Method Needs and Fitness for Purpose Statement –Final draft

Date: January 11, 2006

Project: Determination of neomycin sulfate in animal feeding stuffs

Project Leader:

Project Team:

1.0 Needs:
Neomycin is approved in feed as the sulfate for the treatment and control of colibacillosis (bacterial enteritis) caused by *E.coli* susceptible to neomycin in cattle, swine, sheep, and goats.

Neomycin is used in feed as the base for the prevention and treatment of bacterial enteritis (scours) in swine and calves.

Improved methodology is required to more accurately verify and determine the levels of neomycin sulfate in Type A Medicated Articles and in various animal feeds including milk-replacers and concentrates. Methodology may also be required to determine residual levels to verify clean out procedures of manufacturing equipment for various state programs.

The method of analysis must be able to determine both neomycin B & C content. The majority of neomycin activity for agricultural use is in neomycin B, containing a smaller amount (approximately 9%) of neomycin C. The total potency should be determined as follows:

$$Total\ Neomycin\ Concentration = Neomycin\ B\ peak\ area + \frac{(Neomycin\ C\ peak\ area)}{2}$$

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

Type A medicated articles (drug premixes) and Type B medicated feeds (supplements and mineral premixes): 95 – 105 % of the target value
Type C medicated feed: 90 – 110 % of the target value
Residual analysis: > 80 %

Applicability:

Neomycin sulfate is approved in drinking water for use in cattle, swine, sheep, turkeys, and goats, and in feed for use in cattle, swine, sheep, chickens, turkeys, and goats. Neomycin base is used in combination with oxytetracycline for prevention and treatment of scours in swine in complete feed and in calves in milk-replacer.
Type A medicated articles: 716 g/kg (325 g/lb) in Neomix 325, and 110 g/kg (50 g/lb) in Neo-Terramycin 50/50, and 100/50, 50 and 100 grams per pound neomycin sulfate equivalent to 35 and 70 grams neomycin base.
Type B medicated feeds: maximum concentration of Neomycin sulfate approved in Type B is 100 g/lb or 200,000 g/ton (22 %) or 220 g/kg.
Type C medicated feeds: as Neomycin sulfate for use in cattle, swine, sheep, and goats a) in complete feed 250 – 2,250 g/ton or 275 – 2480 mg/kg, and b) in milk replacer 400 – 2,000 g/ton or 440 – 2200 mg/kg, and c) chickens and turkeys 50 – 200 g/ton or 55 – 220 mg/kg.

Detection Limits:

Medicated products: *1.5* g/ton or *1.6* mg/kg
Residual analysis: *0.3* g/ton or *0.33* mg/kg

Determination Limits:

Medicated products: *5* g/ton or 5.5 mg/kg

Residual analysis: *1* g/ton or *1.1* mg/kg

Precision Repeatability:

Medicated products: CV_r = or < 5 %

Residual analysis: CV_r = or < 10 %.

Precision Reproducibility

Medicated products: CV_R = or < 10 %

Residual analysis: CV_R = or < 20 %.

Range: 0.9 – 650,000 g/ton or 10 – 716,000 mg/kg

Recovery:

Medicated products: 90 – 110 %

Residual analysis: > 80 %

Selectivity:

The method is to be free of interferences from matrix, other drugs, vitamins and minerals. Neomycin is compatible with and sold in combination with oxytetracycline in a Type A Medicated Article or premix.

Linearity of standard curve:

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

Special Considerations:

Two reference standards exist.

1) USP Standard Reference Neomycin Sulfate from USP is base compound. Lot L-2 is 782 ug/mg dry as neomycin sulfate. It only contains Neomycin B. Assay is microbiological.

2) Neomycin sulfate from Sigma-Aldrich USP grade contains Neomycin B and Neomycin C. Lot 061K08921 has a C of A of 701 ug/mg (dry) with the microbiological assay.

The HPLC assay of the Sigma-Aldrich neomycin sulfate will yield 2 peaks on the HPLC system that are well separated. The Neomycin C peak is approximately 9 % of the Neomycin B peak in terms of peak area.

The USP standard reference neomycin sulfate is used as the reference to establish HPLC standard response plot. This response plot is used to calculate the amount of Neomycin in the Sigma material. The same reference neomycin lot is used to calculate the amount of Neomycin C from the Neomycin C peak, which is then divided by 2 for the potency.

Both peaks as noted before are calculated based on the USP Standard Reference compound. The Sigma material has a total potency that is 95.5 % Neomycin B and 4.5 % Neomycin C on a potency basis.

The Neomycin B response is used for both the B and C calculations and can be either the USP reference compound or a working standard based on the Sigma compound.

Performance of this method should be comparable to or exceed that of AOACI plate assays.

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