Method Needs and Fitness for Purpose Statement – Final DRAFT

Date: September 15, 2006

Project: Determination of vitamin A in animal feeding stuffs

Project Leader:

Project Team:

1.0 Needs:

Vitmain A is a fat-soluble vitamin and an essential nutrient required for the growth and maintenance of all vertebrates. As such, vitamin A supplement is added to almost all animal feed for both farmed animals and companion animals. Synthetic vitamin A is normally used to supplement feed and is added as the all-trans retinyl acetate or palmitate.

The current AOACI OMA (974.29) uses noxious reagents for color development, is susceptible to interference from other absorbing species, and is very labor-intensive. An analytical method for determining retinol content of animal feeds using liquid chromatography (LC) is needed to eliminate the use of noxious reagents, improve the accuracy of the method, and improve the throughput and ease-of-use of the method.

The developed methodology should cover a large range of vitamin A levels and should be applicable to feeds for a wide variety of animal species, including farm and companion animals. The method should produce results comparable to the current AOACI OMA (974.29) with the understanding that the OMA may have a high bias at low vitamin A levels. Other desirable characteristics of the method include improved throughput and ease-of-use and the ability to separate the 13-cis and all-trans retinol isomers in cases where there is a significant contribution of the 13-cis isomer to the total retinol content. At this point in time, it is recommended that the 13-cis isomer be separated but not adjusted for activity in the final calculations.

1.1 Performance Needs:

Accuracy: (See Recovery)

Feeds
227 - 30,000 IU/lb (500 – 66,000 IU/kg): 75 – 120 %
30,000 - 75,000 IU/lb (66,000 – 165,000 IU/kg): 80 – 115 %
Feeds premixes
75,000 - 500,000 IU/lb (165,000 – 1,100,000 IU/kg): 85 – 110 %
500,000 – 1,000,000 IU/lb (1,100,000 – 2,200,000 IU/kg): 90 – 108 %
1,000,000 - 10,000,000 IU/lb (2,200,000 – 22,000,000 IU/kg): 92 – 105 %
10,000,000 – 650,000,000 IU/lb (22,000,000 – 1,400,000,000 IU/kg): 95 – 102 %

Applicability:

Feeds: 227 - 75,000 IU/lb (500 – 165,000 IU/kg)
Feed premixes: 75,000 – 650,000,000 IU/lb. (165,000 - 1,400,000,000 IU/kg)

Detection Limits:

Feeds and feed premixes: 100 IU/lb. (220 IU/kg)

Determination Limits:

Feeds and feed premixes: 227 IU/lb. (500 IU/kg)

Precision Repeatability:

Feeds
227 - 30,000 IU/lb (500 – 66,000 IU/kg): 8 %
30,000 - 75,000 IU/lb (66,000 – 165,000 IU/kg): 6 %
Feeds premixes
75,000 - 500,000 IU/lb (165,000 – 1,100,000 IU/kg): 4 %

500,000 – 1,000,000 IU/lb (1,100,000 – 2,200,000 IU/kg): 3 %
1,000,000 - 10,000,000 IU/lb (2,200,000 – 22,000,000 IU/kg): 2 %
10,000,000 – 650,000,000 IU/lb (22,000,000 – 1,400,000,000 IU/kg): 1.5 %.

Precision Reproducibility:

Feeds
227 - 30,000 IU/lb (500 – 66,000 IU/kg): 16 %
30,000 - 75,000 IU/lb (66,000 – 165,000 IU/kg): 11 %
Feeds premixes
75,000 - 500,000 IU/lb (165,000 – 1,100,000 IU/kg): 8 %
500,000 – 1,000,000 IU/lb (1,100,000 – 2,200,000 IU/kg): 6 %
1,000,000 - 10,000,000 IU/lb (2,200,000 – 22,000,000 IU/kg): 4 %
10,000,000 – 650,000,000 IU/lb (22,000,000 – 1,400,000,000 IU/kg): 3 %.

Range:

227 – 650,000,000 IU/lb. (500 – 1,400,000,000 IU/kg)

Recovery:

Feeds
227 - 30,000 IU/lb (500 – 66,000 IU/kg): 75 – 120 %
30,000 - 75,000 IU/lb (66,000 – 165,000 IU/kg): 80 – 115 %
Feeds premixes
75,000 - 500,000 IU/lb (165,000 – 1,100,000 IU/kg): 85 – 110 %
500,000 – 1,000,000 IU/lb (1,100,000 – 2,200,000 IU/kg): 90 – 108 %
1,000,000 - 10,000,000 IU/lb (2,200,000 – 22,000,000 IU/kg): 92 – 105 %
10,000,000 – 650,000,000 IU/lb (22,000,000 – 1,400,000,000 IU/kg): 95 – 102 %

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 limits of the y intercept include zero.

Special Considerations:

Performance of this method should be comparable to AOACI OMA 974.29 colorimetric method (Carr-Price reagent), keeping in mind that the OMA may produce a high bias in the absorbance measurement from absorbing coextracted compounds, especially at lower levels.

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:

Acceptable analytical standard material is available from U.S. Pharmacopeia (USP), catalog number 1716002.

Method Performance:

Fitness for Purpose Review:

Fitness for Purpose Statement: