

Isolation of Vitamin A, E, D, and Cholesterol by ANKOM^{FLEX} (Method: VC R8)**Definition**

This method is used to isolate Vitamin A, E, and D from premixes.

Scope

This method is applicable to premixes where vitamins are encapsulated and need to be dissolved prior to saponification.

A. Apparatus

1. ANKOM^{FLEX} Analyte Extractor
2. Digestion Vessels (FLEX54, FLEX55)
3. High strength magnetic stir-bars (9380) – *for use in digestion vessels*
4. Round Bottom Flasks (9364) – *for recovery on the FLEX instrument*
5. Prepacked SPE Columns (FLEX-SPE-01)
6. Vitamin Filters (FLEX-VF)
7. Analytical Balance – capable of weighing 1mg

B. Reagents

1. Use deionized water (DI) throughout
2. n-Hexane (reagent grade or higher)
3. Ethanol (95 % or higher)
4. Pyrogallol (or equivalent)
5. Potassium hydroxide (KOH)
6. Butylated hydroxytoluene (BHT)
7. 2 % (w/v) pyrogallol in ethanol: Weigh 10 g ± 0.1 g pyrogallol into a 500 ml volumetric flask. Make up to the mark with ethanol. Mix well.
8. 12.7 N Potassium hydroxide (KOH) solution: Slowly add 500 g KOH into 500 g DI water, while continually mixing.
9. 0.05 g/L BHT in hexane: Weigh 0.05 g ± 0.005 g BHT into a 1 L volumetric flask. Make up to the mark with hexane. Mix well.

C. Sample Preparation

Homogenize, grind, or thoroughly mix a representative sample prior to sampling for analysis on the ANKOM^{FLEX}.

D. Procedure (*see the Operator's Manual for more detail*)

1. Assemble digestion vessels (digestion vessel + vessel bottom assembly + vitamin filter) and add a magnetic stir-bar into each digestion vessel before adding sample.
2. Weigh 0-3 g of the premix sample into the digestion vessel and place the vessel on the FLEX instrument.
3. Follow the instructions in the Operator's Manual on how to: Start an Assay
4. Select Method: [VC R8](#)
5. After the FLEX method has ended, the round bottom flasks in the recovery oven will contain the isolated vitamins. Remove the round bottom flasks, cover the top of each flask with aluminum foil or stopper. Cool each flask under cold running water for ~20 seconds, ensuring water does not enter flask.
6. Reconstitute the isolated vitamins with the appropriate solvent for further quantitation on HPLC.