



Chlortetracycline Working Group Update

**AAFCO Laboratory Methods and Services Committee Meeting
2018 Annual Conference**

- **Performance:**
 - Performance of this method should be comparable to or exceed plate assay AOAC 967.39 and turbidimetric assay 977.37.
- **Applicability**
 - Poultry, swine, cattle, lamb and mink feed products
 - Non-medicated feeds, Type C feeds, supplements and premixes
- **Accuracy, Precision and Intermediate Precision**
 - Type A and Type B: 95 – 105 %, $\leq 5\%$ and $\leq 10\%$
 - Type C: 90 – 110 %, $\leq 5\%$ and $\leq 10\%$
 - Contamination analysis (< 10 mg/kg): > 80 %, $\leq 10\%$ and $\leq 15\%$
- **Sensitivity and Selectivity**
 - MQL: ≥ 10 mg/kg, 2 mg/kg; < 10 mg/kg, 0.2 mg/kg
 - The method should be free from interferences

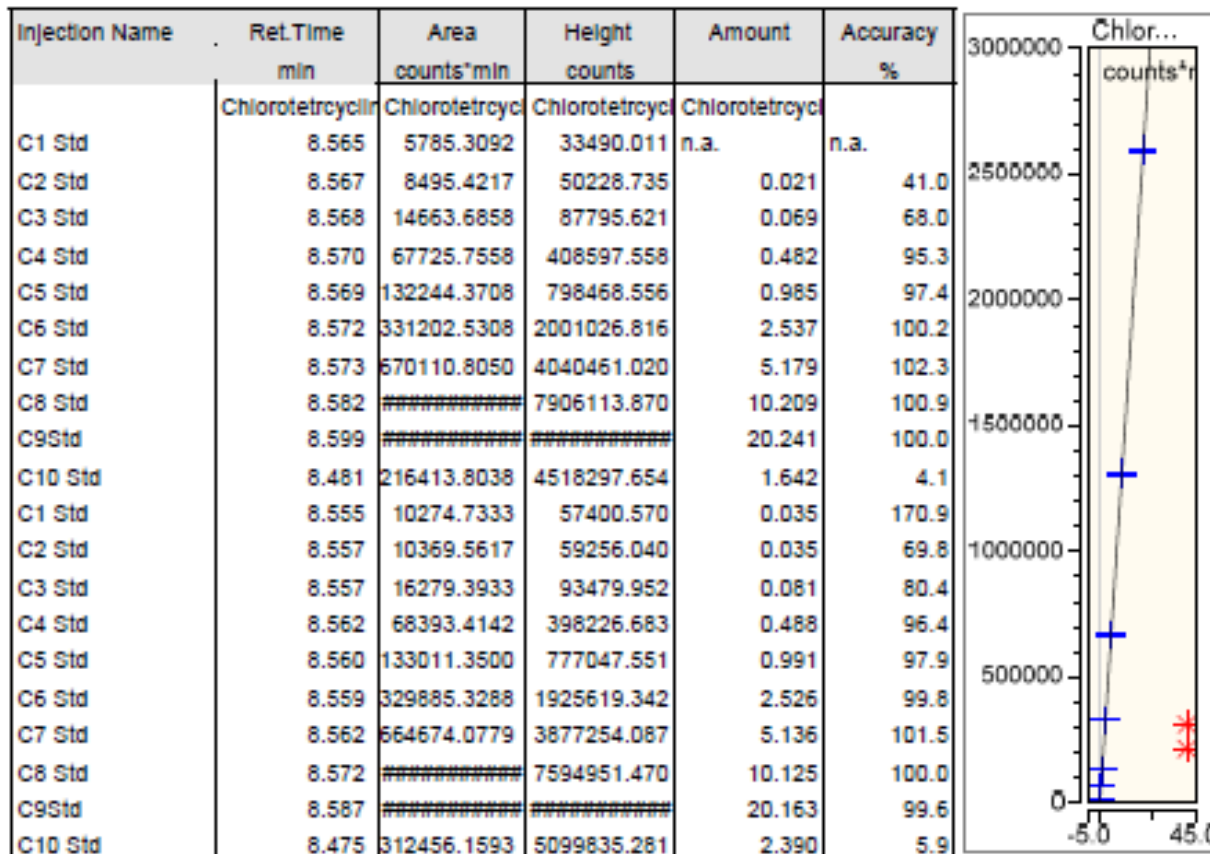
- **Reference Method: AOAC Official Method (Oxytetracycline, FLD)**
- **Method Performance Evaluation completed**
- **Method Performance Evaluation Plan:**

ID	Concentration	Replicates
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

- **Fluorescence Detector: 390 excitation, 512 emission**
- **HPLC Column: ACE Excel C18, 3 μ m, 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 mL/min**
- **Column Temperature: 30°C**
- **Injection Volume: 5 μ L**
- **Run Time: 15 minutes**

Calibration Summary

Peak Name	Eval.Type	Cal.Type	Points	Offset	Slope	Curve	Coeff.Det.
				(C0)	(C1)	(C2)	%
Chlorotetracycline	Area	Lin, WithOffset	18.000	5835.597	128271.110	0.000	99.9947

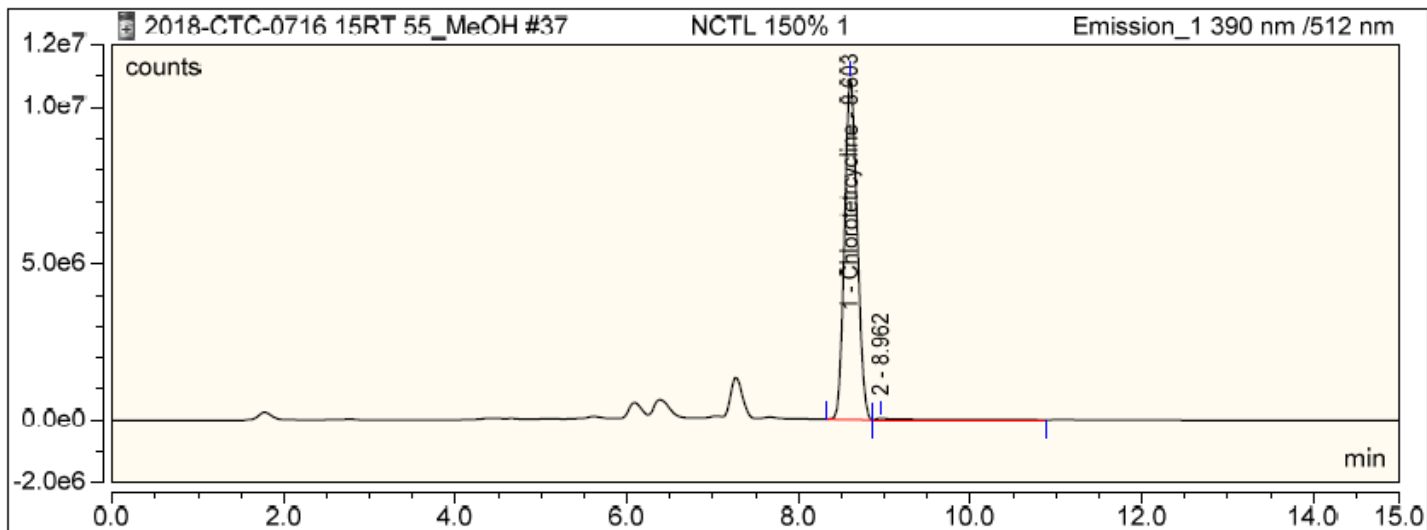
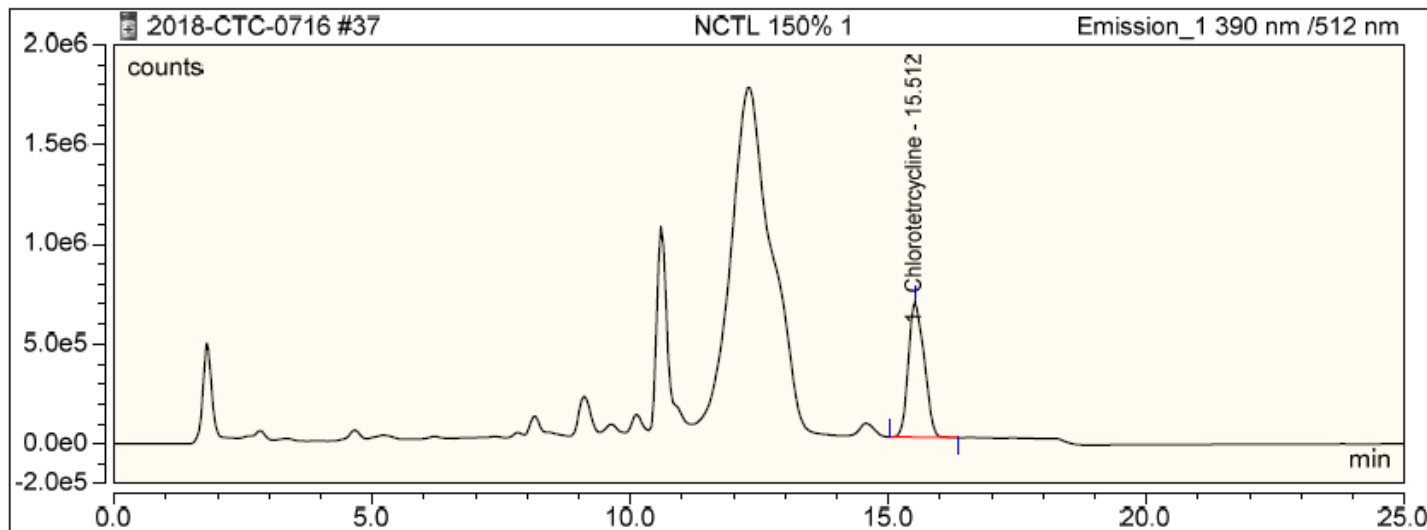


Summary of Accuracy and Precision

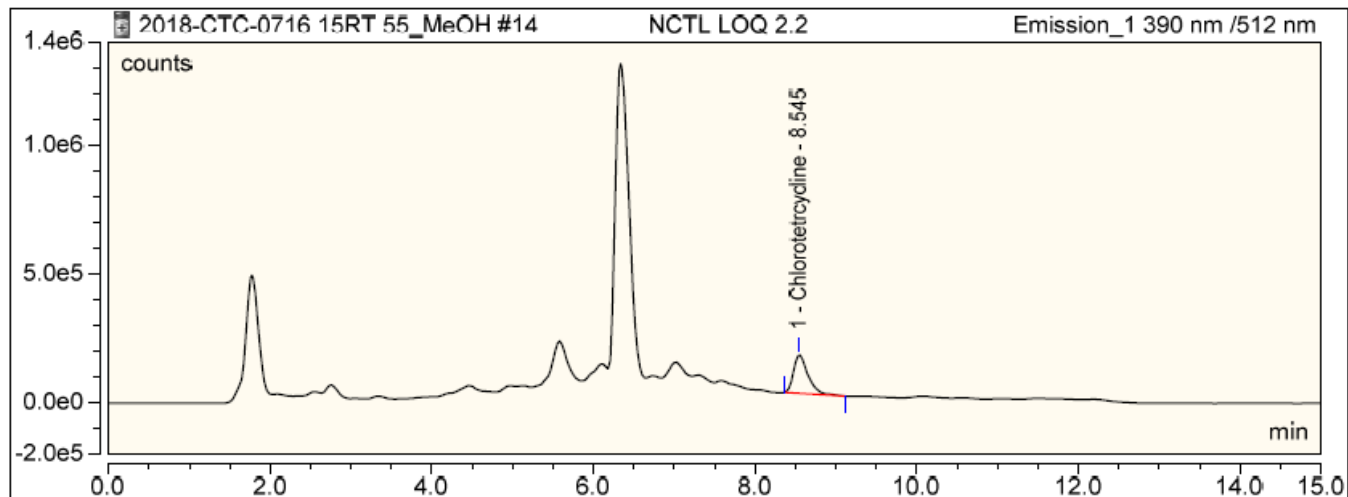
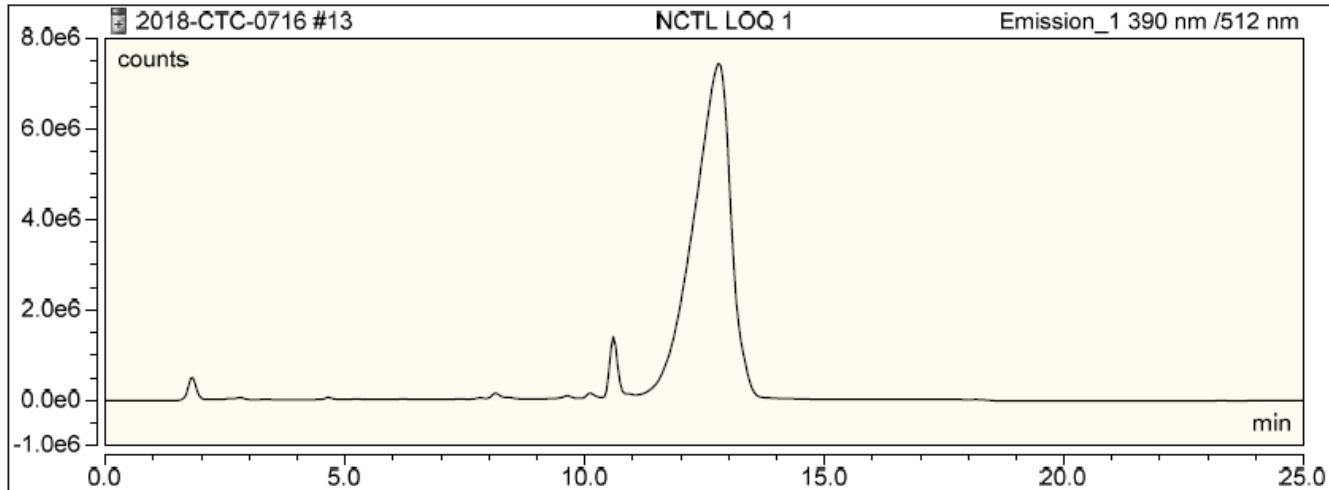
Target Concentration	Average (mg/kg)	Recovery (%)	RSD (%)
LOQ	2	205	14.68
50%	50	99.5	0.82
100% (Low)	100	99.8	0.59
100% (High)	198	99.1	0.21
150%	300	99.9	0.32

1. Injection volume
2. Mobile phase composition
3. Mobile Phase pH
4. Gradient
5. Runtime

Fortified Poultry Feed, 1 mg/kg



Fortified Poultry Feed, 1 mg/kg



- 1. Evaluate method fitness**
- 2. Draft method transfer protocol (to include method familiarization)**
- 3. SLVs for method performance verification in all matrices**