

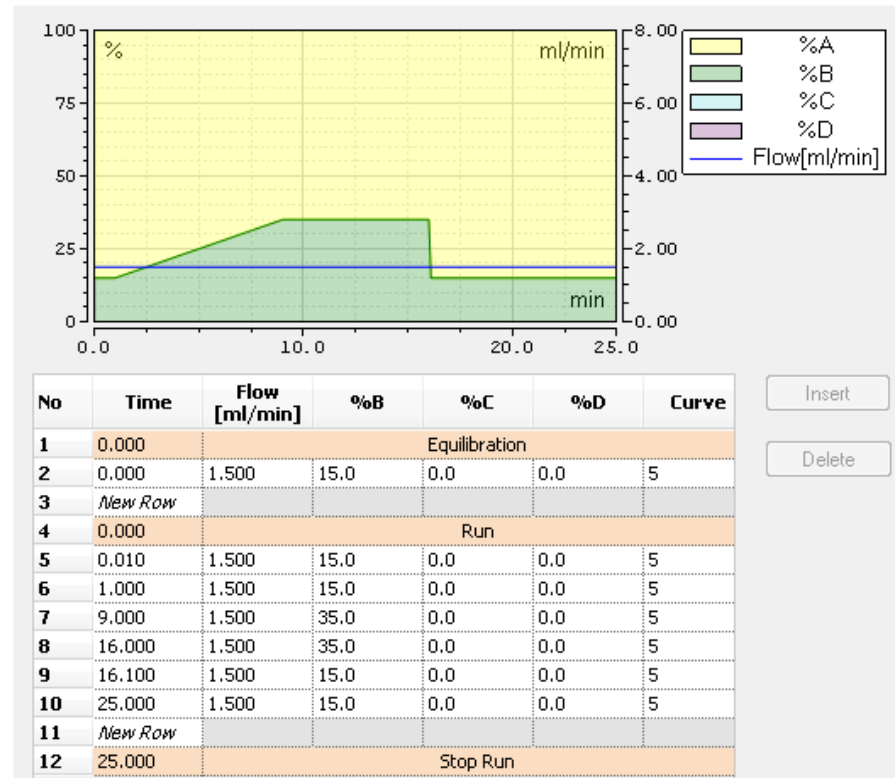


Chlortetracycline Working Group Update

**AAFCO Laboratory Methods and Services Committee Meeting
2019 Annual Conference**

- **Oxytetracycline/Oxytetracycline Hydrochloride in Animal Feed, Fish Feed, and Animal Remedies,**
 - **AOAC OFFICIAL METHOD 2008.09**

- **Fluorescence Detector:** 390 excitation, 512 emission
- **HPLC Column:** Phenomenex Prodigy ODS, 5µm, 150x4.6mm with guard
- **Mobile Phase A:** 0.1M sodium acetate, 55 mM calcium chloride, 20 mM disodium EDTA
- **Mobile Phase B:** Methanol
- **Flow Rate:** 1.5 mL/min
- **Injection Volume:** 20 µL
- **Run Time:** 25 minutes



Reference Method Modifications

Item No.	Item ID	AOAC 2008.09	ECAL Internal Method
1	Analyte	Oxytetracycline	Chlorotetracycline
2	Concentrations of calibration stds	0.2, 0.6, 1.2, 2.4, 4.0, and 5.0 mg/mL	0.2, 0.5, 0.1, 0.5, 1, 2.5, 5, 10, 20, and 40 mg/mL 0.2, 0.5, 0.1,, 1, 2.4, 5, and 10 mg/mL
3	Shaker	Orbital or wrist-action shaker	Eberbach Shaker Model No. E6010.00
4	Intermediate standard 2	10 mg/mL	20 mg/mL
5	250 ml container for extraction	Erlenmeyer flask	HDPE plastic bottles
6	Filter Extract	All glass solvent filter apparatus and 0.45 mm nylon filter, 47 mm, or equivalent.	Whatman 13mm GDX Disposable Filter Device, 0.45µm Syringe filter
7	HPLC Column	Phenomenex Prodigy ODS-3, 4.6 ´ 150 mm, 5 mm, with C18 guard column (Phenomenex, Torrance, CA), or equivalent	Ace Excel 3 C18, 150 x 4.6mm EXL 1111546U
8	Standard solutions	Prepare fresh every 3 weeks.	Prepare fresh monthly.
9	Flow Rate	1.5 mL/min	1 mL/min
10	Injection Volume	20 µL	5 µL + 2.5 µL
11	Run Time	25 min	15 min

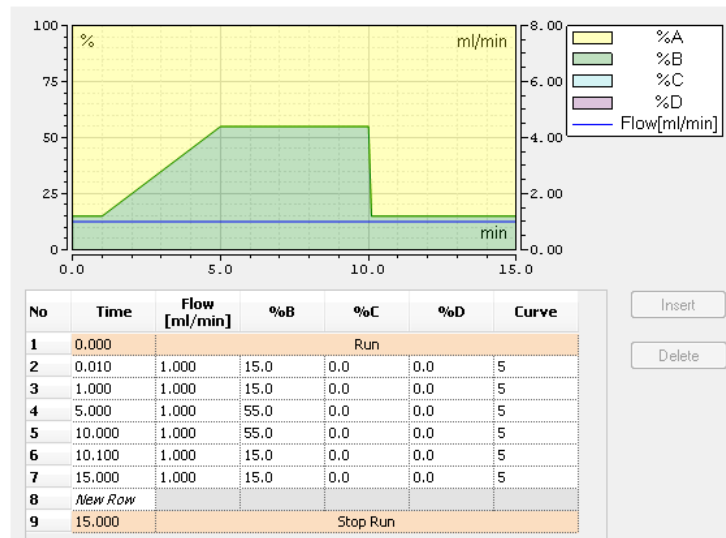
- 1. Oxytetracycline/Oxytetracycline Hydrochloride in Animal Feed, Fish Feed, and Animal Remedies,**
 - AOAC OFFICIAL METHOD 2008.09, Modified

- 2. Assay of Chlortetracycline in Animal Feeds by Liquid Chromatography with Fluorescence Detection**
 - Houghlum ET AL: Journal of AOAC International, Vol. 80, No. 5, 1997, Pages 961-965

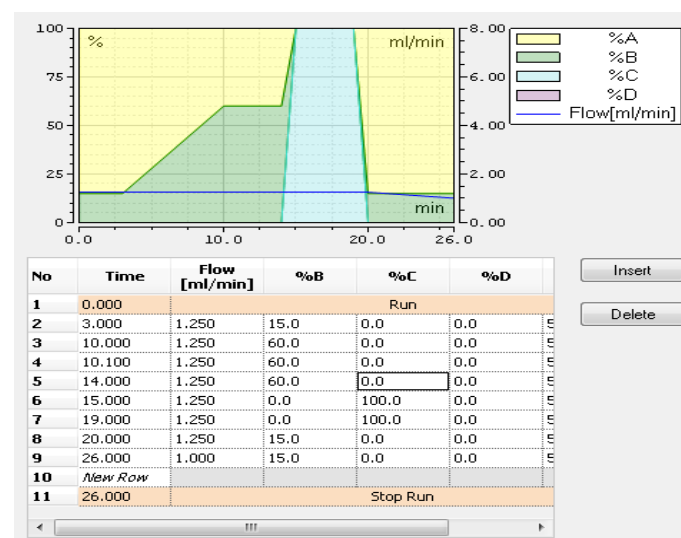
- 3. F.3 FEED – Chlortetracycline**
 - ISDA Plant Industries Laboratory, Feed & Fertilizer Lab Methods Manual
 - Reference: "Assay of Chlortetracycline in Animal Feeds by Liquid Chromatography with Fluorescence Detection," Journal of AOAC, Vol. 80, No. 5, 1997, pp. 961-965, with modifications

Reference Methods

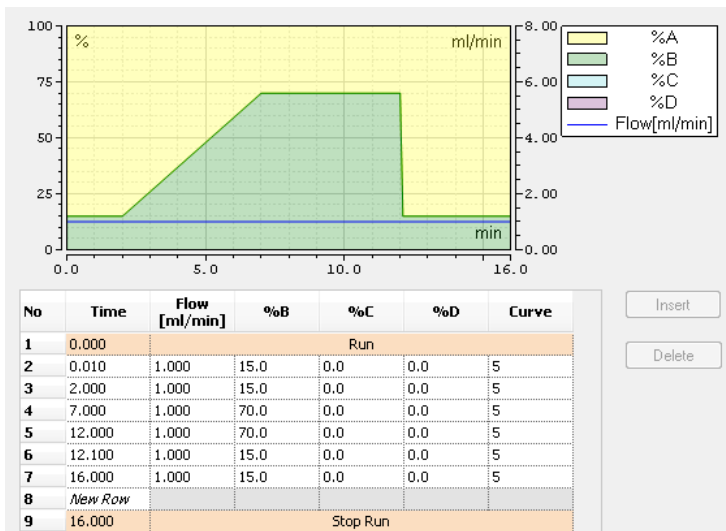
1. ECAL



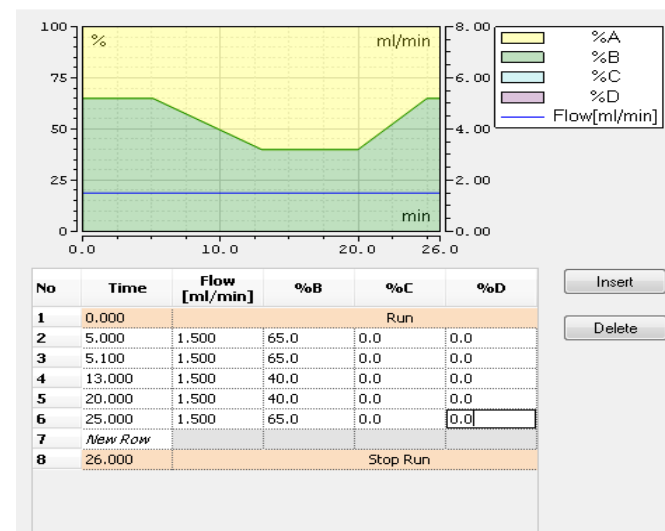
3. F&F Manual



2. MN



4. JAOAC 1997



- **Method Performance Evaluation Plan:**

ID	Concentration	Replicates
Estimated LOQ	1 mg/kg	5
50%	50 mg/kg	5
100% (Low)	100 mg/kg	5
100% (High)	200 mg/kg	5
150%	300 mg/kg	5

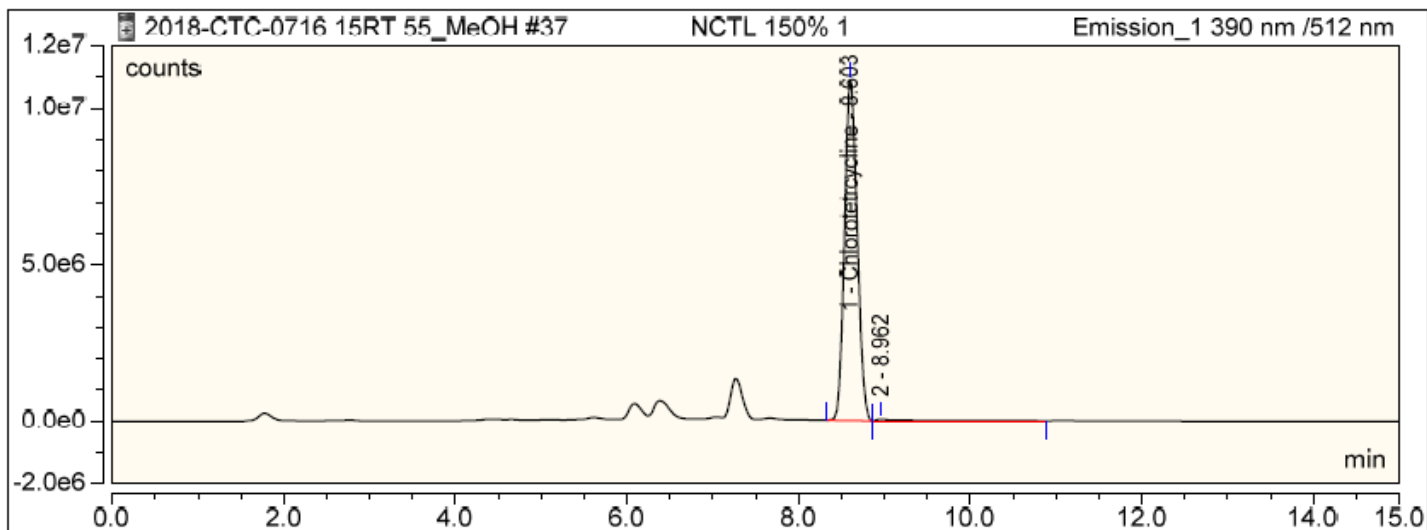
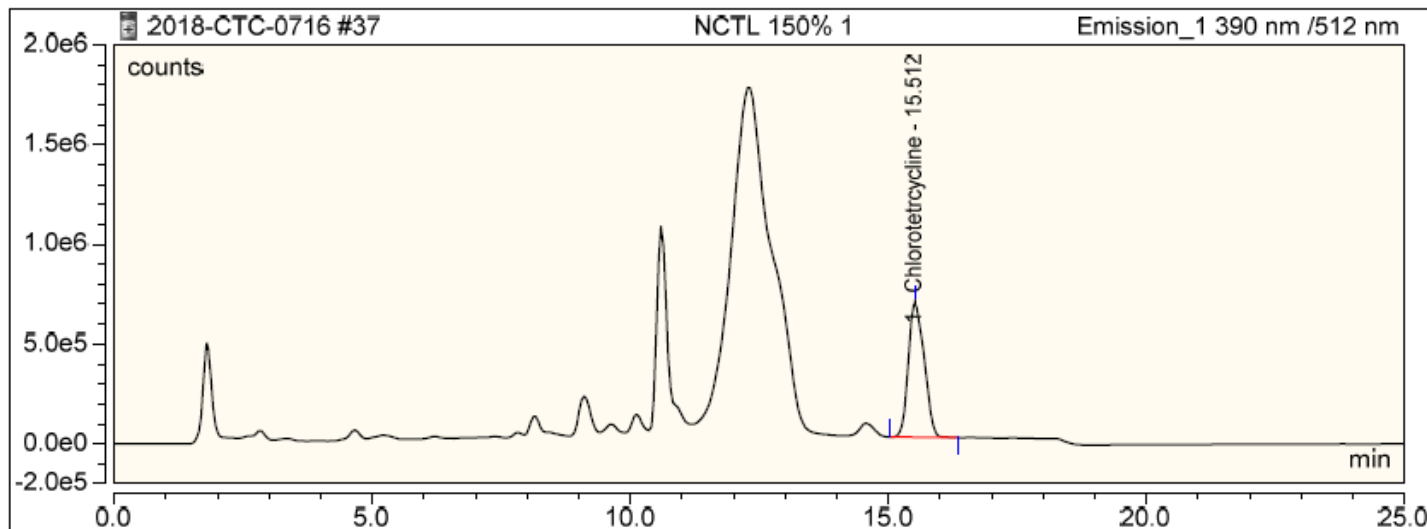
Summary of Accuracy and Precision

Target Concentration	Average, n=5 (mg/kg)	Recovery/Accuracy (%)	RSD (%)
LOQ	0.95	94.5	15.4
50%	50.0	98.7	0.81
100% (Low)	99.8	98.4	0.58
100% (High)	198	97.5	0.20
150%	298	98.2	0.31
CRM 9429	5.93	134	4.85
Sample X	46.4	46	0.79

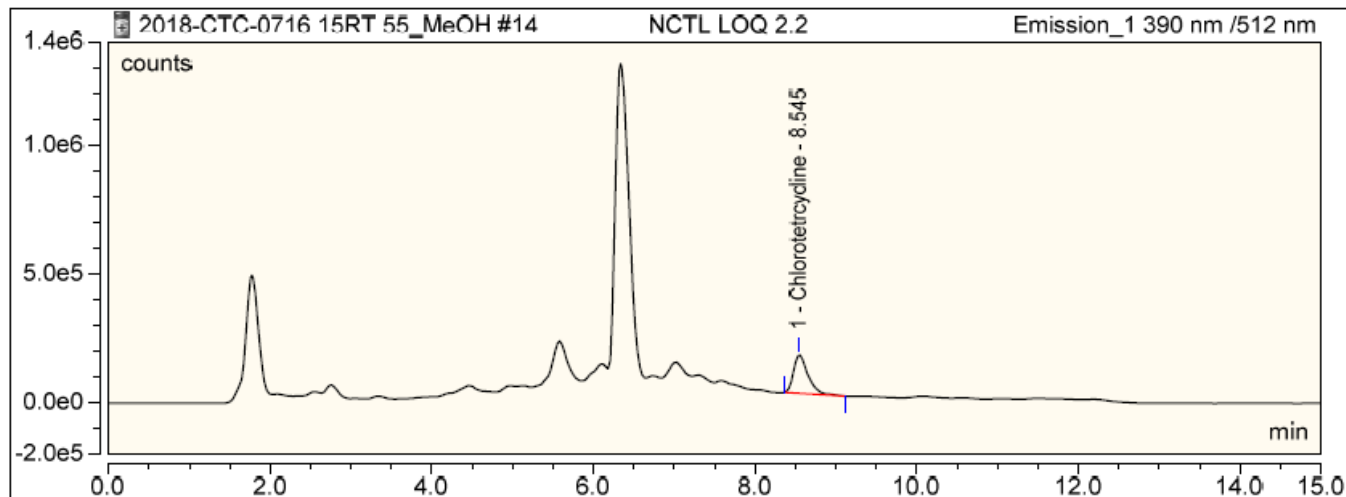
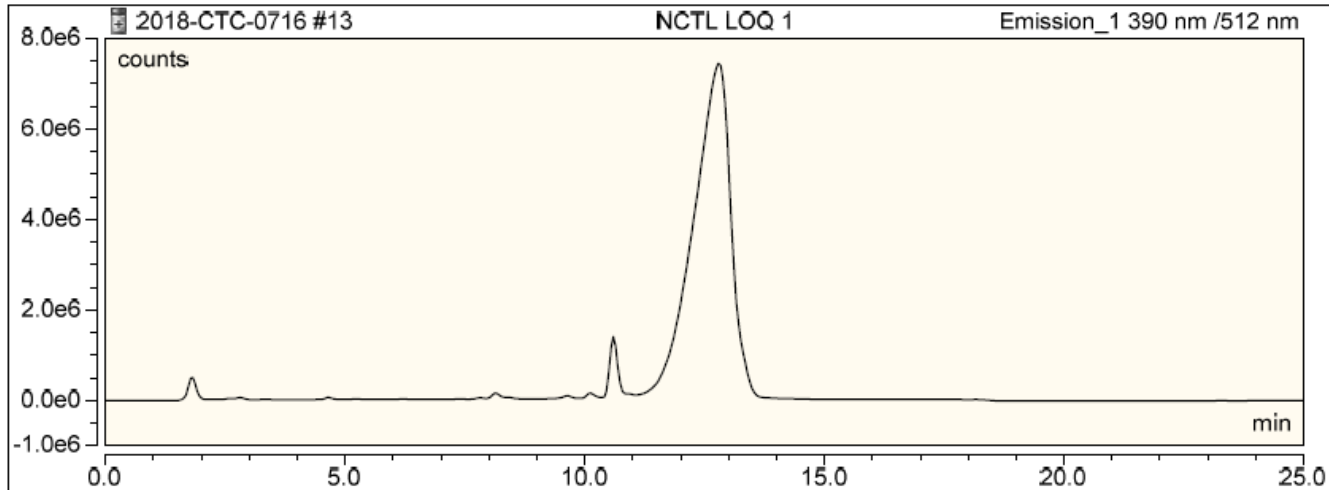
Comparison of Method Gradient Modifications and Injection Volume

Sample ID	Inj. No.	Amount mg/kg	Amount mg/kg	Amount mg/kg
		OTC-modified 1	OTC-modified 1a	OTC-modified 2
468-2019-07020004 a	30	46.803	49.2152	48.2212
468-2019-07020004 b	31	45.6045	47.6056	45.8774
468-2019-07020004 c	32	47.5326	49.2478	47.5437
468-2019-07020004 d	33	45.7721	47.1043	45.1842
468-2019-07020004 e	34	46.4798	47.4876	45.8903
Average:		46.4	48.1	46.5
Std.Dev:		0.70	0.91	1.14

Fortified Poultry Feed, 300 mg/kg



Fortified Poultry Feed, 1 mg/kg



- 1. Method trials using same sample preparation and instrument conditions**
- 2. Revise method**
- 3. SLV for method performance verification in all applicable matrices**
- 4. Issue method transfer protocol to participating laboratories**