



Tylosin Working Group Update

**AAFCO Laboratory Methods and Services Committee Meeting
2017 Annual Conference**

- **Proof of concept / method familiarization**
 - LC-MS/MS experiments indicate comparable performance to AOAC plate assay
- **Several published UV methods exist**
- **Reference materials acquired:**
 - Tylosin certified reference standard
 - Tylosin factor B
 - Tylosin factor C
 - Tylosin factor D
 - Tylosin-urea adduct

HPLC

1. Reverse-phase separation
2. Gradient elution

Detection

1. UV 285
2. Mass spectrometry (ESI)

RT	Analyte	MW (Da)	Transition (Da)	DP (volts)	CE (volts)	CXP (volts)
4.03	Tylosin	916.469	772.5	146	41	12
			174.3	146	55	10
4.02	Desmycosin	774.385	318.4	146	41	12
			174.2	146	55	10
4.43	Macrocin	902.535	758.421	26	39	12
			174.2	26	53	8
4.08	Relomycin	919.474	775.54	26	41	12
			174.1	26	75	8
4.41	Roxithromycin (I.S.)	837.4	679.4	31	10	29
			158.1	31	10	43

- **Performance:**
 - Performance of this method should be comparable to or exceed that of the FDA approved turbidimetric assay.
- **Analytical Range**
 - % to ppb
- **Accuracy**
 - Type A and Type B: 95 – 105 %
 - Medicated complete feeds > 10.0 mg/kg (Type C): 90 – 110 %
 - Contamination analysis < 10 mg/kg: > 80 %
- **Selectivity**
 - Tylosin is comedicated in Type C matrices
- **Sensitivity**
 - Should be able to determine tylosin A, B, C and D content

- UV method lacks sensitivity
- Relative abundance of tylosin factors in batch RS0958
 - Tylosin: (A) 97.4%; (B) 0.44%; (C) 0.02%; (D) 2.2%
- Relative abundance in a 100 ppm Type C feed:
 - Tylosin A: 99.4 ppm
 - Tylosin B: 570 ppb
 - Tylosin C: 20 ppb
 - Tylosin D: 720 ppb
- Biological conversion factors using *Staphylococcus aureus*:
 - 1.00 = tylosin (A)
 - 1.26 = desmycosin (B)
 - 1.01 = macrocin (C)
 - 0.33 = relomycin (D)

- ✓ **Extraction solvent**
- ✓ **Extraction conditions**
- ✓ **Hydrolysis procedure**
- ✓ **Sample cleanup**
- ✓ **LC gradient**
- ✓ **Stability of standards and sample extracts**

- 1. Recruit collaborators!**
- 2. Survey labs using HPLC-UV and HPLC-MS/MS methods**
- 3. Draft method**
- 4. Evaluate method fitness**
- 5. Draft method transfer protocol (to include method familiarization)**
- 6. SLVs for method performance verification in all matrices**



Chlortetracycline Working Group Update

**AAFCO Laboratory Methods and Services Committee Meeting
2017 Annual Conference**

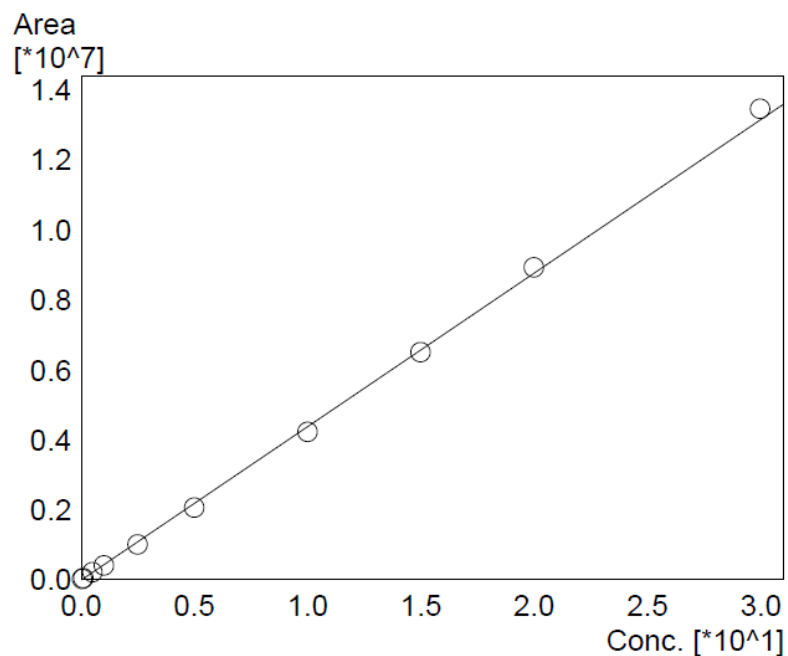
- **Performance:**
 - Performance of this method should be comparable to or exceed AOAC plate assay methods 967.39 and AOAC turbidimetric assay 977.37.
- **Analytical Range**
 - % to ppb
- **Accuracy**
 - Type A and Type B: 95 – 105 %
 - Medicated complete feeds > 10.0 mg/kg (Type C): 90 – 110 %
 - Contamination analysis < 10 mg/kg: > 80 %
- **Selectivity**
 - Chlortetracycline is comedicated in Type C matrices
- **Sensitivity**

- **Reference Method: JAOAC 80(5)961-5, Assay of Chlortetracycline in Animal Feeds by Liquid Chromatography with Fluorescence Detection**
- **Method Performance Evaluation completed**
- **Method Performance Evaluation Plan:**

ID	Concentration	Replicates
LOQ	1 mg/kg	7
50%	50 mg/kg	3
100%	100 mg/kg	5
150%	150 mg/kg	3
LCS	254 mg/kg	5

Linearity

ID# : 1
Name : Chlortetracycline
Quantitative Method : External Standard
Function : $f(x)=438793*x+6893.41$
Rr1=0.9995287 Rr2=0.9990576 RSS=1.472920e+010
MeanRF: 4.621559e+005 RFSD: 7.730133e+004 RFRSD: 16.726244
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/C
Detector Name : Detector A



#	Conc.(Ratio)	Area	Accuracy[%]
1	0.04993867	33026	119.3
2	0.09987734	52296	103.6
3	0.4993867	221285	97.8
4	0.9987734	417060	93.6
5	2.496933	1014099	91.9
6	4.993867	2072325	94.3
7	9.987734	4232204	96.4
8	14.9816	6512592	99.0
9	19.97547	8930978	101.8
10	29.9632	13462212	102.3

Accuracy: 100 mg/kg Target Concentration

Replicate	Expected Conc. (ppm)	Calc. Conc. (ppm)	Recovery	Average Conc. (ppm)	RSD	Average Recovery
LOQ b	1.00	1.76	177.1%	1.59	6.34%	160.4%
LOQ c	0.99	1.65	167.4%			
LOQ d	1.00	1.58	158.8%			
LOQ e	0.99	1.51	152.6%			
LOQ f	0.99	1.53	155.0%			
LOQ g	0.99	1.50	151.6%			
50% Target a	49.7	51.6	103.7%	51.8	0.41%	104.0%
50% Target b	49.9	52.0	104.3%			
50% Target c	49.7	51.7	104.0%			
100% Target a	98.9	99.3	100.4%	99.5	1.62%	100.2%
100% Target b	99.0	101.7	102.7%			
100% Target c	99.5	97.3	97.7%			
100% Target d	99.9	100.0	100.1%			
100% Target e	99.3	99.1	99.8%			
150% Target a	149.4	150.1	100.4%	151	0.83%	101.5%
150% Target b	148.9	152.6	102.4%			
150% Target c	149.0	151.6	101.8%			
LCS 201530 a	254.1	198.1	78.0%	195	2.82%	76.8%
LCS 201530 b	254.1	195.2	76.8%			
LCS 201530 c	254.1	197.1	77.6%			
LCS 201530 d	254.1	199.9	78.7%			
LCS 201530 e	254.1	185.9	73.1%			

- ✓ **Extraction solvent**
- ✓ **Extraction conditions**
- ✓ **Sample cleanup**
- ✓ **LC gradient**
- ✓ **Stability of standards and sample extracts**

- 1. Recruit (more) collaborators!**
- 2. Survey methods in use**
- 3. Draft method**
- 4. Evaluate method fitness**
- 5. Draft method transfer protocol (to include method familiarization)**
- 6. SLVs for method performance verification in all matrices**