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Simple Confirmatory Method for the Determination of Erythromycin Residues in Trout: A Fast Liquid−Liquid Extraction Followed by Liquid Chromatography−Tandem Mass Spectrometry

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In recent years, erythromycin has received considerable attention for its therapeutic efficacy against some bacterial kidney diseases in aquaculture and, therefore, suitable and sensitive analytical methods to monitor erythromycin residues in fish are required. A fast sample treatment followed by an LC-ESI-MS/MS method is described for the purification, identification, and quantification of erythromycin A residues in fish. After two extractions with acetonitrile, samples were defatted with *n*-hexane, filtered, and analyzed by tandem mass spectrometry. Three characteristic transition reactions (*m*/*z* 734 → 716, 734 → 576, and 734 → 558) in multiple reaction monitoring were tested for the determination and confirmation of erythromycin A. The method was in-house validated through the determination of precision, accuracy, specificity, stability, calibration curve, decision limit (CCα), and detection capability (CCβ), in accordance with European Commission Decision 657/2002. The coefficients of variation ranged from 1.8 to 9.4% and from 7.5 to 10.9% for intra- and interday repeatability, respectively. Recovery data were also satisfactory, with values varying from 85 to 97%. The method was specific, stable, and robust enough for the required purposes. The calibration curve showed a good linearity in the whole range of the tested concentrations (0−1000 μg kg^{-1}) with a correlation coefficient (r^2) equal to 0.9956. CCα and CCβ were found to be 220 and 238 μg kg^{-1}, respectively.

KEYWORDS: Antibiotics; macrolides; erythromycin A; fish; trout; quantification; confirmation; LC-MS/MS

INTRODUCTION

In recent decades, the international trade of aquaculture products has been growing continuously, and this positive trend is expected to continue in the future (*1*). The variety of farmed aquatic species has expanded, and it is now diversifying and intensifying. At the same time, the increase of diseases, due to intensive aquaculture growth and market globalization, has required a more widespread use of veterinary drugs and chemicals. Nonetheless, relatively few drugs are approved for their use in aquaculture to date. Therefore, fish farmers might use off-label or banned active substances. Certainly, cooperative efforts should be made in the European Union (EU) to gain needed approvals for drugs to be used in aquaculture.

Fish farming is rapidly expanding in the Mediterranean area, Italy included. As in other countries, such growth has been accompanied by recurrent problems with bacterial infectious diseases. Trout represents 80% of Italian aquaculture production, and it is very susceptible to infection from *Lactococcus garvieae*, an emerging zoonotic pathogen that has been isolated from various species of fish, from cattle, and from humans (*3*). The few antibacterial drugs registered for aquaculture in Italy (i.e., amoxycyllin, flumequine, sulfamerazine, sulfadiazine, oxytetracycline, and chlortetracycline) are efficient against Gram-negative bacteria, but inadequate against Gram-positive cocci such as *L. garvieae*.

Erythromycin is a suitable drug against infection from *L. garvieae* and other systemic bacterial infections. For this reason, in recent years, it has received growing attention for its potential efficacy in fish therapy (*3*). Despite this, in Italy, the administration of erythromycin in aquaculture is possible only in an off-label regimen, the drug not yet being registered for its use in fish.

The use of antibiotics and chemotherapeutic agents in animals reared for human consumption should be based on toxicological and pharmacokinetic data obtained in the specific animal species considered. Some microbiological, metabolic, and pharmacokinetic aspects of erythromycin have been reported in previous studies; however, very few data on erythromycin pharmacokinetics in different fish species are available (*4*, *5*).

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The European Union (EU) Commission has established a maximum residue level (MRL) of 200 μg kg^{-1} erythromycin in tissues (muscle plus skin in natural proportion) for fish in general (*6*).

Within this framework, there is a demand for suitable and sensitive analytical methods to monitor erythromycin residues in fish and to establish withdrawal times in fish after pharmacological treatments.

Several methods for the determination of macrolide residues in food by liquid chromatography with ultraviolet or fluorescence detection are available in the literature (*7*−*10*); however, they showed interfering peaks and/or poor sensitivity when tested in our laboratory. Other chromatographic methods with electrochemical detection have a discrete specificity and sensitivity and a quantitation limit adequate to MRL (*11*, *12*), but they do not fulfill the guidelines, laid down by European Commission Decision 2002/657/EC, concerning the performance of analytical methods and the interpretation of results (*13*).

In recent years, liquid chromatography coupled to mass spectrometry or tandem mass spectrometry (LC-MS or LC-MS/MS) has replaced older techniques for the analysis of macrolides in different food matrices (*12*, *14*−*23*). In particular, LC-MS/MS in multiple reaction monitoring (MRM) mode is a suitable technique for the identification and quantification of macrolides at trace levels. Among the several LC-MS/MS methods developed for the analysis of erythromycin A in food, only one of them has been tested in fish (*14*). The authors of this work conclude their paper by remarking on the need for an improved sample cleanup and a suitable surrogate internal standard for a more accurate quantification of erythromycin A in fish.

Therefore, the aim of the present work was to develop and optimize an LC-MS/MS method to identify and quantify erythromycin A residues in trout. The method was validated according to European Commission Decision 2002/657/EC (*13*).

MATERIALS AND METHODS

Chemical and Reagents. Ammonium acetate, *n*-hexane, and acetic acid were of analytical reagent grade and purchased from J. T. Baker (Florence, Italy). Acetonitrile of HPLC grade was also purchased from J. T. Baker. Water was purified in a Milli-Q system from Millipore (Milan, Italy). Erythromycin A dihydrate (purity 99.1%) was purchased from Sigma (Milan, Italy). Internal standard [$^{13}C_2$]erythromycin A (*N*,*N*-dimethyl[$^{13}C_2$], chemical purity = 96.8%, isotopic purity = 92.1%) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA).

Individual standard stock solutions (1 mg mL^{-1}) were prepared in acetonitrile; the solutions were stored at −20 °C, and they were stable for ∼2 weeks. Individual and composite working standard solutions were prepared weekly by appropriate dilution of the standard stock solutions with acetonitrile; the solutions were stored at 4 °C, and they were stable for at least 1 week.

Sample Treatment. Muscle plus skin in natural proportion from rainbow trout reared in circular tanks was collected and frozen until time for analysis. A rapid and simple liquid extraction and cleanup procedure was developed. In contrast with previously published studies, no solid-phase extraction (SPE) step was found to be necessary (*13*−*15*). The sample pretreatment procedure was similar to the one described by Guyonnet et al. (*16*) except for some modifications. Briefly, a 10 mg kg^{-1} solution (100 μL) of internal standard [$^{13}C_2$]erythromycin and, after a few minutes, 1 mL of distilled water were added to the homogenized tissue sample (∼1 g). After agitation on a vortex mixer for 20 s, 4 mL of acetonitrile was added, and the solution was agitated on a vortex mixer for another 2 min. After sonication for 5 min at room temperature, the solution was centrifuged at 10000*g* for 8 min. The supernatant was transferred into a 12-mL tube, and the residue was extracted with a fresh portion of acetonitrile (5 mL). After agitation, sonication, and centrifugation, the resulting supernatant was added to the first one, and 1 mL of the combined extracts was defatted with 2 mL of *n*-hexane. The sample was filtered through a 0.45-μm-pore-size nylon filter, and the final solution was injected into the LC-MS/MS.

Liquid Chromatography−**Tandem Mass Spectrometry.** Analyses were performed with an Agilent (Palo Alto, CA) liquid chromatograph assembled with an 1100 series LC quaternary pump, a micro vacuum degasser, an autosampler, and a column oven. Chromatographic separation was obtained by means of a reversed-phase Symmetry C18 column 150 × 4.6 mm, 3.5 μm (Waters, Milford, MA) at room temperature. The eluents were 1 mM ammonium acetate containing 0.1% acetic acid (eluent A) and acetonitrile (eluent B), under gradient conditions at a flow rate of 300 μL min^{-1}. The elution gradient consisted of the following steps: at 0 min, A at 80%; at 7 min, A at 0%; at 15 min, A at 0%; at 18 min, A at 80%; at 25 min, A at 80%. The injection volume was 10 μL, and the run time was 20 min.

Mass spectral analyses were performed on an Applied Biosystems API 3000 triple-stage quadrupole mass spectrometer (Toronto, ON, Canada), equipped with a turbo ion spray interface operating in the positive ion selection mode and set at 400 °C, with the spray voltage set at 4 kV.

Detection and quantification of erythromycin A were performed by MRM of the protonated precursor ion and related product ions. For quantification, the internal standard method with peak area ratio was used. Because, to the best of our knowledge, no deuterated erythromycin A standard is available to date, [$^{13}C_2$]erythromycin A was considered as a possible internal standard, rather than roxithromycin, which has been used in other studies. [$^{13}C_2$]Erythromycin A, compared to roxithromycin, has the advantage of coeluting with erythromycin A, so that the two molecules can be analyzed in very similar ionization environments. Indeed, it is well-known that, if analyte and internal standard have even slightly different retention times, they can find very different ionization environments, yielding poor accuracy. On the other hand, the two drawbacks of using [$^{13}C_2$]erythromycin A as internal standard are (1) the interference given by the percent (11.5%) of [$^{13}C_2$]-erythromycin A naturally present in erythromycin A on the peak area signal of the internal standard and (2) the small mass shift (equal to 2) between the internal standard and the analyte. Both problems were overcome by exploiting the natural average abundance (11.5%) of [$^{13}C_4$]-erythromycin A in [$^{13}C_2$]erythromycin A, that is, by using as internal standard the amount of [$^{13}C_4$]erythromycin A naturally present in [$^{13}C_2$]-erythromycin A. In this manner, the interference on the internal standard is minimized to the natural abundance (<1%) of [$^{13}C_4$]erythromycin A contained in erythromycin A. Moreover, the mass shift between internal standard and erythromycin A is now large enough (equal to 4).

A solution of 100 μg L^{-1} erythromycin A in 1 mM ammonium acetate containing 0.1% acetic acid/acetonitrile (80:20, v/v) was directly infused at a flow rate of 10 μL min^{-1}. The protonated precursor ion $(M + H)^+$ was followed at *m*/*z* 734.5 in positive ion mode. The instrument tuning was carried out using the automatic tuning tool of Analyst 1.4 software (MDS-Sciex, Toronto, ON, Canada) to determine declustering, focusing and entrance potentials, fragmentation pattern, collision energy, and collision cell exit potential. Quadrupoles Q1 and Q3 were set on unit resolution. The analytical data were processed by Analyst 1.4 software.

The MS/MS fragmentation pattern obtained from erythromycin A under the described conditions is shown in **Figure 1**. Besides the protonated precursor ion $(M + H)^+$, five diagnostic product ions were produced. The mass transition reactions used for erythromycin A identification and quantification were *m*/*z* 734.5 → 576.4 (collision energy = 26 eV, dwell time = 200 ms) as quantifier, *m*/*z* 734.5 → 558.5 (collision energy = 26 eV, dwell time = 200 ms), and *m*/*z* 734.5 → 716.3 (collision energy = 22 eV, dwell time = 200 ms) as qualifiers. The mass transition reaction *m*/*z* 734.5 → 158.0 was not taken into account because it suffered from a small interference from the matrix. The mass transition reaction used for internal standard [$^{13}C_2$]-erythromycin A was *m*/*z* 738.5 → 580.4 (collision energy = 26 eV, dwell time = 200 ms).

Analytical Method Performance. For identification purposes, the retention times of erythromycin A in the standards and in the samples

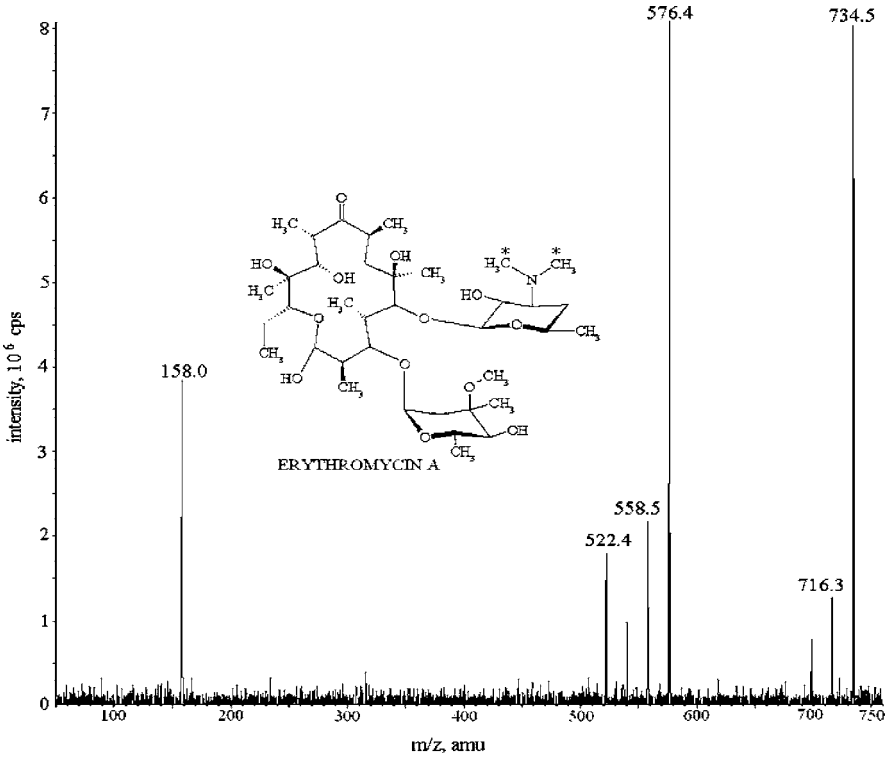

Figure 1. Full scan mass spectrum (positive ion mode) of erythromycin A (100 μg L^{-1}) standard solution directly infused at a flow rate of 10 μL min^{-1}. The two asterisks indicate the positions of the two ^{13}C atoms in the internal standard [$^{13}C_2$]erythromycin A.

were compared at a tolerance of ±2.5%. Moreover, in accordance with the 2002/657/EC European Decision, the relative ion intensities (each daughter ion area signal versus the base daughter ion area signal) of the spiked trout samples were compared with the relative ion intensities of erythromycin A standard solutions, at the same concentration levels used for the construction of the calibration curve.

To achieve the *in-house* validation of the analytical method, the following parameters were considered: recovery, repeatability, specificity, decision limit (CCα), detection capability (CCβ), linearity of the standard response, matrix calibration curve, and stability.

In the absence of any certified reference material (CRM), the recovery was determined by analysis of 18 aliquots of a blank fish tissue fortified at three different levels (six replicates for each level). In this case, the concentrations considered were 100, 200, and 300 μg kg^{-1}, corresponding to 0.5, 1, and 1.5 times the MRL.

Precision, expressed as repeatability, was calculated by repeated analyses on the same sample sets used for recovery tests, with the only difference that independent samples were re-extracted and analyzed on two other occasions for calculating interday repeatability.

Specificity of the LC-MS/MS method was proved following the mass transition reactions chosen to detect erythromycin A and [$^{13}C_2$]-erythromycin A in blank fish tissue samples. Specificity was also tested by analyzing, under the same conditions, a blank fish tissue sample fortified with tilmicosin (50 μg kg^{-1}), tylosin (100 μg kg^{-1}), and spiramycine (200 μg kg^{-1}). These macrolides and fortification levels were chosen considering that the European Union Commission has established MRLs of 50 and 100 μg kg^{-1} in all food-producing species for tilmicosin and tylosin, respectively, and an MRL of 200 μg kg^{-1} in bovine for spiramycine (*6*).

In the 2002/657/EC European Decision, CCα and CCβ replace the detection and quantification limits, respectively (*13*). In the case of erythromycin A, which has an established permitted limit, CCα was calculated by analyzing 20 aliquots of a blank fish tissue, all fortified with the analyte at the maximum permitted limit (200 μg kg^{-1}). The concentration at the maximum permitted limit, plus 1.64 times the corresponding standard deviation, represents the CCα ($\alpha = 5\%$).

Then, CCβ was established by analyzing 20 aliquots of a blank fish tissue, all fortified with the analyte at the calculated CCα. The concentration at the CCα, plus 1.64 times the corresponding standard deviation, equals the CCβ ($\beta = 5\%$)

A calibration curve was constructed using seven levels (fish blank tissue samples fortified at 50, 100, 200, 300, 500, and 1000 μg kg^{-1} of erythromycin A, including 0).

Finally, the stability of erythromycin A, both in solution and in matrix, was estimated. The former was assessed by means of four sets of 10 erythromycin A standard solution aliquots at MRL concentration (200 μg kg^{-1}). The 40 freshly prepared solution aliquots were immediately analyzed, and then the four sets were stored at −20, 4, 20 (dark), and 20 °C (light), respectively. The solution aliquots were reanalyzed after 2, 5, and 8 weeks. The latter was assessed by analysis of one incurred trout. The drug concentration was determined on fresh fish tissue. Then, the sample was stored at − 20 °C, and further aliquots of tissue were analyzed after 1, 2, 4, and 13 weeks.

For the assessment of all the mentioned parameters, the analyte response was always related to the internal standard response.

RESULTS AND DISCUSSION

Under the chromatographic conditions described above, the retention time of erythromycin A was ∼9.80 min, as shown in **Figure 2**.

For LC-MS/MS erythromycin confirmation, **Table 1** shows the ion intensity ratios (relative ion intensities) of the spiked trout tissue samples compared to those of the standard solutions. The daughter ion *m*/*z* 576.4 was chosen as base peak. The relative percent differences between the spiked samples and the standard solutions were 19.0% for *m*/*z* 558.5 versus 576.4 and 44.2% for *m*/*z* 558.5 versus 716.3; these values, considering the respective relative ion abundances, are both within the maximum permitted tolerances required in the 2002/657/EC European Decision (*13*). Consequently, all of the daughter ions considered in the present study can be used for confirmation purposes, and the daughter ions *m*/*z* 558.5 and 576.4 are the most accurate. In this regard, it is opportune to remember that, as stated by the 2002/657/EC European Decision, only three identification points are required for confirmation of authorized veterinary drugs. Two mass transition reactions are sufficient

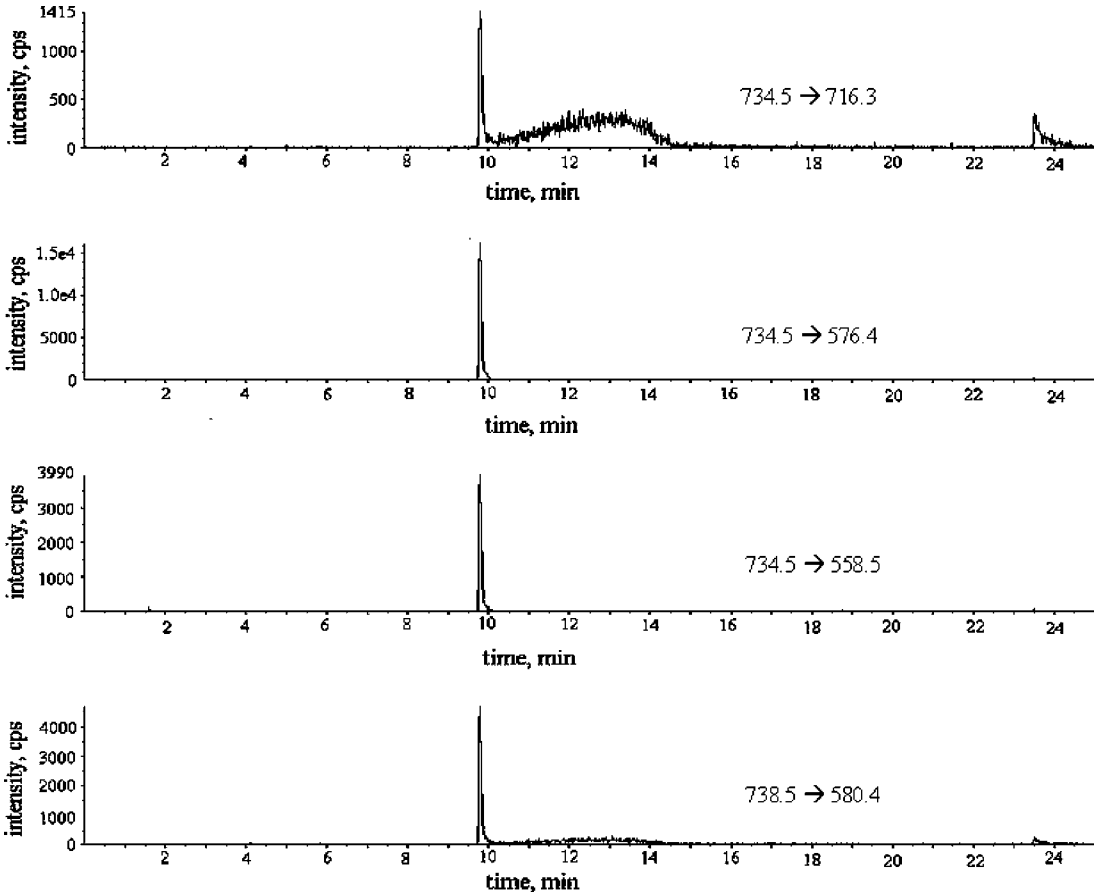

Figure 2. Mass chromatogram (positive ion mode) of a fish tissue sample fortified with erythromycin A (200 μg kg^{-1}) and [$^{13}C_2$]erythromycin A (1000 μg kg^{-1}). These concentrations, considering the dilution factor during the sample treatment, are equivalent to 20 and 100 μg kg^{-1}, respectively. Three transition reactions for erythromycin A and one for [$^{13}C_2$]erythromycin A (bottom row) are shown.

Table 1. Relative Ion Intensities for Erythromycin A Standard Solutions and Trout Tissue Samples Fortified with Erythromycin A at Six Different Levels

erythromycin A standards solutions[a] concn (μg kg^{-1})	intensity ratios (%) of daughter ions vs base daughter ion peak (*m/z* 576.4)	
	m/z 716.3	*m/z* 558.5
50	6.2	29.9
100	5.9	32.2
200	6.2	29.8
300	6.1	30.2
500	6.0	30.8
1000	6.1	29.7
mean ± SD	6.1 ± 0.12	30.4 ± 0.95

spiked trout tissue samples[a] concn (μg kg^{-1})	intensity ratios (%) of daughter ions vs base daughter ion peak (*m/z* 576.4)	
	m/z 716.3	*m/z* 558.5
50	8.7	24.3
100	8.9	24.7
200	8.8	24.9
300	8.7	24.6
500	9.0	24.3
1000	9.1	24.8
mean ± SD	8.8 ± 1.6	24.6 ± 0.25
difference (%)	44.2	19.0

[a] $n = 5$ for both erythromycin A standard solutions and spiked trout tissue samples.

to gain four identification points when working in LC-MS/MS and MRM mode.

Table 2. Performance of the Analytical Method for the Determination of Erythromycin A in Fish Tissues (Muscle plus Skin in Natural Proportion)

fortification level (μg kg^{-1})	measured content[a] (μg kg^{-1})	recovery (%)	intraday repeatability[b] [CV (%)]	interday repeatability[c] [CV (%)]
100	85 ± 9.2	85	9.4	10.9
200	176 ± 19	88	1.8	10.5
300	256 ± 19	97	2.4	7.5

[a] Values are mean ± SD for six samples. [b] Values are referred to six independent samples analyzed in one day. [c] Values are referred to six independent samples analyzed three times on three different days.

The recovery and the precision, expressed as intra- and interday repeatability, of the method are reported in **Table 2**. The coefficients of variation (CV) ranged from 1.8 to 9.4% and from 7.5 to 10.9% for intra- and interday repeatability, respectively. These data indicate that the precision of the method developed in this study is good; indeed, CVs were all below the recommended limit (15%) reported in the 2002/657/EC European Decision (*13*). Recovery data were also satisfactory, with values varying from 85 to 97%; indeed, these values fell within the guideline range (from −20 to 10%) for mass fraction ≥10 μg kg^{-1}, reported in the 2002/657/EC European Decision (*13*).

With regard to specificity of the LC-MS/MS method, no interference was found around the retention time of erythromycin A (**Figure 3**). Only in the case of the mass transition reaction *m/z* 734.5 → 158.0 was there a small peak close to the

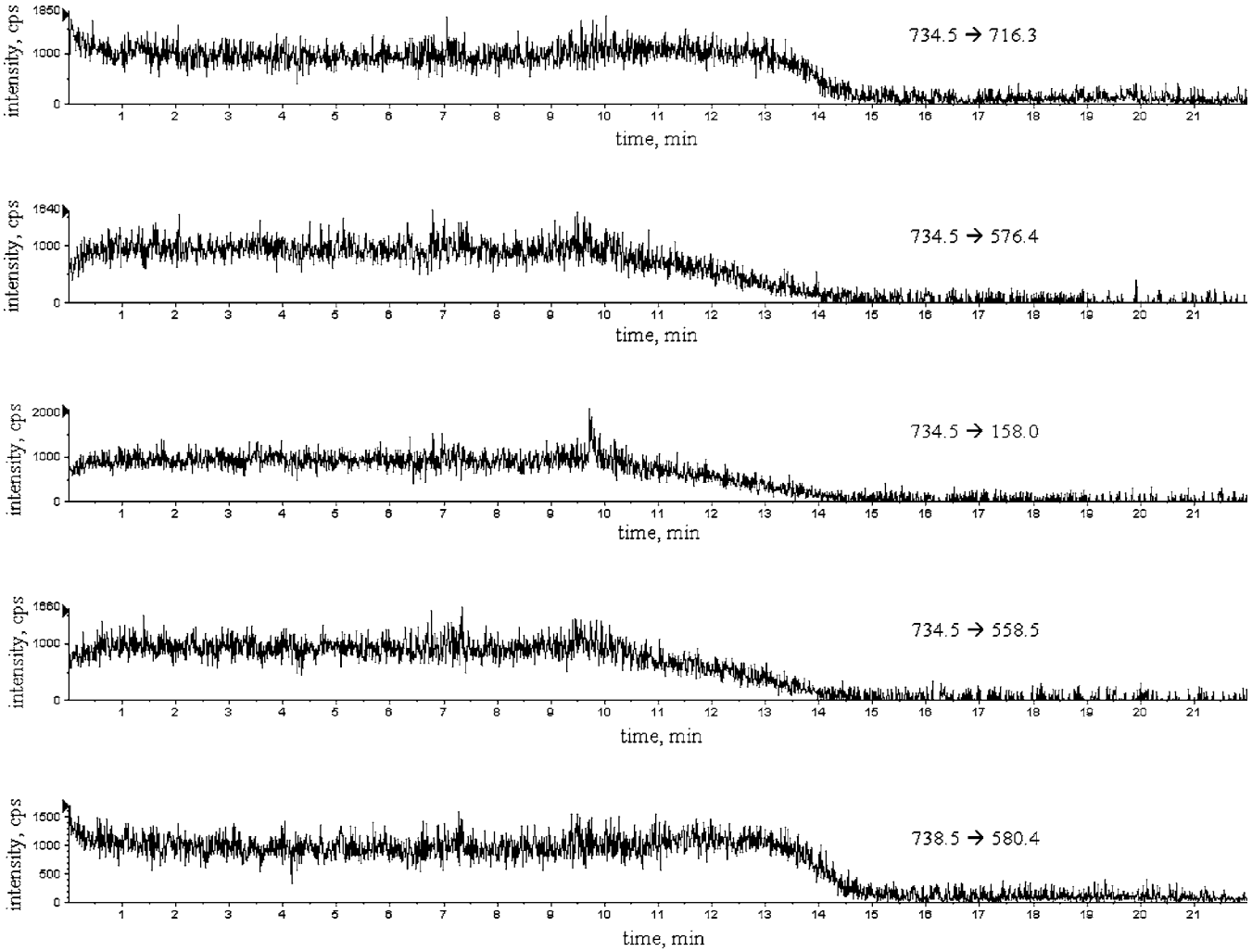

Figure 3. Mass chromatogram (positive ion mode) of a blank fish tissue sample. Four transitions for erythromycin A and one for [$^{13}C_2$]erythromycin A (bottom row) are shown.

Table 3. Stability of Erythromycin A in Solution

		erythromycin A concn in fresh solution			erythromycin A concn during time (%)		
storage condition	*n*	spiked (μg kg^{-1})	found (μg kg^{-1})	%[a]	2 weeks	5 weeks	8 weeks
room temperature (light)	10	200	212 ± 21	100 ± 10	92 ± 15	80 ± 7	65 ± 10
room temperature (dark)	10	200	200 ± 38	100 ± 18	93 ± 10	83 ± 15	70 ± 13
4 ° C	10	200	207 ± 17	100 ± 8	96 ± 10	89 ± 10	77 ± 13
–20 ° C	10	200	206 ± 14	100 ± 6	99 ± 9	95 ± 7	90 ± 6

[a] Concentration normalized at 100%.

analyte retention time (see **Figure 3**). For this reason, the transition reaction *m*/*z* 734.5 → 158.0 was not considered as a suitable quantifier or qualifier reaction. Moreover, no interfering signal was detected when the blank fish tissue sample fortified with tilmicosin, tylosin, and spiramycine was analyzed (data not shown).

With regard to the decision limit (CCα), the standard deviation measured at MRL (200 μg kg^{-1}) on 20 fortified fish tissue samples was equal to 11 μg kg^{-1}, resulting in a CCα of 220 μg kg^{-1}. The detection capability (CCβ) was 238 μg kg^{-1}.

The calculated calibration curve showed a good linearity in the whole range of tested concentrations (0−1000 μg kg^{-1}) with a correlation coefficient (r^2) equal to 0.9956 and CVs that ranged from 1.1 to 18.2%. The resulting equation was $y = 0.0152x + 0.4795$, where y is the analyte/IS peak area ratio and x is the analyte concentration. In addition, the corresponding calibration curve corrected for recoveries ($y = 0.0145x + 0.2433$) showed a good linearity with a correlation coefficient of 0.9981 and CVs ranging from 5.2 to 13%.

Data on erythromycin A stability in solution are shown in **Table 3**. As expected, the better storing condition of standard solution was at −20 °C, followed by 4 °C and then room temperature in the dark and in the light, respectively. However, degradation phenomena already occur in all storing conditions after 2 weeks. After 8 weeks, ∼10% of erythromycin A was lost in the better storing conditions and ∼35% in the worst ones.

Data on erythromycin A stability in matrix show a similar trend: after 2, 5, 8, 10, and 13 weeks of storage at − 20 °C, drug concentrations were 98, 94, 89, 85, and 79% of the solution at time 0, respectively.

In summary, the key aim of this study was to develop and optimize a fast, simple, and suitable confirmatory method for

the determination of erythromycin A residues in fish. The results demonstrate that the method affords sensitivity, precision, and accuracy required for generating validated quantitative data. The extraction and cleanup procedures present many advantages over those currently employed for the isolation of erythromycin from complex matrices (*14*−*23*). It is simple and relatively short (only 45 min for the whole procedure), and it minimizes the use of solvents and glassware. The LC-MS/MS technique allows the selective determination of erythromycin with a fast chromatographic separation.

In conclusion, the analytical strategy developed in this study represents a valid alternative to the older confirmatory methods normally employed for the determination of erythromycin residues in fish. The developed method, being simple, fast, and sensitive, can be employed for regulatory purposes, to increase and improve control and monitoring of antibacterial drug residues in fish products.

LITERATURE CITED

(1) *Yearbook of Fishery Statistics 2002. Aquaculture Production*; Vol. 94/2 FAO Statistics Series 181 and Fisheries Series 67; FAO: Rome, Italy, 2004.

(2) Schmidtke, L. M.; Carson, J. Induction, characterisation and pathogenicity in rainbow trout *Oncorhynchus mykiss* (Walbaum) of *Lactococcus garvieae* L-forms. *Vet. Microbiol.* **1999**, *69*, 287−300.

(3) Bullock, G. L.; Leek, S. L. Use of erythromycin in reducing vertical transmission of bacterial kidney disease. *Vet. Hum. Toxicol.* **1986**, *28*, 18−20.

(4) Moffit, C. M. Oral and injectable applications of erythromycin in salmon fish culture. *Vet. Hum. Toxicol.* **1991**, *33*, 49−53.

(5) Fairgrieve, W. T.; Masada, C. L.; McAuley, W. C.; Peterson, M. E.; Myer, M. S.; Strom, M. S. Concentrations of erythromycin and azithromycin in mature Chinook salmon (*Oncorhynchus tshawytscha*) after intraperitoneal injection, and in their progeny. *Dis. Aquat. Organ.* **2005**, *64* (2), 99−104.

(6) Council Regulation 2377/90/EC of 26 June 1990 laying down a community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Off. J. Eur. Union* **1990**, *L 224* (Aug 8), 1.

(7) Juhel-Gaugain, M.; Anger, B. Multiresidue chromatographic method for the determination of macrolide residues in muscle by high-performance liquid chromatography with UV detection. *J. AOAC Int.* **1999**, *82*, 1046−1053.

(8) Dreassi, E.; Corti, P.; Bezzini, F.; Furlanetto, S. High-performance liquid chromatographic assay of erythromycin from biological matrix using electrochemical or ultraviolet detection. *Analyst* **2000**, *125*, 1077−1081.

(9) Sastre Torano, J.; Guchelaar, H. J. Quantitative determination of the macrolide antibiotics erythromycin, roxithromycin, azithromycin and clarithromycin in human serum by high-performance liquid chromatography using pre-column derivatization with 9-fluorenylmethyloxycarbonyl chloride and fluorescence detection. *J. Chromatogr. B* **1998**, *720*, 89−97.

(10) Edder, P.; Coppex, L.; Cominoli A.; Corvi, C. Analysis of erythromycin and oleandomycin residues in food by high-performance liquid chromatography with fluorometric detection. *Food Addit. Contam.* **2002**, *19*, 232−240.

(11) Janecek, M.; Quilliam, M. A.; Bailey, M. R., North, D. H. Determination of erythtromycin A by liquid chromatography and electrochemical detection, with application to salmon tissue. *J. Chromatogr.* **1993**, *619*, 63−69.

(12) Billedeau, S. M.; Heinze, T. M.; Siitonen, P. H. Liquid chromatography analysis of erythromycin A in salmon tissue by electrochemical detection with confirmation by electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 1534−1538.

(13) Commission Decision (EC) 657/2002. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Communities* **2002**, *L 221* (Aug 17).

(14) Pleasance, S.; Kelly, J.; LeBlanc, M. D.; Quilliam, M. A.; Boyd, R. K.; Kitts, D. D.; McErlane, K.; Bailey, M. R.; North D. H. Determination of erythromycin A in salmon tissue by liquid chromatography with ion-spray mass spectrometry. *Biol. Mass Spectrom.* **1992**, *21*, 657−687.

(15) Delépine, B.; Hurtaud-Pessel, D.; Sanders, P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry. *J. AOAC Int.* **1996**, *79*, 397−404.

(16) Guyonnet, J.; Bromet, N.; Bertaim, T.; Kaltsatos, V. Routine determination of erythromycin in edible pig tissues using a high sensitivity LC-MS-MS system. In *Proceedings of the Euroresidue IV Conference*; van Ginkel, L. A., Ruiter, A., Eds.; National Institute of Public Health and the Environment (RIVM): Veldhoven, The Netherlands, 2000; p 490.

(17) Bajic, S.; Doerge, D. R.; Lu, L.; Hansen, E. B. Analysis of erythromycin by liquid chromatography/mass spectrometry using involatile mobile phases with a novel atmospheric pressure ionization source. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 156−160.

(18) Dubois, M.; Fluchard, D.; Sior, E.; Delahaut, P. Identification and quantification of five macrolide antibiotics in several tissues, eggs and milk by liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. B* **2001**, *753*, 189−202.

(19) Codony, R.; Compano, R.; Granados, M.; Garcia-Regueiro, J.; Prat, M. D. Residue analysis of macrolides in poultry muscle by liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. A* **2002**, *959*, 131−141.

(20) Hwang, Y. H.; Lim, J. H.; Park, B. K.; Yun, H. I. Simultaneous determination of various macrolides by liquid chromatography/mass spectrometry. *J. Vet. Sci.* **2002**, *3*, 103−108.

(21) Horie, M.; Takegami, H.; Toya, K.; Nakazawa, H. Determination of macrolide antibiotics in meat and fish by liquid chromatography-electrospray mass spectrometry. *Anal. Chim. Acta* **2003**, *492*, 187−197.

(22) Yang, S.; Carlson, K. H. Solid-phase extraction-high-performance liquid chromatogreaphy-ion trap mass spectrometry for analysis of trace concentrations of macrolide antibiotics in natural and waste water matrices. *J. Chromatogr. A* **2004**, *1038*, 141−155.

(23) Wang, J.; Leung, D.; Butterworth, F. Determination of five macrolide antibiotic residues in eggs using liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 1857−1865.

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