Liquid Chromatographic Analysis of Incurred Amoxicillin Residues in Catfish Muscle Following Oral Administration of the Drug

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Improper application of antibiotic chemicals to livestock and aquaculture species may lead to the occurrence of residues in food supplies. An appropriate depletion period is needed after the administration of drugs to animals for ensuring that residues in edible tissues are below established tolerance levels. This study was conducted to determine incurred amoxicillin residues in catfish muscle following oral administration. Dosed fish were harvested after four depletion periods, and muscle fillets were analyzed for amoxicillin residues using an HPLC method with precolumn derivatization and fluorescence detection. The residue levels in fish after a 6-h depletion ranged from 40 to 64 ng/g with one exception at 297 ng/g. Average residue levels decreased to 5.4 and 2.8 ng/g after 24- and 48-h depletions, respectively. Residue levels after a 72-h depletion decreased to below the method's limit of quantitation (1.2 ng/g). An LC-MS/MS confirmatory method was developed. Confirmation of the presence of amoxicillin was demonstrated in incurred fish samples containing residues at ~50−300 ng/g.

Keywords: *LC; amoxicillin; incurred residues; catfish; depletion*

INTRODUCTION

Antibiotics have been used in livestock farming for several decades. Improper applications of antibiotics may lead to the occurrence of residues in food supplies. Farm animals treated with antibiotics are required to be held for a specific withdrawal period until all residues are depleted to a safe level before the animal tissue or milk can be used for human consumption. It is equally important to allow a proper depletion period after administration of antibiotic to aquaculture species for ensuring the complete elimination of residues in food supplies. The U.S. official tolerance for amoxicillin residues is 0.01 ppm (10 ppb or 10 ng/g) in milk and uncooked cattle tissue (CFR, 1991). Amoxicillin is potentially useful as a therapeutant in aquaculture. No tolerance has been established for amoxicillin residues in fish tissues at the present time.

Current methods for the routine detection of β-lactam antibiotic residues are primarily microbiological tests. These methods are generally useful for screening purposes. However, these tests lack selectivity and specificity; that is, they cannot distinguish between different forms of β-lactam antibiotics. Analysis of amoxicillin and other β-lactam antibiotic residues in biological samples using high-performance liquid chromatography (HPLC or LC) have been reported, but most of them are applicable for plasma (Lee and Brooks, 1984; Muth et al., 1996; Yuan et al., 1995) and milk (Harik-kahn and Moats, 1995; Luo et al., 1997; Sorensen et al., 1997; Straub et al., 1994). Very few LC methods are available in the literature for animal tissues, especially for amoxicillin residues at trace levels (Ang et al., 1996; Rose et al., 1997). Among these methods, LC analysis with fluorescence detection developed by Ang et al. (1996) offered the most sensitive, accurate, and precise determination of amoxicillin residues in fish muscle. This method has been further verified with a conventional microbial inhibition method for the determination of both fortified and incurred amoxicillin residues in catfish muscle (Ang et al., 1998).

The objectives of the present study were to determine residue levels of amoxicillin in catfish tissue following oral administration of the drug and to assess the previous LC method developed in our laboratory for incurred residue applications. The information concerning the depletion of amoxicillin residues from muscle fillets of channel catfish is useful, especially if this drug is to be approved in aquaculture practices. Potential analytical interference due to other chemicals or drugs was also evaluated. A tandem mass spectrometry method was developed, which could be used to confirm higher levels amoxicillin residues found using LC/fluorescence for quantitation. To demonstrate this, LC electrospray ionization/mass spectrometry/mass spectrometry (LC-ESI/MS/MS) was used to confirm the presence of derivatized amoxicillin in two of the incurred fish samples, on the basis of a second determination of the LC retention time and the measurement of the intensity ratios for signals at masses corresponding to four specific daughter ions from the protonated parent molecule. The criterion for confirmation, four ions with

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a variation in intensity ratios of no more than 10% relative to reference spectra from the authentic compounds, is consistent with the well-known "three ion criterion" for confirmation by mass spectrometry, which requires three ions and a variation of no more than 10%.

MATERIALS AND METHODS

Chemicals and Apparatus. Reference standard of amoxicillin trihydrate, containing 861 μg of amoxicillin/mg, was purchased from the U.S. Pharmacopoeia Convention (USP, Inc., Rockville, MD). Standard stock solutions at 1 mg/mL were prepared in water and stored at 4 °C for up to 1 month. Appropriate dilutions were made daily for preparing intermediate and working solutions. All solvents (methanol, acetonitrile, and ethyl ether) were of LC grade and were supplied by J. T. Baker, Inc. (Phillipsburg, NJ). All chemicals were of ACS reagent grade except where indicated. Granular potassium phosphate, monobasic (KH_2SO_4), and potassium hydroxide were of Fisher reagent grade (Fisher Inc., Fair Lawn, NJ); sodium chloride and citric acid were from J. T. Baker, Inc.; and trichloroacetic acid, formaldehyde, formic acid, and ammonium formate were from Aldrich Chemical Co. (Milwaukee, WI). Water was distilled, deionized, and passed through a carbon filter at >15 ohms/cm (Milli-Q water purification system; Water Corp., Milford, MA). The solid-phase extraction system and the LC system were those described previously (Ang et al., 1996). Essentially they included C_{18} cartridges (Sep-Pak Vac 3 cm^3, 500 mg of sorbent from Waters), a Waters 600E pump, a pump controller, and a Waters 470 scanning fluorescence detector. The analytical column, a Prodigy 5 μm, ODS-3 4.6 mm × 250 mm, was obtained from Phenomenex Co. (Torrance, CA). The tissue homogenizer was an Ultraturrax model T25 (Ika-Labortechnik, Janke & Kunkel GmbH & Co. KG, Staufen, Germany) with an S25N-18G probe.

The LC-MS/MS experiments were conducted using a Finnigan (San Jose, CA) TSQ7000 triple-quadrupole mass spectrometer operated in the selected reaction monitoring (SRM) mode. Four product ions (*m*/*z* 39, 65, 120, and 185) from *m*/*z* 203, the protonated parent molecule ($M + 1^+$), were generated using argon as the collision gas (~1 mTorr) at 75 eV. The experiments were conducted in the electrospray ionization (ESI) mode. The LC separation was accomplished using a Phenomenex Atlantis C-18 column (250 × 2 mm) with a flow rate of 200 μL/min. The solvent flow was provided by a Varian 9012 solvent delivery system. The injector was a Hewlett-Packard 1100 autosampler. An LC detector (Spectrophysics Spectro 100, monitoring at 254 nm) was used to detect UV active components ~0.2 min prior to introduction into the mass spectrometer.

Experimental Catfish and Drug Administration. Channel catfish (*Ictalurus punctatus*) were produced in a hatchery and raised in ponds at the University of Arkansas at Pine Bluff. Fish weighing 0.5−1.0 kg were maintained in indoor circular tanks (250 L) in a flow-through system for one week prior to administration of amoxicillin. Fish were fed a commercial catfish feed (Arkat Feeds, Dumas, AR) at a rate of 2% of body weight once daily during the acclimation periods. Water temperature was 27.5 ± 1.4 °C. Control fish were fish harvested from the same tank before antibiotic treatment.

Each fish was weighed and anaesthetized with a 50 mg/L tricaine methanesulfonate (MS222) solution (Sigma Chemical Co., St. Louis, MO) before the dosing operation. Anesthetized fish were held stationary in a vertical position while a water suspension of amoxicillin (50 mg/mL, Amoxi-inject, Smith Kline Beecham, West Chester, PA) was administered to the fish orally using a plastic pipet (5 mL, graduated). The dosage was 110 mg/kg of fish body weight (i.e., 1 mL of the suspension was fed to fish of 1 lb or 0.45 kg). After the drug administration, the fish were placed back in the tank and maintained on the commercial diet. Five fish were collected at each time interval for various depletion periods up to 72 h postdosing, packed in ice, and transported to the National Center for Toxicological Research (NCTR) laboratory within 1 h. Each fish was then filleted with a fillet knife. Care was taken to avoid gut rupture and contamination of the fillets with gut contents. The work area and all cleaning instruments were rinsed thoroughly with tap water between fish. Fillets were rinsed with tap water, packed in ziplock bags, and frozen immediately at −70 °C. Control fish were similarly processed.

Sample Extraction and LC Determination. Prepared frozen catfish fillets were allowed to thaw partially at 4 °C before blending. Five gram aliquots of blended muscle were homogenized with a phosphate buffer solution following the procedure of Ang et al. (1996). Three or four replicate samples were prepared for each fish. The initial extraction, solid-phase extraction and concentration, derivatization, and LC/fluorescence determination were performed as reported previously (Ang et al., 1996). Residues in fish samples were identified by matching the peak retention time with the standard and further confirmed by photodiode array (PDA) spectrum analysis of the amoxicillin derivative, where the concentrations were adequate for PDA detection. A standard curve was constructed by plotting peak area versus concentration of amoxicillin. Quantitation of amoxicillin was derived from the standard curve using the peak area.

Test of Potential Interferences. A number of commonly used veterinary drugs were tested for their potential interferences with the amoxicillin determination. These included other β-lactam antibiotics (ampicillin, penicillin G, cloxacillin, and cephapirin), lincomycin, streptomycin, erythromycin, acriflavin, tricaine methanesulfonate, 14 sulfa drugs (sulfanilamide, sulfadimethoxine, sulfamerazine, sulfapyridine, sulfadiazine, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfachloropuridazine, sulfamonomethoxine, sulfadoxine, sulfamethoxazole, sulfathiazole, and sulfaquinoxoline), and a laboratory-made amoxicillin penicilloic acid metabolite. These compounds were prepared in acetonitrile or water depending on their solubility at 1000 ng/mL. One milliliter of each solution was used in derivatization, ether extraction, and LC analysis under the same conditions as for amoxicillin. This concentration was equivalent to 200 ng/g in fish.

Sample Extraction and LC Preparation for LC-MS/MS Confirmation. For LC-MS/MS analysis, certain chemicals (nonvolatile salts) could not be used during the sample preparation and isolation steps as they were incompatible in the LC-MS/MS system. For this reason the extraction procedure was modified. Ammonium acetate buffer solution (0.5 M; adjusted to pH 4.5 with acetic acid and ammonia hydroxide solution) instead of phosphate buffer was used for the initial extraction. Sodium chloride was eliminated in the liquid/liquid partition step. A heating block (Thermolyne type 16500 Dribath, Dubuque, IA) instead of a boiling water bath was used for the derivatization process. After heating and cooling of the reaction mixture, the amoxicillin derivatives were extracted with 4.5 mL of ethyl ether five times instead of with 3 mL, three times. The ether layer was evaporated to dryness under a stream of nitrogen at 40 °C, reconstituted in 1 mL of mobile phase, and filtered through a 0.20 μm membrane before injection onto the LC. The mobile phase was 18% acetonitrile in water preadjusted to pH 4.35 with formic acid. The injection volume was 100 or 200 μL, and three injections of each volume were made for each sample extract. Amoxicillin peaks were collected manually, combined, and evaporated to dryness using a vacuum centrifuge concentrator (Speedvac Systems, Savant Instruments, Inc., Holbrook, NY) at room temperature (23 °C). The residue was redissolved in 100 μL of the LC-MS/MS mobile phase (30% acetonitrile in 0.1% formic acid). This prepared solution (corresponding to 4.5 g of fish) was ready for LC-MS/MS analysis.

LC-MS/MS Confirmation. Twenty microliters of prepared, final sample extracts (as described above) was injected onto the LC column of the LC-MS/MS system. Confirmation of the amoxicillin derivative was based on the LC retention time and the intensity ratios for four product ions from *m*/*z* 203 ($M + 1^+$) determined using spiked fish. The expected product ion intensity values were established using the average obtained from duplicate samples (5 g of fish) spiked with 0, 0.5, 1.0,

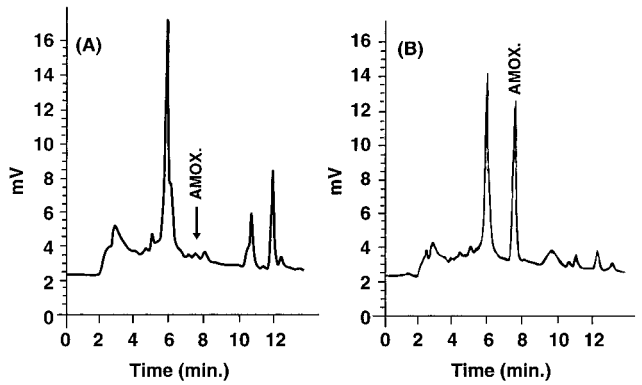

Figure 1. LC chromatograms of (A) control catfish muscle and (B) incurred amoxicillin (10 ng/g) in muscle of dosed catfish.

5.0, or 10.0 μg of amoxicillin (equivalent to 0, 0.1, 0.2, 1.0, and 2.0 μg/g, respectively). A standard solution of derivatized amoxicillin corresponding to 200 ng/g of residues in fish (1 μg of amoxicillin in a 5-g sample) and a blank fish sample were tested first. Incurred samples were analyzed later in the experiment. Confirmation required the correct retention time (within 0.1 min) and a match between the reference and experimental ion intensities to within a tolerance of 10% relative abundance. Because the minimum criterion for confirmation by mass spectrometry within FDA has traditionally required three ions within the prescribed tolerance (10%), we monitored four ions with the expectation that one daughter ion might be obscured by an interference. For the two authentic samples analyzed, as well as control and spiked-fish samples, all four product ion windows have been free of interferences. Thus, although the confirmation step might be considerably more complicated and less sensitive than the quantitative steps, it does provide the high degree of specificity that will be necessary for confirmation of samples containing high levels of residues.

RESULTS AND DISCUSSION

LC Determination of Dosed Fish. Typical LC chromatograms showing the control fish and incurred amoxicillin in dosed fish are presented in Figure 1. No significant interference was encountered in the analysis of these samples. The chromatograms of the control fish muscle were similar to those reported earlier (Ang et al., 1996). Amoxicillin was not detected (<0.5 ng/g) in any of the five control fish.

The amoxicillin contents of individual fish after oral administration of the drug and depletion are shown in Table 1. The concentrations of amoxicillin varied between individual fish within a group. Amoxicillin residues in 6-h-postdosing catfish ranged from 40 to 64.2 ng/g with one exception at 297 ng/g. Data also showed that the average residue levels decreased to 5.4 and 2.9 ng/g after 24 and 48 h postdosing, respectively. The variation ranges were narrower for fish after longer periods of depletion. Residue levels in fish after 72-h postdosing decreased to below the limit of quantitation, 1.2 ng/g (Ang et al., 1996). The standard deviation (SD) of the mean of each group was calculated. As described earlier, three to four subsamples were analyzed for each fish. Variations expressed as relative standard deviation (%RSD) of subsamples were <20% for residue levels >1.2 ng/g. These data indicate that the LC method as used in this study meets the FDA (U.S. FDA, 1986) criteria in precision for animal drug residue analysis. Under the present study conditions, the depletion rate was fastest from 6 to 24 h. However, there were still trace amounts of residues after 72 h of depletion, but they were below the limit of quantitation.

Table 1. Amoxicillin Residues in Catfish Muscle Following Oral Administration of the Drug

depletion time (h)	fish	fish wt (kg)	amoxicillin, ng/g mean	SD[a]	%RSD[b]
6	1	0.76	64.2	6.86	10.7
	2	0.56	50.6	3.62	7.2
	3	0.38	60.5	4.23	7.0
	4	0.48	40.0	7.17	17.9
	5	0.66	297	40.1	13.5
24	6	0.38	<1.2[c]		
	7	0.36	7.3	0.51	7.0
	8	0.32	3.7	0.69	18.6
	9	0.44	7.0	0.12	1.7
	10	0.52	7.9	0.71	9.0
48	11	0.50	<1.2[c]		
	12	0.46	1.4	0.12	8.4
	13	0.54	6.9	0.25	3.6
	14	0.70	2.8	0.21	7.5
	15	0.38	1.9	0.20	10.4
72	16	0.48	<1.2[c]		
	17	0.30	<1.2[c]		
	18	0.44	<1.2[c]		
	19	0.36	<1.2[c]		
	20	0.36	<1.2[c]		

[a] Standard deviation. [b] Relative standard deviation. [c] Limit of quantitation.

Test of Potential Interferences. Data of interference tests demonstrated that ampicillin was the only compound that produced a significantly large peak, but its retention time (17 min) was much later than amoxicillin. Tricaine methanesulfonate had a small peak eluting close to amoxicillin (0.5 min later than amoxicillin), but it was well resolved. One minor peak of sulfaquinoxoline eluted at the same retention time as amoxicillin. Thus, the sulfaquinoxoline solution was subjected to solid-phase extraction and subsequent ether extraction before the derivatization step. The minor peak area was reduced to <1% of the amoxicillin peak at a comparable concentration level.

LC-MS/MS Confirmation. A blank and several spiked fish samples were analyzed by LC-MS/MS first. The LC retention times in LC-MS/MS experiments with spiked fish varied only by ~0.1 min, ranging from 5.4 to 5.5 min, for all eight of the experiments. The ratios of the four product ions were determined by SRM in duplicate experiments at each level, corresponding to 100, 200, 1000, and 2000 ng/g. The intensity ratios for the product ions did not appear to be concentration dependent from 100 to 2000 ng/g. The average relative abundance values were 24.6% (*m*/*z* 39), 14.4% (*m*/*z* 65), 100% (*m*/*z* 120), and 69.5% (*m*/*z* 185). The standard deviations, ranging from 0.8 to 2.1, for the average of the ratios corresponding to the four concentration levels, were much less than the maximum 10% (absolute) deviation often allowed by the FDA and other agencies for confirmation by mass spectrometry. Because four ions, rather than three, were monitored, the method could also be applied to samples having interference at one of the four product ion masses and still meet the minimum ("three ion") criteria for confirmation.

Duplicate tissues from each of two fish (fish 2 and 5), sampled after 6 h of depletion, were selected to demonstrate confirmation by LC-MS/MS. Fish 2 was selected because its amoxicillin residue content (50.6 ng/g) was approximately at the median in the first group (6-h depletion). Fish 5 was selected because its residue

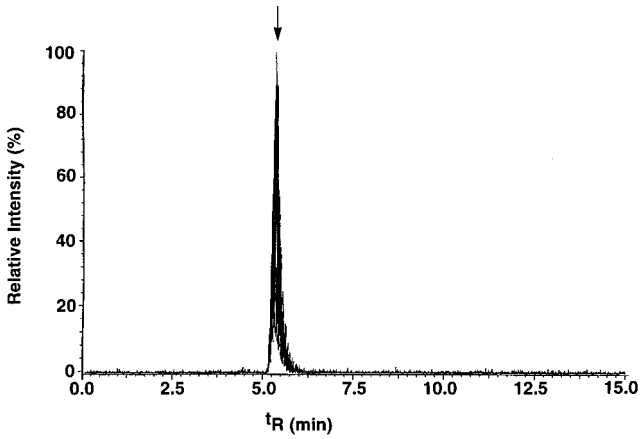

Figure 2. LC SRM MS/MS total ion response trace for a 200 ng/g standard of derivatized amoxicillin.

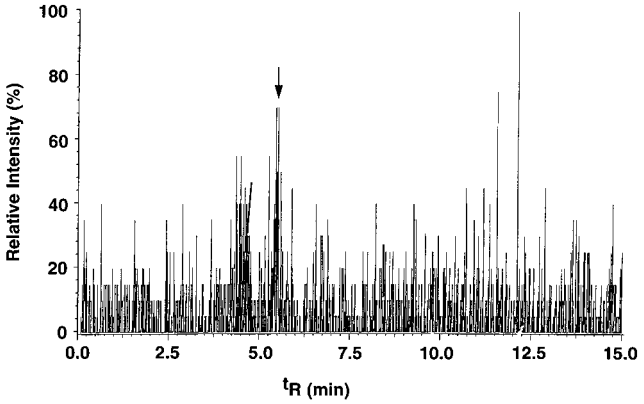

Figure 3. LC SRM MS/MS total ion response trace for a blank fish sample.

content was much higher than those of other fish in the same group. Fish samples with depletion times >6 h were not analyzed by LC-MS/MS because their residue levels were <10 ng/g, the tolerance level established for cattle tissue. A standard corresponding to 200 ng/g showed a single large deflection at 5.3 min in the time/intensity trace (Figure 2), indicating a single component having a parent *m/z* value of 203 and signal for one or more of the four selected product ions. The ratios of these ions were 23.3% (*m/z* 39), 12.3% (*m/z* 65), 100% (*m/z* 120), and 60.5% (*m/z* 185), all within 10% (absolute) of the values obtained in the prior experiment with spiked fish. As shown in Figure 3, no signal was detected from the corresponding blank fish sample. An SRM ion chromatogram for fish 2 is shown in Figure 4. The apparent high-frequency noise in the trace is caused by changes in the product ion mass, plotted as several different masses in the same display. This is an artifact of the display in the SRM mode. The signal on the baseline demonstrates that the actual signal-to-noise level for this sample is about 25:1 or better. The retention time and ion intensity values for the four samples from the two fish (fish 2 and 5) are given in Table 2. The observed retention times, from 5.3 to 5.5 min, are in good agreement with the values for the previously analyzed spiked fish and also for the standard analyzed concurrently. Similarly, the intensity ratios for all of the ions are within 10% (absolute) of the values observed with the spiked fish and the concurrently analyzed standard solution. These data clearly demonstrate confirmation for fish with levels at 50 and 297 ng/g. The present results may encourage

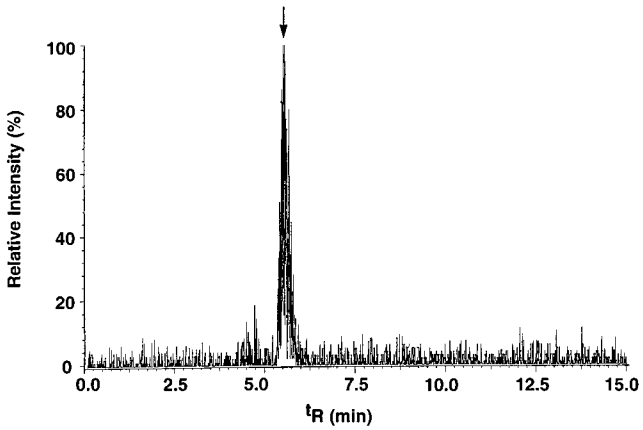

Figure 4. LC SRM MS/MS total ion response for one of two analyses of fish sample 2.

Table 2. LC ESI SRM MS/MS Results for Incurred Fish Samples 2 and 5

m/z (% ra)[a]	standard	fish 2-1	fish 2-2	fish 5-1	fish 5-2
39	26.3	26.3	25	24.1	27.7
65	12.3	15.8	12.5	15.8	16.8
120	100	100	100	100	100
185	60.5	63.1	62.5	66.9	67.2
t_r (min)[b]	5.3	5.5	5.5	5.4	5.3

[a] ra, relative abundance. [b] t_r, retention time.

additional studies involving the application of this methodology using LC-MS/MS systems employing off-axis introduction in the electrospray inlet. These newer systems provide higher sensitivity for ESI/MS and allow more liberal use of nonvolatile salts.

As stated earlier, very limited LC methods are available in the literature for the determination of amoxicillin residues in animal tissues. Besides the method developed in our laboratory (Ang et al., 1996), the only other method reported was by Rose et al. (1997). Their data indicated that the recovery of amoxicillin in cattle muscle was 57% within-day and 50% on two different days with RSDs of 19 and 15%, respectively. Mercuric chloride was used in their procedure. In comparison, the method used in this study as developed in our laboratory earlier showed high recoveries (>80% for catfish and >75% for salmon muscle) with RSDs of <6% for fortified samples. No mercuric chloride was used. Furthermore, this method has been verified with the widely used microbial inhibition method for both spiked and incurred samples (Ang et al., 1998), which was the first reported bridging study for the analysis of amoxicillin residues.

In the present study, additional investigations have been conducted for validating this method for incurred amoxicillin residues over a wide range. Potential interfering compounds, that is, other drugs and chemicals, were tested, and none caused any concerns. The detection by fluorescence was shown to be selective and sensitive. Residues in selected samples containing 50 or 297 ng/g were further confirmed by an LC-MS/MS method.

Conclusions. The present study provided data on the depletion of amoxicillin residues in catfish after oral administration of a single dose of the drug. Amoxicillin residues were depleted from catfish at a fairly rapid rate during the first 24 h. Fish administered 110 mg of amoxicillin/kg of body weight retained <10 ng/g of the antibiotic after 24 h. The residue levels further de-

creased to <1.2 ng/g after 72 h. Large variations were observed between fish within a depletion group. The LC method with fluorescence detection developed in our laboratory previously was satisfactory for the determination of incurred amoxicillin residues within the range tested. No interference was found in the presence of several other chemicals or fish drugs. An LC-MS/MS confirmatory method was developed. Confirmation of the presence of amoxicillin was demonstrated in incurred fish samples containing 50 and 297 ng/g of amoxicillin.

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LITERATURE CITED

Ang, C. Y. W.; Luo, W.; Hansen, E. B., Jr.; Freeman, J. P.; Thompson, H. C., Jr. Determination of amoxicillin in catfish and salmon tissues by liquid chromatography with precolumn formaldehyde derivatization. *J. AOAC Int.* **1996**, *79* (2), 389−396.

Ang, C. Y. W.; Luo, W.; Kiessling, C. R.; McKim, K.; Lochmann, R.; Walker, C. C.; Thompson, H. C., Jr. A bridging study between liquid chromatography and microbial inhibition assay methods for determining amoxicillin residues in catfish muscle. *J. AOAC Int.* **1998**, *81*, 33−39.

CFR. *U.S. Code of Federal Regulations, Part 21. δ 556.38, Amoxicillin*; U.S. GPO: Washington, DC, 1991.

Harik-kahn, R.; Moats, W. A. Identification and measurement of β-lactam antibiotic residues in milk: integration of screening kits with liquid chromatography. *J. AOAC Int.* **1995**, *78*, 978−986.

Lee, T. L.; Brooks, M. A. High-performance liquid chromatographic determination of amoxicillin in human plasma using a bonded-phase extraction. *J. Chromatogr.* **1984**, *306*, 429−435.

Luo, W. H.; Hansen, E. B., Jr.; Ang, C. Y. W.; Deck, J.; Freeman, J. P.; Thompson, H. C., Jr. Simultaneous determination of amoxicillin and ampicillin in bovine milk by HPLC with fluorescence detection. *J. Agric. Food Chem.* **1997**, *45*, 1264−1268.

Muth, P.; Metz, R.; Beck, H.; Bolten, W. W.; Vergin, H. Improved high-performance liquid chromatographic determination of amoxicillin in human plasma by means of column switching. *J. Chromatogr.* **1996**, *729* (1−2), 259−266.

Rose, M. D.; Tarbin, J.; Farrington, W. H. H.; Shearer, G. Determination of penicillin in animal tissues at trace residue concentrations: II. Determination of amoxicillin and ampicillin in liver and muscle using cation exchange and porous graphitic carbon solid-phase extraction and high performance liquid chromatography. *Food Addit. Contam.* **1997**, *14* (2), 127−133.

Sorensen, L. K.; Rasmussen, B. M.; Boison, J. O.; Keng, L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high performance liquid chromatographic method. *J. Chromatogr. B: Biomed. Sci. Appl.* **1997**, *694* (2), 383−391.

Straub, R.; Linder, M.; Voykser, R. D. Determination of β-lactam residues in milk using perfusive particle liquid chromatography combined with ultrasonic nebulization electrospray mass spectrometry. *Anal. Chem.* **1994**, *66*, 3651−3658.

U.S. FDA. *Memorandum, General principles for evaluation of the safety of compounds used in food-producing animals*; U.S. Food and Drug Administration: Rockville, MD, Sept 1986.

Yuan, Z.; Russlie, H. Q.; Canafax, D. M. Sensitive assay for measuring amoxicillin in human plasma and middle ear fluid using solid-phase extraction and reversed-phase high performance liquid chromatography. *J. Chromatogr. B: Biomed. Appl.* **1995**, *674* (1), 93−99.

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