MULTI-RESIDUE SCREENING METHOD FOR THE DETERMINATION OF NON-Steroidal ANTI-INFLAMMATORY DRUG RESIDUES IN COW’S MILK WITH HPLC-UV AND ITS APPLICATION TO MELOXICAM RESIDUE DEPLETION STUDY

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Received for publication July 20, 2009

Abstract

A simple and short HPLC-UV screening method for the determination of residues for seven NSAIDs (carprofen, diclofenac, flunixin, meloxicam, phenylbutazone, tolfenamic acid, vedaprofen) and their three metabolites (4-methylaminoantipyrine, 5-hydroxyflunixin, oxyphenbutazone) in cow’s milk has been developed. The sample preparation was based on liquid-liquid extraction with acetonitrile in the presence of sodium chloride. The separation of analytes was performed on a C18 column with a gradient of acetonitrile and the ammonium acetate buffer pH 5.0. UV-detector wavelength was programmed in order to improve sensitivity. The method was validated according to the CD/2002/657 criteria. For most analytes, relatively high recoveries were observed (76%-98%). Within-laboratory reproducibility levels were in the range of 3.6%-17.8% (CV, %). For phenylbutazone, oxyphenbutazone, and 4-methylaminoantipyrine recoveries were considerably lower (44%-68%) and reproducibility was up to 41.9%, which was probably caused by the instability of analytes. The robustness of the method for different fat content was successfully investigated. The method was verified by its use in the determination of meloxicam residues in milk samples obtained from meloxicam-treated cows. The obtained results confirm the usefulness of the developed method for the analysis of NSAIDs residues in milk.

Key words: milk, NSAIDs, residues, meloxicam, HPLC.

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of compounds which show anti-inflammatory, analgesic, and antipyretic properties. Most of them are not chemically related but have a common mechanism of action based on the inhibition of the metabolism of arachidonic acid. NSAIDs are used in veterinary medicine in the treatment of colic and musculoskeletal disorders in horses, infectious diseases in cattle, and as an aid in the treatment of mastitis agalactia syndrome in sows and mastitis in dairy cows (5). A few NSAIDs are authorised for use in milking cows. In the European Union, for some NSAIDs, maximum residue limits (MRLs) in milk have been established (10): flunixin (marker residue 5-hydroxyflunixin) 40 µg/kg, meloxicam 15 µg/kg, tolfenamic acid 50 µg/kg, and metamizole (marker residue 4-methylaminoantipyrine) 50 µg/kg. For ketoprofen and carprofen MRL value in bovine milk has not been established because of the negligible risk related to its authorised use. Most of NSAIDs used in human medicine (diclofenac, ibuprofen, naproxen, etc.) or approved for pets (vedaprofen, phenylbutazone) are prohibited in the treatment of dairy cows.

According to European Union law (9), residues of NSAIDs in milk have to be monitored because of the potential risk to consumer’s health.

The methods for the determination of NSAIDs in different matrices are widely described in the literature. Sample preparation is based on liquid-liquid extraction (21) with SPE clean-up (23). The determination of these drugs in plasma (6), tissues (4), and in environmental samples (22) is performed using different chromatographic techniques: HPLC-UV (12, 14), HPLC-DAD (16, 18), LC-MS/MS (3, 7, 15), and GC-MS (31). There are also several published methods for the determination of NSAIDs in milk and most of them are single-residue procedures. Single-step liquid extraction was used in the determination of phenylbutazone (13, 24), ketoprofen (25), ketoprofen and flunixin (11) by LC-MS/MS, and metamizole and its metabolites (28) by LC-MS.
Multi-residue methods for the determination of NSAIDs in milk have also been published on. Stoyke et al. (30) developed a multi-residue procedure for the determination of 11 NSAIDs in milk. In this method, samples were extracted with acetonitrile in the presence of sodium chloride solution and cleaned-up with C18 columns. Final extracts were analysed by HPLC-DAD. The same procedure was applied by Gallo et al. (17) for the determination and confirmation of 16 NSAIDs in milk by HPLC-DAD and LC/ESI-MS/MS. Despite the wide range of analytes, none of the multi-residue methods allows the determination of the residues of two important drugs with MRL values in milk: meloxicam and 4-methylaminoantipyrine simultaneously.

The objective of this study, therefore, was to develop a simple screening method allowing simultaneous determination of a wide spectrum of NSAIDs (especially all substances with established MRL values) in milk and to validate it according to the requirements of residue control methods.

**Material and Methods**

**Reagents.** The analytical standards for NSAIDs and some of their metabolites were supplied by the following manufacturers: carprofen (CPF), diclofenac (DC), meloxicam sodium (MEL), phenylbutazone (PBZ), tolfenamic acid (TOL), oxyphenbutazone hydrate (OPB) – Sigma (USA), flunixin meglumine (FLU) – ISP (USA), vedaprofen (VED) - Akzo Nobel (Netherlands), 4-methylaminooantipyrine (4MAA) – Hoechst (Germany), and 5-hydroxyflunixin (5OHFLU) - Xenobiotic Laboratories (USA). CPF, TOL, VED, 4MAA, and 5OHFLU were donated by the Community Reference Laboratory for Drug Residues in Berlin. Acetonitrile (ACN), HPLC grade, was purchased from Merck (Germany). Methanol (MeOH), HPLC grade, and acetic acid (HPLC grade, <99%) was purchased from J.T. Baker (Germany). Sodium chloride (NaCl) and ammonium acetate (CH₃COONH₄) were purchased from POCh (Poland). Ultrapure water (resistance >18 MΩ) was obtained from Mili-Q system (Millipore, France).

**Standard solutions.** All standard solutions were prepared in methanol. Stock-standard solutions (1,000 µg/mL) were prepared by weighing 10.0 (±0.1) mg of standard substances and dissolving in 10 ml of solvent. Stock-standard solutions were stable for 12 months when stored at 2-10°C except for PBZ and OPB solutions, which were stable for 1 month. Intermediate-standard solutions (100 µg/mL) were prepared by diluting suitable aliquots of stock standard solutions. Working-standard solution containing: 1.0 µg/mL of 4MAA, DC, CPF, PBZ, TOL, and VED; 2.0 µg/mL of OPB, 0.8 µg/mL of FLU, and 5OHFLU; 0.3 µg/mL of MEL used for milk sample fortification, was prepared by diluting suitable aliquots of working-standard solutions and was stable for 1 month.

**Milk samples.** For the method development and validation, raw milk (free of NSAIDs residues) was used. Mixed portions of compliant milk samples were prepared from samples obtained and analysed in the residue-control programme. Milk was checked for possible interferences by HPLC, transferred to screw-capped polypropylene tubes, and stored in the freezer at a temperature below −18°C until analysis.

For the verification of the possible influence of the milk’s fat content on NSAIDs recovery, samples (n=2) with different fat percentages: 2.17; 3.01; 4.04; and 5.44 (analysed on milk analyser MilkoScan 4000) were used. For the verification of the developed method, milk samples with incurred meloxicam were used. Six cows received a single intravenous dose of 0.5 mg/kg b.w. of meloxicam (Metacam, Boehringer Ingelheim). The milk samples were collected in the morning and evening milking during six consecutive days. Every yield of milk was mixed and samples of 50 ml were transferred into polypropylene screw-capped tubes and stored at a temperature below -18°C until analysis.

**Sample preparation.** The sample of 5 (±0.01) g was weighed in a polypropylene tube. Five milliliters of ACN and 1 g of NaCl was added and samples were vigorously vortex-mixed for 1 min. Next, the tube was centrifuged (4,500 g, 15 min, -5°C) and the supernatant was transferred to a glass tube and evaporated under a gentle stream of nitrogen at 40°C. The dry residue was reconstituted in 0.5 ml of ACN:MeOH:0.05 M CH₃COONH₄ pH 5.0 (1+1+1, v+v+v) solution, transferred to the autosampler vial (2 ml), centrifuged (4,500 g, 5 min) and injected (50 µl) into the HPLC column.

**Chromatographic conditions.** The instrumental analysis of NSAIDs was performed using the Varian Prostar HPLC system, equipped with quaternary pump, autosampler, column oven, and UV-Vis detector, controlled by Galaxie Workstation software. For the analysis of UV spectra of NSAIDs, the Agilent 1100 system, equipped with quaternary pump, degasser, autosampler, column oven, and diode-array detector (DAD) and controlled by Chemstation software was used. Chromatographic separation of the compounds was performed on Inertsil ODS-3 column (150x4.6 mm, 5 µm, GL Science, Japan) connected with precolumn (4x3 mm, SecurityGuard, Phenomenex, USA). The column oven temperature was controlled at 30°C. The HPLC column was prepared with the mobile phase consisting of 10% of acetonitrile (component A) and 90% of 0.05 M CH₃COONH₄, pH 5.0 adjusted with acetic acid (component B) at flow rate 1.2 ml/min. The following 30 min gradient elution programme was applied: 10% of A was held for 3 min, increased to 60% of A at 15 min and held for 2 min, then increased to 80% of A at 20 min, and reduced to 10% of A at 25 min. For the next 5 min 10% of A was pumped. The UV-Vis detector was programmed to monitor the effluent of the column for absorbance at 265 nm from 0.0 to 11.0 min, 365 nm from 11.0 to 12.4 min, and 290 nm from 12.4 to 30.0 min.

**Validation method.** A validation experiment was performed to fulfil the requirements described in the Commission Decision 2002/657/EC (8). Linearity was verified by the preparation of calibration curves.
Table 1
Spiking levels used in validation of the method

<table>
<thead>
<tr>
<th>Analyte*</th>
<th>MRL (µg/kg)</th>
<th>I spiking level (µg/kg)</th>
<th>II spiking level (µg/kg)</th>
<th>III spiking level (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4MAA</td>
<td>50</td>
<td>25.0</td>
<td>50.0</td>
<td>75.0</td>
</tr>
<tr>
<td>5OHFLU</td>
<td>40</td>
<td>20.0</td>
<td>40.0</td>
<td>60.0</td>
</tr>
<tr>
<td>CPF</td>
<td></td>
<td>25.0</td>
<td>50.0</td>
<td>75.0</td>
</tr>
<tr>
<td>DC</td>
<td></td>
<td>25.0</td>
<td>50.0</td>
<td>75.0</td>
</tr>
<tr>
<td>FLU</td>
<td></td>
<td>20.0</td>
<td>40.0</td>
<td>60.0</td>
</tr>
<tr>
<td>MEL</td>
<td>15</td>
<td>7.5</td>
<td>15.0</td>
<td>22.5</td>
</tr>
<tr>
<td>OPB</td>
<td></td>
<td>50.0</td>
<td>100.0</td>
<td>150.0</td>
</tr>
<tr>
<td>PBZ</td>
<td></td>
<td>25.0</td>
<td>50.0</td>
<td>75.0</td>
</tr>
<tr>
<td>TOL</td>
<td>50</td>
<td>25.0</td>
<td>50.0</td>
<td>75.0</td>
</tr>
<tr>
<td>VED</td>
<td></td>
<td>25.0</td>
<td>50.0</td>
<td>75.0</td>
</tr>
</tbody>
</table>


Table 2
The absorption maxima of NSAIDs and wavelength programme for the UV-Vis detector

| Compound | Maximum absorbance, nm* | Wavelength programme | Retention time, min (+2.5%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>4MAA</td>
<td>245</td>
<td>265 (0.0-11.0 min )</td>
<td>9.20 ±0.23</td>
</tr>
<tr>
<td>MEL</td>
<td>365</td>
<td>365 (11.0-12.4 min )</td>
<td>12.00 ±0.3</td>
</tr>
<tr>
<td>5OHFLU</td>
<td>290</td>
<td></td>
<td>12.74 ±0.32</td>
</tr>
<tr>
<td>FLU</td>
<td>285</td>
<td></td>
<td>14.00 ±0.35</td>
</tr>
<tr>
<td>OPB</td>
<td>242</td>
<td></td>
<td>14.40 ±0.36</td>
</tr>
<tr>
<td>DC</td>
<td>280</td>
<td></td>
<td>15.30 ±0.38</td>
</tr>
<tr>
<td>CPF</td>
<td>300</td>
<td>290 (12.4-30.0 min)</td>
<td>15.60 ±0.39</td>
</tr>
<tr>
<td>PBZ</td>
<td>260</td>
<td></td>
<td>16.55 ±0.41</td>
</tr>
<tr>
<td>TOL</td>
<td>290</td>
<td></td>
<td>17.00 ±0.43</td>
</tr>
<tr>
<td>VED</td>
<td>285</td>
<td></td>
<td>21.00 ±0.53</td>
</tr>
</tbody>
</table>

*Wavelengths obtained by analysis of standard of NSAIDs with HPLC-DAD. Abbreviations are explained in the footnote to Fig. 1.

The mixed-standard solutions on five suitable concentration levels were analysed and recorded peak areas of each analyte were plotted versus concentration. The equations and regression coefficients of the curves were calculated. Linearity of calibration curves was demonstrated with the F-test lack-of-fit and the working range was established.

In order to investigate the specificity of the method (potential interferences from the matrix and other veterinary drugs), 20 blank milk samples from different regions of Poland and standard mixtures of sulfonamides, nitroimidazoles, and tetracyclines were analysed.

The precision and accuracy of the method were evaluated by the analysis of blank milk samples fortified with NSAIDs on three different levels, specific for each analyte. For MRL substances (5OHFLU, MEL, 4MAA, TOL) 0.5×MRL, 1×MRL and 1.5×MRL levels were applied. For other compounds, spiking levels were chosen by the authors (Table 1).

For the repeatability study, three series were analysed (six samples for each spiking level). Standard deviation (SD) and coefficient of variation (CV, %) were calculated for each level. Additionally, two series (on three levels) were analysed in reproducibility conditions (two different occasions, another technician) and overall SD and CV were calculated. The overall mean value of concentration obtained in the reproducibility study was used to calculate recovery expressed as percentage. Decision limit (CCα) and detection capability (CCβ) were calculated based on the procedure described in ISO 11843 (20). The limit of detection (LOD) and limit of quantification (LOQ) were calculated on the basis of the signal to noise ratio (S/N=3 for LOD and S/N=10 for LOQ) on the chromatograms of 20 blank milk samples.

**Results**

Chromatograms of a blank milk sample and a sample fortified at II spiking level are presented in Fig. 1. The gradient elution of the mobile phase, consisting of acetonitrile and ammonium acetate buffer (pH 5.0), allows good separation of NSAIDs from matrix components in 30 min run time (Fig. 1B). Despite the simple and short sample preparation, chromatograms of blank milk samples did not reveal any peaks from the matrix in the retention times of analytes (Fig. 1A).
An analysis of UV spectra showed differences in the absorption maxima of the respective NSAIDs; therefore a programme of wavelengths in the UV detector was introduced (Table 2).

The results of the method validation are presented in Table 3. The calibration curves were characterised by high values of regression coefficients ($r^2 > 0.998$). The results show relatively high recoveries in the range of 44%-98% for most analytes. The precision of the method was acceptable; CV values for repeatability were in the range of 1.2%-20.1% and for within-laboratory reproducibility 3.5%-41.9%. The limits of detection (1.5-10 µg/kg) show the high sensitivity of the method.

Fig. 1. Chromatograms (A) - blank milk sample, (B) - milk sample spiked with NSAIDs on II spiking level.

4MAA - 4-methylaminoantipyrine, 5OHFLU - 5-hydroxyflunixin, CPF - carprofen, DC - diclofenac, FLU - flunixin meglumine, MEL - meloxicam sodium, OPB - oxyphenbutazone hydrate, PBZ - phenylbutazone, TOL - tolfenamic acid, VED - vedaprofen.
Fig. 2. Recoveries of NSAIDs determined in milk with different fat levels. Abbreviations are explained in the footnote to Fig. 1.

Fig. 3. Elimination profile of meloxicam in the milk of treated cows (n=6, average ± SD).

A – full range of concentrations, B – meloxicam concentrations around the MRL, LOD, and LOQ values.
<table>
<thead>
<tr>
<th>NSAID</th>
<th>Spiking level, µg/kg</th>
<th>Linearity</th>
<th>Recovery, %</th>
<th>Repeatability, CV, %</th>
<th>Within-laboratory reproducibility, CV, %</th>
<th>CCα, µg/kg</th>
<th>CCβ, µg/kg</th>
<th>LOD, µg/kg</th>
<th>LOQ, µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>4MAA*</td>
<td>25.0 50.0 75.0</td>
<td>10-100 µg/kg</td>
<td>44.3</td>
<td>52.8 50.7</td>
<td>20.1 14.2 19.8</td>
<td>41.9</td>
<td>22.7</td>
<td>18.8</td>
<td>70.1</td>
</tr>
<tr>
<td>5OHFLU*</td>
<td>20.0 40.0 60.0</td>
<td>8-80 µg/kg</td>
<td>83.1</td>
<td>79.4 76.1</td>
<td>1.5 1.8 1.4</td>
<td>6.4</td>
<td>4.0</td>
<td>9.2</td>
<td>43.6</td>
</tr>
<tr>
<td>CPF</td>
<td>25.0 50.0 75.0</td>
<td>10-100 µg/kg</td>
<td>89.1</td>
<td>82.7 79.6</td>
<td>4.3 2.5 1.8</td>
<td>4.7</td>
<td>3.8</td>
<td>7.8</td>
<td>15.5</td>
</tr>
<tr>
<td>DC</td>
<td>25.0 50.0 75.0</td>
<td>10-100 µg/kg</td>
<td>91.7</td>
<td>85.6 81.2</td>
<td>2.1 2.8 2.5</td>
<td>7.4</td>
<td>3.5</td>
<td>9.4</td>
<td>6.9</td>
</tr>
<tr>
<td>FLU</td>
<td>20.0 40.0 60.0</td>
<td>8-80 µg/kg</td>
<td>90.3</td>
<td>82.9 79.9</td>
<td>1.7 1.9 1.2</td>
<td>5.4</td>
<td>4.6</td>
<td>7.4</td>
<td>6.5</td>
</tr>
<tr>
<td>MEL*</td>
<td>7.5 15.0 22.5</td>
<td>3-30 µg/kg</td>
<td>97.6</td>
<td>82.6 79.8</td>
<td>12.8 4.7 9.5</td>
<td>17.8</td>
<td>6.5</td>
<td>10.8</td>
<td>16.4</td>
</tr>
<tr>
<td>OPB</td>
<td>50.0 100 150.0</td>
<td>20-200 µg/kg</td>
<td>62.6</td>
<td>66.9 68.2</td>
<td>13.8 5.7 5.4</td>
<td>18.6</td>
<td>9.3</td>
<td>11.0</td>
<td>9.5</td>
</tr>
<tr>
<td>PB</td>
<td>25.0 50.0 75.0</td>
<td>10-100 µg/kg</td>
<td>66.0</td>
<td>66.9 66.5</td>
<td>8.2 4.5 3.9</td>
<td>13.2</td>
<td>8.3</td>
<td>11.7</td>
<td>8.5</td>
</tr>
<tr>
<td>TOL*</td>
<td>25.0 50.0 75.0</td>
<td>10-100 µg/kg</td>
<td>86.2</td>
<td>82.4 80.0</td>
<td>5.8 4.9 2.1</td>
<td>6.3</td>
<td>5.6</td>
<td>8.5</td>
<td>55.2</td>
</tr>
<tr>
<td>VED</td>
<td>25.0 50.0 75.0</td>
<td>10-100 µg/kg</td>
<td>91.6</td>
<td>83.1 79.4</td>
<td>5.5 1.9 3.4</td>
<td>6.5</td>
<td>4.3</td>
<td>8.9</td>
<td>6.7</td>
</tr>
</tbody>
</table>

* substance for which MRL in milk was established. Abbreviations are explained in the footnote to Fig. 1.
The results of the experiment on the influence of milk fat content on NSAIDs recovery are presented in Fig. 2. The analysis of spiked milk samples containing different fat levels (2.17%-5.44%) shows similar recoveries of the analytes, and their variability was comparable with those obtained in the validation study.

The developed and validated method was verified by the determination of meloxicam residues in milk from the cows treated intravenously with Metacam. The elimination profile of meloxicam in milk is presented in Fig. 3. The highest concentration of meloxicam determined in milk collected 12 h after the treatment was 361 ±78 µg/kg (average ± SD) (Fig. 3A). The concentration dropped down in further milkings and decreased below the MRL value (15 µg/kg) between days 2 and 3 after the treatment. Residues of meloxicam were undetectable 4 d after the treatment (LOD 1.0 µg/kg) (Fig. 3B).

Discussion

The developed chromatographic conditions allow good separation and quantitative analysis of 7 NSAIDs and their three metabolites. According to the authors’ knowledge, it is one of the first methods allowing simultaneous HPLC determination of 4MAA and the rest of NSAIDs.

The choice of the mobile phase pH was a significant stage in the method optimisation. Some authors (2) used acids solutions or buffers at low pH value (2.0-3.0) for mobile phase, which was useful in the analysis of most NSAIDs, but not suitable for the separation of metamizole metabolites. The buffer at pH 5.0 significantly improved the peak shape of 4MAA and additionally shortened the retention times (RTs) of NSAIDs (1). The obtained RTs were stable for all analytes and they fitted into the 2.5% interval as required by Commission Decision 2002/657/EC (8).

The use of the wavelength programme for the UV-Vis detector greatly improved the sensitivity of the method for MEL and 4MAA (Table 3). Additionally, the use of the mobile phase at pH of 5.0 resulted in a lower LOD value for PBZ because it has a higher absorption maximum at higher pH values (240 nm at pH 3.0 against 264 nm at pH 7.0) (24).

Relatively simple and fast sample preparation was developed. The authors have taken advantage of the experience of Neto et al. (26), who used acetonitrile for the deproteinisation of plasma and Daeselaere et al. (11) for deproteinisation of milk samples. Acetonitrile is a very useful solvent due to its good solubility of drugs and ability to precipitate proteins in the sample. Nevertheless, because of its miscibility with water, sodium chloride was added to allow separation of phases. Stoyke et al. (30) extracted NSAIDs from milk with acetonitrile and NaCl solution. Peck et al. (27) observed that the addition of NaCl (crystal) to serum or plasma before the extraction provides cleaner extracts in comparison to acetonitrile alone. A low temperature (-5°C) during centrifugation additionally improved the separation of phases. For the evaporation of extracts, a relatively low temperature (40°C) was applied because the tendency to degradation of some the analytes (PBZ and OPB) had been previously reported (21). The sample preparation described above allows the extraction of up to 30 samples concurrently. Some described methods are based on solid-phase extraction, e.g. Stoyke et al. (30) and Rupp et al. (29) with C18 cartridges. Our trials with different SPE cartridges (C18, Oasis HLB, anion and cation exchange, mixed mode) indicated problems with developing one clean-up procedure for the “acidic” NSAIDs, MEL, and 4-MAA (non published data). Single-step liquid extraction ensured good sample clean-up without SPE technique and allowed the determination of a wider spectrum of analytes in comparison to the other published methods.

The results of validation show the good precision and accuracy of the described method. However, for the three analytes recoveries were considerably lower: 4MAAA - 44-53%, OPB - 62-68%, and PBZ - 66-67%. In our opinion, it can be explained by the instability of these compounds. Some authors reported the fact of PBZ and OPB degradation (6, 19); however, we have not found any published data on 4MAA stability. This was also indirectly confirmed by the visibly lower precision of determination of the mentioned analytes (CV up to 41.9%). Calculated CCα and CCB values are used in the residue control for the correct interpretation of results, according to Commission Decision 2002/657/EC. The LOD and LOQ values characterised the sensitivity of the method, and are useful in pharmacokinetic and residue elimination studies. The obtained values prove the high sensitivity of the method and its usefulness for the mentioned purposes. CCα and CCB values were comparable to those obtained by Stoyke et al. (30).

Fat content can be an important factor influencing the method’s performance, because of lipophilicity of some drugs. Martin et al. (24) performed a study of phenylbutazone recovery dependence on fat content in milk sample (0.5%, 3.0%, 3.5%-4.4%). The robustness of our method for this factor was investigated for all determined analytes. Stable recoveries of all NSAIDs have confirmed the independence of the method for fat content.

The comparison of the results of meloxicam elimination from milk is difficult because we have not found any data, except for a summary report published by the European Medicine Agency (EMEA) (32). The EMEA report described longer elimination of meloxicam from milk: concentrations above MRL were noticed after 5 d post dosing and residues were detected to 9 d post dosing (LOD 2.5 µg/kg). Meloxicam concentrations in milk collected 6 h post dosing were 347 µg/kg and 325 µg/kg for high and low milk yield, respectively. The values obtained in our study had good correlation with values reported by the EMEA.

In conclusion, the presented method is one of the first published screening multi-residue procedures for the determination of NSAIDs residues in milk. Simple sample preparation and liquid chromatography with UV detection allows determining drugs with
sufficient sensitivity. The method has been successfully validated and verified in the determination of meloxicam in the tested samples of cow’s milk.

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