Method Needs and Fitness for Purpose Statement – Final v2

Date: Sept. 9, 2009

Project: Determination of bacitracin methylene disalicylate (BMD) in animal feeding stuffs

Project Leader:

Project Team:

1.0 Needs:

Bacitracin methylene disalicylate (bacitracin MD) is used for increased rate of weight gain and improved feed efficiency in chickens, turkeys, pheasants, quail, and swine. In chickens, it is also used for improved feed efficiency for egg production, prevention and control of necrotic enteritis. In turkeys, bacitracin MD is used for control of transmissible entertis and in quail for prevention of ulcertative enteritis. In swine, bacitracin MD is used to control porcine proliferative enteritis (ileitis), swine dysentery and clostridial enteritis (when fed to pregnant sows). In cattle, bacitracin MD reduces the number of liver condemnations due to abscesses.

Methodology is required to verify the levels of bacitrracin MD in various feeds, supplements and premixes. Methodology is also required to determine contamination levels to verify clean out of manufacturing equipment for the prevention of cross contamination. Bacitracin MD is compatible with the following drug combinations: amprolium, amprolium with ethopabate, arsanilic acid, clopidol, decoquinate, diclazuril, chlortetracycline, fenbendazole, halofuginone hydrobromide, hygromycin B, ivermectin, lasalocid sodium, lincomycin, monensin, narasin, nicarbazin, nitarasone, robenidine hydrochloride, salinomycin, semduramicin, roxarsone (3-nitro-4-hydroxyphenylarsonic acid), tylosin, and zoalene.

Bacitracin has 1 major isomer (bacitracin A) and 5 other active isomers each having one possible epimer. In addition, oxidation products of both the isomers and epimers exist, and there is 1 major inactive isomer. The active isomers each have approximately the same biopotency (100%), while the epimers and oxidation products have potencies ranging from 50-75% and 0-75% respectively. Any HPLC method development will have to resolve these components in order to arrive at an accurate bacitracin determination. It is therefore necessary to perform an extensive comparison of any HPLC developed method with the current microbiological assay method.

1.1 Performance Needs (based on laboratory sample)

Accuracy: (See Recovery)

Drug premix (Type A): 85 – 110 %
Medicated feeds (Type B & C) $\geq$ 10 mg/kg: 80 – 115 %
Contamination Analysis and medicated feeds < 10 mg/kg: 70 – 125 %

Applicability:

For use feeds for broilers, replacement and laying chickens, turkeys, pheasants, quail, swine, feedlot beef cattle
Premixes: 30 g/lb (66 g/kg), 50 g/lb (110 g/kg), 37.4 g/lb (82.5 g/kg) and 60 grams/lb

(132 mg/kg)
Medicated complete feed for chickens: 4-50 g/ton (4.4-55 mg/kg), 100-200 g/ton (110-220 mg/kg)
Medicated complete feed for turkeys: 4-50 g/ton (4.4-55 mg/kg), 200 g/ton (220 mg/kg)
Medicated complete feed for quail: 5-20 g/ton (5.5-22 mg/kg), 200 g/ton (220 mg/kg)
Medicated complete feed for pheasants: 4-50 g/ton (4.4-55 mg/kg)
Medicated complete feed for feedlot beef cattle: 70 or 250 mg/head/day
Medicated complete feed for swine: 10-30 g/ton (11-33 mg/kg), 250 g/ton (275 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: CV_r = or < 8 %
Contamination analysis: CV_r = or < 10 %.
Precision Reproducibility
Medicated products: CV_R = or < 15 %
Contamination analysis: CV_R = or < 20 %.
Range: 0.1 mg/kg to 110,000 mg/kg (110 g/kg, 11%)
Recovery:
≥ 10 mg/kg: 80 -115 %
< 10 mg/kg: > 70 – 125 %
Selectivity:
The method is to be free of interferences from matrix, other drugs, vitamins, minerals.
Linearity of standard curve:
$r \geq 0.999$, and 95 % confidence limit of the y-intercept includes zero.
Special Considerations:
Performance of this method should be comparable to or exceed that of the FDA approved microbiological assay method.
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:
Standards and acceptable sources are to be identified. Standards are to be provided with assigned purity or potency and uncertainty value (if possible).

Method Performance:

Fitness for Purpose Review

Fitness for Purpose Statement