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Multi-residue liquid chromatography/tandem mass spectrometry method for the detection of non-steroidal anti-inflammatory drugs in bovine muscle: optimisation of ion trap parameters

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A multi-residue liquid chromatography/tandem mass spectrometry method (LC/MS^2) was developed for the detection of the non-steroidal anti-inflammatory drugs acetylsalicylic acid (via the marker residue salicylic acid), flunixin, phenylbutazone, tolfenamic acid, meloxicam and ketoprofen, in bovine muscle. After extraction of the bovine muscle with acetonitrile, the cleanup was performed using a Oasis HLB column. The evaporated eluate was reconstituted and analysed by LC/MS^2. To obtain optimal detection of salicylic acid and phenylbutazone, the ion trap mass spectrometric parameters activation q and maximum ion injection time, respectively, were optimized. The activation q for salicylic acid was increased to obtain reliable detection of both salicylic acid and its product ion. The maximum ion injection time for the time segment containing phenylbutazone was decreased since there were not enough scans across the chromatographic peak of this compound. The multi-residue method was able to detect the different analytes below or at the maximum residue limit (MRL) or minimum required performance limit (MRPL) or, in the case of phenylbutazone and ketoprofen, at 100 and 20 $\mu g\,kg^{-1}$, respectively. Copyright © 2004 John Wiley & Sons, Ltd.

As long as 2500 years ago, Hippocrates recommended willow bark to relieve the pain of childbirth and to reduce fever. These medicinal extracts of barks contained salicylates. The origin of the group of salicylates lies in the naturally occurring compound salicin, that can be found in a number of different plants. The presence of salicin has been documented to occur in willow and poplar species (*Salicaceae*), wintergreen, birch and a variety of rose. Salicylic acid is also found naturally in many herbs, vegetables and fruit.[1] Acetylsalicylic acid, the active ingredient of aspirin, was synthesised by Bayer in 1899. Since that time a number of new anti-inflammatory compounds have been developed. Non-steroidal anti-inflammatory drugs (NSAIDs) have been used routinely in veterinary practice since the early 1970s. They are often the initial therapy for inflammation disorders of several animal species. They are commonly prescribed for musculoskeletal pain, coliform mastitis, pulmonary diseases and enteritis. The use of NSAIDs in veterinary medicine has evolved in a way similar to that in human medicine. In recent years the treatment of pain in animals has become an important issue, even in food-producing animals.

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NSAIDs act by inhibiting the body's ability to synthesise prostaglandins, a family of hormone-like chemicals some of which are made in response to cell injury. Side effects can occur, so a combination of several NSAIDs can be fatal. The major toxicities affect the gastro-intestinal, hematopoietic and renal systems. Other effects associated with use of NSAIDs include hepatotoxicity, aseptic meningitis, diarrhoea, and central nervous system depression. Gastrointestinal erosions and ulcerations are the most common and serious side effects of NSAIDs. Gastrointestinal toxicity is primarily due to the inhibition of prostaglandin E2 activity, which mediates blood flow and mucus secretion. Direct irritation by acidic drugs may be important, and impaired platelet function may contribute to mucosal bleeding. Aspirin irreversibly acetylates the platelet cyclooxygenase and therefore platelet aggregation defects caused by aspirin can last up to one week. Renal toxicities include renal vasoconstriction and renal insufficiency.[2]

NSAIDs may be structurally classified as carboxylic acids or enolic acids. The carboxylic acid derivatives include salicylates (acetylsalicylic acid), acetic acids, propionic acids (ketoprofen), anthranilic acids, aminonicotinic acids (flunixin) and fenamates (tolfenamic acid), while the enolic acids include pyrazolones (phenylbutazone) and oxicams (meloxicam) (Fig. 1).[2,3]

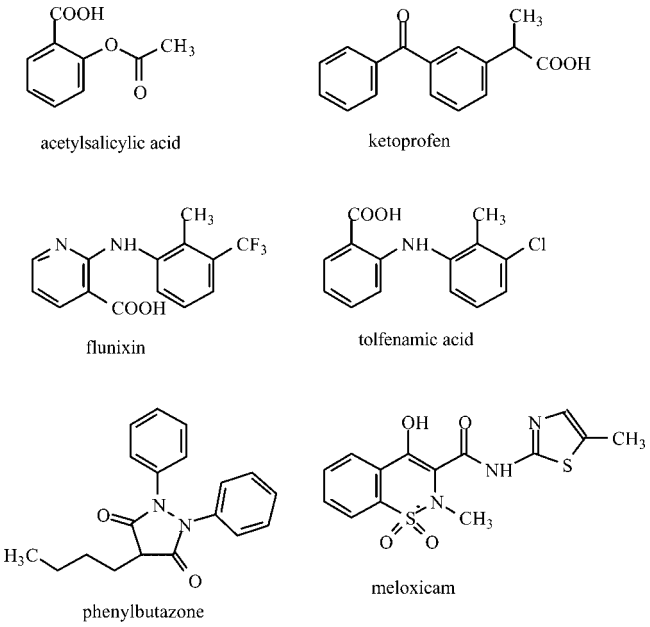

Figure 1. Chemical structures of acetylsalicylic acid, ketoprofen, flunixin, tolfenamic acid, phenylbutazone and meloxicam.

In food-producing animals the use of drugs is restricted to registered products for which a maximum residue limit (MRL) is established. NSAIDs which have an MRL (Annex I) are carprofen (bovine, equine), vedaprofen (equine), flunixin (bovine, porcine, equine), tolfenamic acid (bovine, porcine) and meloxicam (bovine, porcine, equine) (Table 1). Two NSAIDs do not have a MRL (Annex II), namely, ketoprofen and salicylates. However, according to the law in Belgium, drugs that are not registered in Belgium cannot be administered to food-producing animals. Therefore, acetylsalicylic acid cannot be used in Belgium since it is not registered for use in bovine.[4] For acetylsalicylic acid a minimum required performance limit (MRPL) of 40 $\mu g\,kg^{-1}$ is used in Belgium. Ketoprofen is licensed for bovine in Belgium, but a waiting period of 4 days must be respected.

In January 2001, a Belgian research project was started in which injection sites from slaughtered animals were collected at the slaughterhouse. In analysing these samples, an overview could be given of which veterinary medicinal products are frequently used/abused nowadays in practice. In 2002, 29.5% of the injection sites contained a veterinary product at a concentration higher than the MRL. In 6.5% of these injection sites NSAIDs (flunixin, tolfenamic acid and meloxicam) were detected. In fact NSAIDs were the second most detected veterinary drugs in injection sites in 2002. In 2003, 31.6% of the injection sites contained a veterinary product at a concentration higher than the MRL and 7.3% of these injection sites contained NSAIDs. In 2003, NSAIDs were the most detected veterinary drugs.[5,6]

Table 1. Maximum residue limits (MRLs) set for NSAIDs in bovine muscle

Analyte	MRL ($\mu g\,kg^{-1}$)
Carprofen	500
Flunixin	20
Tolfenamic acid	50
Meloxicam	20

Literature data for analysis of NSAIDs indicate extraction and cleanup procedures for the determination of one or two compounds,[3,7–20] with mass spectrometry as the main detection technique. No literature data were found on multi-residue methods in bovine muscle for structurally different compounds. In this study a liquid chromatography/tandem mass spectrometry (LC/MS^2) multi-residue method was developed for bovine muscle to identify salicylic acid, phenylbutazone, flunixin, tolfenamic acid, meloxicam and ketoprofen.

EXPERIMENTAL

Reagents and chemicals

The NSAID standards phenylbutazone, tolfenamic acid and ketoprofen were obtained from Sigma-Aldrich (St. Louis, MO, USA), while salicylic acid was from Acros (Geel, Belgium), meloxicam from ICN Biomedicals (Irvine, CA, USA) and flunixin was a generous gift from the Department of Pharmacology, Pharmacy and Toxicology (Merelbeke, Belgium). The internal standard desoximethasone was obtained from Sigma-Aldrich. All chemicals used were of analytical grade from Merck (Darmstadt, Germany) and Acros.

Stock standard solutions of 1000 ng μL^{-1} were prepared in ethanol. For the preparation of working solutions, 0.4% acetic acid in MeOH/H_2O (60:40) was used. All standard and working solutions were stored at −20°C.

Instrumentation

The HPLC apparatus comprised a 1100 series quaternary pump and an autosampler (Hewlett Packard, Palo Alto, CA, USA). Chromatographic separation was achieved using an Alltima HP C_{18} column (5 µm, 150 × 2.1 mm; Alltech, Deerfield, IL, USA). The mobile phase consisted of a mixture of methanol (A) and water with 0.1% acetic acid (B). A linear gradient was run (60% A for 9 min, increasing to 100% in the next 4 min) at a flow rate of 0.3 mL min^{-1}.

LC/MS^2 detection used an LCQ Deca ion trap (Thermo-Finnigan, San José, CA, USA) with an electrospray ionisation (ESI) interface, in both negative and positive ion modes. Each analyte was evaluated based on the product ions present in the MS^2 spectra (Table 2).

Extraction and clean-up

To a 2 g aliquot of minced tissue, 1000 $\mu g\,kg^{-1}$ desoximethasone was added as internal standard. The NSAIDs were extracted from the tissue using 10 mL acetonitrile. After mixing and centrifugation (5 min, 5500 rpm) the supernatant was evaporated to dryness at 60°C under a stream of nitrogen. The cleanup was performed using an Oasis HLB column (60 mg, 3 mL; Waters, Milford, USA). The columns were conditioned with 1 mL methanol and 1 mL ultrapure water. The residue was reconstituted in 100 µL methanol and 900 µL ultrapure water. After application of this extract, the cartridge was rinsed with 1 mL MeOH/H_2O (5:95) and vacuum-dried. The NSAIDs were eluted from the column with 1 mL methanol

Table 2. Precursor and product ions (*m/z*) used for the evaluation of different NSAIDs and the internal standard desoximethasone

Analyte	Detection mode	Precursor ion	MS^2 First-generation product ions	MS^3 Second-generation product ions
Salicylic acid	Negative	137	93	65
Phenylbutazone	Negative	307	279 131	
Flunixin	Negative	295	251 231	
Tolfenamic acid	Negative	260	216	180
Meloxicam	Negative	350	146 210 286	
Desoximethasone (IS)	Negative	435	355 375	
Ketoprofen	Positive	255	209	105 131 194
Desoximethasone (IS)	Positive	377	321 339 357	

Figure 2. Enzymatic hydrolysis of acetylsalicylic acid to salicylic acid.

and 1 mL 10% acetic acid in hexane. The eluate was evaporated to dryness at 60°C under a stream of nitrogen. The residue was reconstituted in 50 µL methanol and subsequently 100 µL 0.4% acetic acid in MeOH/H_2O (60:40), before injecting 30 µL onto the HPLC column.

RESULTS AND DISCUSSION

Acetylsalicylic acid

Acetylsalicylic acid is rapidly hydrolysed to salicylic acid by aryl esterases, a group of enzymes that are widely distributed in blood, plasma, liver, kidney and certain tissues (Fig. 2). Therefore, the detection of acetylsalicylic acid was performed by detection of the marker-residue salicylic acid.[21]

LC/MS^2 method

The different NSAIDs were detected using a LC/MS^2 method in negative ion mode. The instrument parameters, collision energy and activation q are summarised in Table 3. The isolation width was set to 2 Da for each NSAID. Salicylic acid had an activation q different from the default value 0.25, as discussed in the next paragraph.

The standards of salicylic acid, phenylbutazone, flunixin, tolfenamic acid, meloxicam, and the internal standard desoximethasone, were spiked into blank bovine muscle, at concentrations listed in Table 4. For flunixin, tolfenamic acid and meloxicam the spike concentrations were the maximum residue limit (MRL), for acetylsalicylic acid (spiked as salicylic acid) the minimum required performance limit (MRPL); phenylbutazone is forbidden by law in Belgium and therefore has no MRL. Figure 3 shows the extracted ion chromatograms and the MS^2 spectra for different NSAIDs. Phenylbutazone could only be detected at a concentration of 100 µg kg^{-1}. In the literature, phenylbutazone was most often analysed in plasma; as a result of the limited cleanup necessary for plasma, low concentrations of phenylbutazone could be detected. Clark *et al.* were able to detect phenylbutazone at a concentration of 10 µg kg^{-1} in bovine kidney,[15] but in that case a specific method for phenylbutazone was required. In this study a multi-residue method was developed. No literature data were found for the detection of phenylbutazone in bovine muscle.

Mass spectrometric detection of salicylic acid

The detection of salicylic acid using MS^1 was rather poor, and its fragmentation (loss of the carboxylic acid group as CO_2) was not reproducible. Therefore, attempts were made to derivatise salicylic acid, but this compromised the multi-residue method. So another solution was proposed based on MS^2.

Once isolation of the selected precursor ion in an ion trap is completed, the radio-frequency (rf) amplitude is reduced to obtain a certain q_z-value. Not only the precursor ion but also the product ions to be monitored need to be within the stability region at this q_z-value. By default the q_z-value of a Thermo ion trap mass spectrometer is set to 0.25, corresponding to a certain low mass cutoff (LMCO) value. By increasing the q_z-value, the LMCO value will increase, so possible product ions with *m/z* ratios below this LMCO value will not be stored.

Table 3. Instrument parameters (collision energy and activation q) of the LC/MS^2 method for the detection of NSAIDs

Analyte	Collision energy (%)	Activation q
Salicylic acid	47	0.35
Phenylbutazone	49	0.25
Flunixin	40	0.25
Tolfenamic acid	46	0.25
Meloxicam	35	0.25
Ketoprofen	25	0.25

Table 4. Concentrations at which the NSAIDs were spiked into blank bovine tissue

Analyte	Spiked concentration (µg kg^{-1})
Salicylic acid	40 (MRPL acetylsalicylic acid)
Phenylbutazone	100
Flunixin	20 (MRL)
Tolfenamic acid	50 (MRL)
Meloxicam	20 (MRL)
Ketoprofen	20

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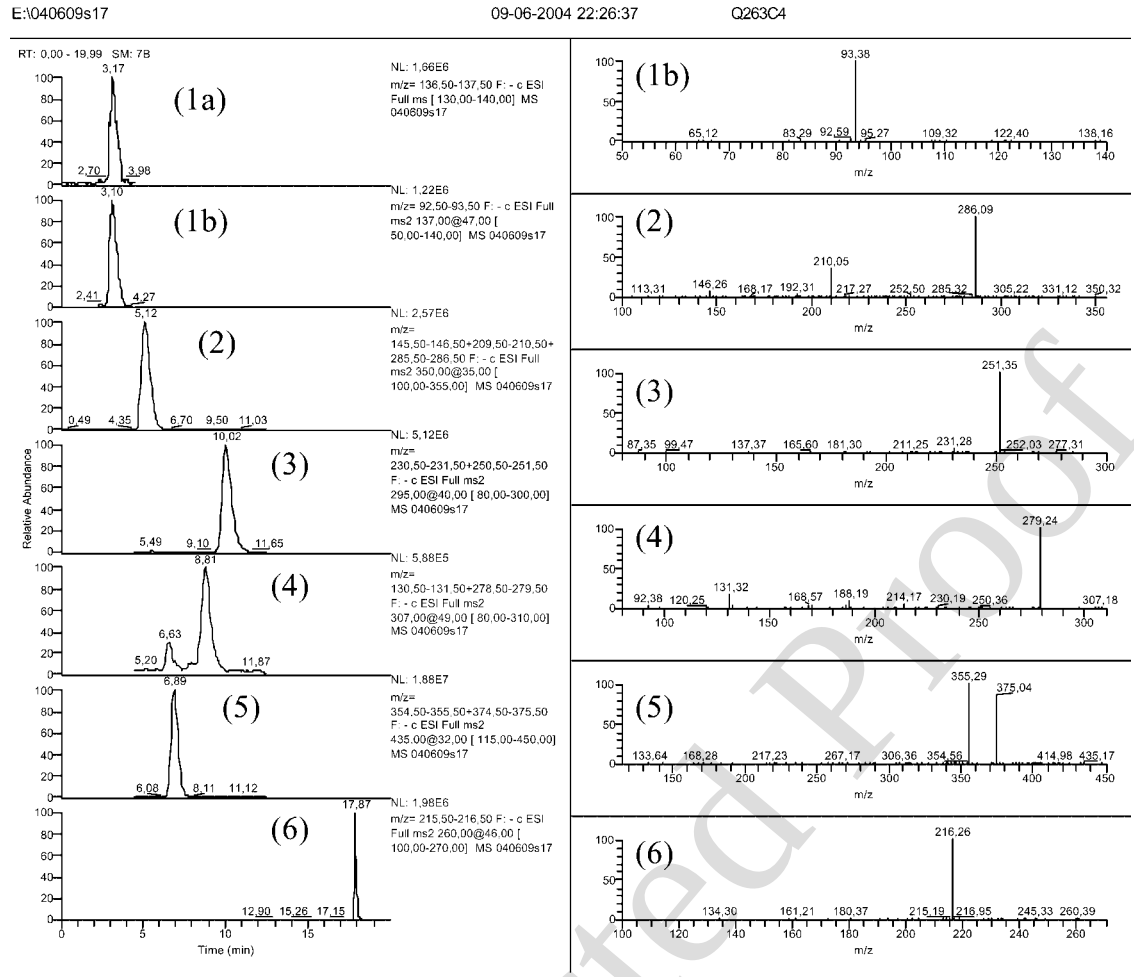

Figure 3. Extracted fragment ion chromatograms and MS/MS spectra of $[M-H]^-$ ions of salicylic acid (1), meloxicam (2), flunixin (3), phenylbutazone (4), desoximethasone (I.S.) (5), and tolfenamic acid (6).

In negative ion mode salicylic acid produced a $[M-H]^-$ ion with m/z 137. During isolation the rf amplitude was ramped in order to eject all ions with $m/z < 137$. Subsequently, a broadband waveform was applied to eject ions with $m/z > 137$. At that point the $[M-H]^-$ ion of salicylic acid was isolated in the ion trap and the rf amplitude was reduced again to position this ion within the stability region along the q_z-axis. At the default q_z-value of 0.25, the precursor ion with m/z 137 was not stable and not every scan contained the product ion with m/z 93, although both the precursor ion and the product ion should have been present within the stability region. Therefore, the q_z-value for m/z 137 was increased. At a q_z-value of 0.35 the $[M-H]^-$ ion of salicylic acid was stable, and a good and reproducible detection of the product ion with m/z 93 was obtained. This q_z-value corresponds to an LMCO value of m/z 50, low enough to detect the product ion.[22–24]

Mass spectrometric detection of phenylbutazone

Detection of phenylbutazone required adaption of another parameter, the maximum ion injection time, the time for which ions are allowed to accumulate in the mass analyser when automatic gain control is on to maintain the optimum quantity of ions for each scan to avoid space charge effects. By default the maximum ion injection time is set by the manufacturer to 200 ms. Too low a value can result in loss of sensitivity because the mass analyser traps fewer than the optimum number of ions, but too high a value can give insufficient data points across the chromatographic peak. The latter is only the case when the number of microscans is already set to its lowest value. (Each microscan is one complete mass analysis cycle, i.e., ion injection and storage followed by scan out of ions, followed by ion detection; a number of microscans is summed to produce one scan. The ion injection time and the number of microscans affect the scan time.)

Since the method discussed here is a LC/MS^2 multiresidue method, MS parameters had to be set within several LC time segments in order to be able to detect all the NSAIDs. In this case three time segments were used in the instrumental method, each time segment corresponding to analysis of different sets of compounds (Table 5). Time segment 2

Table 5. NSAIDs analysed in each LC time segment

Time segment	Analyte
Segment 1	Salicylic acid
	Meloxicam
Segment 2	Phenylbutazone
	Desoximethasone (IS)
	Flunixin
Segment 3	Tolfenamic acid

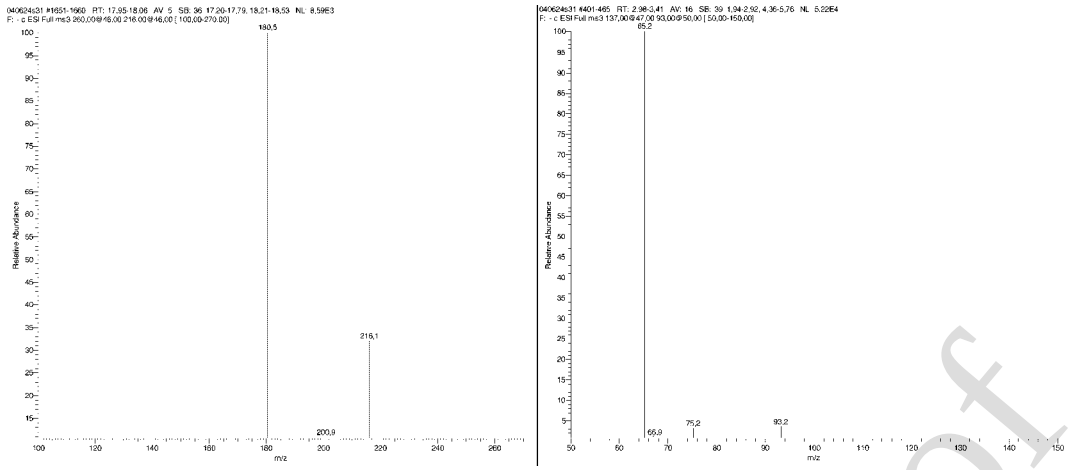

Figure 4. MS^3 mass spectra of ([M−H]$^-$ → *m/z* 216) for tolfenamic acid spiked into blank bovine muscle at a concentration of 50 μg kg^{-1} (left), and of ([M−H]$^-$ → *m/z* 93) for salicylic acid spiked into blank bovine muscle at a concentration of 40 μg kg^{-1} (right).

analysed phenylbutazone and flunixin and the internal standard desoximethasone. The partitioning of the total scan time within LC time segment 2 among three compounds meant that there was a decrease in the number of scans available to detect each analyte. Therefore, the number of microscans was set to 1 to increase the number of scans recorded across the chromatographic peak of each analyte. However, at the default maximum ion injection time of 200 ms, there were still not enough scans obtained across the chromatographic peak for phenylbutazone to obtain a well-defined and intense chromatographic peak. Therefore, the maximum ion injection time was lowered to 50 ms.

Confirmation of salicylic acid and tolfenamic acid

Veterinary drugs that are forbidden in the European Union are group A substances. The minimum number of identification points (IPs) for such forbidden compounds is set to 4. Veterinary drugs that have a MRL (the maximum concentration of a residue which is legally permitted or acceptable in food) are group B substances. The minimum number of IPs for these compounds is set to 3.[25]

Both salicylic acid and tolfenamic acid had one product ion in the MS^2 full scan spectra of their [M−H]$^-$ ions (Fig. 3), so 2.5 IPs were earned (1 precursor ion and 1 product ion). To create more specificity and to achieve enough IPs, full scan MS^3 spectra of the product ions were investigated. Figure 4 shows the MS^3 mass spectrum for tolfenamic acid spiked into blank bovine muscle at a concentration of 50 μg kg^{-1}, containing a second-generation product ion at *m/z* 180, so 4 IPs were earned (1 precursor ion, 1 product ion and 1 second transition product ion). Figure 4 also shows the MS^3 mass spectrum for ([M−H]$^-$ → *m/z* 93 → *m/z* 65) of salicylic acid spiked into blank bovine muscle at a concentration of 40 μg kg^{-1}. At the default q_z-value of 0.25 the product ion at *m/z* 93 was not stable and the spectrum was empty. When the q_z-value for *m/z* 93 was increased to 0.35 again *m/z* 93 was not stable, but a second-generation product ion was observed at *m/z* 65; however, not every scan contained the *m/z* 65 ion. At a q_z-value of 0.45 the first-generation product ion at *m/z* 93 was stable and observable in the MS^3 spectrum, and an intense and reproducible signal for the second transition product ion at *m/z* 65 was obtained.

Thus, for the confirmation of tolfenamic acid and salicylic acid according to the criteria of Commision Decision 2002/657/EEC, MS^3 full scan spectra of the [M−H]$^-$ ions via their major first-generation product ions will be obtained in an extra acquisition for the valid identification of these NSAIDs.

Ketoprofen

Ketoprofen could be detected and identified using the method described above for the previous NSAIDs, but using LC/MS^2 in positive ion mode. A combination of the two polarity modes was not ideal since polarity change (alternating detection in negative and positive ion modes) led to a loss in sensitivity and a loss of information. Therefore, two acquisitions were necessary to detect all NSAIDs although the extraction and cleanup were identical. The key instrumental parameters, collision energy and activation q, are summarised in Table 3; the isolation width was set to 2 Da. The standard ketoprofen and the internal standard desoximethasone were spiked into blank bovine muscle at a concentration of 20 μg kg^{-1} ketoprofen (Table 4). Figure 5 shows the MS^2 and MS^3 extracted ion chromatograms and spectra for ketoprofen.

Carprofen

Carprofen, which has an MRL specified for bovine muscle, is not yet incorporated in the present multi-residue method. There was no demand from the Ministry of Public Health for the detection of carprofen, and this NSAID has not yet been detected in injection sites analysed in the laboratory of chemical analysis at the Faculty of Veterinary Medicine.[14,15] However, this NSAID will be incorporated in the present LC/MS^2 method as soon as possible.

Qualitative method and requirements for quantitation

This multi-residue method is only a qualitative method. The following qualitative validation parameters were tested: specificity, selectivity, decision (CCα) and detection limit (CCβ).

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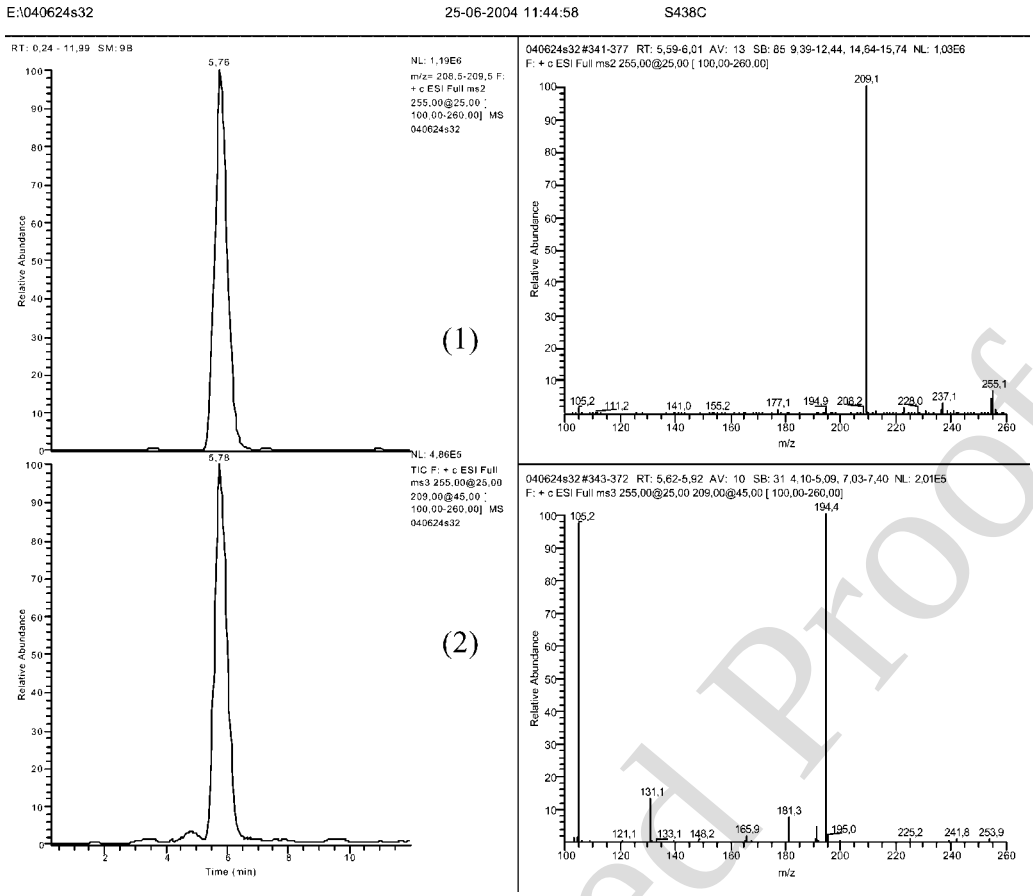

Figure 5. (1) Extracted fragment ion chromatogram and MS^2 spectrum of $[M+H]^+$ ions, and (2) extracted fragment ion chromatogram and MS^3 mass spectrum of ($[M+H]^+ \rightarrow m/z$ 209), for ketoprofen spiked into blank bovine muscle at a concentration of 20 $\mu g\,kg^{-1}$.

The specificity of the method was demonstrated by LC/MS^2 analysis of blank bovine muscle; no interferences were observed in analysis of these blank samples and in analysis of bovine muscle spiked with salicylic acid, flunixin, phenylbutazone, meloxicam and tolfenamic acid (Fig. 3). The minimum number of IPs for forbidden compounds is set to 4, and veterinary drugs with a specified MRL require 3 IPs. Each NSAID detected in this method has at least 4 IPs (Table 2). Thus, blank muscle samples were spiked at the MRL concentrations of flunixin, meloxicam and tolfenamic acid, the MRPL concentration of acetylsalicylic acid (spiked as salicylic acid), and at 100 $\mu g\,kg^{-1}$ for phenylbutazone. For salicylic acid the CCβ was equal to or lower than the spiked concentrations, 40 $\mu g\,kg^{-1}$. The CCα was calculated by subtracting 1.64 times the standard deviation at the CCβ-value. For flunixin, meloxicam and tolfenamic acid, the MRL concentration plus 1.64 times the standard deviation determined at the MRL concentration equalled the decision limit CCα. CCβ was calculated as CCα plus 1.64 times the corresponding standard deviation, supposing that $\sigma_{CC\alpha}$ equals σ_{MRL}. In Table 6 values of both CCα and CCβ are summarised for the different NSAIDs. The CCα of phenylbutazone gave an unacceptably low concentration because the peak area ratios were not reproducible. For ketoprofen not enough data have been collected yet.

Before quantification can be carried out, a suitable internal standard needs to be added to the method and the extraction with acetonitrile needs to be evaluated. Desoximethasone is not an ideal internal standard since its chemical structure is quite different from those of the NSAIDs. However, the NSAIDs have different chemical structures among themselves, so more than one internal standard should be added to the method. Unfortunately, adding extra analytes to the method and to each LC time segment can cause a decrease in the number of scans across the chromatographic peak for each analyte, so some suitable compromise will have to be developed.

The efficiency of the extraction with acetonitrile needs to be evaluated. The methods used for the determination of NSAIDs in edible bovine tissues described in the literature involve an initial acid hydrolysis prior to extraction with acetonitrile or ethyl acetate.[7,16] NSAIDs are most often analysed in plasma and urine.[2,9,11,12,17–20] There are not many methods describing the detection of NSAIDs in bovine

Table 6. Validation parameters CCα and CCβ determined for different NSAIDs in bovine muscle

	CCα ($\mu g\,kg^{-1}$)	CCβ ($\mu g\,kg^{-1}$)
Salicylic acid	$\leq$35.54	$\leq$40
Flunixin	20.58	21.15
Meloxicam	22.45	24.89
Tolfenamic acid	59.10	68.20

muscle. Therefore, further research of possible extraction techniques for the detection of NSAIDs in edible bovine tissues is necessary.

The extraction capability of the described method needs to be evaluated using incurred samples. Several unknown samples have already been extracted and cleaned up according to the described method; the NSAIDs flunixin, phenylbutazone and salicylic acid were determined in these samples of bovine muscle. However, we have no data for the recovery of these NSAIDs since there was no knowledge of the true concentrations of NSAIDs present in the samples. So, samples containing a NSAID will be collected in the future and different extraction methods will be evaluated, including the described extraction with acetonitrile, and an initial acid hydrolysis and the addition of an enzyme to break any protein bonds, since NSAIDs are strongly bound to proteins.

In summary, considerable further research is necessary to develop a quantitative multi-residue method for the NSAIDs salicylic acid, phenylbutazone, flunixin, tolfenamic acid, meloxicam and ketoprofen.

CONCLUSIONS

A LC/MS^2 multi-residue method was developed to identify salicylic acid, phenylbutazone, flunixin, tolfenamic acid, meloxicam and ketoprofen in bovine muscle. Ketoprofen was detected in positive ion mode, while the other NSAIDs were detected in negative ion mode, so two acquisitions were necessary to detect all NSAIDs. In addition, for the confirmation of salicylic acid, tolfenamic acid and ketoprofen, full scan MS^3 spectra of the $[M-H]^-$ or $[M+H]^+$ ions via their major first-generation fragment ions were necessary. Some ion trap parameters (activation q and maximum ion injection time) needed to be adapted for optimal detection of salicylic acid and phenylbutazone. These adjustments were consequences of intrinsic properties of ion traps, including low collision energy (and thus limited numbers of different product ions necessitating MS^3) as well as the low mass cutoff for stable trapping of low-mass product ions.

This multi-residue method is currently only a qualitative method. Before it can be extended to quantification, a suitable internal standard needs to be added to the method and the extraction with acetonitrile needs to be evaluated. Non-compliant samples will be collected and different extraction methods will be evaluated. So, further research is necessary to develop a quantitative multi-residue method for the NSAIDs salicylic acid, phenylbutazone, flunixin, tolfenamic acid, meloxicam and ketoprofen.

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REFERENCES

1. Lawrence JR, Peter R, Baxter[Q1], Robson J, Graham AB, Paterson JR. *J. Clin. Pathol.* 2003; **56**: 651.
2. Baert K. PhD thesis, Ghent University, Faculty of Veterinary Medicine, 2003; 3–18.
3. Daeseleire E, Mortier L, De Ruyck H, Geerts N. *Anal. Chim. Acta* 2003; **488**: 25.
4. European Agency for the Evaluation of Medicinal Products.
5. De Wasch K, Van Hoof N, Poelmans S, Okerman L, Courtheyn D, Ermens A, Cornelis M, De Brabander HF. *Anal. Chim. Acta* 2003; **483**: 387.
6. Van Hoof N, De Wasch K, Poelmans S, De Brabander HF. In *Rapid and On-Line Instrumentation for Food Quality Assurance*, Tothill IE (ed). Woodhead Publishing Ltd.: Cambridge, 2003; 91–115.
7. Boner PL, Liu DDW, Feely WF, Robinson RA, Wu J. *J. Agric. Food Chem.* 2003; **51**: 7555.
8. Boner PL, Liu DDW, Feely WF, Wisocky MJ, Wu J. *J. Agric. Food Chem.* 2003; **51**: 3753.
9. van Eeno P, Delbeke FT, Roels K, Baert K. *J. Pharm. Biomed. Anal.* 2003; **31**: 723.
10. Rupp HS, Holland DC, Munns RK, Turnipseed SB, Long AR. *J. AOAC Int.* 1995; **78**: 959.
11. Stanley SMR, Owens NA, Rodgers JP. *J. Chromatogr. B Biomed. Appl.* 1995; **667**: 95.
12. Singh AK, Jang Y, Mishra U, Granley K. *J. Chromatogr. B Biomed. Appl.* 1991; **106**: 351.
13. Hormazabal V, Yndestad M. *J. Liq. Chromatogr. Relat. Technol.* 2001; **24**: 2477.
14. Abdel-Hamid ME, Novotny L, Hamza H. *J. Pharm. Biomed. Anal.* 2001; **24**: 587.
15. Clark SB, Turnipseed SB, Nandrea GJ, Madson MR, Hurlbut JA, Sofos JN. *J. AOAC Int.* 2002; **85**: 1009.
16. Asea PA, Patterson JR, Korsrud GO, Dowling PM, Boison JO. *J. AOAC Int.* 2001; **84**: 659.
17. Wiesner JL, de Jager AD, Sutherland FCW, Hundt HKL, Swart KJ, Hundt AF, Els J. *J. Chromatogr. B* 2003; **785**: 115.
18. Martin MIG, Gonzales CIS, Hernandez AJ, Cachan MDG, de Cabo MJC, Cuadrado ALG. *J. Chromatogr. B* 2002; **769**: 119.
19. Luo Y, Rudy JA, Uboh CE, Soma LR, Guan F, Enright JM, Tsang DS. *J. Chromatogr. B* 2004; **801**: 173.
20. Kim JY, Kim SJ, Paeng KJ, Chung BC. *J. vet. Pharmacol. Ther.* 2001; **24**: 315.
21. Bakar SK, Niazi S. *J. Pharm. Sci.* 1983; **72**: 1024.
22. [Q2]Hoffmann ED, Charette J, Stroobant V. *Mass Spectrometry: Principles and Applications*, John Wiley: Chichester; 39–59.
23. March RE. *J. Mass. Spectrom.* 1997; **32**: 351.
24. Wong PSH, Cooks RG. *Curr. Sep.* 1997; **16**: 85.
25. Commision Decision 2002/657/EEC *Off. J. Eur. Comm.*, no L 221: 23–33.

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