

AAFCO LM&SC Fat Soluble Vitamins Working Group

Vitamin A Team & Vitamin E Team

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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 (eg. 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 21st 2017

- **Do we need to separate 13-cis-retinol?**
 - ✓ Separation is recommended but not necessary to quantitate
- **Do we need to separate α , β , γ , and δ -tocopherol?**
 - ✓ Regulations are based on α -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 has 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)