPLO).[9,13]

The therapeutic versatility of FX has encouraged its "extra-label" use in different animal species, expanding its use in veterinary medicine. Recent research has investigated its pharmacokinetics and safety in cats, camels, rabbits, and even large mammals such as African elephants (African bush elephant).[14-16] Furthermore, the drug has been used to treat pain in calves and pigs, including novel strategies such as transmammary transfer from sows to piglets via milk during lactation.[17,18]

Despite its market consolidation, FX presents challenges regarding physicochemical stability. Forced degradation studies reveal that the molecule is susceptible to photolysis, oxidation, and acid stress processes, generating degradation products that can compromise the purity of the raw material.[19] Furthermore, there is a lack of official methods in pharmacopoeias for the qualitative and quantitative analysis of raw material batches, highlighting the need for the development of new chromatographic analyses for drug quality control.[20]

In parallel with industrial demands, monitoring residues in livestock and controlling doping in equestrian athletes also require sensitive analytical methodologies. The detection of low concentrations in complex matrices, such as bovine milk, urine, and equine hair, is fundamental to ensuring food safety and the ethical integrity of sporting competitions.[21-23] Such applications require the use of advanced analytical technologies, such as tandem mass spectrometry (LC-MS/MS) and high-precision fluorescence detectors.[24,25]

Considering the expansion of clinical use and the increase in regulatory requirements, as well as the importance of developing and validating analytical methods for drug quality control, this work presents a review of the analytical methodologies described in the literature for the determination of firocoxib. The review was conducted using the SciFinder, ScienceDirect, PubMed, and CAPES Periodicals Portal databases, searching for terms such as firocoxib determination, methods for firocoxib, firocoxib validation, firocoxib by HPLC, firocoxib by LC, and firocoxib pharmacokinetics.

Original articles, methodological studies, pharmacokinetic investigations, stability studies, and regulatory reports involving FX, published between 2005 and 2024, were considered, in addition to searches in official compendia. The inclusion criteria encompassed original articles in English, focusing on chromatographic techniques for FX determination. Studies that did not contain analytical data for the determination of FX,

duplicate publications, conference abstracts without complete methodological information, and studies unrelated to analytical methodologies were excluded.

PHYSICOCHEMICAL PROPERTIES

FX, registered under CAS number 189954-96-9, is a substituted furanone chemically known as 3-(cyclopropylmethoxy)-5,5-dimethyl-4-[4-(methylsulfonyl)phenyl]furan-2-one (Figure 1).[19,20] Its molecular structure is represented by the formula $C_{17}H_{20}O_5S$, with a molar mass of 336.4 g/mol.[4,20]

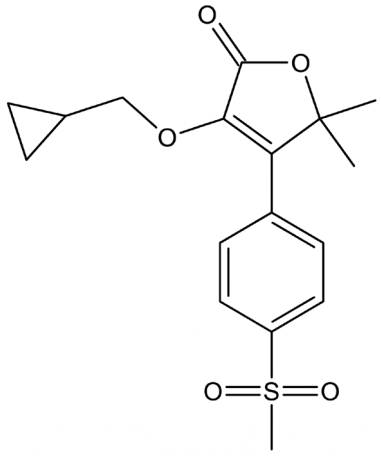

Figure 1. Chemical structure of firocoxib (CAS No. 189954-96-9).
Source: Authors' own figure.

In its pure state, the drug is characterized as a white to off-white crystalline powder with a non-hygroscopic nature.[19,20] From a thermal standpoint, it has a melting point in the range of 78–80 °C, a density of 1.31 g/cm³, and an estimated boiling point of 563.9 °C under normal atmospheric pressure.[4,19]

The solubility of FX is a critical parameter for pharmaceutical development, being considered practically insoluble in aqueous media, with a solubility of only 0.0105 mg mL^{-1}.[19,20] In contrast, it demonstrates significant affinity for polar organic solvents, being highly soluble in dimethyl sulfoxide (67 mg mL^{-1}) and soluble in ethanol.[4,19] This markedly lipophilic nature is responsible for the high volume of distribution observed in various species, which facilitates the crossing of biological membranes and penetration into fluids such as synovial fluid and breast milk.[12,18]

Electrochemically, FX is classified as a neutral and non-ionizable molecule under physiological pH conditions.[4] Although it has a theoretical pKa of 19.69 for its most acidic portion, it does not present functional groups that undergo significant protonation or deprotonation in the pH range commonly used in reversed-phase liquid chromatography.[20]

Its conjugated structure functions as an efficient chromophore, allowing quantitative detection by ultraviolet spectroscopy between 240 nm and 290 nm, as well as by fluorescence detection, a technique that offers superior sensitivity for monitoring trace levels in neonates.[11,24,25]

Regarding stability under environmental stress, FX demonstrates robustness under basic and thermal stress conditions.[19] However, the integrity of the molecule is compromised under strong oxidation, prolonged acid stress, and photolytic exposure in solution, generating up to six main degradation products.[19]

Finally, it is crucial to note that the drug has a high affinity for polymeric surfaces, tending to bind to plastic storage tubes, which necessitates the mandatory use of glass containers to avoid underestimation of analytical results.[16]

PHARMACOLOGICAL PROPERTIES

The pharmacological properties of FX define it as a highly precise therapeutic agent within contemporary veterinary medicine. Its efficacy is supported by superior enzymatic selectivity and a kinetic profile that favors

the treatment of acute and chronic inflammatory conditions in various species.[3,4] It was discovered and its patent was registered in 1998 by Merck Canada, marking the period of its initial chemical innovation.[26] Initial FDA approval for use in dogs occurred in 2004, making the drug available under the trade name Previcox® in chewable formulations.[27,28] In 2007, the drug was introduced for the control of pain and inflammation associated with osteoarthritis in horses, consolidating its clinical application in large animals.[4,29,30]

The efficacy of FX is supported by its high enzymatic selectivity for COX-2, which is significantly superior to that of other non-steroidal anti-inflammatory drugs (NSAIDs), allowing the inhibition of pathological mediators while preserving COX-1-dependent tissue homeostasis.[4,20,31] *In vitro* studies have demonstrated COX-1:COX-2 selectivity ratios of 643 for horses, 384 for dogs, and 58 for cats, indicating a large margin of clinical safety.[3,4]

Pharmacodynamics (mechanism of action)

FX exerts its therapeutic activity primarily by interrupting the arachidonic acid cascade, acting as a highly selective inhibitor of the cyclooxygenase-2 (COX-2) enzyme (Figure 2).[19,32] Unlike conventional NSAIDs, which non-specifically block both COX-1 and COX-2, FX preferentially binds to the inducible isoform (COX-2), which is expressed in response to inflammatory stimuli and tissue injury.[3,20] This inhibition prevents the synthesis of pro-inflammatory prostaglandins, key mediators of pain, fever, and edema.[4,15]

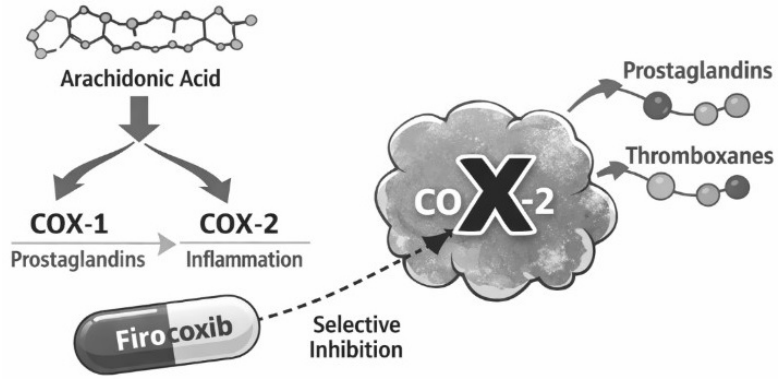

Figure 2. Mechanism of action of firocoxib. Source: Author's own figure.

The pharmacodynamic advantage of this coxib lies in its ability to spare the COX-1 enzyme at therapeutic concentrations.[5,8] COX-1 is a constitutive enzyme essential for maintaining physiological homeostasis, including gastric mucosa protection, platelet aggregation, and regulation of renal blood flow.[32,33] *In vitro* and *ex vivo* studies demonstrate that firocoxib's selectivity for COX-2 is significantly higher than that observed in other NSAIDs, with selectivity ratios (IC_{50} COX-1:COX-2) reaching 643 in horses and 384 in dogs.[3,4]

Clinical applications

Clinically, FX is established as the treatment of choice for controlling pain and inflammation related to chronic osteoarthritis in dogs and horses.[9,34] In horses, the use of the paste formulation demonstrated clinical improvement in approximately 80% of cases of natural osteoarthritis, with rapid results observed as early as the first week of treatment.[35] Multicenter studies indicate that FX is particularly effective in reducing pain on manipulation and improving range of motion, outperforming traditional drugs in specific joint parameters.[34,35]

Its efficacy in improving lameness and mobility is comparable to traditional NSAIDs such as phenylbutazone and carprofen, but with a significantly lower incidence of gastrointestinal side effects.[8,34] In addition to chronic use, the drug is widely employed in postoperative analgesia for orthopedic and soft tissue surgeries.[13,29] The "extra-label" application of FX has expanded to include pain mitigation in livestock handling procedures, such as dehorning in calves and castration in lambs and goats.[36-38]

A relevant therapeutic advance is the transmammary route, where administration of the drug to lactating sows allows the transfer of therapeutic levels to piglets through milk, reducing physiological stress and

improving post-processing weight gain.[18] In wild species, FX has been evaluated for pain management in African elephants, demonstrating potential for the treatment of joint conditions in megafauna.[16]

Pharmacokinetics

The absorption of FX after oral administration is characterized by high efficiency and speed in various animal matrices. In horses, the absolute bioavailability is remarkable, with reported values ranging from 79% for tablets to 111% for the paste formulation.[5,39] Peak plasma concentration (Tmax) generally occurs between 0.77 and 4 h, although this parameter may be delayed by the presence of food in the gastrointestinal tract, which reduces the absorption rate in horses.[12,37] In other species, such as adult sows and goats, oral bioavailability is around 70-71%.[37,40] The distribution of FX is exceptionally wide, reflected in a significantly larger volume of distribution (Vss) than that of other traditional NSAIDs.[12,36] This characteristic is attributed to its high lipophilicity and the absence of ionizable charge at physiological pH.[4,5] Vss values vary considerably between species: horses (1.5 to 3.66 L/kg), dogs (~2.9 L/kg), calves (~6.54 L/kg), and reaching extremes in pigs, with values between 7.75 and 13.8 L/kg.[36,39,40]

The drug's affinity for plasma proteins is high, exceeding 97% in horses, meaning that only a small free fraction is available to exert immediate pharmacological activity.[5,12] However, its lipophilic nature facilitates penetration into deep tissues and biological fluids. FX reaches synovial fluid at concentrations of approximately 30% relative to plasma and efficiently penetrates aqueous humor and breast milk.[5,18,41]

FX metabolism occurs predominantly in the liver through dealkylation and glucuronidation processes.[5,15] The main metabolic pathway involves the removal of the cyclopropylmethyl group, resulting in the metabolite descyclopropylmethylfirocoxib (DFX).[5,19] *In vitro* and *ex vivo* studies confirm that both DFX and its glucuronide conjugates are pharmacologically inactive, exhibiting negligible affinity for the COX-2 enzyme.[3,4]

Urinary excretion is the main route of elimination of the drug and its metabolites. In horses, approximately 68% of the administered dose is recovered in the urine, while only 15% is eliminated via feces.[5,12] Renal clearance of intact FX is low, indicating that hepatic metabolism is an essential prerequisite for the final elimination of the compound.[4,12]

The elimination half-life ($t_{1/2}$) of FX shows extreme variations among the species studied, which directly impacts dosage protocols. In adult horses, the half-life is long, ranging from 30 to 44 hours, and 21.5 hours in goats, allowing once-daily administration.[12,39] This slow elimination profile favors plasma bioaccumulation after multiple doses, resulting in steady-state concentrations 3 to 4 times higher than those obtained after a single dose.[12,42] In contrast, young animals and other species exhibit much faster clearance. In neonatal foals, the half-life is drastically reduced to approximately 11 to 13 hours, possibly due to accelerated maturation of enzymatic systems or differences in volume of distribution.[24,25] Similarly, short half-lives have been documented in camels (~5.75 h), rabbits (~9.1 h), and Greyhound dogs (~6.5 h).[15,23,43] This kinetic diversity reinforces the importance of sensitive analytical methods for therapeutic monitoring, especially in species where rapid elimination requires narrower detection windows. For production animals, such as pigs and goats, the half-life is in an intermediate range of 21 to 31 hours, which raises crucial questions about the withdrawal period and the persistence of residues in edible tissues.[37,40]

Understanding this ADME (Absorption, Distribution, Metabolism, and Excretion) profile is fundamental for the development of new analytical methodologies. The high lipophilicity and metabolic stability in certain matrices, such as equine hair, allow for long-term monitoring of drug use, while its presence in milk requires highly sensitive methods to ensure food safety.[18,22]

ANALYTICAL METHODOLOGIES

The use of FX as a safe therapeutic agent and the monitoring of its use in competition and production animals require the development of rigorous analytical methods. The complexity of biological matrices, which vary from plasma and urine to tissues and hair, coupled with the need to monitor degradation products in pharmaceutical inputs, has driven the evolution of techniques that combine high selectivity with sensitivity.[19,32] In general, the determination of FX is not limited to a single analytical technique; therefore, an overview of the main analytical methodologies applied to different species is presented in Table I.

Table I. Chromatographic methods for the analysis of firocoxib in different matrices

Sample Matrix	Objective of the analysis	Technique	Scan	Ionization	Precursor Ion (*m/z*)	Detection (nm) / Mass Transitions (*m/z*)	State of Charge	Matrix Effect / Interference	Mobile Phase	Stationary Phase	Flow (mL/min)	Concentration Range	RT (min)	LOD / CCα	LOQ / CCβ	Ref
Plasma (Dog / Horse)	Clinical routine and bioequivalence studies	HPLC-UV	N/A	N/A	N/A	290 nm	N/A	No endogenous interference in 20 animals and 7 concomitant substances.	Isocratic: ACN/ Water/TFA (45:55:0.025)	Inertsil ODS-3 (150 x 4.6 mm, 5 µm)	1.0 – 1.2	25 – 10.000 ng mL^{-1} (D) / 25 – 2500 ng mL^{-1} (H)	8.0 – 9.5	10 ng mL^{-1}	25 ng mL^{-1}	[11]
Plasma (Camel)	Pharmacokinetics and Metabolite Identification	LC-HRMS (Orbitrap)	Full Scan (100-550 Da)	HESI+	337.1032	337.1032 (2 ppm tolerance)	z = 1	Irrelevant matrix effect (< 15%)	Gradient: FA 0.1%/Methanol	Agilent Zorbax ZDB C18 (3.5 µm; 2.1 x 50 mm)	0.3	0.5 – 200 ng mL^{-1}	2.62	N/I	0.5 ng mL^{-1}	[15]
Soro (African Elephant)	Pharmacokinetics	HPLC-UV	N/A	N/A	N/A	290 nm	N/A	Drug binding to plastics (using glass)	ACN/water/TFA (45:55:0.025)	Phenomenex Luna PFP(2) (5 µm; 150 x 4.6 mm)	1.0	2 – 100 ng mL^{-1}	9.8	1 ng mL^{-1}	2 ng mL^{-1}	[16]
Plasma (Calf)	Pharmacokinetics in unweaned animals	LC-MS/MS	MRM	ESI+	337.1	337.1 > 130.1 / 129.2 / 55.1	z = 1	Non-matrix-based external curve	Water (0.1% FA)/ ACN (0.1% FA)	Ascentis Express C18 (2.7 µm; 100 x 2.1 mm)	0.5	1.0 – 1000 ng mL^{-1}	5.5	0,10 ng mL^{-1}	1,00 ng mL^{-1}	[17]
Piglets and Tissues	Transmammary Pharmacokinetics and Stress Mitigation (Spaying/Tail Resection)	LC-MS (Ion Trap)	Full Scan	ESI+	337	Fragments 283, 265, 237	z = 1	Use of firocoxib-d6 as standard; ion suppression assessed via post-column infusion.	Gradient: Water (0.1% FA)/ACN (0.1% FA)	Hypersil Gold C18 (100 x 2.1 mm; 3 µm)	0.275	1–500 ng mL^{-1} (Plasma) / 0.05–10 µg g^{-1} (Tissues)	4.9	0.2 ng mL^{-1}	1.0 ng mL^{-1}	[18]
Raw material (Bulk)	Forced Degradation Study and Structural Elucidation	HPLC-DAD LC-HRMS	Full Scan MS-MS	ESI+ / ESI-	337.1108	210, 240, 254 nm / Fragments: 283, 265, 237	z = 1	Elucidation of 6 degradation products (DP1-DP6)	Gradient: Water (0.1% FA)/ACN	HALO C18 (50 x 4.6 mm; 2.7 µm)	0.6	0.4 mg mL^{-1}	5.48	N/I	N/A	[19]
Raw material (Bulk)	Quality control and impurities (High Speed)	HPLC-UV	N/A	N/A	N/A	240 nm	N/A	Separation of 7 impurities and degrading substances	Gradient: H_3PO_4 0,1%/ACN	HALO Biphenyl (30 x 4.6 mm; 2.7 µm)	2.5	0.4 – 480 µg mL^{-1}	1.99	0.12 µg mL^{-1}	0.4 µg mL^{-1}	[20]
Bovine Plasma	Confirmatory residue analysis (Doping)	LC-MS/MS	MRM	ESI+	337	337 > 130 / 337 > 283	z = 1	Matrix-matched curve RSD < 10%	Gradient: Water (0.001 M Acetic Acid)/ACN	Agilent Eclipse Plus C18 (1.8 µm; 3.0 x 50 mm)	N/I	0 – 20 ng mL^{-1}	7.38	0.67 ng mL^{-1}	1.14 ng mL^{-1}	[21]
Hair (Equine)	Long-Term Monitoring	LC-MS/MS	MRM	ESI+	337.02	337.02 > 283.1 (130.1/237.0)	z = 1	Evaluation of matrix effects (n=5); ionic suppression < 18%	Gradient: 5 mM Ammonium Formate Buffer (pH 3)/Methanol	Phenomenex Kinetex C18 (50 x 2.1 mm; 2.6 µm)	0.35	1 – 740 pg mg^{-1}	5.6	0.5 pg mg^{-1}	1 pg mg^{-1}	[22]
Urine (Grey hound)	Pharmacokinetics for medication management in running	LC-MS/MS	MRM	ESI+	337.1	337.1 > 265.1 237.1 / 130.1	z = 1	Use of firocoxib-d4 as an internal standard	Gradient: FA 0.1%/Methanol	Poroshell 120 EC-C18 (2.7 µm; 3 x 50 mm)	N/I	2.5 – 1000 ng mL^{-1}	7.0	N/I	2.5 ng mL^{-1}	[23]
Plasma (Foals)	Pharmacokinetics and Safety (Oral)	HPLC-Fluo	N/A	N/A	N/A	250 nm / 375 nm	N/A	Protein precipitation with ACN (0.2 mL sample)	45:55 (v/v) ACN/ Water + 0.25% TFA anhydride	Zorbax RxC18 (150 x 3 mm; 3.5 µm)	0.5 and 0.8	2.5 – 250 ng mL^{-1}	N/I	2.5 ng mL^{-1}	5.0 ng mL^{-1}	[24]

(continued on next page)

Table I. Chromatographic methods for the analysis of firocoxib in different matrices (continued)

Sample Matrix	Objective of the analysis	Technique	Scan	Ionization	Precursor Ion (*m/z*)	Detection (nm) / Mass Transitions (*m/z*)	State of Charge	Matrix Effect / Interference	Mobile Phase	Stationary Phase	Flow (mL/min)	Concentration Range	RT (min)	LOD / CCα	LOQ / CCβ	Ref
Plasma (Foals)	Pharmacokinetics and Safety (IV)	HPLC-Fluo	N/A	N/A	N/A	250 nm / 375 nm	N/A	Non-matrix-based external curve (Neat standard)	60% Methanol / 40% Water	Zorbax RxC18 (250 x 4.6 mm; 5 µm)	1.0	2.5 – 250 ng mL^{-1}	N/I	2.5 ng mL^{-1}	5.0 ng mL^{-1}	[25]
Plasma (Dog / Horse)	Pharmacokinetic studies	LC-MS/MS	MRM	ESI+	337.0	337.0 > 283.0	z = 1	Ionization efficiency > 72%	Isocratic: ACN/ Ammonium Formate 2 mM (pH 4.0) (45:55)	Phenomenex Luna Phenyl-Hexyl (3 µm; 100 x 2 mm)	0.25	1 – 3000 ng mL^{-1}	4.52	0.25 ng mL^{-1}	1 ng mL^{-1}	[32]
Urine (Dog / Horse)	Doping Control	LC-MS/MS	MRM	ESI+	337.0	337.0 > 283.0	z = 1	Stability in 3 F/T cycles	Isocratic: ACN/ Ammonium Formate 2 mM (pH 4.0) (45:55)	Phenomenex Luna Phenyl-Hexyl (3 µm; 100 x 2 mm)	0.25	5 – 3000 ng mL^{-1}	4.52	1.0 ng mL^{-1}	5 ng mL^{-1}	[32]
Plasma (Calf)	Pharmacokinetics and Cauterization	LC-MS/MS	SRM	ESI+	337	Fragmentos 130, 237, 283	z = 1	Matrix-matched with no internal pattern	Water (0.1% FA)/ ACN	Intersil ODS-4 (3 µm; 75 x 2.1 mm)	N/I	0.005 – 5.0 µg mL^{-1}	7.5	N/I	0,005 µg mL^{-1}	[36]
Plasma (Goat)	Pharmacokinetics and Oral Bioavailability	UPLC-MS/MS	SRM	ESI+	337	337 > 283 / 337 > 130 341>284 – quantification of firocoxib-d4	z = 1	Recovery via Ostro Plate (96-well)	Gradient: Water (0.1% FA)/ACN (0.1% FA)	Waters HSS T3 (1.8 µm; 2.1 x 50 mm)	N/I	5 – 10000 ng mL^{-1}	0.8-1.3	N/I	5 ng mL^{-1}	[37]
Plasma (Lamb)	Pharmacokinetics and Renal Function	UPLC-MS/MS	MRM	ESI+	337	337 > 283 / 337 > 237	z = 1	Use of firocoxib-d6 as an internal standard	Water (0.1% FA)/ Methanol (0.1% FA)	Waters XSELECT HSS T3 (2.5 µm; 2.1 x 50 mm)	0.6	0.02 – 100 ng mL^{-1}	2.2	0.6 ng mL^{-1}	1 ng mL^{-1}	[38]
Plasma (Porcine)	Pharmacokinetics and Residues	UPLC-MS/MS	MRM	ESI+	337.10	337.10 > 282.9 / 337.10 > 129.8	z = 1	SPE Extraction (Oasis HLB Prime)	Ammonium Formate 2 mM (pH 4.0)/ACN	Acquity UPLC HSS T3 (1.8 µm; 2.1 x 50 mm)	0.4	0.1 – 1000 ng mL^{-1}	3.0	N/I	0.1 ng mL^{-1}	[40]
Tissue (Porcine)	Waste Depletion	LC-MS (Ion Trap)	Full Scan	ESI+	N/A	Fragments 283, 265, 237	z = 1	Muscle, Kidney, Liver and Skin/Fat	Water (0.1% FA)/ ACN	Hypersil Gold C18 (3 µm; 100 x 2.1 mm)	0.275	0.05 – 10 µg g^{-1}	4.9	N/I	0.01 µg g^{-1}	[40]
Plasma (Rabbit)	Pharmacokinetics and Pharmacodynamics	UPLC-MS/MS	MRM	ESI+	337.10	337.10 > 282.9 / 337.10 > 129.8	z = 1	S/N > 10 no LLOQ	Ammonium Formate 2 mM (pH 4.0)/ACN	Acquity UPLC HSS T3 (1.8 µm; 2.1 x 50 mm)	0.4	0.1 – 1000 ng mL^{-1}	3.0	0.05 ng mL^{-1}	0.1 ng mL^{-1}	[43]
Cow's Milk	Confirmatory residue analysis	RRLC-MS/MS	MRM	ESI+	337.2	337.2 > 283.2 337.2 > 237.0	z = 1	Without interference from other NSAIDs	Gradient: Water (Acetic Acid 0.001 M)/ACN	Agilent Eclipse Plus C18 (1.8 µm; 2.1 x 50 mm)	0.75	0 – 20 ng mL^{-1}	2.57	1.18 ng mL^{-1}	2.02 ng mL^{-1}	[44]
Plasma (Equine)	High-Resolution Pharmacokinetics	LC-HRMS (Orbitrap)	Full Scan	ESI+	337.1098	337.1098 (10 ppm tolerance)	z = 1	LLE extraction with methyl tert-butyl ether	Gradient: ACN/ Water (0.2% FA)	ACE 3 C18 (3 µm; 50 x 2.1 mm)	N/I	0.1 – 200 ng mL^{-1}	~2.5	N/I	0.1 ng mL^{-1}	[45]
Plasma (Equine)	Clinical Pharmacokinetics	HPLC-UV	N/A	N/A	N/A	290 nm	N/A	Liquid-liquid extraction (EtOAc:Hexane)	Isocratic: Water (0.025% TFA)/ ACN (50:50)	Waters Sunfire C18 (5 µm; 150 x 4.6 mm)	1.1	5 – 1500 ng mL^{-1}	6.9	2.5 ng mL^{-1}	5 ng mL^{-1}	[46]
Plasma, PRP and APS (Equine)	Impact of NSAIDs on Cytokines and GFs	LC-MS/MS	MRM	ESI+ / ESI-	337	337 > 283	z = 1	LLE extraction with methyl tert-butyl ether; use of ketoprofen-d3 as a standard	Ammonium Formate 5 mM/ ACN	ACE C18 (75 x 2.1 mm; 5 µm)	N/I	1.0 – 250 ng mL^{-1}	5.0	N/I	1.0 ng mL^{-1}	[47]

Table notes on the following page.

Table I (continued). Notes
ACN: Acetonitrile; APS: Autologous protein solution; CCα: Decision Limit; CCβ: Detection Capability; ESI: Electron Spray Ionization; GFs: Growth Factors; HESI: Heated Electrospray Ionization; FA: Formic Acid; HPLC-DAD: High-Performance Liquid Chromatography with Diode Array Detector; HPLC-Fluo: High-Performance Liquid Chromatography with Fluorescence Detector; HPLC-UV: High-Performance Liquid Chromatography with Ultraviolet Detector; IV: Intravenous; LC-MS: Liquid Chromatography coupled with Mass Spectrometry; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry; LC-HRMS: Liquid Chromatography–High-Resolution Mass Spectrometry; LLE: Liquid-Liquid Extraction; LOD: Limit of Detection; LOQ: Limit of Quantification; MRM: Multiple Reaction Monitoring; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; N/I: Not Identified; N/A: Not Applicable; Ref: References; PRP: Platelet-rich plasma; RRLC-MS/MS: Fast Resolution Liquid Chromatography with Tandem Mass Spectrometry; RT: Retention Time; SPE: Solid Phase Extraction; SRM: Selected Reaction Monitoring; TFA: Trifluoroacetic Acid; UHPLC-MS/MS: Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry.

Sample preparation and clean-up

Sample preparation is the crucial step for the success of quantitative analysis, aiming at the elimination of endogenous interferents and the concentration of the analyte. For FX, Solid Phase Extraction (SPE) has become established as the preferred technique, predominantly using hydrophilic-lipophilic copolymer cartridges (Oasis HLB).[11,32] Automated protocols in 96-well plates allow the processing of up to 200 samples per day, increasing laboratory efficiency.[32] Alternatively, Liquid-Liquid Extraction (LLE) with mixtures of ethyl acetate and hexane (40:60) or diethyl ether have been successfully applied, offering a low-cost alternative with recoveries greater than 90%.[23,46]

In highly complex matrices, innovative strategies have been validated. In the case of bovine milk, the use of acetonitrile followed by purification in Evolute ABN cartridges ensured the detection of residues at nanogram levels.[44] For the analysis of equine hair, segments are subjected to decontamination with acetone and extraction with methanol under sonication, allowing retrospective monitoring of the drug for months.[22]

A critical factor identified in pre-analytical handling is the tendency of FX to bind to plastic surfaces by adsorption; therefore, the use of glassware or acidification of the sample with 5% acetic acid is mandatory to prevent losses and ensure the accuracy of the results.[16,32]

Stability in biological matrices and manipulation

FX demonstrates excellent stability in equine and canine plasma samples, withstanding up to eight freeze-thaw (F/T) cycles and remaining intact for up to two years when stored at -20 °C.[11,32] In urine, stability is guaranteed for at least three F/T cycles.[32]

However, a critical factor for “physical” stability is adsorption: the drug has a high affinity for polymeric surfaces and can bind to plastic tubes.[16] To avoid underestimating analytical results, the use of glass containers or acidification of samples is mandatory.[16,32] Furthermore, reconstituted urine extracts should be kept refrigerated (10 °C or less), as they become unstable if left at room temperature for 72 hours.[32]

Chromatographic techniques

High-performance liquid chromatography (HPLC)

HPLC has established itself as the primary tool for ensuring the conformity of FX on both industrial and clinical scales. For quality control of bulk raw material batches, the use of fused-core biphenyl phase columns has enabled the development of high-speed methods capable of separating the drug from seven impurities and degradants in a retention time of only 1.99 minutes.[20] This protocol was performed with a high flow rate of 2.5 mL/min and detection at 240 nm, ensuring operational efficiency in routine monitoring.[20]

In detailed studies of stability and forced degradation, the use of HPLC coupled to diode array detectors (DADs) is vital for the structural elucidation of byproducts.[19] Research has identified six main degradation products (DP1-DP6) generated under acid, oxidative, and photolytic stress, using HALO C18 columns and mobile phase gradients composed of water with formic acid and acetonitrile.[19] Under these conditions, the

main analyte exhibits an approximate retention time of 5.48 minutes, allowing for adequate resolution of all degradants.[19] In the context of therapeutic monitoring and bioequivalence in dogs and horses, ultraviolet (UV) detection at 290 nm is widely used due to its robustness and cost-effectiveness.[11,46]

Isocratic methods employing Inertsil ODS-3 or Waters Sunfire C18 columns achieve limits of quantification (LOQ) between 5 and 25 ng mL^{-1}, which is sufficient to cover the therapeutic plasma range in these species.[11,46]

Furthermore, chromatographic selectivity is maintained even in the presence of concomitantly administered substances, such as other anti-inflammatory drugs or antibiotics.[11,46] For matrices that require extreme sensitivity with low sample volume, such as neonatal foal plasma, fluorescence detection (Excitation 250 nm/Emission 375 nm) emerges as the preferred strategy.[24,25] This approach uses Zorbax Rx C18 columns and achieves a LOQ of 5 ng mL^{-1} from only 0.2 mL of plasma, being fundamental for documenting the accelerated clearance of the drug in young animals.[24,25]

Finally, the versatility of HPLC-UV has been proven in complex matrices of wild megafauna, such as the serum of African elephants (*Loxodonta africana*).[16] In these studies, analytical robustness was maintained through the use of Phenomenex Luna PFP columns, achieving a LOQ of 2 ng mL^{-1}. A critical physicochemical aspect highlighted for this technique is the need to use glassware during sample preparation and storage, aiming to mitigate losses due to adsorption in polymeric containers.[16]

Liquid chromatography coupled to mass spectrometry (LC-MS/MS)

Tandem mass spectrometry, in multiple reaction monitoring (MRM) mode, has become established as the platform for biological and regulatory analyses of FX due to its high selectivity.[21,32] Positive-mode electrospray ionization (ESI+) with isocratic separation on Phenyl-Hexyl columns was used in analyses with dogs and horses.[32] Monitoring is performed through the *m/z* transition 337.0 > 283.0, presenting a retention time (RT) of approximately 4.52 minutes.[19,32]

For doping control in greyhounds, the method with Poroshell 120 EC-C18 columns under a methanol and formic acid gradient was used, monitoring the *m/z* transition 337.1 > 265.1 for quantification and the 237.1/130.1 fragments for identity confirmation.[23]

In the context of food safety, confirmatory analysis in bovine plasma used Agilent Eclipse Plus C18 columns, focusing on the *m/z* transitions 337 > 130 and 337 > 283.[21]

For bovine milk, rapid resolution liquid chromatography (RRLC-MS/MS) provided extremely high sensitivity, with a decision limit (CCα) of 1.18 ng mL^{-1}, based on the *m/z* fragments 283.2 and 237.0 to ensure the absence of interference from other anti-inflammatory drugs.[44]

Research in calves has used fused-core technology columns, such as Ascentis Express C18, or ODS-4 stationary phases.[17,36] These methods monitored transitions such as *m/z* 337.1 > 130.1 and 55.1, ensuring limits of detection (LOD) of up to 0.10 ng mL^{-1}, which is vital for studies in neonatal or unweaned animals.[17]

For retrospective detection in equine hair, the method employed Kinetex C18 columns and monitored transitions to ions *m/z* 283.1, 130.1, and 237.0.[22] This technique allowed the quantification of levels on the order of picograms (LOQ 1 pg mg^{-1}), making it possible to identify drug use for several months after the last administration.[22]

Orbitrap high-resolution mass spectrometry (HRMS)

Orbitrap high-resolution mass spectrometry is a methodology used when the determination of exact mass and the elucidation of complex chemical structures are required. It is most often used as a detector coupled to ultra-high performance liquid chromatography (UHPLC) systems.[15]

For example, studies conducted with camel samples used a configuration consisting of the ThermoFisher Accela UHPLC system, an Agilent Zorbax ZDB C18 column (3.5 µm; 2 mm x 50 mm) connected to an Exactive Orbitrap mass spectrometer via a heated electrospray ionization source (HESI-II) in positive mode. Data acquisition was performed in full scan mode (100–550 Da) with a resolution of 25,000, allowing the identification of FX (*m/z* 337, 1032) and its diagnostic fragments (*m/z* 283, 265, 237) with a mass error of less than 2 ppm.[15,45]

In the analysis of raw material (bulk), the Orbitrap operating in Full Scan MS/MS mode was fundamental for the identification and structural characterization of six main degradation products (DP1-DP6) generated under stress conditions.[19,20] Monitoring was based on the exact precursor ion at *m/z* 337, 1108, with diagnostic fragments observed at *m/z* 283, 265, and 237.[19]

In the study conducted with horses, the Waters Acquity UPLC system was employed with an ACE 3 C18 column (3 µm; 2.1 mm x 50 mm) coupled to an LTQ Orbitrap XL. Ionization was performed by positive electrospray (ESI), operating with a resolution of 60,000. Accurate mass detection in full-scan mode focused on the *m/z* range 250–350, monitoring the precursor ion at m/z 337.10982 with a tolerance window of 10 ppm. The use of Orbitrap XL allowed for an extremely sensitive limit of quantification (LOQ) of 0.1 ng mL^{-1} in plasma.[45] Detection was performed with a mass tolerance window of 10 ppm (*m/z* 337,1098) and a resolution of 60,000, which ensured high analytical specificity for drug monitoring in high-performance animals.[45]

Although FX undergoes relatively limited hepatic biotransformation, its metabolism generates analytically relevant compounds that must be considered during method development and validation. The primary metabolic pathway involves demethylation of the cyclopropylmethyl group, producing desalkyl-firocoxib (DFX), followed by glucuronidation reactions. Minor metabolic routes include hydroxylation and the formation of phase II conjugates. Although these metabolites are considered pharmacologically inactive, their presence may influence analytical performance, particularly in biological samples collected during the elimination phase.[4,15,19]

From an analytical perspective, the increased polarity of these metabolites poses important challenges for chromatographic selectivity. In conventional HPLC-UV methods, DFX may exhibit retention behavior similar to that of the parent drug because both compounds share the same furanone core structure, increasing the risk of co-elution and compromising quantitative accuracy. Consequently, careful optimization of chromatographic conditions and the use of highly selective detection systems are required to ensure reliable discrimination between FX and its metabolites, especially in pharmacokinetic, residue depletion, and doping-control studies involving trace-level concentrations.[4,15,19]

In high-performance animals, the technique enabled the detection of FX in plasma for up to five days after intravenous administration.[4,15] The method used the monitoring of the exact monoisotopic mass at *m/z* 337.1032, maintaining a mass error of less than 2 ppm.[15] In addition to sensitivity, the high resolution allowed the tentative identification of the desalkyl-firocoxib metabolite (*m/z* 283) and the performance of retrospective data mining on archived samples.[15]

Ultra-performance liquid chromatography (UPLC-MS/MS)

This methodology uses columns with sub-2 µm particles to maximize chromatographic resolution in reduced run times, generally less than 3 minutes.[40,43]

UPLC analyses predominantly used the Waters Acquity H-Class system coupled to triple quadrupole spectrometers (such as Xevo TQ-S or Waters TQD).[37,43] The stationary phase of choice was Waters HSS T3 (1.8 µm; 2.1 x 50 mm), valued for its ability to retain lipophilic compounds under reversed-phase conditions.[37,40] Alternatively, columns with slightly larger particles, such as Waters XSELECT HSS T3 (2.5 µm), were employed to ensure robustness in lamb plasma analyses.[38]

The mobile phases were binary systems of acidified water (0.1% formic acid or 2 mM ammonium formate) and organic modifiers such as acetonitrile or methanol.[37,43] The typical gradient started with a high proportion of aqueous phase (60–80%), with rapid ramps to 95–100% organic solvent, operating at flow rates of 0.4 to 0.6 mL/min.[37,38] This resulted in retention times (RT) ranging from 0.8 to 3.0 minutes, depending on the species and gradient optimization.[37,43] Positive-mode electrospray ionization (ESI+) is universally adopted, monitoring FX via the precursor ion $[M+H]^+$ at *m/z* 337.1.[37,40] The multiple monitoring reaction (MRM or SRM) transition to the *m/z* 283.0 fragment (loss of the cyclopropylmethyl group) serves as a quantifier, while the transitions to *m/z* 130.1 and *m/z* 237.0 are used for diagnostic confirmation.[37,40]

The technique achieves extremely low limits of quantification (LOQ), situated at 0.1 ng mL^{-1} for rabbits and pigs,[40,43] 1 ng mL^{-1} in lambs,[38] and 5 ng mL^{-1} in goats.[37] The use of stable internal standards, such as

firocoxib-d4 or d6, is essential to control ion suppression and ensure accuracy in bioavailability and tissue residue studies.[37,38]

Stability and forced degradation

The chemical stability and forced degradation profile of FX are fundamental to ensuring the integrity of the active pharmaceutical ingredient (API) and the efficacy of its veterinary formulations. Unlike other base-sensitive compounds, FX demonstrates remarkable robustness under basic and thermal conditions, but is vulnerable to acidic, oxidative, and photolytic stresses in solution.[19] Studies based on ICH guidelines reveal that the FX molecule can generate up to six major degradation products (DP-1 to DP-6) when subjected to extreme conditions.[19,20]

The structural elucidation of FX degradation products is robustly performed through a combination of high-resolution mass spectrometry (LC-HRMS) and nuclear magnetic resonance (NMR). While LC-HRMS allows for the identification of the exact mass and molecular formula of the degradants, NMR is essential for the definitive confirmation of chemical connectivity and structural rearrangements.[19]

Acidic degradation

Under acidic stress conditions (such as exposure to 0.3 N HCl at 105 °C), the ether bond in the allylic position of the molecule is susceptible to hydrolysis.[19] This process involves the formation of an oxonium salt followed by the breaking of the bond, resulting in DP-1 (3-hydroxy-5,5-dimethyl-4-[4-(methylsulfonyl) phenyl]-2(5H)-furanone), identified by LC-HRMS in acidic medium through the ion with exact mass *m/z* 283.0644.[19] It is important to highlight that DP-1 is also identified as a process impurity originating during drug synthesis.[19,20]

Oxidative degradation

FX exhibits different degradation pathways depending on the oxidizing agent. In the degradation with hydrogen peroxide (H_2O_2), through a mechanism initiated by free radicals, the furanone ring undergoes oxidation and cleavage, leading to the formation of DP-2 (p-(methylsulfonyl)benzoic acid), whose structure was confirmed by 1H and 13C NMR studies. LC-HRMS detected this product in negative ionization mode (*m/z* 199.0074), confirming the cleavage of the furanone ring by a free radical mechanism.[19]

While in degradation with potassium permanganate ($KMnO_4$), being a stronger oxidant with affinity for electron-rich double bonds, $KMnO_4$ attacks the lactone portion, generating diketone derivatives identified as DP-3, DP-4, and DP-5.[19] LC-HRMS identified DP-5 as a diketone derivative of FX (*m/z* 386.1274), while DP-3 and DP-4 are byproducts resulting from the subsequent hydrolysis of the ester bonds of DP-5.[19]

Photodegradation and thermal stability

Although FX is photostable in its solid state, it is highly unstable under UV radiation when in solution. This degradation pathway is highly relevant for liquid pharmaceutical formulations and biological extracts, where light exposure must be avoided to prevent underestimation of analytical results, as UV radiation promotes the transition of π-electrons to non-bonding orbitals, generating radicals that attack adjacent carbons and close the furanone ring, resulting in the DP-6 degradation product.[19]

Therefore, prolonged exposure triggers a cis-stilbene type photocyclization, generating a cyclized product where carbon-15 is bonded to carbon-2 of the original structure. This solution-photodegradation product was isolated and subjected to comprehensive 1D and 2D NMR studies, including COSY, HSQC, and HMBC.[19]

In biological matrices such as equine and canine plasma, FX remains stable for up to two years at -20 °C and through multiple freeze-thaw cycles, provided that pre-analytical management takes into account its high affinity for plastic surfaces.[11] The drug is thermally stable, both in the solid state and in solution at 105 °C for seven days, without measurable degradation.[19,20] The use of advanced methods ensures that the stability profile of FX is accurately monitored, directly assisting in industrial quality control.[19,20]

REGULATORY AND FUTURE PERSPECTIVES

FX has been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of pain and inflammation associated with osteoarthritis in dogs and horses, as well as for the control of postoperative pain in dogs.[11,32,42] In the European Union, all veterinary medicinal products must be included in the Annex to Regulation (EU) No 37/2010, which establishes maximum residue limits (MRLs).[21]

Currently, MRLs are defined only for equine tissues, resulting in a "zero tolerance" policy for bovine tissues in several jurisdictions.[21,44] In the United States, "extra-label" use (ELDU) is permitted under AMDUCA guidelines when animal health is at risk and there is no approved alternative, provided that no residues result in the food chain.[37,48]

The expansion of the clinical use of firocoxib to a diverse range of species, ranging from companion animals and athletes to production animals and wild megafauna, poses unique analytical challenges that shape the development of new methodologies.[14-18]

Interspecies metabolic and pharmacokinetic variability is the main driver of this technical evolution.[4] While in adult horses the long half-life (~30 – 44 h) allows monitoring by robust HPLC-UV methods,[5,12,39,46] the significantly faster elimination observed in neonatal foals (~11 h), camels (~5.75 h) and greyhounds (~6.5 h) requires the use of techniques with greater sensitivity and selectivity, such as LC-MS/MS and Orbitrap.[15,23-25,32] In these species, achieving extremely low limits of quantification (LOQ) (0.1 to 1.0 ng/mL) is vital to adequately describe the terminal elimination phase and establish precise detection windows for doping and medication control.[15,23,40,43,45]

Furthermore, "extra-label" use in production animals, such as swine, goats, and cattle, under AMDUCA guidelines, requires methodologies capable of detecting residues in highly complex matrices to ensure food safety.[17,36,37,40] This has driven innovations such as the development of confirmatory methods for bovine milk (CCα of 1.18 ng/mL) and tissue depletion techniques in matrices such as liver, kidney, and muscle, where the interference of endogenous components requires more rigorous clean-up processes, such as automated solid-phase extraction (SPE).[21,32,40,44]

The expansion to new matrices has also enabled advances in long-term monitoring. The development of methods for equine hair (LOQ 1 pg/mg) exemplifies how the need for retrospective surveillance in athletes has driven analytical sensitivity to picogram levels.[22] Finally, the cross-species finding that FX undergoes adsorption on polymeric surfaces has refined universal pre-analytical handling, making the use of glassware or immediate acidification of samples a gold standard for ensuring the integrity of quantitative data, regardless of the matrix studied.[16,32]

Thus, the expansion of FX use not only diversifies clinical applications but also acts as a catalyst for the development of more sensitive, rapid, and sustainable analytical tools, fundamental for global regulatory compliance.[4]

REGULATION IN SPORT AND PERFORMANCE

In competition horses, FX is classified as a controlled substance by the USEF and the RMTC with plasma limits of 20 ng mL^{-1} for racehorses and 240 ng mL^{-1} for performance horses.[42,45]

Recent studies indicate that prolonged administration of FX in horses does not negatively impact cytokine or growth factor concentrations in regenerative therapies, such as platelet-rich plasma (PRP) and autologous protein solution (APS), allowing the concomitant clinical use of these modalities.[47]

CLINICAL PERSPECTIVES AND RESEARCH

Future clinical applications of FX include expansion to neonates, small ruminants, and swine.[25,37] In piglets, the transmammary route via medicated sows has been shown to be effective in reducing post-processing stress and improving average daily weight gain.[18,40] In feline medicine, FX was used to validate the MI-CAT(V) clinical metrology instrument, demonstrating efficacy in detecting the response to treatment for osteoarthritis pain.[14] Furthermore, studies in mares with placentitis suggest that the drug has suppressive effects on the production of inflammatory cytokines and prostaglandins.[49]

CHEMICAL AND SYNTHETIC INNOVATIONS

In the field of industrial synthesis, novel green chemistry approaches have been developed for the construction of the methyl sulfone moiety of FX, employing inorganic sulfur dioxide and environmentally friendly methylating agents.[20]

Additionally, modular multicomponent fluoromethyl sulfonation strategies have been proposed to generate derivatives with enhanced metabolic stability and lipophilicity, potentially improving the bioavailability of future generations of selective COX-2 inhibitors.[50]

These advances in synthetic chemistry have important implications for analytical science, as the introduction of alternative synthetic routes requires rigorous monitoring of process-related impurities, intermediates, and degradation products.[1,4] Consequently, the development of rapid and reliable stability-indicating methods becomes essential to ensure product quality, structural integrity, and regulatory compliance throughout the manufacturing process.[20]

A major analytical challenge lies in distinguishing synthesis-related impurities from degradation products generated during storage or stress conditions. This is particularly relevant for compounds such as DP-1, which has been identified both as an acid degradation product and as a synthetic intermediate, requiring highly selective methodologies for accurate characterization and quantification.[19]

To address these demands, advanced chromatographic approaches have been employed. High-speed methods using fused-core biphenyl stationary phases have demonstrated the ability to separate FX from up to seven impurities and degradation products in less than two minutes, providing efficient tools for routine quality control.[20] Furthermore, comprehensive characterization of impurities and novel derivatives relying on the complementary use of high-resolution analytical techniques, particularly LC-HRMS (Orbitrap) and NMR spectroscopy, which enable precise molecular identification and confirmation of structural connectivity.[15,19]

Therefore, innovations in FX synthesis not only contribute to the development of more sustainable manufacturing processes and potentially improved therapeutic agents but also drive the continuous evolution of analytical methodologies. The increasing demand for sensitivity, selectivity, and rapid impurity profiling reinforces the central role of modern chromatographic and spectrometric techniques in pharmaceutical quality control and regulatory assessment.[1,4,19,20]

CONCLUSION

This review described the chemical and pharmacological properties of FX, highlighting its high selectivity for COX-2 and its lipophilic profile. It also presented the chromatographic methodologies used for the analysis of FX. Among the methodologies, high-performance liquid chromatography (HPLC) continues to be widely used for routine quality control of raw materials and pharmaceutical formulations due to its robustness, accessibility, and cost-effectiveness. In contrast, more advanced methodologies, including LC-MS/MS, UHPLC-MS/MS, and high-resolution mass spectrometry (HRMS) with an Orbitrap analyzer, offer superior sensitivity, selectivity, and structural characterization capabilities, making them essential for pharmacokinetic studies, residue monitoring, doping control, impurity profiling, and identification of degradation products.

Therefore, this review provides an updated overview of the analytical landscape of FX and can serve as an aid to researchers and quality control laboratories in developing more effective and sustainable methodologies for food safety, therapeutic monitoring in various species, pharmaceutical development, and veterinary anti-doping programs.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Use of artificial intelligence tools

The authors declare that no artificial intelligence tools were used in the preparation of this article.

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