Conversion of Cephapirin to Deacetylcephapirin in Milk and Tissues of Treated Animals

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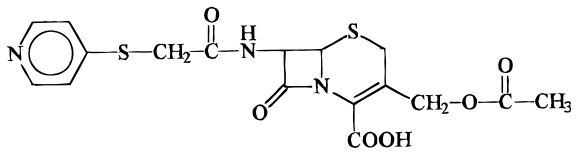
Cephapirin is one of six β-lactam antibiotics approved for use in the treatment of food-producing animals in the United States. When used for treatment of mastitis by intramammary infusion, it is partially converted to a microbiologically active metabolite identified as deacetylcephapirin (DACEP). The degradation was followed in four cows with naturally acquired mastitis which were treated with cephapirin. DACEP persisted longer than the parent compound in the milk. When a calf was treated with cephapirin by intramuscular injection, the compound was almost completely converted to DACEP in tissues. The deacetyl form must be considered in the determination of residues in treated animals.

Keywords: *Cephapirin; deacetylcephapirin; determination; liquid chromatography; β-lactam screening tests; milk; tissues*

INTRODUCTION

Cephapirin (CEP) is one of six β-lactam antibiotics approved for use in the treatment of food-producing animals in the United States (U.S. Food and Drug Administration, 1997). Tolerances of 100 and 20 ppb have been established for residues of CEP in tissues and milk, respectively (U.S. Code of Federal Regulations, 1998). These tolerances do not include any provision for the presence of biologically active metabolites. CEP is widely used for the treatment of dairy cows for mastitis. In a recent study (Moats, 1999), we found that CEP was the second most frequently found residue (after penicillin G) in commercial milk samples testing positive for the presence of β-lactam antibiotics by screening tests. Cabana et al. (1976) found that when CEP was administered intravenously to animals and humans, it was partially converted to a microbiologically active metabolite identified as deacetylcephapirin (DACEP). The structures of CEP and DACEP are shown in Figure 1. DACEP was also identified by Tyczkowska et al. (1991) in the milk of cows treated with CEP by intramammary infusion. Moats (1993) found that, in milk of cows treated with CEP, >50% of the residue was in the deacetyl form as determined by liquid chromatographic (LC) analysis and that DACEP could be detected for a longer period than the parent form. Seymour et al. (1988) and Oliver et al. (1990) found that, after administration of CEP to cows with mastitis, milk sometimes tested positive for antibiotics for longer than the speci-



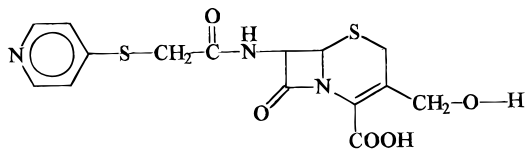


Figure 1. Structures of cephapirin (CEP) and deacetylcephapirin (DACEP).

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fied 96 h withdrawal time using, respectively, the *Bacillus stearothermophilus* disc assay (BSDA) and the Delvotest, which is based on inhibition of the growth of *B. stearothermophilus*. Van Eenennaam et al. (1993) reported that milk from cows with clinical mastitis frequently tested positive for antibiotics by a number of screening tests prior to administration of antibiotics and also 21 days after treatment. The issues of testing milk for antibiotics are discussed by Cullor (1996).

The occurrence of CEP residues in tissues has not been reported nor has any method been described for the determination of residues in tissues. The studies of Cabana et al. (1976) suggest that a considerable portion of tissue residues would be in the deacetyl form. As of this writing, CEP is not sold in a form suitable for injection into large animals. However, a tolerance has been established for CEP residues in tissues.

The present study includes a comparison of results of LC analysis and six β-lactam screening tests on milk from cows with naturally acquired mastitis which were treated with CEP by intramammary infusion. The LC method (Moats, 1993) was adapted to the determination of residues in tissues, and the application to the determination of residues in a calf treated by intramuscular injection of CEP is described.

MATERIALS AND METHODS

Treatment of Animals. *Intramammary Treatment.* Dairy cows that had spontaneously developed mastitis were selected for the study. Milk from the cows selected tested negative for antibiotics (with one exception) by each of the six screening tests used and by LC analysis. The cows were treated using a commercial CEP formulation (Cefa-Lak, Aveco Laboratories, Fort Dodge, IA) which consisted of 200 mg of CEP in peanut oil and which was infused into the affected quarter of the udder. A second treatment was given 12 h after the first. Composite milk samples from all four quarters were collected for analysis. These were tested by the six screening tests at North Carolina State University and were shipped frozen by overnight express to Beltsville for LC analysis.

Intramuscular Treatment. An 84 kg calf was treated intramuscularly with 30 mg/kg of CEP sodium (Cefadyl brand, Apethocon, a BristoL-Myers Squibb Co., Princeton, NJ). The calf was euthanized after 4 h. Blood and tissue (muscle, liver, and kidney) were collected immediately after slaughter. The tissues were frozen and maintained at −70 °C until they were shipped frozen by overnight express to Beltsville for analysis. Blood was centrifuged the next day, and the serum was frozen at −70 °C and shipped to Beltsville along with the tissues.

Screening Test Kits. Six commercial screening test kits that used a variety of approaches for detection of antibiotics were selected for the present study. Although not approved for use on milk samples from individual cows, it was of interest to compare results of screening tests and LC analysis. The Delvotest-P (Gist-Brocades Food Ingredients Inc., Menomonee Falls, WI) is based on inhibition of the growth of *B. stearothermophilus* in a tube with an indicator dye, bromcresol purple, which changes to yellow if no antibiotic is present in the sample. Two tests were receptor enzyme assays. These were the Delvo-X-Press β-lactam residue test kit (Gist Brocades Food Ingredients Inc.) and the SNAP β-lactam test kit (IDEXX Laboratories Inc., Westbrook, ME). The Penzyme milk test kit (Cultor Food Science Group, New York, NY) is based on inhibition of an enzyme by β-lactam antibiotics. An ELISA test procedure, the Lac-Tek B-L milk screening kit (IDEXX) was included. An assay based on competitive binding to bacterial receptors, the Charm II Tablet β-lactam test for penicillin G, amoxicillin, ampicillin, ceftiofur, and cephapirin (Charm Sciences, Malden, MA) was also included. The tests were all conducted according to the manufacturers' instructions, except that positive samples were not subjected to confirmatory testing as described by Anderson et al. (1998).

LC Analysis. *Chemicals and Reagents.* Acetonitrile was of HPLC grade (EM Omnisolv or equivalent). Tetraethylammonium chloride (Et_4NCl), 1-decanesulfonic acid (sodium salt, 98%), and dodecyl sulfate (sodium salt, 98%) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). CEP was purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were of reagent grade from several sources. A stock solution of CEP was prepared at 1 mg/mL and stored frozen at −20 °C until needed. Working dilutions of 100, 10, and 1 μg/mL were prepared biweekly or as necessary and stored at 4 °C. The 10 μg/mL standard was stored at room temperature.

Glassware and Other Equipment. Glassware required included graduated cylinders, 25 and 50 mL; conical graduated centrifuge tubes, 15 mL, calibrated to 1 and 4 mL; glass-stoppered sidearm flasks, 250 mL; and conical flasks, 125 mL. All glassware was cleaned in a special detergent (MICRO, International Products, Trenton, NJ, or equivalent) at ∼60 °C for 30 min (longer may etch glassware), then rinsed in deionized water, then rinsed for 5 min or longer in a dilute acid bath (∼0.01 M HCl or H_2SO_4), and then again with deionized water.

Other equipment required included a blender, Waring type, base, with 100 or 300 mL stainless steel jars with covers; a vortex evaporator (Buchler Instrument Co., Fort Lee, NJ); a thermostated hot plate with a shallow tray; and plastic-coated lead rings (I^2R Corp., Cheltenham, PA) to weight flasks during evaporation.

Extraction/Deproteinization. (a) Milk. Milk (10 mL) was measured into a 125 mL conical flask and mixed with 2 mL of 0.1 M Et_4NCl. Then, 40 mL of acetonitrile was added slowly with continual stirring (final volume = 50 mL). After standing for 10 min, the supernatant was decanted through a plug of glass wool in the stem of a funnel; 40 mL of filtrate (= 8 mL of milk) was collected and transferred to a 250 mL glass-stoppered sidearm flask, and 2 mL of 0.01 M, pH 6, buffer (5:1 KH_2PO_4/Na_2HPO_4) was added. The flasks were connected to a water pump vacuum. After the contents had stopped boiling, the flasks were weighted with lead rings and placed in a shallow (1−2 cm) water bath heated to 40−50 °C. The contents were evaporated to 1−2 mL, but not to dryness, and were rinsed into graduated tubes with several small portions of water to a final volume of 4 mL. This was filtered through a 25 mm, 0.45 μm, PVDF syringe filter into a 4 mL autosampler vial. This procedure was also used with blood serum.

(b) Tissue, Procedure I. Tissue was cut into small pieces, and 5 g was transferred to a small (100−300 mL) blender jar. Then, 5 mL of water, 2 mL of 0.1 M Et_4NCl (for liver and kidney, 1 mL of 0.2 M Et_4NCl and 1 mL of 0.005 M KH_2PO_4, respectively), and 40 mL of acetonitrile were added, and the mixture was blended for 1 min at half full power as measured by a variable resistance transformer (final volume = 50 mL). After standing for 10 min, the supernatant was decanted through a small plug of glass wool in the stem of a funnel; 40 mL of filtrate (20 mL for liver and kidney) was collected, which was equivalent to 4 g of tissue (2 g for liver and kidney). The filtrate was transferred to a 250 mL sidearm flask, and 5 mL of water and 5 mL of *tert*-butyl alcohol (to suppress foaming) were added. The filtrate was concentrated by evaporation as described for milk. If foaming persisted, more *tert*-butyl alcohol was added, always with an equal volume of water.

(c) Tissue, Procedure II. Tissue was cut into small pieces, and 15 g was weighed into a 100−300 mL blender jar and blended with 45 mL of water for 1−2 min at low power until the tissue was thoroughly broken up. Ten milliliters of homogenate was treated as described for milk. For evaporation, *tert*-butyl alcohol and water were added to the filtrate to suppress foaming.

HPLC Fractionation. The HPLC system used for cleanup consisted of a Varian (Sugarland, TX) model 9012 pump, a Waters (Milford, MA) WISP 712 autosampler with a 2000 μL loop, an ISCO (Lincoln, NE) FOXY fraction collector, a Waters 990 diode array detector, and a Supelcosil LC-18 column (4.6 × 150 mm, 5 μm particle size) (Supelco, Bellefonte, PA). When

Table 1. Depletion of Cephapirin from Cow 1 after Treatment by Intramammary Infusion[a]

sample	day (time)	CEP[b] (ppb)	DACEP (ppb)	Delvo-test-P	Delvo-X-Press	Penzyme	SNAP	Charm II	Lac-Tek B-L
0[c] (control)	1 (a.m.)	0[d]	0	−	−	−	−	−	−
1[c]	1 (p.m.)	1700	4620	+	+	+	+	+	+
2	2 (a.m.)	1670	2930	+	+	+	+	+	+
3	2 (p.m.)	146	500	+	+	+	+	+	+
4	3 (a.m.)	11	39	+	+	+	+	+	+
5	3 (p.m.)	2.8	9.3	+	+	+	+	+	+
6	4 (a.m.)	0	3	−	+	−	+	+	−
7	4 (p.m.)	ND[e]	0	−	+	−	+	-	−
8	5 (a.m.)	ND	0	−	−	−	−	−	−

[a] Milk samples analyzed were composites of four-quarters except as indicated. [b] CEP, cephapirin; DACEP, desacetylcephapirin. [c] Two hundred milligrams of cephapirin in peanut oil (Cefa-Lak) administered by intramammary infusion following this milking. [d] 0 = none detected. The detection limit is ~1 ppb. [e] ND, not done.

Table 2. Depletion of Cephapirin from Cow 2 after Treatment by Intramammary Infusion[a]

sample	day (time)	CEP[a] (ppb)	DACEP (ppb)	Delvo-test-P	Delvo-X-Press	Penzyme	SNAP	Charm II	Lac-Tek B-L
0[b] (control)	1 (p.m.)	0	0	−	−	−	−	−	−
1[b]	2 (a.m.)	1600	1850	+	+	+	+	+	+
2	2 (p.m.)	2000	3400	+	+	+	+	+	+
3	3 (a.m.)	96	330	+	+	+	+	+	+
4	3 (p.m.)	15	41	+	+	+	+	+	+
5	4 (a.m.)	1.3	7.7	+	+	+	+	+	+
6	4 (p.m.)	0	1.3	+	+	+	+	+	−
7	5 (a.m.)	0	0	+	+	+	+	+	+
8	5 (p.m.)	0	0	−	+	+	+	+	+
9	6 (a.m.)	0	0	−	+	+	+	−	−
10	6 (p.m.)	0	0	−	+	−	+	−	−
11	7 (a.m.)	0	0	−	+	−	−	−	−
12	7 (p.m.)	0	0	−	−	−	−	−	−

[a] Footnotes, see Table 1. [b] Two hundred milligrams of cephapirin in peanut oil (Cefa-Lak) administered by intramammary infusion following this milking.

a sample was injected, the autosampler started the other components of the system. Two milliliters of sample extract was loaded onto the HPLC column with a flow of 100% 0.01 M KH_2PO_4, at a flow rate of 1 mL/min. After 3 min, an acetonitrile gradient was started to 60% acetonitrile at 40 min. The column was returned to starting conditions at 41 min and was ready to load another sample at 55 min. The 10 μg/mL standard stored at room temperature was run initially to determine the CEP and DACEP retention times. The fraction collector was set to collect 1.5−2.0 time windows centered on the retention time of these analytes. The DACEP fraction was collected for use as a reference.

Analysis of Fractions. Prior to evaporation, 0.2 mL of 0.01 M KH_2PO_4, 0.01 M H_3PO_4, and 0.01 M sodium decanesulfonate was added to each fraction. The fractions were evaporated to <1 mL under reduced pressure in the vortex evaporator, and the volume was adjusted to 1 mL with water. The HPLC system used for analysis consisted of a Varian model 9012 pump, a Varian 9090 autosampler with a 200 μL loop, a Waters 481 UV−vis detector, and a Varian model 654 data system, with a flow rate of 1 mL/min and UV detection at 290 nm. For CEP, a Supelcosil LC-18 column, 4.6 × 150 mm, 5 μm particle size (Supelco) was used with a mobile phase of 0.015 M H_3PO_4, 0.0075 M sodium dodecyl sulfate/acetonitrile (65:35). For DACEP, a Polymer Laboratories (Amherst, MA) PLRP-S column, 4.6 × 150 mm, 5 μm particle size, was used with a mobile phase of 0.01 M H_3PO_4, 0.01 M KH_2PO_4, 0.01 M sodium decanesulfonate/acetonitrile (82:18). The mobile phases were premixed and were stable indefinitely.

RESULTS AND DISCUSSION

Previous studies (Cabana et al., 1976; Tyczkowska et al., 1991; Moats, 1993) have shown that deacetylcephapirin (DACEP) is an important metabolite of cephapirin (CEP) in treated animals. Cabana et al. (1976) reported that they prepared a standard of DACEP. However, no standard is currently available. A compound with chromatographic behavior identical to that of the DACEP metabolite was formed when an aqueous CEP

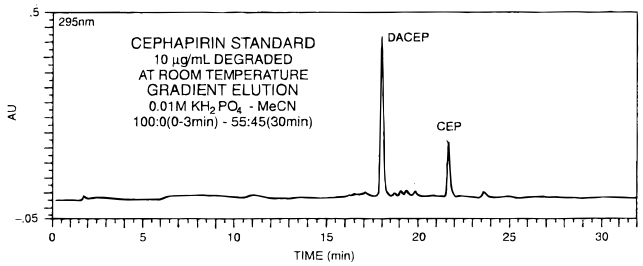

Figure 2. Gradient elution of an aged aqueous CEP standard.

standard solution was allowed to stand at room temperature for several weeks. This was separated from the parent compound by gradient elution (Figure 2) and was isolated by LC fractionation. This was used as a reference to identify DACEP on chromatograms. However, because the purity was unknown, it could not be used for quantitation of DACEP. For quantitation, it was therefore assumed that DACEP had the same molar UV absorption as the parent compound.

For the present study, cows that had spontaneously developed mastitis were selected for treatment. Milk from each cow was analyzed by HPLC and by six screening tests that incorporated a variety of approaches for detection of antibiotics including microbial inhibition (Delvotest-P), immunoassay (Lac-Tek), competitive binding to bacterial receptors (Charm II), enzyme inhibition (Penzyme), and receptor enzyme assays (SNAP, Delvo-X-Press). For this study, cows in which the test results were negative prior to treatment were selected to avoid ambiguity in interpretation of the results after treatment. However, with cow 4 (Table 4) the Charm test was positive. This milk was grossly abnormal (watery) in appearance. It should be noted that the tests are not approved for testing milk from individual cows and, in fairness to the manufacturers, obviously abnormal milk should not be tested (Anderson et al., 1998). The

Table 3. Depletion of Cephapirin from Cow 3 Following Treatment by Intramammary Infusion[a]

sample	day (time)	CEP[a] (ppb)	DACEP (ppb)	Delvo-test P	Delvo-X-Press	Penzyme	SNAP	Charm II	Lac-Tek B-L
0[b] (control)	1 (a.m.)	0	0	-	-	-	-	-	-
1[b]	1 (p.m.)	1810[c]	7700[c]	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]
2	2 (a.m.)	690	880	+	+	+	+	+	+
3	2 (p.m.)	28	140	+	+	+	+	+	+
4	3 (a.m.)	0	2.7	+	+	+	+	+	+
5	3 (p.m.)	2.1	0	-	+	+	+	+	-
6	4 (a.m.)	0	0	-	+	-	+	-	-
7	4 (p.m.)	0	0	-	-	-	-	-	-
8	5 (a.m.)	0	0	-	-	-	-	-	-

[a] Footnotes, see Table 1. [b] Two hundred milligrams of cephapirin in peanut oil (Cefa-Lak) administered by intramammary infusion following this milking. [c] Milk from affected quarter only.

Table 4. Depletion of Cephapirin from Cow 4 Following Treatment by Intramammary Infusion[a]

sample	day (time)	CEP (ppb)	DACEP (ppb)	Delvo-test P	Delvo-X-Press	Penzyme	SNAP	Charm II	Lac-Tek B-L
0[b] (control)	1 (a.m.)	0	0	−	−	−	−	+[c]	−
1[b]	1 (p.m.)	85	4470	+	+	+	ND[c]	+	+
2	2 (a.m.)	36	830	+	+	+	+	+	+
3	2 (p.m.)	14[d]	670[d]	+	+	+	+	+	+
4	3 (a.m.)	0	5	+	+	+	+	+	+
5	3 (p.m.)	0	2.4	−	−	+	+	+	−
6	4 (a.m.)	0	0	−	−	+	+	−	−
7	4 (p.m.)	0	0	−	−	−	−	−	−
8	5 (a.m.)	ND	ND	−	−	−	−	−	−

[a] Footnotes, see Table 1. [b] Two hundred milligrams of cephapirin in peanut oil (Cefa-Lak) administered by intramammary infusion following this milking. [c] Abnormal milk. [d] Milk from affected quarter only.

treatment consisted of two successive treatments at 12 h intervals by intramammary infusion of 200 mg each of a commercial CEP formulation. The results are summarized in Tables 1−4. CEP was mainly converted to the deacetyl form in the milk of treated cows. The DACEP/CEP ratio varied greatly, ranging from <2:1 in cow 2 to as much as 50:1 in cow 4. DACEP could be measured by LC analysis for one or more milkings longer than the parent compound. The screening tests all were positive with milk containing violative (>20 ppb) (U.S. Code of Federal Regulations, 1998) levels of CEP. Some of the screening tests were positive after residues had depleted below levels detectable by LC analysis (~1 ppb). This was particularly true with cow 3. In time, milk from all of the cows tested negative by all of the screening tests, which indicated that the positive test results probably resulted from trace levels of CEP and/or metabolites. These results are consistent with those of Seymour et al. (1988) and Oliver et al. (1990), who found that milk from cows treated with CEP eventually tested negative by the BSDA and the Delvotest-P, respectively, although a few tested positive after the prescribed withholding time of 96 h. The U.S. Food and Drug Administration (1996) has defined positive screening test results on milk containing residues below tolerance levels as "false-violative". The term "false-positive" is reserved for test results that are positive when no antibiotic is present. Thus, with the one exception noted, none of our positive screening test results are "false-positives" by the FDA definition. Our results are not consistent with those of Van Eenennaam et al. (1993) and Cullor (1996), who reported that milk from cows with clinical mastitis gave a high incidence of "false-positive" tests with three of four screening tests evaluated as well as the BSDA. Their conclusions were based on tests run prior to treatment with antibiotics and repeated 21 days following treatment. The presence or absence of residues was not confirmed by more specific chemical procedures.

Some comparative studies of the sensitivity of screening tests (U.S. Food and Drug Administration, 1997) have shown that levels of CEP detected by screening tests with 95% confidence ranged from 3.0 ppb (SNAP) to 18.7 ppb (Lac-Tek B-L), but a significant number tested positive at 2 ppb. These were spiked rather than incurred samples. The response of screening tests to DACEP has not been reported. Cabana et al. (1976) reported that DACEP had 54% of the antimicrobial activity of the parent compound when *Sarcina lutea* was used as the test organism. The sensitivity of the BSDA to DACEP has not been reported.

All of the screening tests reliably detected violative levels of CEP. However, some were quite sensitive and, if the results from testing individual cows are indicative of what might be found when testing comingled milk, could result in rejection of milk containing levels of CEP or metabolites far below violative levels. Although the present study did not confirm the presence of true false-positives as reported by van Eenennaam et al. (1993) and Cullor (1996), their possible occurrence must be considered. Confirmation by LC analysis would therefore prevent economic losses resulting from rejection of milk shipments based on "false-positve" or "false-violative" screening tests. The LC analysis procedure should be able to determine DACEP as well as CEP because DACEP may produce a positive screening test result in the absence of detectable levels of CEP. This information may be useful as a guide to producers who are seeking causes for positive screening test results. The regulatory significance of DACEP residues is not clear. Some analyses of milk samples from commercial sources that had been rejected because they tested positive for β-lactam antibiotics by one or more screening tests (Moats, 1999) indicated that they had been rejected because of the presence of low levels of DACEP.

Because a tolerance of 100 ppb has been established for CEP in edible tissues of animals, it was of interest to determine if the LC method developed for residues in milk could be adapted to the analysis of residues in tissues. This was mainly of theoretical interest because CEP is not, as of this writing, sold in a form suitable for treatment of large animals by intramuscular injec-

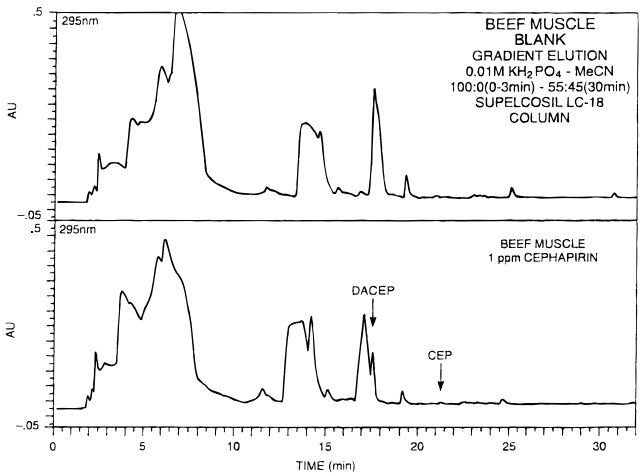

Figure 3. Conversion of CEP to DACEP in a beef muscle homogenate.

Table 5. Recovery of Cephapirin from Tissues and Serum of a Calf Treated by Intramuscular Injection

tissue	CEP (ppm)	DACEP (ppm)
injected muscle	2.74	11.1
muscle	0.0034	0.67
liver	0[a]	2.23
kidney	0.015	67.3
blood serum	0.56	0.75

[a] 0 = none detected.

Table 6. Recovery of Cephapirin as Deacetylcephapirin from Beef Muscle and Kidney

	% recovery (DACEP calcd as CEP) with CEP added at		
tissue	0.01 ppm	0.1 ppm	1.0 ppm
muscle	146	87, 84	82, 83
kidney	152, 445	86, 83	91, 76

tion. Our initial approach was to spike the tissue sample with CEP, prepare a water homogenate, and then process the homogenate as described for milk (procedure II). However, CEP was rapidly degraded to DACEP in the homogenate. Figure 3 shows gradient elution of an extract of a muscle homogenate spiked with CEP. The chromatographic peak corresponding to CEP was not present, and a peak corresponding to DACEP was clearly visible as a shoulder on a large interference. It thus seemed likely that CEP would be degraded to DACEP when injected intramuscularly. To confirm this, a calf was treated by intramuscular injection and slaughtered 4 h after treatment. A modified extraction procedure was developed in which tissues were blended directly in acetonitrile to reduce the possibility of degradation during the extraction process (Moats and Romanowski, 1998). The results are summarized in Table 5 and confirm that CEP residues in tissues, if present, will be mainly in the deacetyl form. However, when tissues were spiked with CEP, some degradation to the deacetyl form was observed even with the direct extraction procedure, so the results in Table 5 may underestimate the actual levels of parent compound present in tissues. The ratio of DACEP/CEP in blood serum was similar to that observed by Cabana et al. (1976). It is, therefore, probably preferable to determine total tissue residues of CEP and metabolites as DACEP using extraction procedure II. Table 6 summarizes the recovery of added CEP as DACEP from calf muscle and kidney using extraction procedure II. The recovered DACEP was calculated as CEP. At 0.01 ppm, quantitation was poor. Recoveries were good and consistent at higher levels. DACEP, if present in tissues, would undoubtedly be detected by screening tests based on microbiological inhibition. It would thus be useful to distinguish it from other possible β-lactam antibiotics even though the regulatory status is uncertain.

LITERATURE CITED

Anderson, K. L.; Moats, W. A.; Rushing, J. E.; O'Carroll, J. M. Detection of Milk Antibiotic Residues by Use of Screening Tests and Liquid Chromatography after Intramammary Administration of Amoxicillin and Penicillin G to Cows with Clinical Mastitis. *Am. J. Vet Res.* **1998**, *59*, 1096−1100.

Cabana, B. E.; Van Harken, D. R.: Hottendorf, G. H. Comparative Pharmokinetics and Metabolism of Cephapirin in Laboratory Animals and Humans. *Antimicrob. Agents Chemother.* **1976**, *10*, 307−317.

Cullor, J. S. Dilemmas Associated with Antibiotic Residue Testing in Milk: Choices, Problems and Issues. In *Veterinary Drug Residues Food Safety*; Moats, W. A., Medina, M. B., Eds.; ACS Symposium Series 636; American Chemical Society: Washington, DC, 1996; pp 44−57.

Moats, W. A. Determination of Cephapirin and Desacetylcephapirin in Milk Using Automated Liquid Chromatographic Cleanup and Ion-pairing Liquid Chromatographic Cleanup. *J. AOAC Int.* **1993**, *76*, 535−540.

Moats, W. A. Confirmatory Test Results on Milk from Commercial Sources which Tested Positive by β-Lactam Screening Tests. *J. AOAC Int.* **1999**, *82*, 1071−1076.

Moats, W. A.; Romanowski, R. D. Multiresidue Determination of β-Lactam Antibiotics in Milk and Tissues with the Aid of High-performance Liquid Chromatographic Fractionation for Cleanup. *J. Chromatogr. A* **1998**, *872*, 237−247.

Oliver, S. P.; Maki, J. L.; Dowlen, H. H. Antibiotic Residues in Milk Following Antimicrobial Therapy During Lactation. *J. Food Prot.* **1990**, *53*, 693−696.

Rajkowski, K. T.; Peeler, J. T.; Messer, J. W. Detectability levels of four β-Lactam Antibiotics in Eight Milk Products Using the AOAC *Bacillus stearothermophilus* Disc Assay. *J. Food Prot.* **1986**, *49*, 687−690.

Seymour, E. H.; Jones, G. M.; McGilliard, M. L. Persistence of Residues in Milk Following Antibiotic Treatment of Dairy Cattle. *J. Dairy Sci.* **1988**, *71*, 2292−2296.

Tyczkowska, K. L.; Voyksner, R. D.; Aronson, A. L. Development of an Analytical Method for Cephapirin and its metabolite in bovine milk and serum by liquid chromatography with UV−Vis Detection and Confirmation by Thermospray Mass Spectrometry. *J. Vet. Pharmacol. Ther.* **1991**, *14*, 41−60.

U.S. Code of Federal Regulations, Sec. 21, Par. 556.115, April 1, 1998.

U.S. Food and Drug Administration. *Evaluation and Use of Milk Antimicrobial Drug Screening Tests*; CVM/FDA, Office of Science: Rockville, MD, Jan 1996.

U.S. Food and Drug Administration. *Beta Lactam Test Methods for Use under Appendix N of the PMO*; Memo M-a-85 Revision 6; CFSAN: Washington, DC, July 14, 1997.

Van Eenennaam, A. L.; Cullor, J. S.; Perani, L.; Gardner, I. A.; Smith, W. L.; Dellinger, J.; Guterbock, W. M.; Jensen, L. Evaluation of Milk Antibiotic Residue Screening Tests in Cattle with Naturally Occurring Clinical Mastitis. *J. Dairy Sci.* **1993**, *76*, 3041−3053.

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