Comparative Bioavailability Study of a Generic and Pioneer Oxytetracycline Veterinary Drug Formulation in Beef Calves

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Objectives of the Study

- **Compare the steady state plasma concentrations of a generic oxytetracycline formulation with those of the pioneer drug**
- **Verify whether the generic drug formulation would leave persistent residues at injection sites and in edible tissues to cause human health concerns**
- **Verify whether the drug will cause undue discomfort and distress to the animal**

Anticipated Results of the Study

- **Comparative Bioavailability and Pharmacokinetic Parameters for the Two Drug Formulations to be submitted to Health Canada for Drug Evaluation, Licensing and Market approval.**
- **Edible Tissue Residue profile of the drugs after administration and observation of label withdrawal periods.**
- **Demonstrate whether the administration of the drug will affect the health status of the experimental animals (irritation, hemorrhage, necrosis, fibrosis, etc)**

Experimental Design to Achieve *Objective 1*

- **House 12 healthy beef calves out of 10 heifers (H) and 10 steers (S) shown to have not been treated with any OTC for the experiment.**
- **Randomly assign 5 calves (3H&2S) to be treated with the pioneer drug formulation (Group A), and another 5 (3S&2H) to be treated with the generic drug (Group B).**
- **The other 2 calves will serve as control.**

Clinical Phase of Study - 1st Leg of Crossover Study (Brandon Research Station, Brandon, Manitoba)

- Collect plasma from each of the 12 calves at time zero.
- Administer each of the Groups A and B calves IM with the appropriate drug and collect plasma at 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, 84, 96, and 108h after drug administration into heparinized vacutainers.
- Harvest plasma and ship samples (cooled) to CVDR, Saskatoon, for chemical analysis

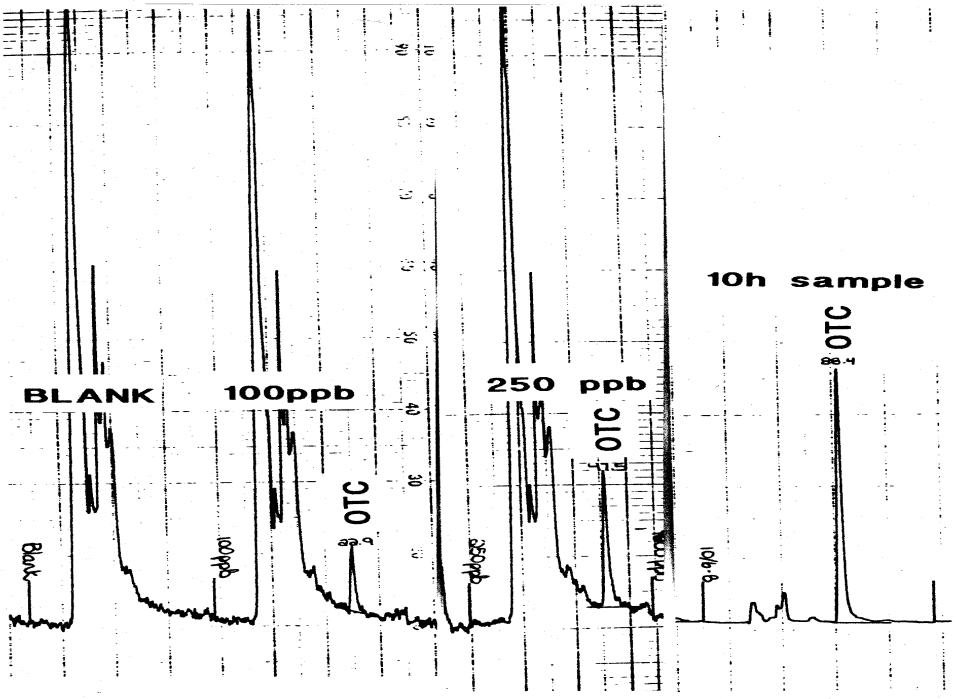
Clinical Phase of Study - 2nd Leg of Crossover Study (Brandon Research Station, Brandon, Manitoba)

- **Allow a 28-day washout period.**
- **Repeat the experiment in the 1st Leg with the exception that the Group of calves that were administered the pioneer drug before will now be administered the generic drug and vice versa.**
- **Collect and harvest plasma at time 0, and 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108 h after drug administration into heparinized vacutainers.**

Chemical Analysis Phase of the Study - Drug Analysis at CVDR, Saskatoon

- **Analytical Method for Oxytetracycline (OTC) Residues in Calf Plasma**
- **Precipitate plasma proteins with trifluoroacetic (TFA) acid.**
- **Filter the supernatant containing OTC and analyse by isocratic HPLC with UV detection at 350 nm.**
  - **[Iverson, Aanesrud, & Kolstad (1989) J. Chrom., 493 217-221]**

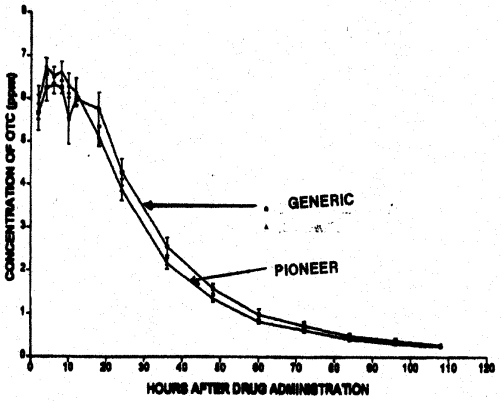
TYPICAL CHROMATOGRAMS FOR OTC IN PLASMA ANALYSIS



Analytical Parameters for The Plasma Method

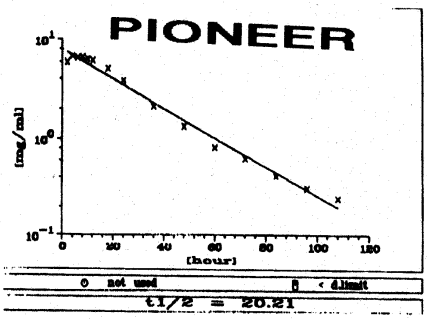
- Method will also detect TTC, CTC, and Demeclocyclone (DMC)
- Calibration Range - 100 - 1000 ng/mL
- Mean Per Cent Recovery - 72%
- Limit of Quantification - 100 ng/mL
- Limit of Detection - 25 ng/mL

BIOAVAILABILITY

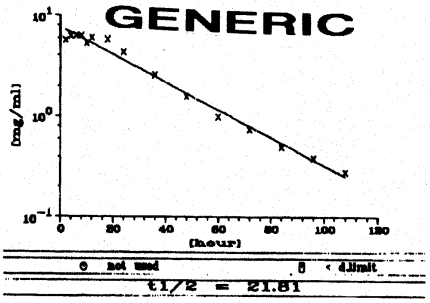


HALF LIFE

PIONEER



GENERIC



Pharmacokinetic Parameters Calculated For Pioneer & Generic OTC Vet Drugs

Parameter	Units	Generic	Pioneer
Max Data	mg mL	6.32	6.74
Peak Time	h	6.00	4.00
Term. elim rate (λ)	$L/h*10^{-2}$	3.18	3.43
Half-life	h	21.81	20.21
AUC[2h-108h]	mg h/mL	233	218
AUC[extrapolated]	mg h/mL	247	231
Mean Residence time	h	31.80	21.50
Plasma Clearance	$mL/min*10^{-5}$	6.74	7.23
Vol of Distribution	$L*10^{-4}$	1.27	1.26

End-Point Tissue Depletion Study - 28, 35, and 42 Days After IM Drug Administration *Objective 2*

- 3 calves (2 steers and 1 heifer) administered the generic drug and 1 steer administered the pioneer drug were sacrificed at a local abattoir. At slaughter, 500 g of normal muscle, fat, liver, kidney and 1kg sample of injection site muscle were collected and shipped on dry ice to CVDR, Saskatoon, for OTC analyses.
- Two other calves also given the generic drug were sacrificed after 35 and 42 days withdrawal. For these animals only the injection site muscle tissues were collected for analyses.

Tissue and Fat Analyses of OTC Residues at CVDR, Saskatoon

- **The Validated Tissue and Fat Method**
- **[Oka, Matsumoto, & Uno (1985). J. Chrom., 325, 265-274].**
- **Oxytetracycline is extracted from 5 g tissue and fat with a pH = 4.0 buffer. The extract is filtered and then cleaned up on a C_{18} solid phase extraction (SPE) cartridge. After rinsing the cartridge with water, OTC is eluted with methanolic oxalic acid.**
- **The eluate is filtered and an aliquot injected into an HPLC equipped with a C_8 analytical column and detected by UV at 350 nm.**

Analytical Parameters for the Tissue Method

- **Method also detects TTC, CTC, and DMC**
- **Calibration Range -** **50 - 1000 ng/g**
- **Mean Per Cent Recovery -** **64 %**
- Limit of Quantitation - 50 ng/g
- **Limit of Detection -** **25 ng/g**
- Repeatability: **CV<20%**

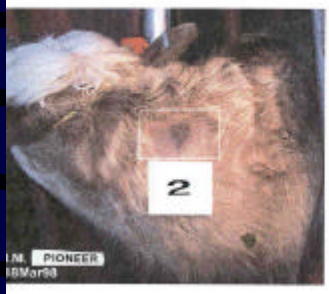
Tissue & Fat Residue Depletion Study for OTC in Beef Calves

			Concn of OTC (ppb) found in				
Animal ID	Drug Given	Wdr Days	Norm Musc	Inj.Site Musc	Kidney	Liver	Fat
235G	P	28	ND	ND	ND	ND	ND
544G	G	28	ND	ND	ND	ND	ND
243G	G	28	ND	ND	ND	ND	ND
423G	G	28	ND	ND	ND	ND	ND
426G	G	35		ND			
303G	G	42		ND			

Wdr. - Withdrawal; ND - Not detectable

Injection Site Reactions After IM Drug Administration

- 1 Steer administered the pioneer drug and
- 2 Steers administered the generic drug were sacrificed 28 days after drug administration and used for post-mortem analysis of tissue irritation
- a 1 kg tissue (8” x 8” x 4” slice) around the injection site was cut and used for observations of gross lesions.
- where obvious indications such as hemolysis or bruising were observed, tissue samples were taken and fixed in buffered formalin for histopathological analysis.



2. No visible injection site reaction.
Histopathology -chronic myositis & fascitis

9 : No visible injection site reaction.
Histopathology -chronic myositis & fascitis

10. No visible injection site reaction.
Histopathology - mild myonecrosis

Photographs of injection sites where either the pioneer OTC (# 2) or generic OTC (# 9 and # 10) was given intramuscularly (200 mg oxytetracycline per 10 kg body to calves.

Injection Site Muscle Tissues Subjected to Histopathological Analysis

Tag ID #	Wdr Days	Drug Admin	Histopathological Analysis & Injection Site Reactions
2	28	Pioneer	Chronic myositis and fascitis No visible injection site rxn
9	28	Generic	Chronic myositis and fascitis No visible injection site rxn
10	35	Generic	Mild myonecrosis No visible injection site rxn

Conclusion

- A Generic OTC Drug Formulated by a Canadian Company was shown to display equivalent plasma absorption elimination distribution profile to the Pioneer Drug Formulation when administered intramuscularly to beef calves.

- It did not leave any detectable residues in edible tissues of beef calves at slaughter and did not produce any adverse responses from the animals.

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