Chlortetracycline: A Single Laboratory Validation

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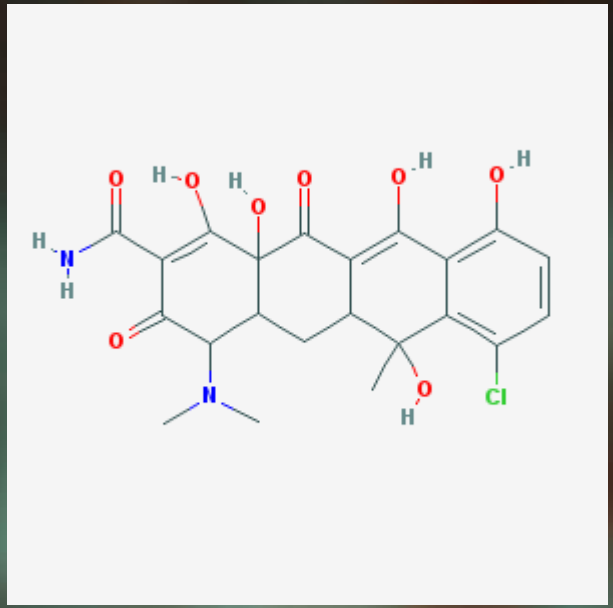
Chlortetracycline (CTC)



Molecular Formula: $C_{22}H_{23}ClN_2O_8$
Molecular Weight: 478.87962
Tetracycline antibiotic
Can be used in feed, or as a topical
Manufactured by Zoetus

Chlortetracycline (CTC)

Uses in Cattle:

- Growth promotion
- Treatment of anaplasmosis
- Treatment of bacterial enteritis
- Treatment of pneumonia associated with shipping fever

Uses in Swine:

- Control of porcine ilietis
- Treatment of bacterial enteritis

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Uses in Chickens:

- Growth promotion
- Control of infectious synovitis
- Control of chronic respiratory disease
- Reduction of mortality due to *E. coli* infections

Uses in Sheep:

- Growth promotion

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Uses in Turkeys:

- Control of complicating bacterial organisms associated with Blue Comb (transmissible enteritis)

Uses in Ducks:

- Control and treatment of fowl cholera

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Use in Psittacine birds:

- Control of psittacosis caused by *Chlamydia psittaci*

Use levels in feeds:

- Type A: 50 gm in 0.45 kg
- Type B: Approximately 8000 gm/ton max
- Type C: 10 to 500 gm/ton

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Method considered for the analysis of CTC

- Houglum, JE, and Larson, RD, "Assay of Chlortetracycline in Animal Feeds by Liquid Chromatography with Fluorescence Detection", JAOACI 80(5): 961 – 965, 1997

- There are two SOP's based upon this publication

- **Principle of method:**
  - Acidified acetone is added to feed samples and shaken to extract chlortetracycline into solution. Samples are filtered to remove particles and then run by HPLC to determine the concentration of chlortetracycline in the feed. Calibration is done by external standards.

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The method:

- Weigh out Feed according to the following table:

Guarantee (g/t)	App. Sample wt (gm)	Initial Volume (ml)
< 200	10.00 ± 0.05	50.00
201 – 500	10.00 ± 0.05	100.00
501 – 1000	5.00 ± 0.05	100.00
1001 – 6500	1.00 ± 0.05	100.00
➢ 6500	1.00 ± 0.05	100.00

  - Dilutions of the above are made as necessary in order to bracket the sample with the standards.
- Add Extraction Solution according to the above table, cap the bottles, and sonicate for approximately 5 minutes.
- Shake on a horizontal shaker for at least 50 minutes (SCS did 60 minutes)

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Method cont'd:

- Let contents settle for a few minutes, then filter through filter paper (SCS centrifuged then placed a 30 ml aliquot into 50-ml PPCT)
- Pour filtrate into a 3 ml syringe fitted with a 0.45 μ syringe filter (Nylon®) (SCS used a 0.20 μ Nylon® syringe filter)
- Chromatography:
  - MP: 65:18:17 5 mM Oxalic acid in water : MeOH : ACN
  - Flow: 0.90 ml/min (SCS flow = 1.0 ml/min)
  - Column: Phenomenex Luna ODS(3) 5 μ 120 Å 250 x 4.6 mm
  - Column Temperature: 35° C
  - Injection Volume – 10 μl
  - Analytical Wavelength: 372.0 ± 2.0 nm
  - Approximate R_t = 7.2 min

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Method Cont'd

- Standard Curve
  - Ranges from 0.010 ppm to 306.6 ppm

Method Needs and Fitness for Purpose Statement

- Performance Needs
  - Accuracy:
    - Type A and B: 95 – 110 %
    - Type C – 90 – 110 %
  - Applicability:
    - 5.5 – 220 mg/kg in the feed
  - Detection Limit:
    - 0.6 mg/kg in the feed
  - Determination Limit:
    - 2.0 mg/kg in the feed

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Method Needs and Fitness for Purpose Statement – (Cont'd)

- Repeatability:
  - RSD_r = < 5.00 %
- Reproducibility:
  - RSD_R = < 10.00 %
- Range:
  - 0.2 mg/kg to 220 g/kg
- Recovery:
  - 90 – 110 %
- Selectivity:
  - The method is to be free of interferences from matrix, other drugs, vitamins, and minerals.
- Linearity:
  - Curve: R $\geq$ 0.999 with a 95% CI of the Y intercept including zero.

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Test Samples:

- Type A
  - 2000 g/t
  - 4000 g/t
  - 8000 g/t
  - 20000 g/t
- Type B
  - 400 g/t
  - 450 g/t
  - 560 g/t
  - 500 g/t x 2
  - 700 g/t
- Type C
  - 50 g/t
  - 75 g/t
  - 100 g/t x 2
  - 150 g/t x 2
  - 200 g/t
- Blanks
  - Swine feed, sheep feed, cattle feed, dry dog food

Chlortetracycline (CTC)

SLV Blank Feeds used for spikes

These will also be used for the residue portion of the slv

ID	Label (g/t)	Grand mean (g/t)	RSD Within day	RSD Bet. Day	Total RSD	Pred. RSD	HorRat	F-Value	F-Critical	P-Value
Swine Blank	n/a	< 0.50	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Cattle Blank	n/a	< 0.50	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Sheep Blank	n/a	< 0.50	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Dog Food	n/a	< 0.50	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

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Test Sample Results:

ID	Label (g/t)	Grand mean (g/t)	RSD Within day (%)	RSD Bet. Day (%)	Total RSD (%)	Pred. RSD (%)	HorRat	F-Value	F-Critical	P-Value
CTC – 01	50	52.8	5.99	5.04	7.83	2.20	3.56	0.708	3.490	0.565
CTC – 02	100	97.5	3.65	0.80	3.73	2.01	1.86	1.192	3.490	0.354
CTC – 03	150	148.1	1.88	0.75	2.03	1.03	1.97	1.637	3.490	0.233
CTC – 04	200	200.6	1.82	0.99	2.07	1.80	1.15	2.171	3.490	0.144
CTC – 05	150	155.3	2.20	1.43	2.62	1.03	2.54	2.697	3.490	0.093
CTC – 06	500	505.8	1.58	1.30	2.04	1.57	1.30	3.681	3.490	0.043
CTC – 07	500	503.0	1.14	0.65	1.31	1.57	0.84	2.287	3.490	0.131
CTC – 08	400	408.6	3.48	2.13	4.08	1.62	2.52	0.374	3.490	0.773
CTC – 09	100	106.8	2.87	1.51	3.24	1.98	1.64	2.11	3.490	0.152
CTC – 10	560	559.6	2.61	0.68	2.70	1.54	1.75	1.273	3.490	0.328

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Test Sample Results:

ID	Label (g/t)	Grand mean (g/t)	RSD Within day (%)	RSD Bet. Day (%)	Total RSD (%)	Pred. RSD (%)	HorRat	F-Value	F-Critical	P-Value
CTC – 11	75	87.5	3.78	2.04	4.30	2.04	2.11	2.162	3.490	0.146
CTC – 12	2000	2031	4.76	3.42	5.86	1.15	5.11	0.516	3.490	0.679
CTC – 13	700	700.4	2.23	1.44	2.65	1.49	1.78	2.654	3.490	0.096
CTC – 14	450	466.1	5.13	4.43	6.78	1.59	4.27	0.746	3.490	0.545
CTC – 15	4000	4066	6.06	1.40	6.22	1.15	5.43	1.214	3.490	0.347
CTC – 16	20^5	18700	3.35	1.20	3.56	0.91	3.91	0.128	3.490	0.941

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Sample Notes:

- CTC – 01 contains roxarsone @ 40 g/t
- CTC – 02 contains amprolium @ 72 g/t
- CTC – 03 contains clopidol @ 104 g/t
- CTC – 04 contains decoquinate @30 g/t
- CTC – 05 contains hygromycin B @ 12 g/t
- CTC – 06 contains monensin @ 120 g/t
- CTC – 07 contains salinomycin @ 57 g/t
- CTC – 08 contains bacitracin @ 32 g/t
- CTC – 09 contains penicillin G @ 63 g/t
- CTC – 10 contains decoquinate @ 16 g/t
- CTC – 11 contains lasalocid @ 22 g/t
- CTC – 12 contains lasalocid @ 32 g/t
- CTC – 13 contains lasalocid @ 675 g/t
- CTC – 14 contains zoalone @ 132 g/t
- CTC – 15 contains lasalocid @ 202 g/t
- CTC – 16 contains decoquinate @ 525 g/t

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Experimental conditions that were varied:

- Column (A)
  - Column switched to a C8, with same particle size, pore sized, etc.
- Mobile Phase
  - Oxalic acid concentration:
    - 0 mM Oxalic Acid (B1)
    - 20 mM Oxalic Acid (B2)
- Column Temperature
  - Changed to Room Temp (C1)
  - Changed to 45° C (C2)
- Extraction Solvent Acidifier
  - Changed to HOAc – 1.0 M (D1)
  - Changed to TFA – 0.1 M (D2)
- Extraction Solvent
  - Methanol, acidified with HCl (E1)
  - Acetonitrile, acidified with HCl (E2)

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ID	Grand Mean (g/t)	A (C8)	B1 (no Buffer)	B2 (4x Buffer)	C1 (RT)	C2 (45° C)	D1 (HOAc)	D2 (TFA)	E1 (MeOH)	E2 (ACN)
CTC – 01	53	51	62	50	52	50	35	50	22	51
CTC – 02	98	102	112	101	96	102	69	110	45	102
CTC – 04	201	205	225	195	195	202	141	225	96	211
CTC – 06	506	515	475	496	515	510	329	510	325	525
CTC – 12	700	685	715	700	695	710	476	705	330	715
CTC – 13	2031	1985	1505	2002	1958	1985	901	2014	1102	2030
CTC – 15	4066	4102	3150	4004	4000	4005	3000	4095	2025	4100

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Comparison with Method Needs Statement:

- Accuracy vs. label claim
  - Type A – 95 – 110 %
    - 101.6 % @ 2000 g/t
    - 101.6 % @ 4000 g/t
    - 93.5 % @ 20000 g/t
  - Type B – 95 – 110 %
    - 102.2 % @ 400 g/t
    - 101.2 % @ 500 g/t
    - 100.0 % @ 700 g/t
  - Type C – 90 – 110 %
    - 105.6 % @ 50 g/t
    - 116.7 % @ 75 g/t
    - 97.5 % @ 100 g/t
    - 100.3 % @ 200 g/t
- Applicability
  - Met
- Detection Limit
  - < 0.5 mg/kg
- Determination Limit
  - < 1.10 mg/kg

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Comparison with Method Needs Statement:

- Repeatability
  - 50 – 399 g/t – Mean = 3.17 %
  - 400 – 700 g/t – Mean = 2.70 %
  - ≥ 2000 g/t – Mean = 4.72 %
- Reproducibility
  - 50 – 399 g/t – 1.79 %
  - 400 – 700 g/t – 1.77 %
  - ≥ 2000 g/t – 2.01 %
- Range
  - Met
- Selectivity
  - Met with drugs listed
- Linearity of Curve
  - Mean r^2 = 0.9987

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Conclusions:

- Method as run by SCS meets or exceeds requirements
- Experimental conditions do have an impact on results
  - Column – no difference
  - Buffer – buffer needed for mineral premixes
  - Column temperature – no difference
  - Acidification agent – HOAc is not a strong enough acid for extraction of CTC.
  - Extraction Solution – MeOH is not a suitable extraction solvent, even when acidified.

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Alpharma

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GOT QUESTIONS?

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