Isolation of Vitamin A, E, D, and Cholesterol by ANKOM^{FLEX} (Method: VC R5)

Definition
This method is used to isolate Vitamin A, E, D, and Cholesterol from beverages.
Scope
This method is applicable to beverages, where sample size of 20 g is required.

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 1L volumetric flask. Make up to the mark with hexane. Mix well.

C. Sample Preparation

Homogenize or thoroughly mix a representative sample prior to sampling for analysis on the ANKOM^{FLEX}. It is important to not exceed the specified sample size as that could result in digestion filters plugging or SPE columns overloading.

D. Procedure

1. Assemble digestion vessels (digestion vessel + vessel bottom assembly + vitamin filter) and add a stir-bar into each digestion vessel before adding sample.
2. Weigh 20 g of the beverage sample into the digestion vessel by dripping it onto the stir bar or allowing it to run down the side of the digestion vessel. Do not squirt it directly onto the filter, as this action will break the surface tension of the filter and sample will leak through the filter.
3. Install digestion vessels on the ANKOM^{FLEX}.
4. Follow the instructions in the operating manual on how to: Start an Assay.
5. Select Method: VC R5
6. After the ANKOM^{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.
7. Reconstitute the isolated vitamins with the appropriate solvent for further quantitation on HPLC.

If Limit of Quantitation (LOQ) is a concern, please contact ANKOM for analytical support.