CEFTIOFUR

First draft prepared by
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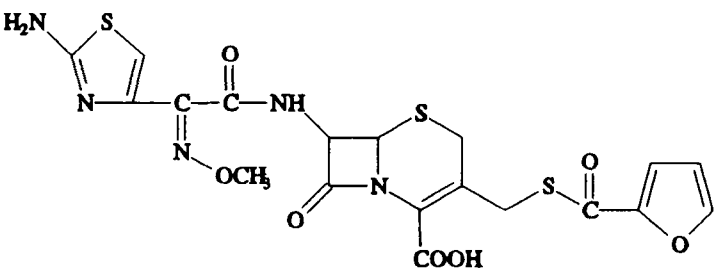
IDENTITY

Chemical names: Ceftiofur; IUPAC name: [6R-[6α,7β(Z)]]-7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[(2-furanylcarbonyl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Synonyms: Ceftiofur sodium, Na ceftiofur, Naxcel, Excenel, CM 31-916, U64279E (Product Trade Names)

Structural formula:



Ceftiofur

Molecular formula: $C_{19}H_{17}N_5O_7S_3$

Molecular weight: 523.55

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Melting point: Degrades on heating (as sodium salt, at 190 to 300°).

Solubility: Freely soluble in water as sodium salt (>400 mg/mL in buffered solutions above pH 5.5); found in solutions as free acid at pH <3.2 with solubility of approximately 0.25 mg/mL. The sodium salt is slightly soluble in organic solvents such as iso-octane (0.006 mg/mL) and chloroform (0.008 mg/mL) and solubility increases for such solvents as ethyl acetate (0.038 mg/mL), acetonitrile (0.072 mg/mL), toluene (0.078) and methylene chloride (0.114 mg/mL) to higher values for tetrahydrofuran (0.2 mg/mL) and n-butyl chloride (0.76 mg/mL).

Optical rotation: As sodium salt, 67° for a 1% w/v aqueous solution at 589 nm.

Other properties: Ceftiofur sodium is hygroscopic. X-ray diffraction patterns indicate an amorphous structure.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Ceftiofur is a third-generation cephalosporin antibiotic developed exclusively for veterinary use. It is available commercially as the sodium salt and exhibits a broad spectrum of activity against both gram-positive and gram-negative bacteria, including β-lactamase-producing strains. It is also active against some anaerobic bacteria. Ceftiofur is bactericidal *in vitro*, inhibiting cell wall synthesis. It is rapidly metabolized *in vivo* to desfuroylceftiofur, which is also microbiologically active. Ceftiofur sodium is off-white to tan in colour and the formulated product may be stored frozen for up to 8 weeks without loss of potency. Storage of the unreconstituted product at a refrigerator temperature of 2 to 8°C is recommended. The reconstituted product is stable under these conditions for up to 7 days, or for 12 hours at room temperature. The product should be protected from light during storage as it is light sensitive. Direct contact with the skin and mucous membranes should be avoided by humans handling the drug to minimize the possibility of a reaction in sensitized individuals. Ceftiofur has not been previously reviewed by the Committee.

Dosage

Ceftiofur sodium is formulated as a dry, lyophilized sterile powder which is re-constituted in sterile or bacteriostatic water for injection. It is available in 1- and 4-gram vials containing ceftiofur sodium equivalent to 50 mg/mL ceftiofur. The recommended dose for cattle is 1.0 to 2.2 mg ceftiofur free acid equivalents (CFAE) per kilogram of body weight, repeated at 24-hour intervals on three consecutive days. When necessary, the treatment may be extended to days 4 and 5. For swine, the recommended dose is 3.0 - 5.0 mg ceftiofur (CFAE) per kilogram of body weight at 24 hour intervals on three successive days. Ceftiofur formulated product is intended only for administration by intramuscular injection in cattle and swine.

METABOLISM AND PHARMACOKINETICS

General

Ceftiofur has been observed *in vivo* to have a more complex metabolism than other cephalosporins, primarily due to the cleavage of its thioester bond, which yields desfuroylceftiofur (DFC) and furoic acid. Studies comparing the metabolism of ceftiofur by the S-9 microsomal enzyme fraction from liver and kidney tissues of various species (rats, pigs, cattle, chickens) demonstrated that ceftiofur was converted *in vitro* first to DFC, with the primary metabolite in kidney being desfuroylceftiofur cysteine disulfide (DCD) and, in liver, being DFC and 3,3'-desfuroylceftiofur disulfide dimer (3,3'-DFD) (Gilbertson *et al*, (1990). Qualitatively, results observed were the same for all species, but metabolism was slower in S-9 fractions from liver and kidney of rats than for the other species tested. Highest concentrations of 3,3'-DFD were observed in chicken liver and kidney S-9 fractions in the *in vitro* studies, while lowest concentrations of this compound were found in rat tissue fractions.

Rat

Following a single oral dose of 200 mg/kg BW ^{14}C-ceftiofur sodium salt, a similar pattern of metabolic breakdown was observed in rats to that described below for cattle receiving the drug by intramuscular injection (Jaglan & Arnold, 1986a,b). The initial metabolite was identified as DFC. Urine contained desfuroylceftiofur thiolactone and DCD (Jaglan & Roof, 1989). Oral administration of ^{14}C-ceftiofur to rats at a rate of 800 mg/kg BW/day for 5 successive days confirmed that DFC was the central metabolite and demonstrated that a similar pattern of metabolites was found in the livers and kidneys of the rats as that observed in tissues of cattle

receiving an intramuscular injection of 44 mg/kg BW (Jaglan *et al*, 1987b). Additional polar metabolites were produced in rats that were not found in studies with cattle and sex related differences in these metabolites were observed only in rats. The primary urinary metabolite in rats was ceftiofur sulfoxide cysteine thioester, shown to be produced by enteric metabolism after oral dosing (Jaglan & Arnold, 1987a). Additional studies using improved LC separations for the metabolites confirmed that the thioester was the primary urinary metabolite in rats, in both 8-hour and 24-hour urine samples after oral dosing (Jaglan & Roof, 1990).

Ceftiofur sulfoxide cysteine conjugate was not detected at low oral doses of 15 mg/kg BW in rats, but was found at dosage rates of 100 - 1000 mg/kg BW (Jaglan, 1987). This compound was not found in the studies conducted with S-9 fractions of liver and kidney from rats and other species (Gilbertson *et al*, 1990), suggesting that this compound is formed uniquely in the gastrointestinal tract of rats. Intramuscular injection of rats with ceftiofur sodium salt produced the same metabolic profile as was seen for this treatment in cattle, with the exception that urine from the rats contained 4.4-21% unmetabolized ceftiofur (Jaglan & Arnold, 1987).

In rats, the primary metabolite in plasma, DFC, was present in a bound form, while DFC was in a free form in cattle plasma (Jaglan & Arnold, 1986b). Further research in rats injected intramuscularly with ^{14}C-ceftiofur sodium demonstrated that, in serum, covalent binding of DFC occurred primarily to albumin and α-1-antitrypsin through the formation of a thioester bond at the HS-group (Jaglan *et al*, 1991). Oral treatment with either the sodium or the hydrochloride salt of ceftiofur produced the same metabolic results (Jaglan *et al*, 1987a).

Incubation of ^{14}C-ceftiofur (119 mg/l) for 15 minutes with cofactor-augmented media containing the S-9 fraction of rat liver microsomal supernatants from Arochlor-1254 induced Fischer 344 rats resulted in 98.5% conversion to DFC. When the initial concentration of ceftiofur was raised to 857 mg/L, 52.5% conversion to DFC was observed at 15 minutes, with complete conversion in 60 minutes (Jaglan *et al*, 1987c).

Cattle

In an early study of the distribution and elimination of ^{14}C-ceftiofur in cattle (Krzeminski *et al*, 1985), LC analyses demonstrated the presence of two apparent metabolites ("C1", "C") in plasma. At one hour post-treatment, C1 reached a maximum concentration, but was not detected at 16 hours after treatment. Further work indicated that the compound designated as "C" in the report was the metabolite and that "C1" was formed on exposure of "C" to trifluoroacetic acid used in sample dilution prior to storage. Two major radioactive peaks, as well as several minor ones, were found in urine samples. Subsequent studies described above comparing metabolism in the rat and the bovine provided identification of the major urinary metabolites and demonstrated that 3,3'-DFD was a major urinary metabolite in cattle (Jaglan & Arnold, 1987b; Jaglan, Kubicek & Gilbertson, 1987). The proposed metabolic pathway for cattle which receive ceftiofur by intramuscular injection is shown in Figure 1. This pathway is also considered to be representative for other mammalian species studied to date for intramuscular administration.

Analyses were conducted on samples collected following treatment of 6 lactating dairy cattle with 5 consecutive intramuscular injections (24-hour intervals) of 2.29 mg ^{14}C-ceftiofur sodium/kg BW (Jaglan *et al*, 198^{0}$_{d}$). It was observed that most of the ceftiofur was eliminated in the urine (62.8 $\pm$ 7.6%) and faecal matter (35.7 $\pm$ 9.7%) within 24 hours of treatment. Only 0.15 $\pm$ 0.03% of the ceftiofur was accounted for as residues in milk. Tissues collected from the animals at slaughter 5 days after the last injection again demonstrated, on analysis, that the highest residues were present in kidney (2.50 $\pm$ 0.67 mg/kg). Residues in liver were 0.37 $\pm$ 0.03 mgk/g, while those in fat and muscle were $<$ 0.1 mg/kg. Maximum concentrations of ceftiofur were in blood samples taken 1 - 2 hours after each treatment (5.1 - 12.1 μg/mL). Residues were essentially in the plasma fraction and were essentially bound to macromolecules, as observed in experiments with rats and pigs. The two main metabolites found in urine samples collected 6 hours after the last treatment were 3,3'-desfuroylceftiofur disulfide (dimer) and DCD.

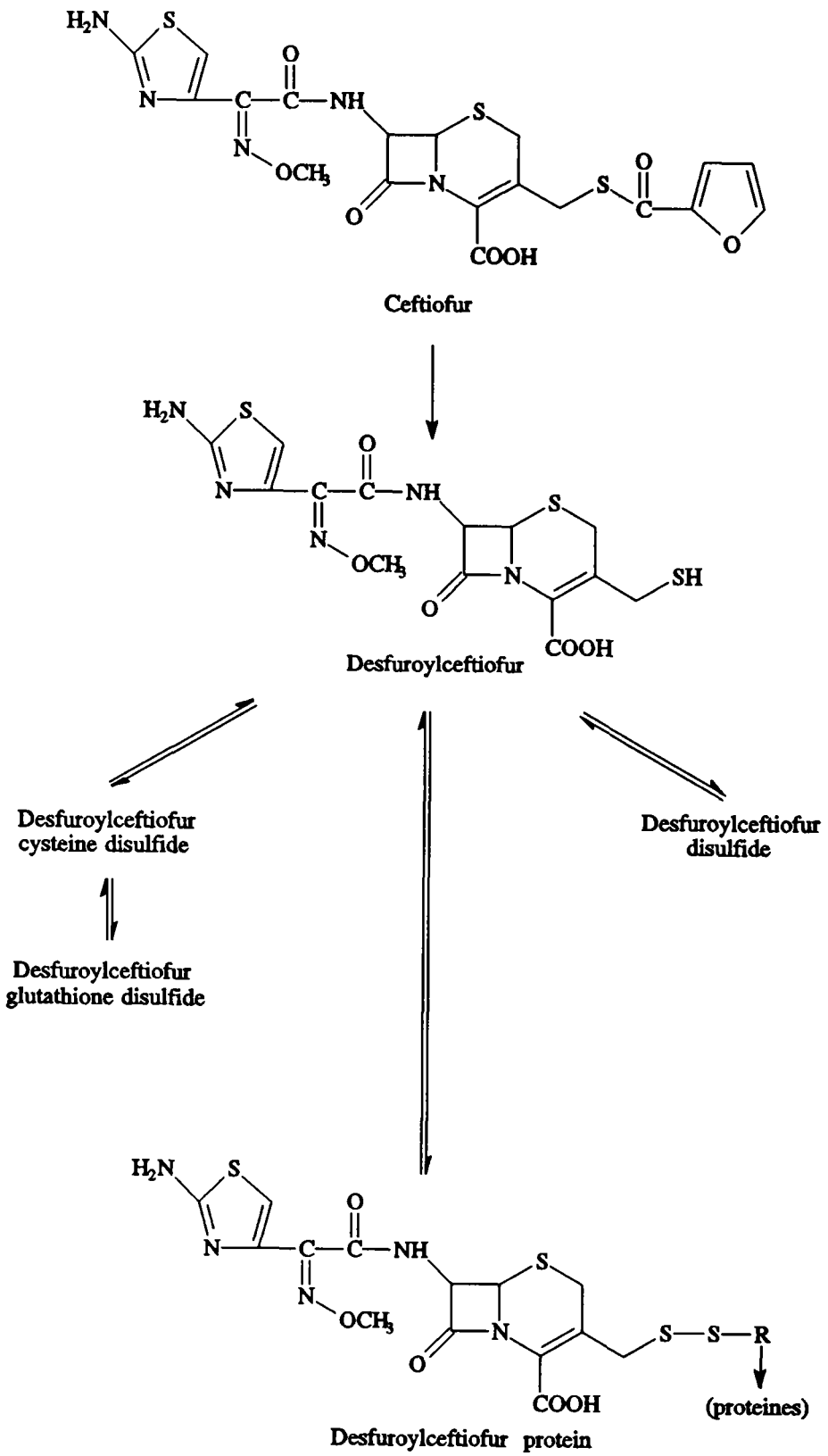
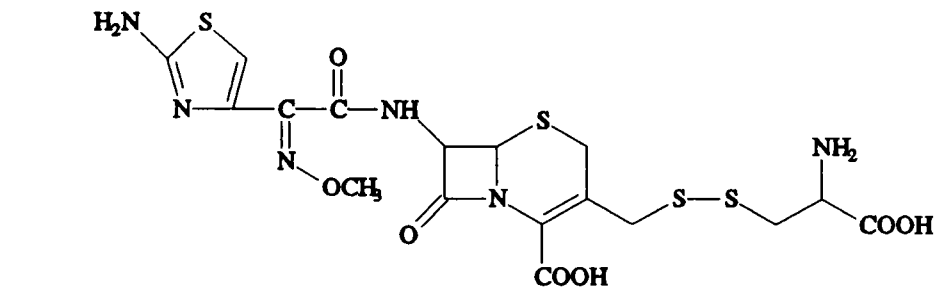
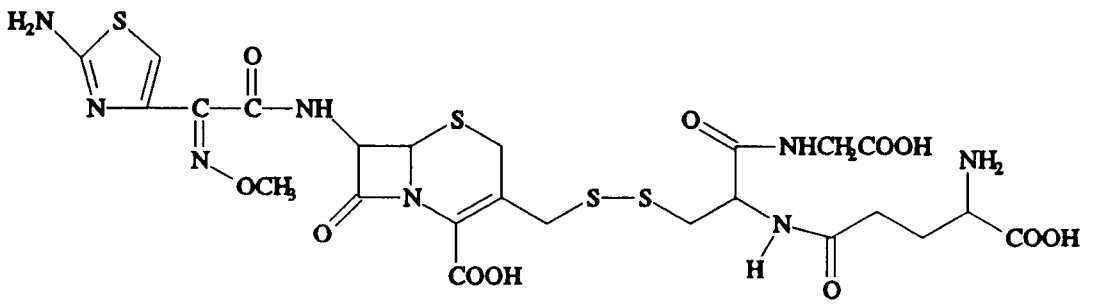


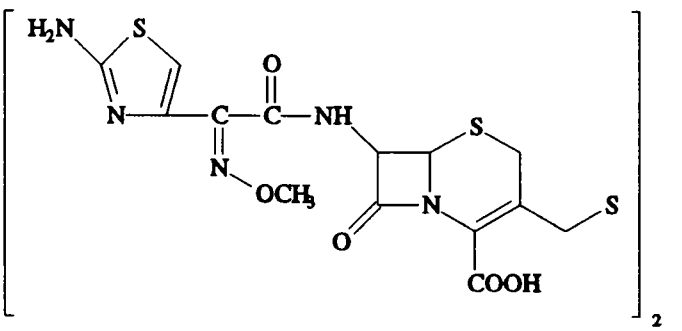
Figure 1. Proposed metabolism for ceftiofur in cattle.



Desfuroylceftiofur
cysteine disulfide



Desfuroylceftiofur
glutathione disufide



Desfuroylceftiofur
disulfide

Figure 2. Proposed metabolites in cattle

Residues in urine were about 10 μg/mL at 36 hours following the last injection and gave a positive result when assayed with the Live Animal Swab Test (LAST).

The half-life for ceftiofur sodium administered to dairy cattle has been calculated to be 212 minutes for intramuscular injection and 217 minutes for intravenous injection (Soback *et al*, 1989). This study demonstrated good absorption of the drug and a high estimated bioavailability, with no active residues detected in any milk samples collected (2, 4, 6 and 8 hours after drug administration, bioassay with *B. stearothermophilus* with detection limit of 0.1 mg/L).

Table 1. Pharmacokinetics of ceftiofur in the bovine.

Variables	Calves[a]	Calves[a]	Lactating cows[b]	Calves[c]
Dose (mg/kg)	1.0	1.0	2.0	2.2
Route of Administration	IM	IV	IV	IM
Method of Assay	HPLC-DCA[d]	HPLC-DCA	Bioassay	Bioassay
C_{max}(μg/mL)	4.12±0.84	7.09±1.59	ND[e]	8.78±2.47
t_{max}(h)	0.75±0.27	0.30±0.59	ND	1.82±0.89
AUCα(μg.h/mL)	34.3±6.29	35.9±11.6	27.2±5.42	66.2±21.6
$t_{½}$(h)	9.65±1.97	8.63±1.28	3.60±0.76	3.58±0.70
MRT(h)	11.4±1.75	10.3±1.72	5.07±1.10	ND
Vd_{ss}(L/kg)	ND	ND	0.39±0.16	ND
Cl_B(mL/min/kg)	ND	ND	1.27±0.26	ND

[a] Banting *et al*, 1989b.
[b] Soback *et al*, 1989.
[c] Halstead, 1990.
[d] HPLC-DCA refers to total desfuroylceftiofur, assayed as desfuroylceftiofur acetamide.
[e] ND means "not detected".

Data reported in a recent study where calves received ^{14}C-ceftiofur sodium intravenously at doses of 0.55, 2.2 and 8.8 mg CFAE/kg BW were consistent with those presented in the above table and demonstrated a proportional relationship between peak plasma concentrations and dosage rate (Brown *et al*, 1993). Based on the data for urinary excretion, there was also no indication of saturation of excretory pathways at the dosages tested. As this report was only available in draft form at the time of this evaluation, actual data from the study have not been included.

The distribution of ^{14}C-ceftiofur sodium was also studied in calves (94.0 - 136.4 kg), which each received three intramuscular injections of 2.2 mg/kg BW at 24-hour intervals (Johnson *et al*, 1985a), following which calves were sacrificed at 8 hours and 3, 21 and 39 days, respectively, and samples were analyzed by total radioactivity. Maximum residue levels (5 - 8.5 mg/L) in blood were at 0.5 - 1.0 hours post-treatment, declining rapidly to about 0.5 mg/L at 24 hours post-treatment. Average total excretion of residues ranged from 76.5% at 8 hours to 103.1% at day 39, with an average for the four calves of 87.2%. Overall, 95% of excreted residues were eliminated in the first day of withdrawal, with urinary excretion being 1.2 to 2.5 times that of faecal excretion. At 8 hours, residues in kidney, liver, fat and muscle were 3.51, 1.29, 0.32 and 0.21 mg/kg, respectively, while residues in lung tissue were 0.92 mg/kg. Injection sites contained 1.5 to 3.9 mg/kg of ceftiofur residues. At day 3, all samples contained less than 1.0 mg/kg of residues, with concentrations in muscle being 0.02 mg/kg. No residues were detectable in muscle at 21 and 39 days of withdrawal, but low concentrations (0.01 - 0.16 mg/kg) persisted in liver and kidney. Injection sites contained 0.01 - 0.40 mg/kg of residues at these withdrawal times. Residue depletion curves for the liver, kidney and lung were biphasic.

Further research was conducted in which 8 Holstein calves received 5 intramuscular injections at 24-hour intervals with ^{14}C-ceftiofur sodium (2.2 mg/kg BW), after which 2 calves were killed following each of 3, 20,

40 and 119 days withdrawal (Johnson *et al*, 1988). These data were combined with the 8-hour data from a related experiment using the same treatment regimen (Johnson *et al*, 1986) to demonstrate that depletion followed a biphasic curve in kidney, liver, muscle and lungs of the treated calves. Furthermore, they demonstrated a crossover at 20 days for the liver and kidney curves, so that while the kidney was identified as the target tissue up to 20 days withdrawal, the liver was recommended as the target tissue at longer withdrawal times. Values for k and $t_{½}$ calculated for these curves are given in Table 2.

Table 2. Residue decline rates in tissues calculated for calves treated with ^{14}C-ceftiofur sodium.

	Phase 1		Phase 2		
Tissue	k ($days^{-1}$)	$t_{½}$(days)	k ($days^{-1}$)	$t_{½}$(days)	Corr.
Kidney	0.42	1.66	0.022	31.5	0.999
Liver	0.41	1.69	0.012	57.8	0.931
Muscle[1]	0.60	1.16	0.008	86.6	0.836
Lung	0.55	1.26	0.013	53.3	0.988
Blood	1.24	0.56	0.033	21.0	0.995

[1]Calculation of muscle depletion rate for phase 2 compromised by analytical detection limit of 0.01 mg/kg.

Swine

The metabolic profile of ceftiofur in the urine and kidney of pigs treated with ^{14}C-ceftiofur sodium by intramuscular injection (Yein *et al*, 1990a) was compared with results obtained for oral administration of the drug to rats (Jaglan & Roof, 1990), demonstrating a match in the metabolite profiles in the kidneys of both the rats and the pigs (Jaglan *et al*, 1990a). In pig kidney, 62.6 ± 4.6% of the total residues were bound to macromolecules and the remainder was free residues, with DCD being the principal free metabolite. DFC is the major bound metabolite, as observed in other species. Residues of DFC were present in samples of fat (0.28 mg/kg), kidney (0.69 mg/kg), and liver (0.93 mg/kg) of 2 pigs slaughtered 24 hours after treatment and in the liver of one of two pigs slaughtered 48 hours post-treatment (0.36 mg/kg). The half-life for ceftiofur, measured as desfuroylceftiofur acetamide was calculated as 13.5 ± 2.61 hours after intramuscular injection and 12.2 ± 1.91 hours after intravenous dosing (Yein *et al*, 1990a).

Pharmacokinetic parameters for DFC in the blood of pigs treated with 3 mg CFAE/kg BW by intramuscular injection, on three successive days at 24-hour intervals, have been calculated and are given in Table 3 (Banting *et al*, 1991b). The table also lists these parameters which have been calculated for pigs receiving a single treatment of 3 mg CFAE/kg BW by intramuscular and by intravenous injection (Banting *et al*, 1991a). The reasons for the differences in calculated parameters for the single dose and 3-dose treatments are not clear, but may relate, in part, to differences in sampling times post-treatment used in the two studies.

Table 3. Pharmacokinetic parameters of desfuroylceftiofur (DFC) in swine.

Variables	Swine[a]	Swine[b]	Swine[b]
Dose (mg/kg)	3 (times 3)	3 (times 1)	3 (times 1)
Route of Administ.	IM	IM	IV
Method of Assay	HPLC-DCA[c]	HPLC-DCA	HPLC-DCA
C_{max}(μg/mL)	12.46 ± 3.53	19.23 ± 7.88	19.62 ± 8.34
t_{max}(h)	2.33 ± 0.82	0.58 ± 0.20	1.11 ± 1.50
$AUC_{0\text{-}24h}$ (μg.h/mL)	136.63 ± 40.58	203.16 ± 121.06[d]	156.63 ± 58.32[d]
$t_{½}$(h)	15.74 ± 0.84	13.46 ± 2.61	12.24 ± 1.91
C_{min}, 24 h (μg/L)	2.62 ± 0.96	-[e]	-[e]

[a] Banting *et al*, 1991b, parameters calculated after last of 3 injections
[b] Banting *et al*, 1991a
[c] HPLC-DCA refers to total desfuroylceftiofur, assayed as desfuroylceftiofur acetamide
[d] $AUC_{0\text{-}\infty}$, μg.h/mL
[e] not calculated

The tissue and fluid distribution of DFC after administration of a single intramuscular injection of ceftiofur,

Table 4. Distribution of desfuroylceftiofur (DFC) in tissues (mg/kg) and fluids (μg/mL) of pigs treated with a single IM injection of 3 mg CFAE/kg BW.

Tissue	2.5 hr.	7.5 hr.	24 hr.
Plasma	13.64	8.31	3.25
Apical Lung	1.91	1.26	0.49
Diaphragmatic Lung	1.64	1.51	0.46
Bronchial Epithelium	1.65	1.49	0.62
Tracheal Epithelium	2.03	1.88	0.60
Tonsils	1.21	1.12	0.30
Kidney	4.33	3.02	1.06
Skin	1.73	1.17	0.57
Jejunum	1.22	1.00	0.41
Mesenteric Lymph Node	1.39	1.09	0.41
Synovial Fluid			
Right Stifle	1.07	0.46	0.29
Left Stifle	0.97	0.74	0.29
Cerebro-Spinal Fluid	<LOQ	<LOQ	<LOQ

Assay limits of quantification (LOQ): plasma, 0.14 mg/kg; kidney, 0.10 mg/kg; muscle, 0.03 mg/kg.

3 mg CFAE/kg BW, to swine has also been investigated for a 24-hour period following injection (Scantox & Cephac, 1994). This experiment indicated that, in healthy pigs, ceftiofur does not cross the blood brain barrier. Results of the residue analyses are presented in Table 4.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Lactating Cattle

Data were considered from studies in lactating cattle administered ^{14}C-ceftiofur by intramuscular administration which demonstrated the time from treatment to the appearance of peak concentrations of total residues in milk and also total residue depletion during and following last treatment (Jaglan *et al*, 1989a,b). In the pilot study (Jaglan *et al*, 1989b), a single cow (Table 5) was treated with 5 successive daily intramuscular doses of ^{14}C-ceftiofur sodium, 1 mg/kg BW (doses 1-3 at 21-hour intervals, doses 4 & 5 at 24-hour intervals).

Table 5. ^{14}C-ceftiofur residues in milk following 5 successive daily intramuscular injection of 1.1 mg/kg body weight of ceftiofur to a cow[a]

Time Post-treatment (hrs.)	Total residues (μg/L[b]) ceftiofur equivalents)
3	15.3
6	34.1
9	38.0
12	43.6
15[c]	33.9
18[c]	20.4
21[c]	19.7
24[c]	18.3

[a] Based on samples from only one animal.
[b] Recalculated from mg/kg to μg/L using a specific gravity of 1.032 for milk.
[c] Includes one milking prior to these milking times.

Six Holstein cows (470-632 kg BW) were injected intramuscularly on five successive days at 24-hour intervals with 2.29 $\pm$ 0.02 mg ^{14}C-ceftiofur free acid-equivalents per kg BW (Jaglan et al, 1989b). Milk was collected at intervals of 12 and 24 hours following each treatment. Three screening tests which would detect the presence of microbiologically active residues in the milk samples (*Bacillus stearothermophilus* disc assay, or BSDA; *micrococcus luteus* cylinder plate assay, or C/P; Delvotest-P) were applied to samples listed in Table 6, with all tests yielding negative results. Actual concentrations of ^{14}C-ceftiofur equivalents present in the samples are shown in Table 6. LOD's claimed for the test kits were, respectively, BSDA, 0.08 - 0.1 mg/L; Delvotest-P, 0.05 mg/L; C/P, 0.015 mg/L.

Table 6. **^{14}C-ceftiofur equivalents (mg/L[a]) in milk collected from dairy cattle administered ceftiofur sodium via intramuscular injection on 5 consecutive days, at 24 hour intervals, at 2.3 mg/kg BW.[b]**

Time Post-treatment (Di + h)[d]	^{14}C Ceftiofur Equivalents (μg/L)[c] Total	Free[c]	Major Free Metabolite[c]
D1 + 12	79.9	28.2	6.9
D1 + 24	39.0		
D2 + 12	98.2	35.6	9.3
D2 + 24	45.1		
D3 + 12	105.4	36.0	9.3
D3 + 24	49.1		
D4 + 12	105.4	35.9	8.8
D4 + 24	51.3		
D5 + 12	111.8	37.1	9.8
D5 + 24	57.9	19.9	4.0
D5 + 36	31.5		
D5 + 48	19.4		
D5 + 60	10.1		
D5 + 72	12.9		
D5 + 84	10.7		
D5 + 96	11.4		
D5 +108	10.9		
D5 +120	9.2		

[a] Recalculated from mg/kg to μg/L using a specific gravity of 1.032 for milk.
[b] Mean value for milk collected from six cows.
[c] ^{14}C ceftiofur equivalents total residues present in milk as determined by total radioactivity (includes free metabolites that are not covalently bound to protein and the major free metabolite, desfuroylceftiofur cysteine disulfide, which is microbiologically active).
[d] (Di + h) refers to ith Dose (Di), where i = 1 to 5, and h hours after last treatment. For example, D1 + 12 refers to the sample taken at 12 hours after the first treatment.

Cattle

In a preliminary study (Johnson *et al*, 1985b), two calves and (124.5, 126.0 kg), two heifers (181.5, 181.8 kg) and a young bull (192.5 kg) were each given 5 successive treatments with 2.2 mg ^{14}C-ceftiofur sodium by intramuscular injection, with a 24-hour interval between injections. No significant differences in residues, as determined by total radioactivity (detection limit 0.02 mg/kg), were found in tissues collected from the calves and two of the heavier animals sacrificed after 8 hours withdrawal. Analysis of the normal edible tissues revealed that residues in kidney were highest (3.9 - 5.9 mg/kg), followed by liver (1.06-1.53 mg/kg), fat

(0.30-1.21 mg/kg) and muscle (0.16-0.30 mg/kg). Highest levels were found in the final (treatment 5) injection sites, ranging from 4.98-10.54 mg/kg, but were generally lower in the injection sites for the earlier treatments, although one injection site from treatment 4 contained 8.67 mg/kg. Injection sites from treatment 3 contained 2.0 mg/kg of residues, or less. In the remaining animal, sacrificed at 75 days after final injection with ceftiofur, traces of ceftiofur residues (<0.02 mg/kg) remained in three injection sites, while residues in the liver and kidney were 0.02 and 0.04 mg/kg, respectively.

The experiment was repeated using 6 calves (124.5-191.5 kg) which received the same dosage and treatment pattern, following which all six animals were sacrificed after 8 hours withdrawal (Johnson *et al*, 1986). Analysis by total radioactivity indicated the highest residue levels in normal tissues to be in kidney (5.54 $\pm$ 1.30 mg/kg), while the average residue found in muscle was 0.23 $\pm$ 0.05 mg/kg. Residues in injection sites were similar to those reported previously (Johnson *et al*, 1985b). Elimination of ceftiofur through urine and faecal clearance was rapid, with 80.5-90.2% of the dose eliminated after 8 hours and maximum concentrations were noted in the blood (5-10 μg/mL) 0.5 to 2 hours post-treatment, confirming earlier results (Johnson *et al*, 1985a).

A comparison of results obtained for the analysis of residues of ^{14}C-ceftiofur in kidney by total radioactivity and liquid chromatography demonstrated that much of the residue present does not include an intact cephalosporin nucleus (Jaglan & Gilbertson, 1991). Zero withdrawal kidney containing incurred residues of 7.2 mg/kg by total radioactivity contained 2.5 mg/kg ceftiofur equivalents, measured as DFC residues by HPLC. Residues were detectable in kidney after up to 3 days withdrawal for calves treated at 1.1 mg CFAE/kg BW/day on 5 successive days, but over 90% of the residues present did not contain an intact cephalosporin nucleus. Nonetheless, kidney is recommended as the target tissue.

Swine

Twelve swine (6 male, 6 female), 4-5 months old, weighing 35-46 kg, were treated by intramuscular injection on three successive days at 24 hour intervals with 5.108 $\pm$ 0.09 mg ^{14}C-ceftiofur free acid equivalents/kg BW and killed at 12 hours following the final injection (Yein *et al*, 1990a). Samples collected included the blood and urine, stomach contents at slaughter, liver, kidneys, lungs and tonsils, each as total organs, muscle from the right ham (about 100 g), an area 10 cm in diameter and 10 cm deep centred on the injection site, 200 g of abdominal fat and 100 g of skin with a small amount of adhering fat. The G.I. tract was washed clean and ground into a composite sample. Approximately 100 g of brain tissue and the mesentery gland (50 g) were also collected. All tissues were sub-sampled for analysis by liquid chromatography, with detection by both diode array and radioactive flow detectors.

Total ceftiofur concentrations in blood were in the range of 20 mg/L 2 hours after each injection, declining to about 10 mg/L after 12 hours and to 4-5 mg/L 24 hours after treatment. Analyses conducted on excreta revealed that 61.82 $\pm$ 4.70 % of the drug was eliminated in the urine and 10.75 $\pm$ 5.07 % was eliminated in the faeces prior to slaughter (collections at 24-hour intervals after treatments 1 and 2, 12 hours after treatment 3). Residues found in edible tissues collected at slaughter are given in Table 7. Residues found in the G.I. tract and gland tissues were similar to those found in liver, while lung tissue contained residues of 2.93 mg/kg total ceftiofur equivalents. Brain tissue contained 0.50 mg/kg total ceftiofur equivalents, the lowest residues found in the various tissues tested.

Table 7. Total residues of ^{14}C-ceftiofur equivalents found in tissues of swine at slaughter (mg/kg).

Tissue	Total Residues ^{14}C-ceftiofur, mg/kg[1]
Injection site	2.90 ± 1.28
Fat	1.49 ± 0.54
Kidney	4.47 ± 0.81
Liver	1.55 ± 0.18
Muscle	0.76 ± 0.24
Skin	1.22 ± 0.52

[1] Mean of determinations for twelve animals.

Other Residue Depletion Studies (with unlabelled drug)

Swine

A study was conducted in which 24 pigs (12 male, 12 female) were divided into four groups, each with 6 animals, and were dosed on three successive days at 24-hour intervals with 3 mg CFAE/kg BW by intramuscular injection (Banting *et al*, 1991b). The groups were sacrificed at 12 hours, 2 days, 5 days and 10 days, respectively, after the final injection. As shown in Table 8, analysis of tissue samples revealed that DFC residues were detectable in liver up to 2 days after the last injection. No detectable residues were found in tissue samples collected at 5 and 10 days post-treatment. Urine samples collected from 3 of the 6 animals slaughtered at 12 hours withdrawal were tested. One contained no detectable residues (LOD 0.1 mg/L), while the other two samples contained 4.29 and 1.87 mg/L DFC, respectively. Urine samples collected at days 2 (4/6), 5 (6/6) and 10 (6/6) contained no detectable residues of DFC.

Table 8. DFC residues (mg/kg) in tissues collected from swine treated with three successive doses (24-hour interval) of CFAE at 3 mg/kg BW[a].

Time from last injection	Fat	Injection Site	Liver	Kidney	Muscle
12 hours	0.40 ±0.08	0.19±0.11	1.51±0.72	2.17 ±1.02	N.D.
2 days	N.D.	N.D.	*	N.D.	N.D.

[a] Detection limit of LC method for DFC is 0.2 mg/kg in tissues.
N.D means not detected.
* Liver sampled from one animal contained 0.21 mg/kg DFC; the other 5 livers contained no detectable residues.

Calves

Calves were treated with 1.1 mg CFAE/kg BW by intramuscular injection on 5 successive days (Jaglan & Cox, 1988). Kidneys collected at slaughter were analyzed and contained residues as follows (number of samples follows sampling time in brackets): 24 hours (6), 0.62 ± 0.12 mg/kg; 36 hours (7), 0.35 ± 0.07 mg/kg; 48 hours (7), 0.14 ± 0.04 mg/kg.

Twenty-five veal calves (39-68 kg), allocated into groups of five, each received 1 mg/kg BW ceftiofur by intramuscular injection on 5 successive days at 24-hour intervals (Banting *et al.*, 1989a). Blood was collected at intervals following the final injection of 0, 2, 4, 8, 16, 24, 48, 72 and 96 hours from 5 randomly selected calves. Groups of 5 calves each were slaughtered at intervals of 5, 10, 15 and 20 days after the final treatment and samples of injection site, muscle, liver, kidney, and fat were collected. Urine was collected from two calves 24 hours after the last treatment and also was collected from the bladder at slaughter, when possible. Samples were analyzed for DFC using a liquid chromatographic method of analysis with limits of quantitation as follows: plasma, 0.1 mg/L; urine, 1.0 mg/L; tissues, 0.2 mg/kg. Highest levels of DFC (2.3-5.0 mg/L) were found in the plasma samples taken at 2 and 4 hours, with concentrations declining rapidly to 0.39-0.74 mg/L at 24 hours, and to 0.00-0.17 mg/L at 48 hours. DFC was not detected at 72 and 96 hours following the final injection. DFC was present in one of two samples collected from each of two calves within 24 hours of treatment, but most urine samples collected at slaughter were negative. All tissue samples were negative at 5 and 10 days post-treatment. Fat and muscle samples were not analyzed for the 15- and 20-day withdrawal animals, but injection sites and livers contained no detectable residues. Kidney collected from one of 5 calves slaughtered after 15 days withdrawal contained 0.40 mg/kg DFC and kidney collected from one of 5 calves slaughtered at day 20 contained 0.27 mg/kg DFC, while the corresponding urine samples collected from these animals at slaughter contained 25.3 and 2.03 mg/L DFC, respectively.

Six healthy calves (54-73 kg) received a single intramuscular injection of ceftiofur sodium at a dosage of 1 mg CFAE/kg BW (Banting & Hewett, 1991). Three calves were killed after 2.5 hours withdrawal and the remaining three were killed at 7.5 hours after treatment. Results of the analyses of tissues collected from these animals are presented in Table 9.

Table 9. DFC residues in tissues from calves treated with a single dose of 1 mg CFAE/kg BW, mg/kg.

Tissue	t = 2.5 hrs.	t= 7.5 hrs.
Liver	1.42 ± 0.34	1.05 ± 0.53
Kidney	1.28 ± 0.07	1.20 ± 0.12
Injection site	1.86 ± 0.70	0.60 ± 0.29

Lactating Dairy Cattle

Forty-eight lactating Holstein dairy cows were assigned to six groups, with each animal receiving 2.2 mg CFAE/kg BW by intramuscular injection on 5 successive days (Robb *et al*, 1992). Milk was collected from the various groups at 1, 2, 4, 6, 8, 10, 12 or 14 hours after the first and last of the five daily injections. Results of HPLC analyses of the milk samples are presented in Table 10, while test kit results are discussed in the next section.

Table 10. DFC residues in milk collected at intervals as shown after the first and fifth injections, from cows treated with 2.2 mg CFAE/kg BW/day for 5 days, μg/L[a].

Collection Interval After Injection (hours)	Collected After First Injection		Collected After Fifth Injection	
	n	Mean	n	Mean
1	3	32.9	5	22.2
2	3	29.2	4	30.0
4	6	29.9	6	29.5
6	6	33.1	6	43.4
8	6	61.0	6	59.3
10	6	72.1	6	71.5
12	6	52.4	6	39.9
14	6	52.3	6	36.4

[a] Only values above the limit of detection (LOD) of 15 μg/L were included in the calculation of means; limit of quantification (LOQ) for the method is given as 50 μg/L.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Screening Tests for Milk

Milk collected from ten lactating cows which were treated with 1.1 mg CFAE/kg BW by intramuscular injection on five successive days was tested using the *Bacillus stearothermophilus* Disk Assay (BSDA), the Delvotest-P and the Charm Test II (Jaglan *et al*, 1988). Milk samples were collected at 12 and 24 hours after each injection and at 12-hour intervals to 144 hours after the final treatment. All samples tested negative by the BSDA, and the Delvotest-P. The Charm Test II detected residues in some samples taken at 12 hours after treatment.

The performance of the (BSDA), the Delvotest-P and the Charm Test II for the detection of ceftiofur in milk was determined using antibiotic-free control milk fortified with ceftiofur, using penicillin G as a positive control (Yein *et al*, 1990b). Under the conditions of the experiment, the detection limits for the three tests were established to be 50, 50 and 5 μg/L CFAE, for the BSDA, Delvotest-P and the Charm Test II, respectively. Most samples collected in a subsequent study with treated dairy cattle (Robb *et al*, 1992) gave a negative response to the Delvotest-P and BSDA, except for 1 cow whose milk tested positive at collection intervals of 2, 6, 10 and 14 hours after the first injection, for both assays. The Charm Test II gave negative results for all samples except those from one cow at the 2, 8 and 14 hour intervals and two cows at 10 hours. For milk collected following the fifth injection, samples from one cow were positive by the Delvotest-P at 6 and 14 hours, while the milk from one cow was positive at 6 hours by the BSDA. All other samples were negative by these tests. Milk samples from one cow were positive by the Charm II assay at 2, 6, 8 and 14 hours. Mean assay results for DFC residues in milk collected from the cows in this study have been reported in Table 10, and individual sample results are available which would permit comparison of test kit and HPLC assay results.

Thirty healthy lactating dairy cows in three herds of 10 animals each were given a daily dose of 1 mg CFAE/kg BW by intramuscular injection on 5 successive days (Jaglan *et al*, 1990b). Milk was collected pre-treatment and 12 and 24 hours after each dosing, plus at intervals of 12 hours up to 120 hours following the final injection. Samples were tested using the BSDA, Delvotest-P and the Charm Test II, plus by Cylinder Plate Assay (CPA) and an immunoassay, the Cite Probe. The microbial inhibition tests, the BSDA, Delvotest-P and the CPA, with detection limits of 50, 50 and 20 μg/L, respectively, gave negative results for 564 out of the total 568 samples collected. The remaining four samples were positive by all three tests. The Cite Probe, with a detection limit

of 25 μg/L, gave 33 positive results out of 209 samples tested after the final dose, but the reliability of the results with this kit are questionable as it was used on previously thawed samples and after the expiry date of the kit. Most samples collected while the animals were on treatment were positive with he Charm Test II, which has a detection limit of 5 μg/L, with the rate of positives declining to 17/30 at 36 hours post-treatment. Urine samples collected during the treatment period and tested with the Live Animal Swab Test (LAST), a microbial growth inhibition test, were also mostly positive, but were generally negative after 36 hours withdrawal. However, there did appear to be a significant number of false positives with the LAST, so its reliability as a predictive test for clearance of ceftiofur residues was questioned.

Six lactating dairy cows (mean weight 493 kg, mean milk production 23.2 kg/day) each received a single intramuscular injection of 2.2 mg ceftiofur active ingredient/kg BW to test the performance of an LC assay and of sixteen screening tests for β-lactam antibiotics for the detection of ceftiofur and its active metabolites (Belschner *et al*, 1994). Milk was collected pre-treatment and at approximately 14 and 24 hours after treatment, as composite samples from each cow for each sampling time. The average concentration of ceftiofur and metabolites detected by LC in the 14-hour samples was 0.030 mg/L (range 0.022-0.038 mg/L), while none of the 24-hour samples tested positive by the LC method (LOD 0.015 mg/L). The Idexx SNAP test kit (LOD 0.02 - 0.04 mg/L) gave positive results for 2 of 6 14-hour samples and 1 of 6 24-hour samples, while the Delvo-X-Press (LOD 0.004 - 0.02 mg/L) gave positive results for all 6 of the 14-hour samples and 1 of 6 24-hour samples. Estimated LOD's for the Idexx SNAP test and the Delvo-X-Press were based on the results of these experiments by comparison with HPLC results. The Charm Quantitative test gave positive results for all milk samples at both 14 and 24 hours after treatment, but all other tests, including the Disk Assay (BSDA) gave negative results for all samples. It has been observed that problems with false positive test kit results may occur when tests designed for application to bulk milk samples are used on samples from individual cows (Cullor, 1992; Van Eenenaam *et al*, 1993).

Screening Tests for Tissue and Urine

An agar cylinder plate assay using M. luteus as the test organism was tested for the detection of ceftiofur residues in fortified samples of bovine muscle, fat, plasma and urine (Stahl, 1985). Recoveries of 75 - 106% were reported for the 20 ng/g and 20 μg/L concentration range in the tissue and fluid samples, respectively. A cylinder plate assay for ceftiofur in plasma using *Providentia alcalifaciens* as the test organism was subsequently validated to an LOD of 0.007 μg/mL and an LOQ of 0.02 μg/mL, with average recoveries for samples fortified at 0.025 and 0.30 μg/mL being 105 and 92%, respectively (Fate *et al*, 1993). Sequential freeze-thaw cycles of the samples resulted in decreases of 15% (1 cycle) and 35% (2 cycles).

Samples of kidney, liver, muscle and injection site were collected from 10 piglets (21-30 kg BW), treated at 24-hour intervals on 3 successive days with 5 mg CFAE/kg BW by intramuscular injection, which were slaughtered 24 hours following the last treatment. The samples were tested using the European Union Four Plate Test (FPT) and the New Dutch Kidney Test (NDKT), which have detection limits of 4 mg/kg for tissue, with the exception that the FPT will detect 1 mg/kg of ceftiofur residue in muscle tissue. All samples tested negative using these two tests (Guelen *et al*, 1989a). In a similar study with calves which received 1 mg CFAE/kg BW at 24-hour intervals on five successive days by intramuscular injection, no residues were detected at 22 hours or 5 days withdrawal using the FPT or the NDKT (Guelen *et al*, 1989b).

Twenty-four healthy pigs (99-117 kg) were divided into two groups, each consisting of 6 males and 6 females, with the two groups being treated, respectively, with 3 mg CFAE/kg BW/day and 5 mg CFAE/kg BW/day, by intramuscular injection for 3 successive days at 24-hour intervals (Yein *et al*, 1992a). The pigs were slaughtered at 11 - 12 hours after the final treatment and samples of liver and kidney were collected from each animal for analysis by the Swab Test on Premises (STOP) and the Calf Antibiotic and Sulfa Test (CAST). Kidney from one animal in each treatment group tested positive by the STOP and all livers tested negative. Using the CAST, the only positive test result was for one kidney from the 5 mg/kg BW treatment group. Kidney samples were also tested using the Four Plate Test (FPT), yielding positive results on plate 3, which uses *M. luteus* as the test organism, for 7 of the 12 samples from the 5 mg/kg BW treatment group (Yein *et al*, 1992b). No positive results were obtained on the other plates or for the animals treated at 3 mg/kg BW. Actual residues of CFAE were determined by HPLC analysis. Mean concentrations for the kidneys from the two treatment groups were 3.35 ± 1.25 mg/kg (3 mg/kg BW) and 5.98 ± 2.21 mg/kg (5 mg/kg BW).

Six female calves (120-180 kg) each received five consecutive daily doses of 1.0 mg CFAE/kg BW by intramuscular injection and were slaughtered at 8 hours following the last injection (Stahl *et al*, 1992). Kidneys and final injection sites were collected and analyzed by the Four Plate Test (FPT) and by HPLC. While no microbiologically active residues were detected in the kidneys by the FPT, HPLC analysis revealed a mean concentration of 2.76 $\pm$ 0.40 mg/kg CFAE. One muscle injection site tested positive by the FPT, while the others tested negative. Injection sites were not assayed by HPLC. It was concluded that for the treatment regimen followed, slaughter following 8 hours withdrawal would not result in a large number of positive FPT results.

Chemical Methods

The stability of ceftiofur residues in milk has been investigated under various conditions of storage (Roof & Jaglan, 1991). Using control raw milk fortified at concentrations of 0.21 - 1.6 μg/mL and analyzed by HPLC, it was shown that residues are stable for 1 day at room temperature, 3 days under normal refrigeration and remained unchanged after 15 days storage at -20°C. A previous study with ^{14}C-ceftiofur residues demonstrated that residues of the ceftiofur metabolite DCD remained unchanged, as analyzed by HPLC, after 7 months storage at -20°C (Roof & Jaglan, 1989).

An HPLC method has been developed for the determination of DFC, the primary metabolite of ceftiofur, in cattle plasma which is based on release of bound residues using dithioerythritol in phosphate buffer (ph 8.7), followed by clean-up on C18 and SAX solid phase extraction cartridges (Jaglan *et al*, 1990c). The DFC is derivatized to form desfuroylceftiofur acetamide and analyzed on a C8 HPLC column under gradient conditions with UV detection at 254 nm. Recoveries were in the 90 - 100% range with a sensitivity of 0.1 mg/L. The methodology has also been applied to the determination of ceftiofur and desfuroylceftiofur related metabolites in bovine kidney, with a limit of quantification of 0.05 mg/kg and recoveries of 87 - 89 % from samples fortified at 0.11 - 5.35 mg/kg (Roof *et al*, 1994). The LOD was calculated to be 0.02 mg/kg for residues in bovine kidney samples.

In bovine muscle, the LOQ and LOD for the LC methodology were calculated to be 0.05 and 0.015 mg/kg, respectively, with recoveries in the 88 - 97% range for samples fortified in the range 0.1 - 10.0 mg/kg (Beconi-Barker *et al*, 1994a). The method has also been applied to bovine liver, with LOD and LOQ of 0.05 and 0.1 mg/kg, respectively, used as actual figures for the study (Beconi-Barker *et al*, 1994b). Recoveries obtained for samples fortified at 0.1, 1.0 and 10.0 mg/kg were 92.0, 87.5 and 93.5%, respectively. When applied to bovine fat, the same LOD and LOQ were determined as for bovine liver and recoveries were again similar (89.4 - 92.2%) in the 0.1 to 10.0 mg/kg fortification range (Beconi-Barker *et al*, 1994c). The methodology was tested for interferences from the cephalosporin antibiotics cefquinone, cefoperazone, cephapirin and cephacetril, all of which were removed by the derivatization and purification process in bovine muscle and kidney, except for cephapirin, which appears in the chromatogram at a retention time separated by about 1 minute from that of the derivatized DFC (Beconi-Barker *et al*, 1994d). Other antibiotics tested in the study , including neomycin, dihydrostreptomycin, spectinomycin, tetracycline and penicillin G, did not cause any interferences. No incurred tissues were tested in any of these validation studies for the HPLC method for tissues.

The HPLC method has been validated using fortified tissues for swine muscle and kidney. The LOQ was assigned as the lowest point on the calibration curve, which was 0.03 mg/kg for muscle and 0.1 mg/kg for kidney (Mignot *et al*, 1994). Recoveries reported were 88.0, 83.1, 79.6 and 74.7% for fortification levels of 0.1, 0.5, 2.0 and 10.0 mg/kg in kidneys, while in muscle the recoveries were 85.0, 80.9, 71.6 and 69.4%, respectively at fortification levels of 0.03, 0.1, 2.0 and 10.0 mg/kg.

A similar approach has been used to develop an HPLC assay for ceftiofur residues in milk (Roof & Jaglan, 1992; Hornish *et al*, 1994). The procedure employs basically the same steps as that described above for plasma, except that an SCX solid phase extraction cartridge is employed instead of the SAX cartridge. The LOD and LOQ given for DFC residues in milk are 15 and 50 μg/L, respectively, with a linear response from 0-560 μg/L and a correlation coefficient of 0.999 for the standard curve. DFC is identified as the marker residue for milk, corrected to total residue by multiplication by a factor of 1.3, based on recoveries from incurred samples containing ^{14}C-ceftiofur.

A method for the simultaneous determination of six β-lactam antibiotics, including ceftiofur, as residues in milk has recently been reported (Tyczkowska *et al*, 1994). In this method, the milk sample is diluted with acetonitrile, purified by ultrafiltration and separated on a phenyl HPLC column using ion-pairing reagents, following which parent compounds are quantitatively determined by their UV absorption at 210 (penicillin G, ampicillin, cloxacillin), 230 (amoxicillin) or 290 nm (cephapirin, procaine, ceftiofur). The eluent from the UV detector was directed into a splitter to reduce the flow rate from 300 μL/min to 4.3 μL/min prior to introduction into an electrospray source connected to a quadrupole mass spectrometer for the simultaneous determination of the compounds tested, except ceftiofur, for which no mass spectral data were reported.

A confirmatory procedure for total residues of DFC in milk has been developed which uses the derivatized DFC residues from the HPLC quantitative procedure for further analysis by thermospray HPLC/MS (Hornish *et al*, 1990). The method uses the pseudomolecular ion at m/z 487 (MH^+) and two fragment ions with m/z 326 and 243 for confirmation and was tested for quantitation based on monitoring of MH^+ from 0.1 to 0.8 mg/L, providing a linear correlation of 0.994, with an estimated limit of detection of 0.05 mg/L. The method requires as a minimum a quadrupole-based mass spectrometer with a suggested mass range of 2000 amu, with a thermospray interface and a low pulse HPLC pump suitable for use with a mass spectrometer. The method was successfully tested on incurred samples.

APPRAISAL

Ceftiofur is well-absorbed following intramuscular injection and has a relatively complex metabolism in mammals, rapidly forming both non-polar and polar metabolites, a significant proportion of which are bound to complex molecules in plasma and tissue. A number of the metabolites are biologically active, with desfuroylceftiofur being the primary metabolite found and the marker residue compound recommended for analysis. Excretion is primarily through the urine and elimination is rapid from both tissues and plasma, even with repeated doses, as demonstrated in a number of studies which were evaluated.

Studies conducted at therapeutic dose levels in swine and cattle indicate that residues are generally below 1 mg/kg in muscle within 12 hours after the last treatment, falling below detection limits in tissues within 2-3 days following the last treatment for most animals tested. Similarly, no residues were detected in milk samples taken at 24 to 48 hours following the last treatment. Depletion has been observed to follow a biphasic curve, with kidney being the target tissue for samples collected up to 20 days post-treatment. Beyond that date, liver may be the preferred target tissue, but residues detected will be negligible. There was no indication of significant persistence of residues at injection sites. Based on these considerations, kidney is recommended as the target tissue for national monitoring programs or investigations involving suspect animals. It is also recognized, however, that muscle is the primary tissue available for examination in international trade and thus may be the choice of tissue for monitoring of imported samples.

A number of bioassay screening tests are commercially available which should be suitable for the monitoring of milk, tissue, plasma and urine samples. For quantification, an HPLC method is available which converts desfuroylceftiofur related metabolites to desfuroylceftiofur acetamide for analysis. The detection limits for the methodology are in the range of 0.02 - 0.05 mg/kg for tissues and 15 μg/L in milk. A HPLC/MS confirmatory method has also been demonstrated for residues in milk.

Maximum Residue Limits

In reaching its decision on the MRLs for ceftiofur sodium, the Committee took into account the following:

- An ADI of 0-50 μg/kg of body weight was established based on a microbiological endpoint. This is equivalent to a maximum daily intake of 3000 μg for a 60-kg person;

- kidney and muscle were considered to be the appropriate target tissues;

- ceftiofur is used in lactating cows;

- a significant proportion of the residues is present as non-extractable protein bound material that does not include an intact β-lactam structure;

- desfuroylceftiofur is the appropriate marker residue and accounts for all residues that include an intact β-lactam structure; and

- limits of quantification for the HPLC method for desfuroylceftiofur are 50 μg/kg for kidney and muscle, 100 μg/kg for liver and fat and 50 μg/l for milk in cattle, and 30 and 100 μg/kg for pig muscle and kidney, respectively.

The Committee recommended the following MRLs for cattle and pigs, expressed as desfuroylceftiofur:

Muscle:	200 μg/kg
Liver:	2000 μg/kg
Kidney:	4000 μg/kg
Fat:	600 μg/kg
Milk (cattle):	100 μg/l

Based on these MRLs, the maximum theoretical daily intake would be 640 μg per person (Table 11).

Table 11. Theoretical maxium daily intake (TMDI) of residues of ceftiofur sodium (DFC)

Tissue	MRL as DFC (μg/kg)	Tissue Consumption (g)	TMDI (μg)
Muscle	200	300	60
Liver	2000	100	200
Kidney	4000	50	200
Fat	600	50	30
Milk	100 μg/L	1.5 L	150
Total			**640**

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