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Validation of a liquid chromatography–tandem mass spectrometric method for the quantification of eight quinolones in bovine muscle, milk and aquacultured products

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Abstract

Quinolones are a group of structurally related antibacterial agents. Over the present decade there has been a significant and progressive increase in the use of this class of antibiotics in animal production. As a consequence the increased use of quinolones can promote the resistance of bacteria. To protect the consumers health, Maximum Residue Limits (MRL) have been established in edible animal matrices by the European Union.

A liquid chromatography–tandem mass spectrometric ($LC–MS^2$) method was developed and validated for the simultaneous quantification of eight quinolones at MRL level in bovine muscle, milk and aquacultured products. The studied quinolones were enrofloxacin, ciprofloxacin, sarafloxacin, danofloxacin, oxolinic acid, flumequine, difloxacin and marbofloxacin. The method involved a single solid-phase extraction followed by the analysis of all quinolones in a single chromatographic run using $LC–ESI–MS^2$. Quinine was selected as internal standard. This paper consists of two parts: the discussion of the analytical method and the discussion of the different validation parameters according to Commission Decision 2002/657/EEC.

Keywords: Quinolones; Validation; Bovine muscle; Aquacultured products and milk

1. Introduction

Quinolones are a group of structurally related antibacterial agents, which are used in human and veterinary medicine. Their general structure consists of a 1-substituted-1,4-dihydro-4-oxopyridine-3-carboxylic moiety combined with an aromatic or heteroaromatic ring (Fig. 1). Quinolones are used in veterinary medicine for the treatment of pulmonary infections, urinary infections and digestive infections [1]. They exert their therapeutic effects by inhibiting DNA gyrase within the bacterial cell. The carboxylic acid at position 3 and the ketone group at position 4 are necessary for DNA gyrase inhibition, whereas substitutions at position 1 and 7 influence the potency and biological spectrum of activity of the drugs [2]. The administration of quinolones to animals, which are destined for human consumption can result in the presence of residues in food products. These residues represent a potential hazard for the consumer and are a concern due to the emergence of drug-resistant bacteria. Over the present decade there has been a significant and progressive increase in the use of quinolones in animal production [3,4]. The European Union has set Maximum Residue Limits (MRL) for quinolones [5], with the aim of minimising the

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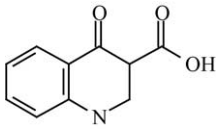

Fig. 1. Basic structure of the quinolones.

risk to human health associated with their residue consumption.

For the determination of quinolones in biological matrices several spectroscopic techniques, such as ultraviolet (UV), fluorescence or mass spectrometry (MS) are used in combination with liquid chromatography (LC). Earlier methods used UV almost exclusively [6], but more recent systems use fluorescence detection [6–19]. These procedures are, however, restricted to a limited number of quinolones. Since several years LC with MS detection has been used for confirmatory analysis because this detection method is more sensitive, selective and allows rapid and multiresidue determination in complex matrices and gives structural information [1,4,6,20–24].

In this work a LC–ESI–MS^2 multiresidue method was developed allowing the detection of eight quinolones: enrofloxacin, ciprofloxacin, sarafloxacin, danofloxacin, oxolinic acid, flumequine, difloxacin and marbofloxacin. All quinolones were analysed in a single chromatographic run at MRL level in bovine muscle, milk and tissue of aquacultured products. Previous studies only dealt with one matrix or similar matrices. Quinolones could be detected in aquacultured products or chicken tissue or milk, but no extraction and clean-up method was described which could be used for all these matrices. So each matrix required a specific method development. In this paper a simple and rapid extraction and clean-up method was developed for the different matrices bovine muscle, milk and tissue of aquacultured products. An ion trap mass spectrometer was used as identification as well as confirmation method instead of the more commonly used quadrupole mass spectrometer [1,4,20–22]. A validation was performed for each matrix and the validation parameters selectivity, linearity, accuracy, precision and decision (CCα) and detection limit (CCβ) are discussed.

2. Experimental

2.1. Reagents and chemicals

The quinolone standards, enrofloxacin and ciprofloxacin were obtained from ICN Biomedicals (Irvine, CA, USA) while flumequine and oxolinic acid were from Sigma–Aldrich (St. Louis, MO, USA), marbofloxacin from Vetoquinol (Aartselaar, Belgium) and sarafloxacin from DVK-CLO (Melle, Belgium). No standards were available for danofloxacin and difloxacin, therefore the veterinary drugs Advocin (Pfizer, UK) and Dicural 50 mg (Fort Dodge Animal Health, The Netherlands), respectively, were used. All chemicals used were of analytical grade from Merck (Darmstadt, Germany).

Stock standard solutions of 1000 ng μl^{-1} were prepared in ethanol for enrofloxacin, danofloxacin, difloxacin and marbofloxacin; in HPLC–water for ciprofloxacin and in 0.1 M NaOH for flumequine, oxolinic acid and sarafloxacin. For the preparation of working solutions HPLC–water was used. All standard and working solutions were stored at −20 °C.

2.2. Instrumentation

The HPLC apparatus comprised of a 1100 series quaternary pump and an autosampler of Hewlett Packard (Palo Alto, CA, USA). Chromatographic separation was achieved using a Symmetry C_{18} column (5 μm, 150 mm × 2.1 mm, Waters, Milford, USA). The mobile phase consisted of a mixture of methanol with 0.1% trifluoroacetic acid (A) and water with 0.1% trifluoroacetic acid (B). A linear gradient was run (20% A for 5 min and increasing to 100% in the next 10 min) at a flow rate of 0.3 ml min^{-1}.

Liquid chromatography–tandem mass spectrometric (LC–MS^2) detection was carried out with a ThermoFinnigan LCQ Deca ion trap with electrospray ionisation (ESI) interface in positive ionmode (San José, CA, USA). The MS detector was operated in three segments each divided in different scan events (Table 1), so the quinolones were separated both chromatographically and massspectrometrically.

Table 1
Instrument parameters precursor ion, isolation width, collision energy and mass range of the the LC–MS^2 method for the detection of quinolones

	Precursor ion, isolation width, collision energy	Analyte
Segment 1		
Scan event 1	325, 2, 37 (100–330)	Quinine = IS
Scan event 2	363, 2, 30 (200–370)	Marbofloxacin
Segment 2		
Scan event 1	360, 2, 30 (200–370)	Enrofloxacin
Scan event 2	332, 2, 30 (200–340)	Ciprofloxacin
Scan event 3	386, 2, 35 (200–390)	Sarafloxacin
Scan event 4	358, 2, 30 (200–365)	Danofloxacin
Segment 3		
Scan event 1	262, 2, 30 (200–280)	Flumequine, oxolinic acid
Scan event 2	276 (200–500)	Flumequine, oxolinic acid

2.3. Extraction and clean-up

2.3.1. Bovine muscle/aquacultured products

To an amount of 2 g of minced tissue 100 μg kg^{-1} quinine was added as internal standard. The quinolones were extracted from the tissue using 20 ml ultrapure water. After mixing and centrifugation (5 min, 5500 rpm) only 10 ml supernatant was used for further clean-up. The clean-up was carried out using a Isolute 500 mg C_{18} SPE Cartridge (IST International, Mid Glamorgan, UK). The columns were conditioned with 2 ml MeOH and 4 ml water. After application of the extract, the cartridge was rinsed with 2 ml MeOH/water (20:80), 2 ml hexane and vacuum dried. The quinolones were eluted from the column with 3 ml 1% trifluoroacetic acid in acetonitrile. The eluate was evaporated to dryness at 45 °C under a stream of nitrogen. The residues were reconstituted in 30 μl methanol with 0.1% trifluoroacetic acid and 120 μl water with 0.1% trifluoroacetic acid before injecting 15 μl on the HPLC column.

2.3.2. Milk

To an amount of 2 ml milk 100 μg kg^{-1} quinine was added as internal standard. To precipitate the proteins present in the milk, 2.5 ml trichloroacetic acid (20% in methanol) was added. After mixing and centrifugation (10 min, 5500 rpm) the quinolones were extracted from the supernatant using 10 ml ultrapure water. The entire supernatant was used for further clean-up after mixing and centrifugation (10 min, 5500 rpm). The clean-up was analogous to the one described for muscle and aquacultured products.

3. Results and discussion

3.1. LC–MS^2 method

Most methods for the detection of quinolones have been designed for the analysis of individual quinolones or for only two or three compounds, although more recently a number of multiresidue methods have been developed. The method described in this paper is a multiresidue method able to simultaneously detect eight quinolones.

Since most quinolones are fluorescent, liquid chromatography with fluorescence detection is mainly used as determination method for routine residue analysis. Fluorescence depends strongly on the pH of the medium. The highest fluorescence is obtained at a pH value from 2.5 to 4.5, whereas the anionic species do not generally show native fluorescence. Marbofloxacin has a poor native fluorescence and therefore has almost exclusively been determined with UV detection. In this paper the more sensitive, specific and selective detection method ion trap mass spectrometry was chosen. Eight different quinolones, in which marbofloxacin, could be determined with this detection method in a single chromatographic run. Most mass spectrometry methods for the identification of quinolones used a quadrupole mass spectrometer that only monitored specific transitions (precursor ion–product ion) of each quinolone. In this paper an ion trap mass spectrometer was used as identification as well as confirmation method. So the full scan MS^2 mass spectrum of each quinolone was recorded which gave more structural information.

The standards enrofloxacin, ciprofloxacin, sarafloxacin, danofloxacin, oxolinic acid, flumequine, difloxacin, marbofloxacin and the internal standard quinine were spiked to blank tissue (bovine muscle and shrimp) and blank milk at the MRL concentration of each quinolone (Table 2). Fig. 2 shows the ion chromatograms of the different quinolones in milk. Similar ion chromatograms were obtained for the matrices bovine muscle and shrimp. Fig. 2 shows all quinolones at their MRL concentration.

3.2. Specificity

The specificity of the method could be demonstrated by LC–MS^2 analysis of blank bovine muscle, blank shrimp muscle and blank milk. No interferences were observed after analysis of these blank samples and after analysis of spiked matrices with all eight quinolones.

Table 2

Pharmacologically active substance	Animal species	MRL (μg kg^{-1})	Target tissue
Enrofloxacin (enrofloxacin + ciprofloxacin)	Bovine	100	Muscle
		100	Milk
	Ovine	100	Muscle
	Porcine	100	Muscle
	Poultry	100	Muscle
Sarafloxacin	Salmonidae	30	Muscle
Danofloxacin	Bovine	200	Muscle
		30	Milk
	Porcine	100	Muscle
	Chicken	200	Muscle
Oxolinic acid	Bovine	100	Muscle
	Porcine	100	Muscle
	Chicken	100	Muscle
	Fin fish	100	Muscle
Flumequine	Bovine	200	Muscle
		50	Milk
	Ovine	200	Muscle
	Porcine	200	Muscle
	Chicken	400	Muscle
	Turkey	400	Muscle
	Salmonidae	600	Muscle
Difloxacin	Bovine	400	Muscle
	Porcine	400	Muscle
	Chicken	300	Muscle
	Turkey	300	Muscle
Marbofloxacin	Bovine	150	Muscle
		75	Milk
	Porcine	150	Muscle

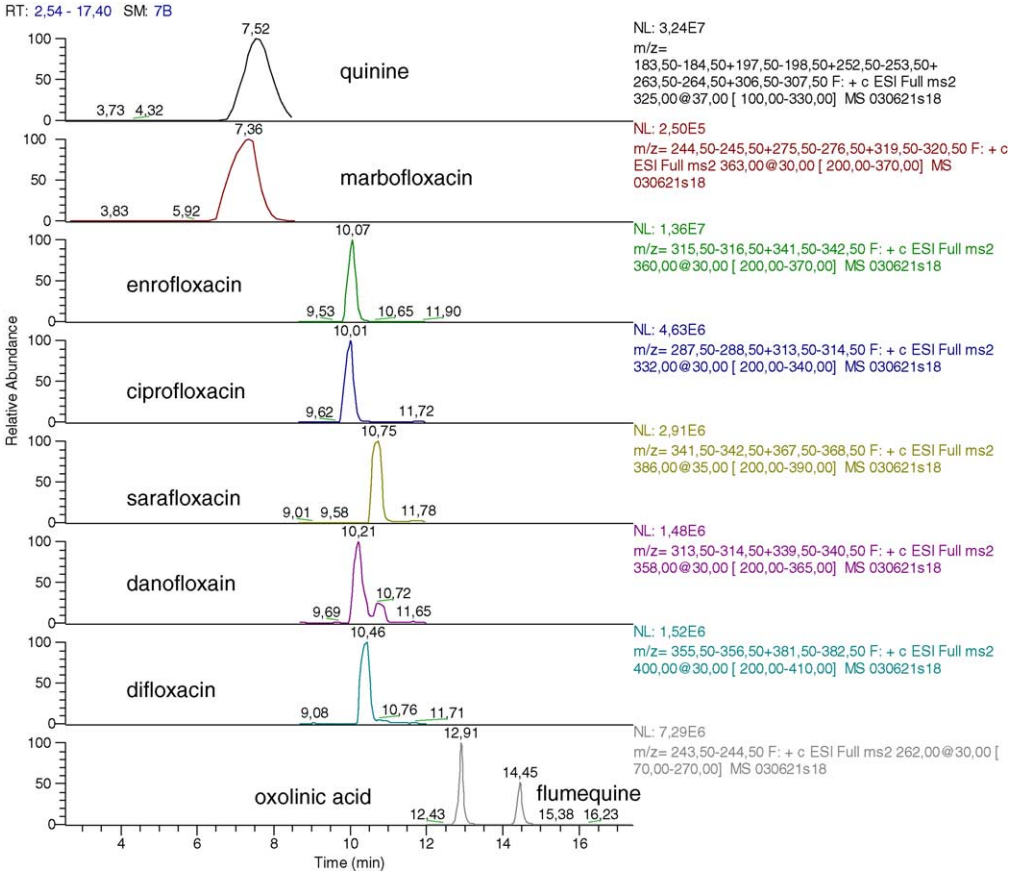

Fig. 2. Ion chromatograms of quinine (IS), marbofloxacin, enrofloxacin, ciprofloxacin, sarafloxacin, danofloxacin, oxolinic acid and flumequine in milk.

3.3. Selectivity

Quinolones are veterinary drugs with a MRL, so the minimum number of identification points (IP) is set to three. LC–MS^n precursor ions earn 1 IP and LC–MS^n product ions earn 1.5 IP [25].

MS^2-full scan of the pseudo-molecular ion of the quinolones enrofloxacin, ciprofloxacin, sarafloxacin, danofloxacin and difloxacin each showed two product ions; a loss of 18 due to the loss of water and a loss of 44 due to the loss of COO^- (Fig. 3). Fragmentation of the pseudo-molecular ion *m*/*z* 262 of the quinolones oxolinic acid and flumequine only showed the product ion *m*/*z* 244, due to the loss of water (Fig. 4). So 2.5 IP were earned. Therefore the ion with *m*/*z* 276 in MS-full scan was also used as a precursor ion, so 3.5 IP were earned. The ion with *m*/*z* 276 is an adduct ion of the pseudo-molecular ion with *m*/*z* 262. A mass of 14 was added to the pseudo-molecular ion. The origin of this adduct ion is unclear. The addition of mass 14 has not yet been mentioned in the literature. MS^2-full scan of the ion with *m*/*z* 276 showed the ion with *m*/*z* 262, so after fragmentation of the adduct ion the pseudo-molecular ion was revealed. Hence, the ion with *m*/*z* 276 is clearly an adduct ion and not an impurity since fragmentation of this ion revealed the same product ions as fragmentation of the pseudo-molecular ion with *m*/*z* 262. If a sample contains flumequine or oxolinic acid MS^3-full scan of the ion with *m*/*z* 262 will be obtained in an extra run for the identification of these quinolones (Fig. 5). MS^2-fragmentation of the

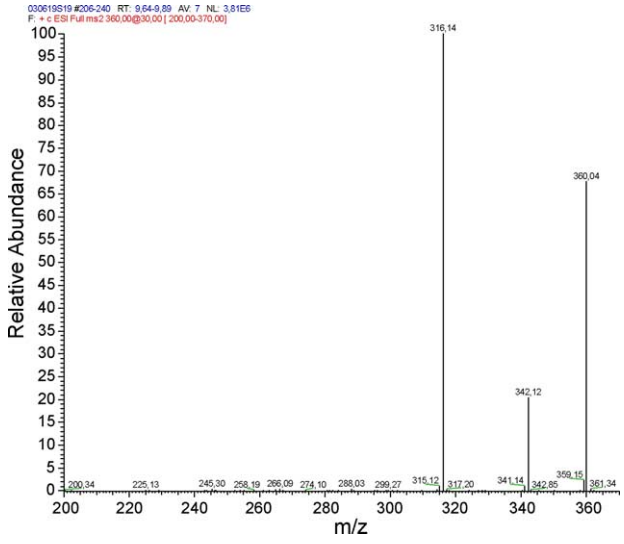

Fig. 3. MS^2-full scan of enrofloxacin spiked to blank milk at a concentration of 100 μg kg^{-1}.

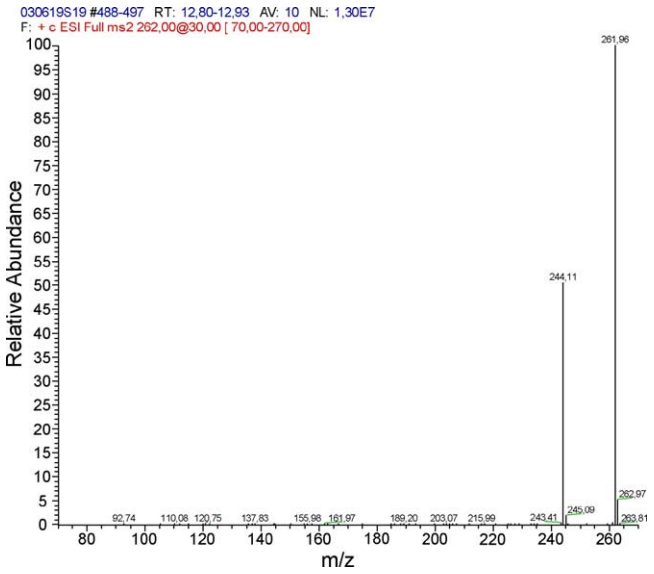

Fig. 4. MS^2-full scan of oxolinic acid and flumequine spiked to blank milk at a concentration of 50 μg kg^{-1}.

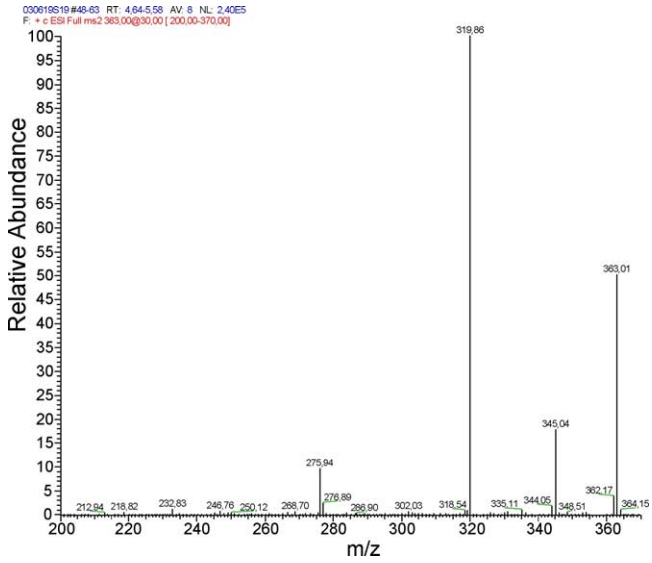

Fig. 6. MS^2-full scan of marbofloxacin spiked to blank milk at a concentration of 75 μg kg^{-1}.

ion *m/z* 363 of the quinolone marbofloxacin had a typical MS^2-mass spectrum with three product ions, *m/z* 276, 320 and 345 (Fig. 6). In the MS^2-mass spectra of all the quinolones the precursor-ion was still clearly present. There was no improvement by increasing the collision energy. This phenomenon could not be explained.

In Table 3 the specific precursor ions, product ions and the IP of each quinolone are summarised.

3.4. Calibration curve

The chromatographic peak areas, used for the quantification were calculated from the extracted ion chromatograms of the most abundant product ions. These product ions are shown in the legend of Fig. 2.

The calibration curves obtained for the spiked bovine muscle, aquaculture and milk samples were linear in the concentration range 1/2 MRL to 2× MRL for the eight quinolones. However, flumequine in shrimp was an exception. The MRL in aquaculture was 600 μg kg^{-1}. This high concentration can cause space charging in the ion trap. A possible consequence is a non-linear calibration curve. Therefore, samples containing flumequine need to be diluted before quantification. The coefficients of determination were higher than 0.98 for bovine muscle, 0.96 for shrimp (except enrofloxacin, 0.91; and difloxacin, 0.94) and 0.97 for milk.

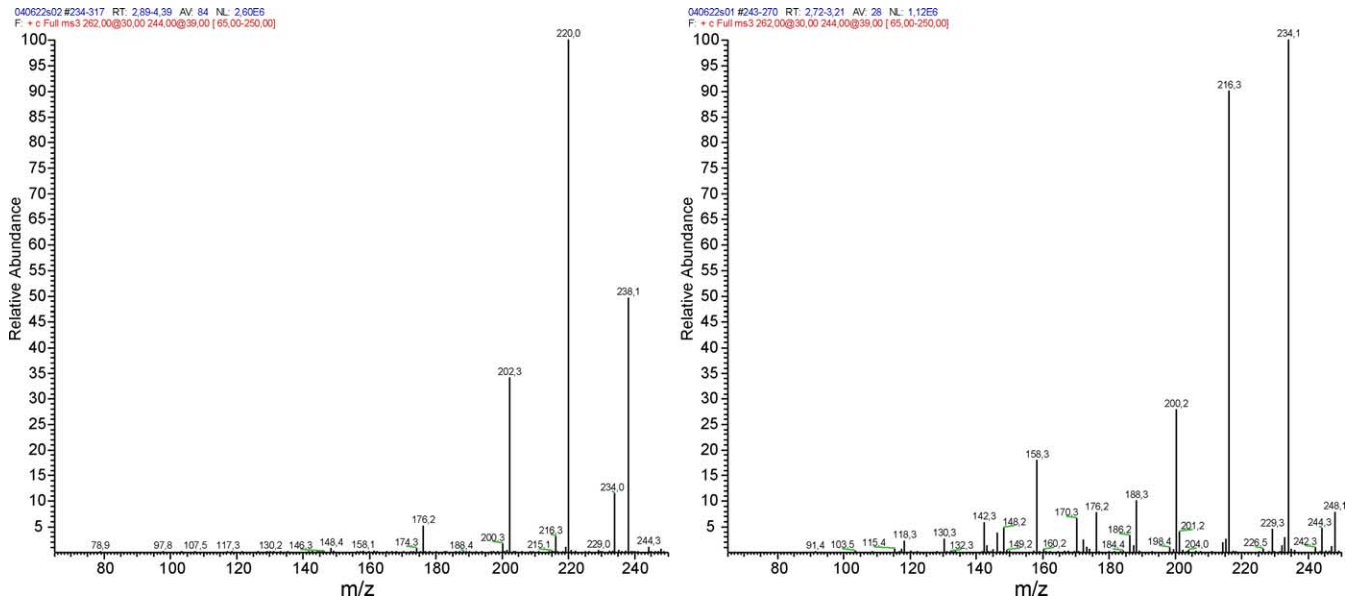

Fig. 5. MS^3-full scan of flumequine (left) and oxolinic acid (right).

Table 3
Summary of the selectivity criteria of the different quinolones

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Identification points
Enrofloxacin	360	316, 342	4
Ciprofloxacin	332	288, 314	4
Sarafloxacin	386	342, 368	4
Danofloxacin	358	314, 340	4
Oxolonic acid	262, 276	244	3.5
Flumequine	262, 276	244	3.5
Difloxacin	400	356, 382	4
Marbofloxacin	363	276, 320, 245	5.5

3.5. Accuracy

The accuracy of the method was evaluated at the MRL concentration. For samples spiked at a concentration above 10 $\mu g\,kg^{-1}$, the accuracy of a confirmation method should range from 80 to 110% [25]. Five blank samples were spiked at the MRL concentration for each quinolone and for each matrix. All these samples had an accuracy within the permitted range. In Tables 4–6 the accuracies are summarised for the different quinolones in each matrix. In bovine muscle the accuracies lay within the acceptable range 93–110%, in shrimp between 86 and 107% and in milk between 86 and 102%.

3.6. Precision

The precision of the method was evaluated at the MRL concentration. The coefficient of variation (CV) for the repeated analysis of spiked material, should not exceed the level calculated by the Horwitz equation [25]. For mass fractions lower than 100 $\mu g\,kg^{-1}$ the application of the Horwitz equation gave unacceptable high values. Therefore the CV for concentrations lower than 100 $\mu g\,kg^{-1}$ should be as low as possible. In that case 23% was taken as a guideline for the coefficient of variation (CV at 100 $\mu g\,kg^{-1}$ = 23%). So, 30 spikes for each matrix were analysed and their concentration was determined with the calibration curve. The coefficient of variation was calculated and was lower than the permitted CV. In Tables 4–6 the CV's are summarised for the different quinolones in each matrix. In bovine muscle the CV's for the different quinolones were lower than 17%; the CV's of enrofloxacin and ciprofloxacin were even 7%. All the coefficients of variation in shrimp were lower than 18%. In milk very low CV's were obtained (lower than 11%), except for marbofloxacin (CV 13%) and difloxacin (CV 16%).

3.7. Decision limit (CCα)

The decision limit is the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant. The calculated average concentration of the 30 samples used to determine the precision, plus 1.64 times the corresponding standard deviation equalled the decision limit (α = 5%). In Tables 4–6 the CCα's are summarised for the different quinolones in each matrix.

The CCα of danofloxacin and difloxacin in bovine muscle can seem rather low looking at their MRL-values in Table 2. In these cases bovine muscle was spiked with the lowest MRL concentration of muscle in general. So danofloxacin was spiked at 100 $\mu g\,kg^{-1}$ (MRL porcine) and difloxacin was spiked at 300 $\mu g\,kg^{-1}$ (MRL turkey, chicken). In the meanwhile a mini-validation was performed for bovine

Table 4
The validation parameters recovery, coefficient of variation, CCα and CCβ for the different quinolones in muscle

Muscle	Enrofloxacin	Ciprofloxacin	Sarafloxacin	Danofloxacin	Oxolinic acid	Flumequine	Difloxacin	Marbofloxacin
MRL ($\mu g\,kg^{-1}$)	100	100	30	100	100	200	300	150
Accuracy (%)	98	97	93	110	108	110	102	103
CV (%)	7	7	14	16	17	14	14	15
CCα ($\mu g\,kg^{-1}$)	111	113	36	123	121	239	361	181
CCβ ($\mu g\,kg^{-1}$)	123	125	43	150	147	285	432	218

Table 5
The validation parameters recovery, coefficient of variation, CCα and CCβ for the different quinolones in aquaculture

Aquaculture	Enrofloxacin	Ciprofloxacin	Sarafloxacin	Danofloxacin	Oxolinic acid	Difloxacin	Marbofloxacin
MRL ($\mu g\,kg^{-1}$)	100	100	30	100	300	300	150
Accuracy (%)	103	102	98	88	107	88	86
CV (%)	14	7	11	14	11	18	13
CCα ($\mu g\,kg^{-1}$)	125	112	36	124	350	412	95
CCβ ($\mu g\,kg^{-1}$)	148	124	41	148	404	505	111

Table 6
The validation parameters recovery, coefficient of variation, CCα and CCβ for the different quinolones in milk

Milk	Enrofloxacin	Ciprofloxacin	Sarafloxacin	Danofloxacin	Oxolinic acid	Flumequine	Difloxacin	Marbofloxacin
MRL ($\mu g\,kg^{-1}$)	100	100	50	30	50	50	50	75
Accuracy (%)	102	101	94	95	90	99	82	86
CV (%)	6	6	7	11	9	8	16	13
CCα ($\mu g\,kg^{-1}$)	110	110	56	36	58	57	65	95
CCβ ($\mu g\,kg^{-1}$)	120	119	62	42	65	63	78	111

muscle, porcine muscle and chicken muscle, each at their corresponding MRL concentrations. On the other hand, oxolinic acid has a very high CCα in aquaculture. At the moment this validation was performed, the MRL for oxolinic acid in fin fish was 300 $\mu g\,kg^{-1}$. This concentration is now lowered till 100 $\mu g\,kg^{-1}$.

For those quinolones that do not have a MRL for one of the matrices discussed the same concentration was applied for bovine muscle and shrimp. For milk a concentration of 50 $\mu g\,kg^{-1}$ was applied.

The CCα's for all quinolones gave acceptable values looking at their MRL concentration, only difloxacin was rather overrated in the different matrices.

3.8. Detection limit (CCβ)

The detection capability is the smallest content of the compound that may be detected, identified and quantified with an error probability of β. CCβ was calculated as the decision limit CCα plus 1.64 times the corresponding standard deviation (β = 5%), supposing that $\sigma_{CC\alpha}$ equals σ_{MRL}. In Tables 4–6 the CCβ's are summarised for the different quinolones in each matrix.

4. Conclusion

A LC–ESI–MS^2 multiresidue method was developed that was able to simultaneously identify and quantify eight quinolones in bovine muscle, tissue of aquacultured products and milk. A simple and rapid extraction and clean-up method was used for the three different matrices. All quinolones were detectable at their MRL concentration and lower. Mass spectrometry was chosen as detection method because this detection method is more sensitive and selective than fluorescence detection. An ion trap mass spectrometry was used as identification as well as confirmation method.

The different validation parameters discussed in this paper were determined for the matrices bovine muscle, shrimp and milk. Meanwhile, a mini-validation was performed for chicken muscle, porcine muscle and different fish types to expand our range of matrices. Quantification was possible in the concentration range 1/2 MRL to 2× MRL because the calibration curves for most quinolones were linear in this range. Only the coefficients of determination for enrofloxacin and difloxacin in shrimp were rather low, less than 0.95. The specificity and selectivity of the LC–MS^2 method were studied. The accuracy and precision of the method were demonstrated since all accuracies were present in the permitted range from 80 to 110% and none of the coefficients of variation did exceed the level calculated by the Horwitz equation or 23 for mass fractions lower than 100 $\mu g\,kg^{-1}$. Using the data of the precision measurements, the decision limit and detection limit could be calculated.

The multi-residue method was validated for the identification and quantification of eight quinolones at MRL-level in bovine muscle, shrimp and milk in correspondence with the criteria of Commission Decision 2002/657/EEC.

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References

[1] B. Delépine, D. Hurtaud-Pessel, Proceedings of the Euroresidue IV, Veldhoven, The Netherlands, 2000, pp. 350–355.
[2] C.K. Holtzapple, S.A. Buckley, L.H. Stanker, J. Chromatogr. B 754 (2001) 1–9.
[3] D. Barron, E. Jiménez-Lozano, S. Bailac, J. Barbosa, Anal. Chim. Acta 477 (2003) 21–27.
[4] B. Toussaint, G. Bordin, A. Janosi, A.R. Rodriguez, J. Chromatogr. A 976 (2002) 195–206.
[5] Annexes I to IV of Council Regulation n°2377/90.
[6] J.A. Hernandez-Arteseros, J. Barbosa, R. Compaño, M.D. Prat, J. Chromatogr. A 945 (2002) 1–24.
[7] B. Roudaut, J.-C. Yorke, J. Chromatogr. B 780 (2002) 481–485.
[8] M. Ramos, A. Aranda, E. Garcia, T. Reuvers, H. Hooghuis, J. Chromatogr. B. 789 (2003) 373–381.
[9] I. Pecorelli, R. Galarina, R. Bibi, Al. Floridi, E. Casciarri, A. Floridi, Anal. Acta Chim. 483 (2003) 81–89.
[10] H. Pouliquen, M.L. Morvan, Food Addit. Contam. 19 (2002) 223–231.
[11] J.C. Yorke, P. Froc, J. Chromatogr. A 882 (2000) 63–77.
[12] S.M. Plakas, K.R. El-Said, F.A. Bencsath, S.M. Musser, C.C. Walker, J. AOAC Int. 82 (1999) 614–619.
[13] O.R. Idowu, J.O. Peggins, J. Pharm. Biomed. Anal. 35 (2004) 143–153.
[14] J.H. Shim, J.Y. Shen, M.R. Kim, C.J. Lee, I.S. Kim, J. Agric. Food Chem. 51 (2003) 7528–7532.
[15] J.E. Roybal, C.C. Walker, A.P. Pfenning, S.B. Turnipseed, J.M. Storey, S.A. Gonzales, J.A. Hurlbut, J. AOAC Int. 85 (2002) 1293–1301.
[16] M.A. Garcia, C. Solans, E. Hernandez, M. Puig, M.A. Bregante, Chromatographia 54 (2001) 191–194.

[17] M.J. Schneider, D.J. Donoghue, J. AOAC Int. 83 (2000) 1306–1312.
[18] J.E. Roybal, A.P. Pfenning, S.B. Turnipseed, C.C. Walker, J.A. Hurlbut, J. AOAC Int. 80 (1997) 982–987.
[19] M.D. Marazuela, M.C. Moreno-Bondi, J. Chromatogr. A 1034 (2004) 25–32.
[20] L. Johnston, L. Mackay, M. Craft, J. Chromatogr. A 982 (2002) 97–109.
[21] B. Delépine, D. Hurtaud-Pessel, P. Sanders, Analyst 123 (1998) 2743–2747.
[22] G. van Vyncht, A. Janosi, G. Bordin, B. Toussaint, G. Maghuin-Rogister, E. De Pauw, A.R. Rodriguez, J. Chromatogr. A 952 (2002) 121–129.
[23] S.B. Turnipseed, J.E. Roybal, A.P. Pfenning, P.J. Kijak, Anal. Chim. Acta 483 (2003) 373–386.
[24] M.J. Schneider, D.J. Donoghue, J. Chromatogr. B 780 (2002) 83–92.
[25] Commision Decision 2002/657/EEC, Off. J. Eur. Comm. no L 221, 23–33.