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Simultaneous determination of thirty non-steroidal anti-inflammatory drug residues in swine muscle by ultra-high-performance liquid chromatography with tandem mass spectrometry

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ABSTRACT

An ultra-high-performance liquid chromatography with tandem mass spectrometric detection (UHPLC–MS/MS) method was established for the simultaneous determination of residues of thirty non-steroidal anti-inflammatory drugs (NSAIDs) in swine muscle. The samples were extracted with acetonitrile and phosphoric acid. The extracts were defatted with *n*-hexane, and then purified by HLB solid-phase extraction cartridge. Analysis was carried out on UHPLC–ESI-MS/MS working with multiple reaction monitoring mode with polarity switching. Limits of detection were between 0.4 μg/kg and 2.0 μg/kg, and limits of quantification were between 1.0 μg/kg and 5.0 μg/kg. The recoveries of NSAIDs were between 61.7% and 125.7% at spiked levels of 1.0–500 μg/kg. The repeatability was less than 8% and the within-laboratory reproducibility was not more than 12.3%. The method was reliable, convenient and sensitive.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the group of drugs that give therapeutic efficacy of treating pain and inflammation, due to conjugate with cyclooxygenase isomers which transform arachidonic acid to prostaglandin [1]. Owing to their effectiveness in suppressing or preventing inflammation, NSAIDs are becoming the most commonly used medicines around the world.

The most common side-effect of NSAIDs is the tendency of inducing gastric or intestinal ulceration. Other side-effects are including disturbance of platelet function, prolongation of gestation or spontaneous labor, changes of renal function, and inducement of kidney or liver tumors [2]. However, the usage of NSAIDs has recently increased significantly, such as meclofenamic acid, carprofen, tolfenamic acid, ketoprofen, has been permitted to administer in animals. To protect consumers from health-threatening residues of veterinary drugs and their metabolites, maximum residue limits (MRLs) of pharmacologically active substances administered to food-producing animals must be assigned. European Union (EU) regulated MRLs of carprofen, firocoxib, tolfenamic acid, meloxicam, metamizole, flunixin, diclofenac, vadeprofen, and listed acetylsalicylic acid, salicylic acid, paracetamol, ketoprofen, as pharmacologically active substances [3]. Japan regulated MRLs of carprofen, ketoprofen, flunixin, meloxican and tolfenamic acid in positive list system [4]. The US Food and Drug Administration (FDA) monitored residues of flunixin, phenylbutazone, and metamizole sodium [5]. In China, aspirin and acetaminophen have been listed in residue monitoring program [6]. Although the MRL procedure has not yet been completed for all NSAIDs, it can be assumed that more strict systems and measures would prevent NSAIDs from misusing in animals.

For the safety of food, more and more analytical techniques have been used for analyzing NSAIDs, such as chromatography (high

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Table 1
Parameters of MRM condition for thirty NSAIDs.

Compound	Ionization mode (ESI)	Precursor ion (m/z)	Daughter ions (m/z)	Cone voltage (V)	Collision energy (eV)	Compound	Ionization mode (ESI)	Precursor ion (m/z)	Daughter ions (m/z)	Cone voltage (V)	Collision energy (eV)
Salicylic acid	ESI (−)	137.0	65.0	32	26	Indoprofen	ESI (+)	282.2	77.1	48	59
			93.0[a]	32	15				236.2[a]	48	20
Loxoprofen	ESI (−)	245.3	83.0[a]	26	18	Rofecoxib	ESI (+)	315.2	269.2	40	20
			201.2	26	7				297.2[a]	40	14
Sasapyrin	ESI (−)	257.2	93.0	16	30	Sulindac	ESI (+)	357.3	233.3[a]	45	45
			137.1[a]	16	10				340.3	45	20
Fenbufen	ESI (−)	253.2	153.2	26	23	Naproxen	ESI (+)	231.2	170.1	30	25
			209.3[a]	26	15				185.2[a]	30	15
Nimesulide	ESI (−)	307.2	79.0	38	25	Ketoprofen	ESI (+)	255.2	105.1	35	25
			229.2[a]	38	16				209.2[a]	35	14
Etodolac	ESI (−)	286.3	212.3	38	25	Tolmetin	ESI (+)	258.2	91.0	28	40
			242.3[a]	38	18				119.1[a]	28	20
Acetaminophen	ESI (+)	152.1	93.0	38	21	Flunixin	ESI (+)	297.2	264.2	45	34
			110.1[a]	38	14				279.2[a]	45	23
Aminoantipyrine	ESI (+)	204.2	56.1[a]	35	19	Firocoxib	ESI (+)	315.2	132.1[a]	39	26
			83.0	14	15				220.2	39	25
Ketorolac	ESI (+)	256.3	77.1	38	38	Meloxicam	ESI (+)	352.2	115.1[a]	30	20
			105.1[a]	38	20				141.1	30	20
Acetophenetidine	ESI (+)	180.2	110.1[a]	38	20	Zomepirac	ESI (+)	292.2	111.0	36	40
			138.1	38	15				139.0[a]	36	17
Mepirizole	ESI (+)	235.2	123.1	43	25	Oxaprozin	ESI (+)	294.2	103.1[a]	40	28
			220.2[a]	43	22				276.3	40	17
Tenoxicam	ESI (+)	338.2	95.1[a]	38	20	Nabumetone	ESI (+)	229.2	128.1	30	37
			121.1	38	27				171.2[a]	30	12
Etoricoxib	ESI (+)	359.2	243.9	60	52	Indomethacin	ESI (+)	358.2	111.0	35	49
			280.2[a]	60	33				139.0[a]	35	16
Piroxicam	ESI (+)	332.2	95.0[a]	32	19	Acemethacin	ESI (+)	416.2	139.0[a]	38	22
			121.0	32	25				174.1	38	18
Formylaminoantipyrine	ESI (+)	232.2	83.0	35	24	Benzydamine	ESI (+)	310.3	86.1[a]	33	44
			104.1[a]	35	23				245.2	18	6

[a]The ion for quantification.

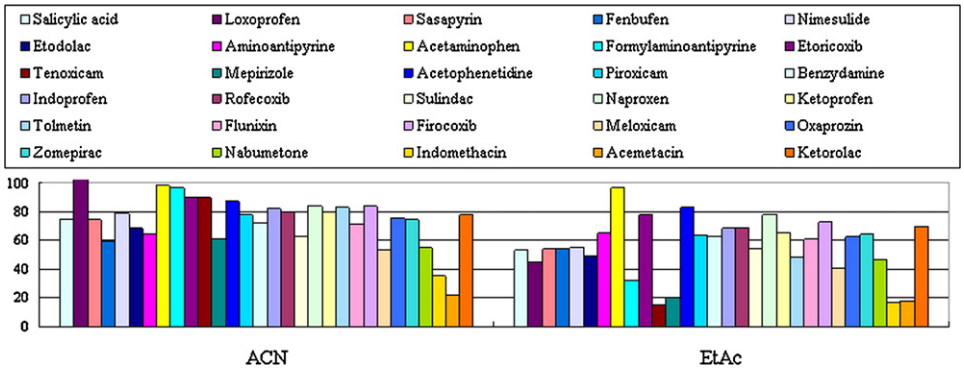

Fig. 1. Comparison of extraction recoveries of acetonitrile (ACN) and ethyl acetate (EtAc).

performance liquid chromatography [7–17], thin-layer chromatography [18] and gas chromatography [19], capillary electrophoresis [20]), spectrophotometry [1], fluorimetry [1], voltamperometry [1]. Earlier studies were focused on determination of NSAIDs in blood [7–13,21], urine [13–15,22–24] and milk [25–28], and the number of analytes was small. As we all know, although liquid chromatography with tandem mass spectrometric detection (LC–MS/MS) method is now being developed in some labs, it is still a great challenge to establish an analysis method for simultaneous determination of NSAIDs in animal original food products. In recent years, a few methods have been published. Boner et al. [28], Igualada et al. [29], Van Hoof et al. [30], and Jedziniak et al. [31] developed methods for simultaneous determination of one, four, six, and ten NSAIDs, respectively. Tao et al. [32] established a UHPLC–MS/MS method which could simultaneously analyze eighteen NSAIDs in swine liver, and trace level (ppb) residues could be detected. However, no previously published method is capable of analyzing all the EU licensed NSAIDs [3] in animal original food products.

This paper is a follow-up of our previous study [32], where we were exploring extraction and purification conditions in order to increase the number of analytes for multi-residue analysis in animal edible tissues, and established a UHPLC–MS/MS method for simultaneous determination of thirty NSAIDs in swine muscle. The major advantages of the method presented in this study are that it can simultaneously analyze more NSAIDs in edible animal tissues, and is easily able to detect these analytes at trace level. The proposed method was validated by measuring selectivity, linearity, accuracy, precision, limits of detection (LODs) and quantification (LOQs). To confirm the effectiveness of the proposed method, real samples from abroad were analyzed. From the results, it was shown that the method was reliable, convenient and sensitive.

2. Experimental

2.1. Reagents and materials

Salicylic acid, loxoprofen, sasapyrin, fenbufen, nimesulide, etodolac, acetaminophen, aminoantipyrine, formylaminoantipyrine, mepirizole, tenoxicam, etoricoxib, acetophenetidine, piroxicam, benzydamine, ketorolac, indoprofen, rofecoxib, sulindac, firocoxib, tolmetin, flunixin, ketoprofen, naproxen, meloxicam, zomepirac, oxaprozin, nabumetone, indomethacin and acemetacin, standards purity ≥ 99%, were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Formic acid and ammonium acetate of HPLC grade were purchased from Acros Organics (Brussels, Belgium). HPLC grade acetonitrile, *n*-hexane, methanol, and analytical reagent grade ammonium hydroxide (25–28%), phosphoric acid, methyl tert-butyl ether, anhydrous sodium sulfate were obtained from Beihuajingxi Corp. (Beijing, China). Water was purified with a Millipore water purification system (resistivity, 18.2 MΩ cm, Millipore, Bedford, MA, USA). *n*-Hexane, saturated with acetonitrile, was prepared by adding 10 mL of acetonitrile to 50 mL of *n*-hexane, and the separated upperlayer after shaking was used. Oasis HLB cartridge (150 mg, 6 cm^3) was purchased from Waters Corp. (Milford, MA, USA). Stock solutions containing 100.0 mg/L of individual chemical were prepared in acetonitrile. 100 μL of each stock solution was transferred into a 10.0-mL volumetric flask to prepare a mixed standard solution with a concentration of 1.0 mg/L of each chemical.

2.2. Instruments

Chromatographic separation was performed on an Acquity™ Ultra performance LC system. Detection was performed on a TQ triple quadrupole mass spectrometric detector with an electrospray ionization (ESI) interface. The instrument control, data acquisition and data treatment were performed with Masslynx 4.1 Analyst software. An Acquity™ UPLC BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm) was used for chromatographic separation. All instruments mentioned above were purchased from Waters Corp. (Milford, MA, USA). Column temperature was set at 30 °C, and injection volume was 10 μL. The mobile phase consisted of acetonitrile (solution A) and 0.1% formic acid solution containing 0.5 mmol/L ammonium acetate (solution B). The gradient elution program: 0–3 min, 90–70% B; 3–5 min, 70% B; 5–6 min, 70–50% B; 6–10 min, 50% B; 10–12 min, 50–10% B; 12–14 min, 10% B; 14–15 min, 10–90% B; 15–16 min, 90% B. Flow rate was 0.2 mL/min.

Ionization of analytes was carried out by an ESI source running in both positive and negative mode. Interface conditions were as follows: capillary voltage was 3.0 kV in ESI^+ mode and 2.5 kV in ESI^- mode; source temperature was 120 °C; desolvation temperature was 350 °C; cone gas was nitrogen with flow rate of 100 L/h; desolvation gas was nitrogen with flow rate of 600 L/h; collision gas was argon with pressure of 2.40×10^{-6} Pa; monitoring mode was multiple reaction monitor (MRM), and parameters are shown in Table 1.

2.3. Sample extraction and purification

The homogenized sample (5 g) was weighed into a 50-mL centrifuge tube and 15 mL of acidic acetonitrile (acetonitrile–phosphoric acid, 80 + 1, v/v) was added followed by anhydrous sodium sulfate (2 g). The sample was shaken (20 min), ultrasonic water bathed (10 min), and centrifuged (4000 rpm,

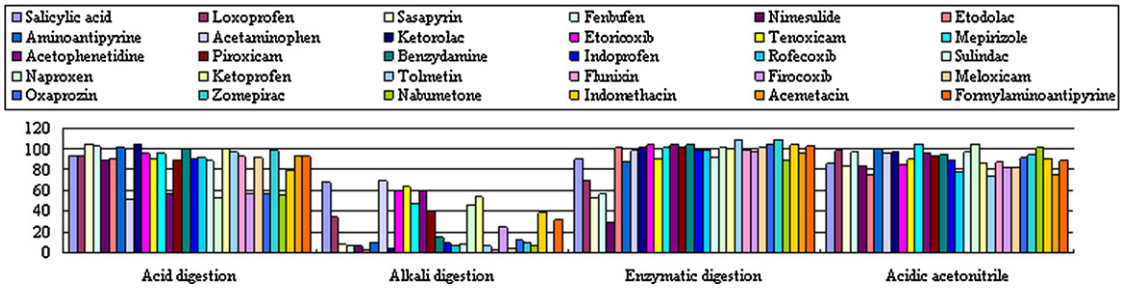

Fig. 2. Comparison of extraction recoveries after acid, alkali, enzymatic digestion and acidic acetonitrile.

5 min). The supernatant was transferred into another 50 mL tube. The above process was then repeated. The supernatants were combined and *n*-hexane saturated with acetonitrile (15 mL) was added. The tube was shaken (5 min) and centrifuged (4000 rpm, 2 min). The supernatant was discarded off and the underlayer was dried (40 °C, N_2), then reconstituted in 5 mL of methanol–phosphoric acid (0.02 M) (5 + 95, v/v). *n*-Hexane saturated with acetonitrile (10 mL) was added and centrifuged (4000 rpm, 2 min) again. The supernatant was discarded off and the underlayer was loaded on a HLB cartridge preconditioned with methanol (5 mL), water (5 mL) and 0.02 M phosphoric acid (5 mL). The cartridge was rinsed with 5 mL of water. The analytes were eluted with the mixture of ammonium hydroxide–acetonitrile–methyl tert-butyl ether (5 + 95 + 1, v/v/v) (4 mL) followed by methyl tert-butyl ether (4 mL) and evaporated to dryness (40 °C, N_2). The residue was reconstituted in acetonitrile–0.1% formic acid (10 + 90, v/v) (1 mL), and then vortexed for 30 s, filtrated through a membrane filter and transferred into the vial.

2.4. Qualitative and quantitative methods

The qualitative methods utilized the abundance ratios of the qualitative ion pairs together with the retention time of chromatographic peaks of the target chemicals. Matrix-matched calibration curves were prepared for quantification. Standard solution was diluted by the negative sample extract prepared as given in Section 2.3, in accordance with spiked levels of 1.0, 5.0, 10.0, 50.0, 100.0, 150.0, 250.0, 500.0 μg/kg. The calibration curves were established by using the linear regression of concentration of each standard against the peak area of each.

3. Results and discussion

3.1. Sample preparation

3.1.1. Optimization of extraction procedure

In order to improve the extraction recoveries of thirty NSAIDs from swine muscle, the extraction procedure was optimized by investigating different organic solvents and extraction conditions. In most residue analytical studies of NSAIDs, acetonitrile or ethyl acetate was selected as the extraction solvent. In this research, after the same extraction process, compared the peak areas of analytes extracted by acetonitrile or ethyl acetate with standards (Fig. 1), the higher recoveries were obtained by using acetonitrile, owing to its good solubility of NSAIDs and capability for precipitation of sample proteins.

According to the references, several extraction methods were reported. Some emphasized relatively high percentage of residues bound with glucuronic or sulfuric acid [33], the enzymatic hydrolysis was performed by Jedziniak et al. extracting nine NSAIDs and one metabolite in swine, horse and chicken muscle [31]. And other reported that NSAIDs were drugs bind to protein, and it was necessary to deconjugate analytes with chemical hydrolysis, the good results were obtained by Clark et al. extracting phenylbutazone in bovine kidney tissue with alkali hydrolysis [34]. Igualada et al., after comparing chemical hydrolysis with enzymatic hydrolysis, decided to extract meloxicam, flunixin meglumine, carprofen, and tolfenamic acid in muscle and liver of bovine, equine, porcine with acidic hydrolysis [22,29]. However, without hydrolysis, the good performance was obtained by Van Hoof et al. extracting acetylsalicylic acid, flunixin, phenylbutazone, tolfenamic acid, meloxicam and ketoprofen with acetonitrile in bovine muscle [30]. Generally speaking, the influence of hydrolysis and type of hydrolysis reagent were concerned. On the basis of all the above results and our previous study [32], and in order to investigating the influence of hydrolysis reagent on recovery in multi-residue extraction, different extraction conditions including acidic (0.5 M, hydrochloric acid), alkali (10%, ammonium hydroxide), enzymatic (β-glucuronidase/arylsulfatase, acetate buffer, pH 4.5) hydrolysis, and acidic acetonitrile (acetonitrile–phosphoric acid, 80 + 1, v/v) without hydrolysis were tested, respectively, by using spiked sample. The hydrolysis samples were incubated for 1 h at 37 °C, then extracted with acetonitrile, and the acidic acetonitrile sample was treated as given in Section 2.3. Results (Fig. 2) showed that extraction recoveries after alkali hydrolysis, due to the influence of saponification, were bad, and those after acidic or enzymatic hydrolysis were good, except for acetaminophen, naproxen, nimesulide and sasapyrin, may be a proof of acidolysis or enzymolysis effect. Acidic acetonitrile, though the extraction recoveries of a few analytes were not as good as those after acidic or enzymatic hydrolysis, was effective for all thirty analytes. Therefore, in order to ensuring the extraction recovery without concerning the influence of bound drug, acidic acetonitrile (acetonitrile–phosphoric acid, 80 + 1, v/v) was selected in our method.

3.1.2. Optimization of clean-up procedure

Many kinds of SPE cartridges have been used due to the different structures of NSAIDs. Boner et al. [28,35] used BondElut SCX; Tao et al. [32] and Clark et al. [34] used BondElut Silica; Chrusch et al. [36] used Oasis MAX; Van Hoof et al. [30] and Feely et al. [37] used Oasis HLB. All cartridges mentioned above were tested in this study. According to our previous study [32], silica cartridge was good performance at retention and purification of eighteen NSAIDs, but not effective for the strong polar drug (i.e. aminoantipyrine). Oasis MAX and Oasis MCX cartridges, owing to their contradistinction of retention mechanism and usage condition, were hard to purify thirty NSAIDs at the same time. While Oasis HLB cartridge, with a hydrophilic–lipophilic balanced copolymer of *n*-vinylpyrrolidone and divinylbenzenes, was good performance at retention and purification of thirty NSAIDs. Most analytes exited in ionized forms in ammonium hydroxide, and acetonitrile was better than methanol due to its stronger elution, but still not strong enough for all analytes. This limitation was overcome by adding

methyl tert-butyl ether. After optimizing the percentage of three elutes, finally 4 mL of ammonium hydroxide–acetonitrile–methyl tert-butyl ether (5 + 95 + 1, v/v/v) and 4 mL of methyl tert-butyl were selected as the elutes.

3.2. Liquid chromatography–mass spectrometry

Acetonitrile and 0.1% formic acid solution (containing 0.5 mmol/L ammonium acetate) were selected as mobile phase. According to our previous study [32], formic acid could improve the ionization of NSAIDs under ESI^+ mode and ammonium acetate could change the pH of mobile phase to improve the ionization of NSAIDs under ESI^- mode. ESI source is particularly suitable for their MS detection because of the polar nature of NSAIDs. Some NSAIDs respond in negative-ion mode following the deprotonation of the carboxylic functional group, while the same site of others can be protonated, allowing the detection of those compounds in positive-ion mode. Gradient, capillary voltage, cone voltage, collision energy were optimized, and MRM was divided into eight scan functions, which could increase the scan spot at the

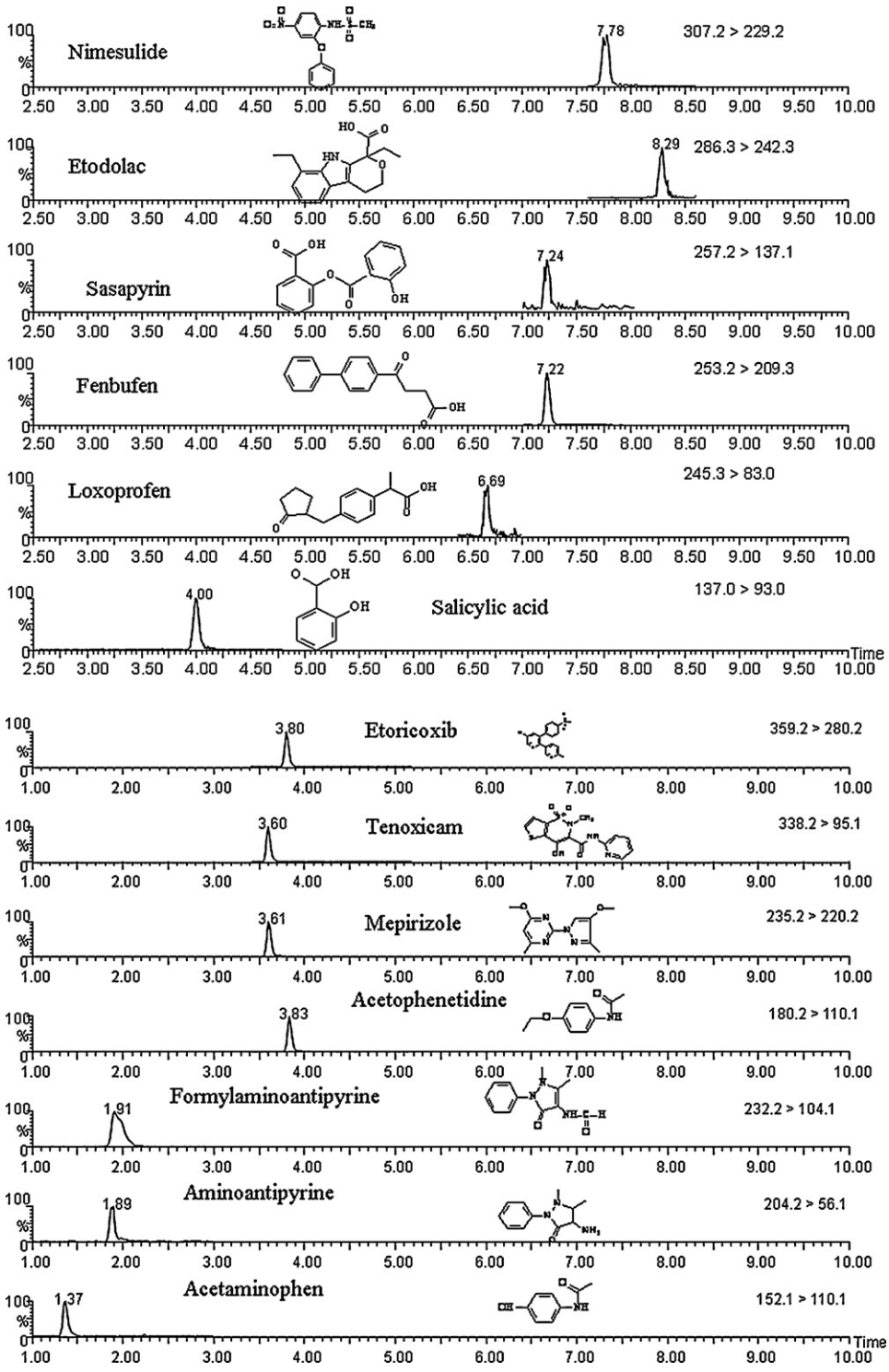

Fig. 3. LC–MS/MS chromatograms of swine muscle samples fortified with thirty NSAIDs, each spiked at the LOQ level.

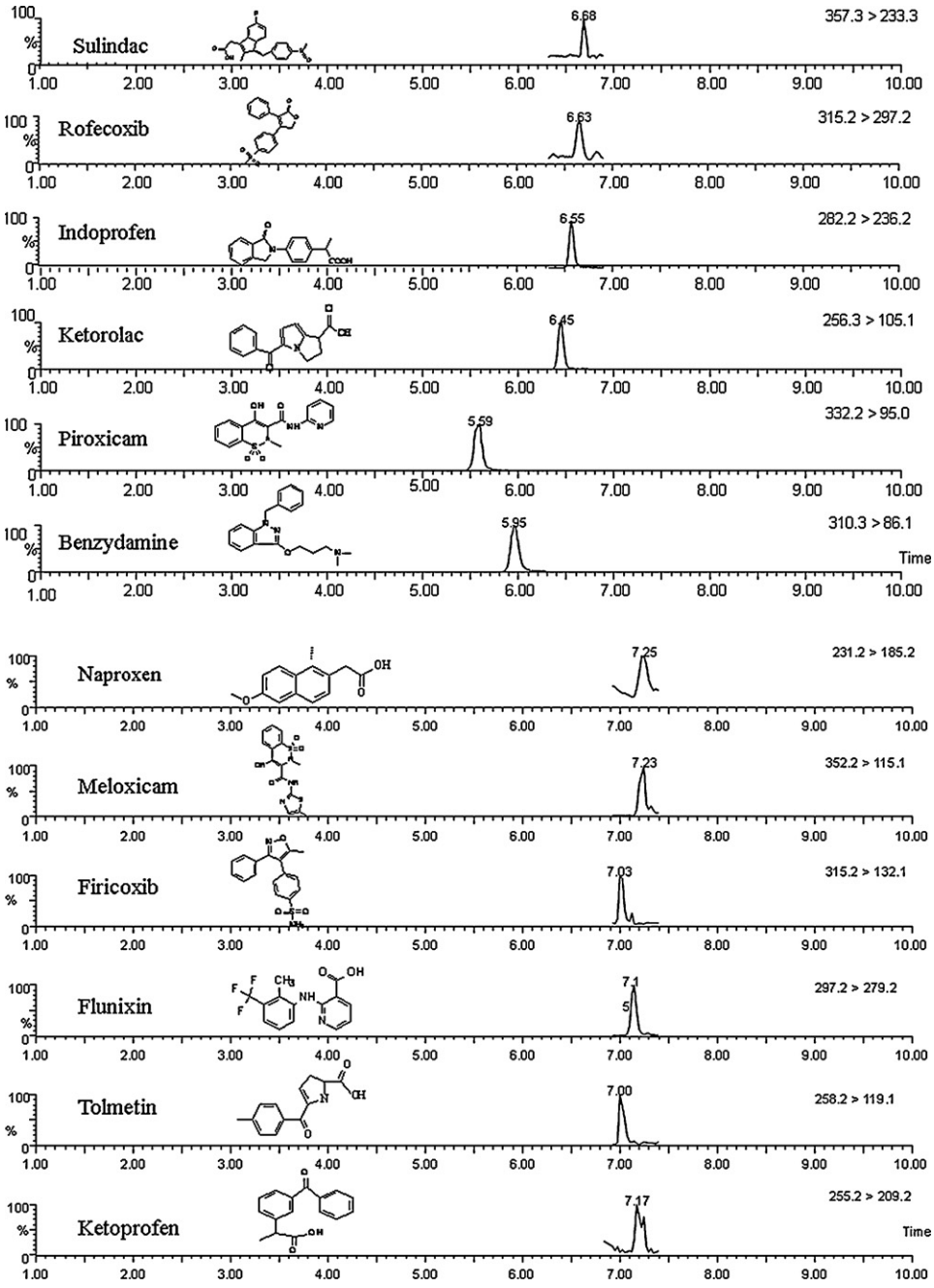

Fig. 3. (*Continued*).

chromatographic peak in both ionization modes at the same scanning speed. With the condition described above, the symmetric and sharpened peaks were retained. Typical MRM chromatograms of fortified samples are shown in Fig. 3.

3.3. Validation

3.3.1. Liquid chromatography–mass spectrometry

The criteria for retention times and ion ratios were examined. The retention time of the analyte peaks compared with standards was within the tolerance of 2.5%. Furthermore two transition ions were monitored for each of the thirty analytes. The most intense ion was used for quantitation. All ion ratios of samples were within the required tolerances of Commission Decision 2002/657/EC [38].

3.3.2. Selectivity

In order to investigate selectivity of this method according to the EC criteria [38], twenty blank muscle samples were analyzed. Blank samples of swine muscle were prepared as given in Section 2.3, then spiked with standard solution and analyzed. Compared the peak areas of analytes with standards, the majority of drugs were ion suppression due to the adverse influence of matrix effect [39], except for acetaminophen (Table 2). The signal of acetaminophen was suppressed (52.7%) without acetophenetidine, it may be a proof that acetophenetidine could hydrolyze to be acetaminophen. Finally, the matrix matched standard curves were used to

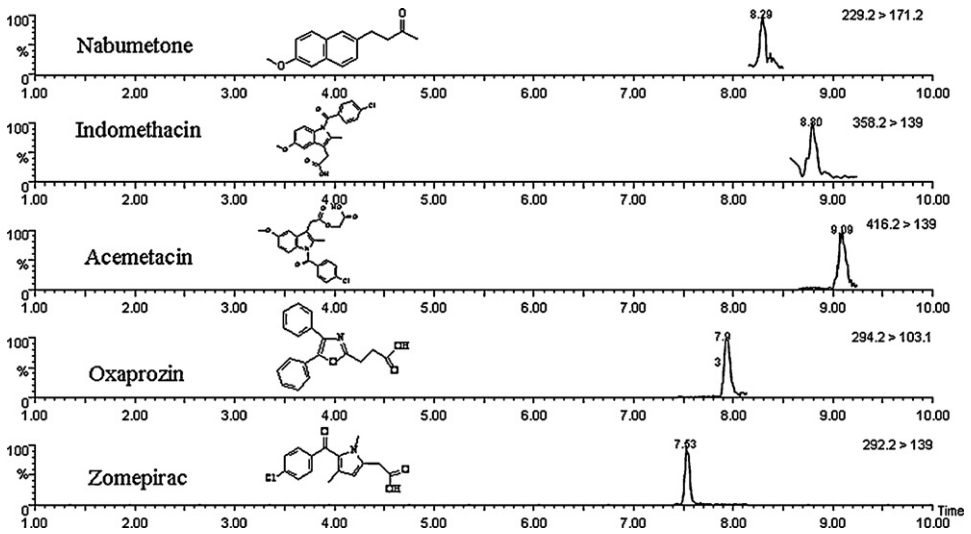

Fig. 3. (*Continued*).

Table 2
Matrix effect.

Compound	S_1 (100 μg/L)	S_2 (100 μg/L)	ME (%)	Compound	S_1 (100 μg/L)	S_2 (100 μg/L)	ME (%)
Salicylic acid	35742.2	23196.7	64.9	Rofecoxib	2619.7	1587.5	60.6
Loxoprofen	13276.5	7408.3	55.8	Sulindac	2223.0	144.5	6.5
Sasapyrin	30325.1	7490.3	24.7	Naproxen	7243.7	1318.4	18.2
Fenbufen	19818.6	11891.2	60.0	Ketoprofen	27306.3	14281.2	52.3
Nimesulide	45379.8	17561.9	38.7	Tolmetin	32020.1	19276.1	60.2
Etodolac	71498.5	9509.3	13.3	Flunixin	256755.2	170742.2	66.5
Aminoantipyrine	636466.0	77648.9	12.2	Firocoxib	18560.1	8036.5	43.3
Acetaminophen	52585.9	68046.2	129.4	Meloxicam	369874.4	80632.6	21.8
Formylaminoantipyrine	396931.0	135750.4	34.2	Oxaprozin	860142.2	141063.3	16.4
Etoricoxib	140009.3	102486.8	73.2	Zomepirac	173783.9	49876.0	28.7
Tenoxicam	43328.1	30633.0	70.7	Nabumetone	8348.9	1845.1	22.1
Mepirizole	223019.2	191796.5	86.0	Indomethacin	141687.6	62059.2	43.8
Acetophenetidine	245698.2	176657.0	71.9	Acemetacin	110196.4	16309.1	14.8
Piroxicam	65611.7	45534.5	69.4	Indoprofen	277781.4	78056.6	28.1
Benzydamine	5016752.4	3070252.5	61.2	Ketorolac	272092.8	117544.1	43.2

Note: S_1 is the peak area of standard and S_2 is the peak area of analyte. ME, absolute matrix effect; ME = $S_2/S_1 \times 100\%$.

quantification, which can effectively compensate the adverse influence of matrix constituents and perform a reliable quantitative analysis.

3.3.3. Linearity

To test the linearity of the method, matrix-matched calibration curves were prepared on each day to give 8-point calibration curves ranging from 0 to 500.0 μg/L concentration of each compound (Table 3). Correlation coefficients (r) were more than 0.9900.

3.3.4. Accuracy

The accuracy was determined through the analysis of negative samples fortified in seven replicates at the 1, 50, and 100 times of the LOQ of each compound on three separate days. Within the linearity range, three levels (1, 50 and 100 times the LOQ, Table 3) were spiked in samples. The recoveries are shown in Table 3. The results met the requirement [40].

3.3.5. Precision

Repeatability results were obtained at three spiked levels (1, 50 and 100 times of the LOQ) under uniform conditions by the same operator on one day. The within-laboratory reproducibility results were obtained at three spiked levels on three separate days, the repeatability was less than 8%, and the within-laboratory reproducibility was not more than 12.3%. The results are shown in Table 3 and within required tolerance [40].

3.3.6. Limit of detection (LOD) and limit of quantitation (LOQ)

To test LODs and LOQs of the method, twenty duplicated negative samples were fortified, pretreated according to Section 2.3, and analyzed. The LODs were the concentrations of analytes fortified, when responses of both transitions on the chromatograms were better than 3 times of signal to noise ratio (S/N), and the LOQs were 10 times. The results are shown in Table 3 and the LC–MS/MS chromatograms are shown in Fig. 3.

3.4. Application of method

The effectiveness of the presented method was proved by analyzing over 100 samples of import swine muscles. Presences of trace residues of acetaminophen, salicylic acid, ketoprofen and aminoantipyrine were confirmed in some samples. The positive rate was about 7%.

Table 3
Mean recoveries, repeatability, reproducibility, linearity range, correlation coefficients, regression equations, LOD, LOQ and retention time (RT) of NSAIDs in swine muscle ($n = 7$).

Compound	Added (μg/kg)	Mean recoveries (%)	Repeatability (%)	Reproducibility (%)	Linearity range (μg/kg)	Correlation coefficients (r^2)	Regression equation	LOD (μg/kg)	LOQ (μg/kg)	RT (min)
Salicylic acid	1.0	125.7	3.2	7.7	1.0–100.0	0.9995	$y = 693.84x - 254.314$	0.5	1.0	4.00
	50.0	85.6	4.3	6.3						
	100.0	104.2	3.1	6.6						
Loxoprofen	1.0	61.7	5.4	7.3	1.0–100.0	0.9975	$y = 379.799x + 660.811$	0.5	1.0	6.69
	50.0	109.6	5.3	12.3						
	100.0	98.3	6.5	11.5						
Fenbufen	5.0	64.0	5.9	5.9	2.0–200.0	0.9963	$y = 122.46x - 457.253$	0.5	2.0	7.22
	100.0	96.4	5.0	6.9						
	200.0	99.2	6.7	7.1						
Sasapyrin	2.0	108.4	4.2	11.2	2.0–200.0	0.9991	$y = 609.387x - 884.097$	0.5	2.0	7.24
	100.0	82.9	5.4	6.8						
	200.0	81.3	2.1	7.5						
Nimesulide	5.0	110.1	5.3	4.7	5.0–200.0	0.9961	$y = 545.256x - 2114.5$	2.0	5.0	7.78
	100.0	82.9	4.3	6.4						
	200.0	82.5	5.8	7.3						
Etodolac	2.0	103.4	6.1	6.8	2.0–200.0	0.9946	$y = 231.46x - 457.246$	0.5	2.0	8.29
	50.0	74.5	6.7	7.9						
	100.0	93.7	6.2	4.1						
Acetaminophen	1.0	99.0	3.3	7.5	1.0–100.0	0.9953	$y = 2633.76x + 8312.91$	0.4	1.0	1.37
	50.0	94.4	3.6	8.6						
	100.0	102.1	4.5	5.7						
Aminoantipyrine	1.0	95.6	7.5	8.2	1.0–100.0	0.9891	$y = 18011.9x + 128389$	0.4	1.0	1.89
	50.0	95.3	4.8	6.7						
	100.0	97.5	4.9	6.6						
Formylaminoantipyrine	1.0	67.5	5.6	5.9	1.0–100.0	0.9944	$y = 2378.13x + 6523.88$	0.4	1.0	1.91
	50.0	96.0	6.7	5.0						
	100.0	105.0	6.8	6.7						
Tenoxicam	1.5	89.4	5.3	7.8	1.0–150.0	0.9993	$y = 392.809x + 450.198$	0.5	1.5	3.60
	75.0	102.6	4.3	7.1						
	150.0	84.2	5.8	6.6						
Mepirizole	1.0	77.9	5.3	5.6	1.0–100.0	0.9892	$y = 3471.35x + 21711$	0.4	1.0	3.61
	50.0	84.5	4.3	7.2						
	100.0	86.0	5.8	5.8						
Etoricoxib	1.0	113.5	7.2	5.4	1.0–100.0	0.9953	$y = 4355.49x + 12166.3$	0.4	1.0	3.80
	50.0	105.0	4.3	7.9						
	100.0	88.9	4.5	8.2						
Acetophenetidine	1.0	95.0	5.7	7.1	1.0–100.0	0.9889	$y = 5055.49x + 31653.7$	0.4	1.0	3.83
	50.0	97.5	6.5	8.1						
	100.0	94.7	6.9	6.8						
Piroxicam	1.0	76.4	7.9	7	1.0–100.0	0.9876	$y = 369.206x + 1220.73$	0.4	1.0	5.59
	50.0	101.2	5.7	7.4						
	100.0	92.8	7.1	5.7						
Benzydamine	1.0	87.9	4.9	7.9	1.0–100.0	0.9976	$y = 66153.9x + 23171.4$	0.4	1.0	5.95
	50.0	88.5	4.2	5.2						
	100.0	93.5	6.3	6.1						

Table 3 (*Continued*)

Compound	Added (μg/kg)	Mean recoveries (%)	Repeatability (%)	Reproducibility (%)	Linearity range (μg/kg)	Correlation coefficients (r^2)	Regression equation	LOD (μg/kg)	LOQ (μg/kg)	RT (min)
Ketorolac	1.0	87.8	7.5	6.7	1.0–100.0	0.9985	$y = 4393.47x - 4196.69$	0.4	1.0	6.45
	50.0	102.4	4.8	4.7						
	100.0	91.5	4.9	6.5						
Indoprofen	1.0	77.4	5.9	7.3	1.0–100.0	0.9971	$y = 6155.42x - 15377.4$	0.4	1.0	6.55
	50.0	78.0	4.6	6.2						
	100.0	108.2	5.7	5.0						
Rofecoxib	1.0	96.4	2.6	5.7	1.0–100.0	0.9933	$y = 67.1288x + 202.092$	0.4	1.0	6.63
	50.0	101.8	2.1	6.5						
	100.0	104.8	2.7	6.9						
Sulindac	4.0	80.6	3.5	8	4.0–400.0	0.9956	$y = 66.4848x - 310.659$	1.0	4.0	6.68
	200.0	105.3	4.2	5.7						
	400.0	98.3	7.8	7.1						
Tolmetin	1.0	69.6	4.1	7.5	1.0–100.0	0.9884	$y = 368.585x - 163.176$	0.4	1.0	7.00
	50.0	98.3	7.5	4.8						
	100.0	73.5	8.6	4.9						
Firocoxib	1.5	91.8	4.6	4.9	1.0–200.0	0.9896	$y = 342.562x + 152.882$	0.5	1.5	7.03
	75.0	79.7	6.8	4.2						
	150.0	85.8	7.9	6.3						
Flunixin	1.0	106.3	5.7	5.6	1.0–100.0	0.9970	$y = 699.22x - 15.6205$	0.4	1.0	7.15
	50.0	73.1	8.2	6.7						
	100.0	86.5	6.7	6.8						
Ketoprofen	1.0	73.7	6.6	5.3	1.0–100.0	0.9974	$y = 478.866x - 1481.14$	0.4	1.0	7.17
	50.0	80.9	5.9	4.3						
	100.0	91.1	5.0	5.8						
Meloxicam	1.0	94.1	5.8	9.2	1.0–100.0	0.9788	$y = 611.111x - 3721.97$	0.4	1.0	7.23
	50.0	101.3	7.8	4.3						
	100.0	89.3	7.1	4.5						
Naproxen	1.0	78.7	7.7	5.3	1.0–100.0	0.9953	$y = 788.878x - 8761.14$	0.4	1.0	7.25
	50.0	89.9	6.6	5.3						
	100.0	93.1	5.2	4.8						
Zomepirac	1.0	75.1	6.6	7.2	1.0–100.0	0.9983	$y = 2675.52x - 7426.86$	0.4	1.0	7.53
	50.0	88.3	6.8	7.1						
	100.0	105.2	5.3	7.1						
Oxaprozin	1.0	74.2	4.3	9.2	1.0–100.0	0.9915	$y = 10721.6x - 57406.6$	0.4	1.0	7.93
	50.0	98.3	5.8	6.4						
	100.0	91.6	5.3	6.4						
Nabumetone	4.0	85.3	4.3	6.4	4.0–500.0	0.9928	$y = 164.254x - 803.974$	1.0	4.0	8.29
	200.0	86.5	5.8	9.9						
	400.0	108.4	6.3	5.4						
Indomethacin	5.0	99.2	4.2	6.6	5.0–500.0	0.9702	$y = 130.132x - 783.01$	2.0	5.0	8.80
	250.0	102.8	5.4	5.9						
	500.0	101.9	5.7	4.6						
Acemetacin	5.0	70.2	4.3	5.7	5.0–500.0	0.9928	$y = 2988.14x - 16360$	1.5	5.0	9.09
	250.0	105.5	2.3	2.6						
	500.0	98.2	4.3	6.9						

4. Conclusions

This study applied an ultra-high-performance liquid chromatography with tandem mass spectrometric detection (UHPLC–MS/MS) to establish a method for simultaneous determination of multi-residue of thirty non-steroidal anti-inflammatory drugs in swine muscle. Extraction and purification conditions for multi-residue analysis in animal original food products were in-depth discussed, based on the previous research. Proved by the actual sample analysis, this method was reliable, convenient and sensitive. Moreover, it met the requirement of residue analysis for NSAIDs.

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