**Method Needs and Fitness for Purpose Statement**

**Date: February 2, 2009**

**Project: Determination of tylosin in animal feeding stuffs**

**Project Leader:**

**Project Team:**

**1.0 Needs:**
Tylosin is approved in feed for increased weight gain and improved efficiency in chickens and swine and for the treatment and control of chronic respiratory disease in chickens, prevention of swine dysentery in swine, prevention and/or control of ileitis in swine and for reduction in the incidence of liver abscesses in cattle.

Improved methodology is required to more accurately verify and determine the levels of tylosin in Type A Medicated Articles and in various animal feeds (Type B and C Medicated Feed) including liquid feed supplement and concentrates. Methodology is also required to determine contamination levels to verify clean out of manufacturing equipment for the prevention of cross contamination.

The method of analysis should be HPLC and must be able to determine tylosin A, B, C & D content. The majority of tylosin activity for agricultural use is in tylosin A, containing a smaller amount of tylosin B, C, & D. The correlation between the microbiological responses of A, B, C & D must be determined and included in the final calculation to determine a total microbiological potency.

| Tylosin Factor | BCF* | |
|---|---|---|
| A | 1.00 | A = tylosin |
| B | 1.26 | B = desmycosin |
| C | 1.01 | C = macrocin |
| D | 0.33 | D = relomycin |

*based on conversion factors using *Staphylococcus aureus*

Based on the biopotency conversion factors (BCF) listed above, determination of tylosin in animal feed by HPLC is as follows:

Tylosin content = [factor A] + 1.26*[factor B] + 1.01*[factor C] + 0.33*[factor D]

**1.1 Performance Needs** (based on laboratory sample)
Accuracy: (See Recovery)

Drug premix (Type A), supplements and mineral mixes (Type B): 95 – 105 %
Medicated complete feeds ≥ 10.0 mg/kg (Type C): 90 – 110 %
Contamination analysis and medicated complete feeds < 10 mg/kg: > 80 %

Applicability:

Tylosin is approved in feed for use in cattle, swine, and chickens.
Combinations
Chickens

Tylosin with monensin.
Tylosin with narasin.

Tylosin with salinomycin

Swine

Tylosin with Pyrantel tartrate
Tylosin with ractopamine HCl

Cattle

Tylosin with monensin
Tylosin with MGA
Tylosin with Decoquinate and Monensin
Tylosin with MGA and monensin
Tylosin with MGA and Lasalocid
Tylosin with Monensin and Ractopamine HCl
Tylosin with Monensin, ractopamine HCl and MGA
Tylosin with Monensin, Zilpaterol and MGA

Type A medicated articles:

40 g Tylosin per pound in Tylan 40 (8.8%, 88000 mg/kg)
40 g Tylosin per pound in Tylan 40 Sulfa G (8.8%, 88000 mg/kg)
100 g Tylosin per pound in Tylan 100 (22%, 220000 mg/kg)

Type B medicated feeds: maximum concentration of Tylosin approved in Type B is 10 g/lb (2.2%, 22 g/kg))

Type C medicated feeds: as Tylosin for use in cattle, swine, chickens and turkeys

Chickens

4- 50 g/ton (4.4 – 55 mg/kg)
20-50 g/ton (layers) (22 – 55 mg/kg)
800-1000 g/ton (broilers) (880 – 1100 mg/kg)

Swine

10-100 g/ton (11 – 110 mg/kg)

Cattle

8.0-360 g/ton (8.8 – 397 mg/kg)

Detection Limits:

Medicated products: 0.3 mg/kg
Contamination analysis: 0.03 mg/kg

Determination Limits:

Medicated products: 1.0 mg/kg
Contamination analysis: 0.1 mg/kg

Precision Repeatability:

Medicated products $\geq$ 10 mg/kg: $CV_r$ = or < 5 %
Contamination analysis and medicated products < 10 mg/kg: $CV_r$ = or < 10 %.

Precision Reproducibility:

Medicated products $\geq$ 10 mg/kg: $CV_R$ = or < 10 %
Contamination analysis and medicated products < 10 mg/kg: $CV_R$ = or < 20 %.

Range:

0.1 – 220,000 mg/kg (22%, 100 g/lb)

Recovery:

Medicated products ≥ 10 mg/kg: 90 – 110 %
Contamination analysis and medicated products < 10 mg/kg: > 80 %

Selectivity:

The method is to be free of interferences from matrix, other drugs, vitamins and minerals.

Linearity of standard curve:

$r \geq 0.999$, and 95 % confidence limit of the y intercept includes zero.

Special Considerations:

Two reference standards exist.
USP Standard Reference
Eli Lilly and Company

Performance:

Performance of this method should be comparable to or exceed that of the FDA approved turbidimetric assay.
The method is to be rugged/robust and critical parameters are to be identified and controlled.
Method performance criteria are to be defined. Familiarization plan is to be suggested which will demonstrate that the laboratory analyst can capably perform the method prior to analyzing samples.
Quality control plan is to be suggested along with warning and out of control limits.

Traceability:

Reference standards and acceptable sources are to be identified. Standards are to be provided with assigned purity or potency and uncertainty value.

**Method Performance**:

**Fitness for Purpose Review**

**Fitness for Purpose Statement**