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Rapid method for the determination of non-steroidal anti-inflammatory drugs in animal tissue by liquid chromatography–mass spectrometry with ion-trap detector

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Abstract

A rapid and new liquid chromatography–mass spectrometry with ion-trap detection method for the determination of meloxicam (MLX), flunixin meglumine (FLU), carprofen (CPF), and tolfenamic acid (TOLF) in animal tissue is described.

MRLs between 10 and 500 μg kg^{-1} in muscle and between 65 and 1000 μg kg^{-1} in liver, from different animal species have been established in the EU for these compounds.

After chemical hydrolysis, an organic extraction from homogenised tissue was performed. Final extract was injected in a liquid chromatograph with an ion-trap mass spectrometer with electrospray interface.

Four identification points (one precursor and two product ions) and a minimum of one ion ratio was monitored for each compound.

For quantitative purposes flunixin-D3 (FLU-D3) was used as internal standard.

The method was validated using fortified blank muscle and liver from different animal species according to the 2002/657/EC European decision criteria.

The decision limits (CCα) and detection capabilities (CCβ) were determined and their values were at concentrations near the MRL for each substance.

Keywords: Meloxicam; Carprofen; Flunixin meglumine; Tolfenamic acid; Non-steroidal anti-inflammatory drug; Mass spectrometry

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) constitute a very wide group of compounds which are, generally, organic acids. They can be classified according to their chemical structures in enolic acids; pyrazolon derivatives; salicylic acid derivatives; *p*-aminophenol derivatives; indole indene derivatives; heteroaryl acetic acids; aryl propionic acids; anthralinic acids and alkanones [1].

They are used extensively in veterinary medicine for their anti-inflammatory, analgesic and antipyretic properties.

The major effect of all NSAIDs is to decrease the synthesis of prostaglandins by reversibly inhibiting cyclooxygenase; this enzyme catalyses the formation of prostaglandin from the precursor, arachidonic acid [1].

Although only in some isolated case serious toxicity has been documented, after acute overdose or prolonged treatment, some adverse effect might occur. NSAIDs are associated with gastrointestinal ulceration, renal effects, skin reactions, central nervous system effects, headaches, hallucinations, among others [2].

In order to protect consumer health, the European Union (EU) has set maximun residue limits (MRLs) for these substances. The MRLs range from 10 to 1000 μg kg^{-1}, depending on the compound and matrix. Table 1 shows the MRLs established in equine, porcine and bovine liver and muscle tissues.

Earlier studies were restricted to plasma, urine and milk using UV detection [3,4] and mass spectrometry [5–7] combined with liquid chromatography.

Nevertheless, few methods for animal tissues were previously reported [8–10] and, they were only applied to bovine species. It

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Table 1
MRL concentration of meloxicam (MLX), flunixin (FLU), carprofen (CPF) and tolfenamic acid (TOLF) in liver and muscle from different animal species

	Meloxicam ($\mu g\,kg^{-1}$)	Flunixin ($\mu g\,kg^{-1}$)	Carprofen ($\mu g\,kg^{-1}$)	Tolfenamic acid ($\mu g\,kg^{-1}$)
Liver				
Bovine	65	300	1000	400
Equine	65	100	1000	[a]
Porcine	65	200	[a]	400
Muscle				
Bovine	20	20	500	50
Equine	20	10	500	[a]
Porcine	20	50	[a]	50

[a]Not established MRL yet.

is interesting to note that previous studies dealt only with tissue matrices for which the EU had established MRLs and carprofen was usually not included in such studies.

We have previously reported a method which can determine two banned substances, phenylbutazone and its active metabolite oxyphenbutazone in animal urine by ion trap liquid chromatography–mass spectrometry [11]. But, in this paper, we report the development and validation of a rapid method for some of the approved NSAIDs, such as meloxicam (MLX), flunixin (FLU), carprofen (CPF) and tolfenamic acid (TOLF) in bovine, porcine and equine liver and muscle.

The molecular structures of MLX, FLU, CPF and TOLF are shown in Fig. 1.

2. Experimental

2.1. Standards

Meloxicam, flunixin, carprofen and tolfenamic acid was purchased from Sigma®. Flunixin-D3 used as internal standard was purchased from Witega. Stock standard solutions were prepared in methanol at concentrations of 325 $\mu g\,mL^{-1}$ (MLX), 500 $\mu g\,mL^{-1}$ (FLU), 500 $\mu g\,mL^{-1}$ (CPF), 500 $\mu g\,mL^{-1}$ (TLF) and 400 $\mu g\,mL^{-1}$ (FLU-D3) and they were stored at −20 °C. Intermediate mixture solutions were also prepared in methanol at 1.3 $\mu g\,mL^{-1}$ (MLX), 20 $\mu g\,mL^{-1}$ (CPF), 8 $\mu g\,mL^{-1}$ (TLF) for spiked liver samples and 4 $\mu g\,mL^{-1}$ (MLX), 10 $\mu g\,mL^{-1}$ (CPF), 10 $\mu g\,mL^{-1}$ (TLF) for spiked muscle samples, and 10 $\mu g\,mL^{-1}$ (FLU), 2 $\mu g\,mL^{-1}$ (FLU-D3) for both matrix samples and they were stored at 5 °C. Working solutions were prepared daily by diluting 10-fold and 100-fold the intermediate solution in a 50:50 formic acid 10 mM/methanol mixture.

2.2. Reagents

All chemicals were of analytical-reagent grade. Methanol used as mobile phase was hypergrade quality from Merck®. Water and ethyl acetate were HPLC grade from Merck®. Hydrochloric acid 37% and formic acid GR for analysis were from Merck®. A 0.25 M hydrochloric acid solution was prepared. Sodium phosphate tri-basic 12-hydrate (p.a.) and sodium hydroxide 97% pellets for analysis were bought from Panreac®. A 10 mM formic acid solution was prepared and filtered through a 0.20 μm hydrophilic polypropylene membrane (Pall®) and used as mobile phase.

2.3. Apparatus

The LC system consisted of a LC 1100 series Agilent Technologies® with a G1311A quaternary pump, G1313A autosampler, G1322A solvent desgasser, G1316A column oven and G2440A ion trap-mass spectrometer. Separations

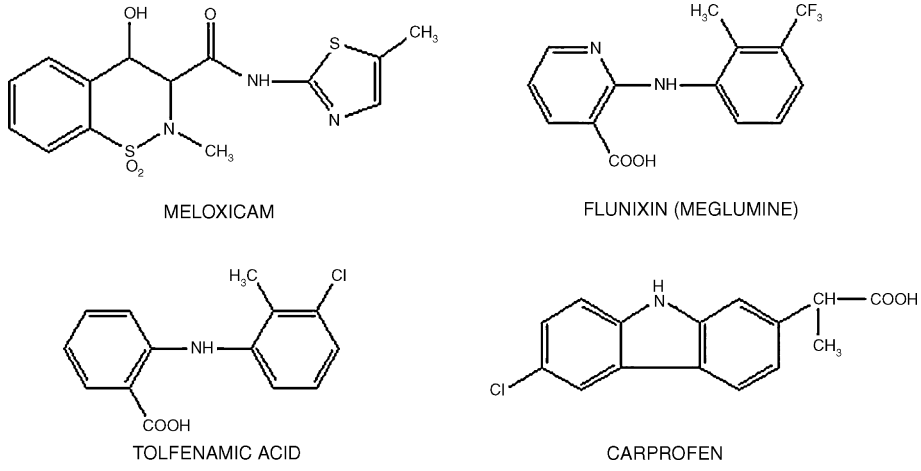

Fig. 1. Molecular structures of the investigated NSAIDs.