1. SCOPE AND PURPOSE:

1.1. **AULC:** This method measures Vitamin A activity from retinol or retinol esters in foods, feeds, ingredients, vitamin premixes, and concentrates. Vitamin A activity from carotene and other vitamin A active components are not detected by this method. Results are reported as International Units of Vitamin A.

1.2. **VITE:** This procedure measures tocopherol and tocopherol esters such as tocopherol acetate (Vitamin E) in foods, feeds, ingredients, vitamin premixes, and concentrates. Values are reported as d,l-alpha-tocopherol acetate.

1.3. There is no assurance that matrices other than those listed can be assayed using this method.

2. PRINCIPLE:

NOTE: Vitamin A and vitamin E are extracted by the same method. One extract can be used to analyze both vitamins on the HPLC. If a UV detector and fluorescence detector are set up in series, one injection can be used to analyze both vitamins.

2.1. Samples and standards are saponified with an alcoholic KOH solution in the presence of an antioxidant (pyrogallol) to convert vitamin A esters to vitamin A alcohol (retinol) and/or tocopherol acetate or tocopherol esters to the alcohol (tocopherol). An aliquot of the extract is shaken with hexane, partitioning the retinol and/or tocopherol into hexane. An aliquot of hexane is evaporated and the extract reconstituted in methanol. The reconstituted extract is injected on a High Performance Liquid Chromatograph (HPLC) equipped with an ultraviolet detector for vitamin A analysis and a fluorescence detector for vitamin E analysis. Vitamin A and vitamin E are quantitated using a set of external standard solutions of known concentration that are taken through the method.

2.2. Using a 5-g sample and a 20 mL aliquot of the 50 mL hexane extract evaporated and reconstituted in 2 mL methanol, the limit of quantitation of this method is 600 IU Vitamin A activity per pound. Using a 5-g sample and a 20 mL aliquot of the 50 mL hexane extract evaporated and reconstituted in 2 mL methanol, the limit of quantitation of this method is 0.4 mg tocopherol acetate/100 g.

2.3. There are no known interferences for this method that have not been addressed.

3. PRECISION:

Records of method precision based on Method Validation and/or known control summaries are located in the QA Master file for this test method. Assay precision may vary with test matrix and analyte level. Terms used to describe method precision are defined in NPSOP3000, *Validation of Quantitative Chemical Tests*.

4. REFERENCES:

4.1. LI-75.214-01 PET FOOD VITAMINS A & E by HPLC-UV/FLD.

4.2. Official Methods of Analysis of AOAC, Methods 2001.13

4.3. “Sources of Error in Vitamin A Analysis”, N. Thiex et al, Journal of AOAC International, Volume 79, No. 6, 1996, 1269-1275.

4.4. "Influence of Extraction Techniques on Determination of Alpha-Tocopherol in Animal Feedstuffs," C. McMurray, W. J. Blanchflower, D. A. Rice; J. Assoc. Off. Anal. Chem.; Vol 63, No. 6, (1980).