AAFCO LM&SC Fat Soluble Vitamins Working Group

Vitamin A Team & Vitamin E Team

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Anaheim, CA January 2018
Review
2017 Round-Robin Study

• 8 Methods for Vitamin A
• 6 Methods for Vitamin E
• 4 Samples prepared by Able Lab
• Although differences between the methods were found, overall, the methods produced similar results.
• Results exhibited much improved within-lab precision and overall precision
• Samples appeared to be relatively stable over the time period studied
Validation Data Requested

- Linearity – Linear range of standards
- LOQ – Including how it was determined
- Repeatability – Within-day precision
- Reproducibility (Intermediate) – Between-day precision (include matrix)
- Matrices for which the method was validated
- Trueness/Recovery – Comparison to known sample (e.g., NIST-SRM) or recovery of standard spikes (include matrix information)
Validation Data Received

- Labs 1-5 submitted relatively complete data for Vit A
- Labs 1-3 submitted relatively complete data for Vit E
- None of the labs submitted data for all matrices that are desired to be covered.
Method Considerations

Conference call on December 21\textsuperscript{st} 2017

• Do we need to separate 13-cis-retinol?
  ✓ Separation is recommended but not necessary to quantitate

• Do we need to separate $\alpha$, $\beta$, $\gamma$, and $\delta$-tocopherol?
  ✓ Regulations are based on $\alpha$-tocopherol only
Collaborative Study Considerations

• Single laboratory to study the effect of sample weight
• Single laboratory to compare hot vs room temperature saponification (data exist)
• Single laboratory study to compare liquid-liquid vs SPE vs. dilution-neutralization – does anyone have this kind of data?
• Selection of sample matrices to include in the collaborative study
• Single method for Vit A and Vit E not necessary
Immediate Path Forward

• Single-laboratory study to determine the minimum sample size needed
  ✓ Minnesota Dept. of Ag. has volunteered to perform the study
  ✓ Will work closely with the Sample Preparation WG on study design

• Need to work closely with the Sample Preparation WG on sample preparation for single-laboratory studies
Methods – Vitamin A

• **Saponification**
  ✓ Ethanol + KOH + Antioxidant
  ✓ Heated (30 – 60 min) or Room Temp (Overnight)

• **Cleanup**
  ✓ Liquid-Liquid Partitions (4)
  ✓ SPE (2)
  ✓ Only Filtration (2)

• **HPLC (RP, UV)**

• **Internal Standard (2)**
Methods – Vitamin E

• Extraction
  ✓ Saponification (3)
  ✓ Methanol Sonication (1)
  ✓ Hexane Homogenization (1)

• Cleanup
  ✓ Liquid-Liquid Partitions (2)
  ✓ Only Filtration (3)

• Internal Standard (1)

• HPLC (-FL ?)
  ✓ Reverse Phase (4), Normal Phase (2)