

Isolation of Vitamin A, E, D, and Cholesterol by ANKOM^{FLEX} (Method: VC R6)

Definition

This method is used to isolate Vitamin A, E, D, and Cholesterol within a given sample using Bromelain for homogenization.

Scope

This method is the primary method for compound feed (not incl. pet food) analysis.

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
8. 300-ml beaker

B. Reagents

1. Use deionized water (DI) throughout
2. Bromelain, $\geq 2,400$ GDU/g (Part number, TBD)
3. Ascorbic acid (or equivalent)
4. n-Hexane (reagent grade or higher)
5. Ethanol (95 % or higher)
6. Pyrogallol (or equivalent)
7. Potassium hydroxide (KOH)
8. Butylated hydroxytoluene (BHT)
9. 2 % (w/v) pyrogallol in ethanol: Weigh 10 g \pm 0.1 g pyrogallol into a 500 ml volumetric flask. Make up to the mark with ethanol. Mix well.
10. 12.7 N KOH solution: Slowly add 500 g KOH into 500 g DI water, while continually mixing.
11. 0.05 g/L BHT in hexane: Weigh 0.05 g \pm 0.005 g BHT into a 1L volumetric flask. Make up to the mark with hexane.
12. DI water, pH 8, 50C. Adjust DI water to pH 8 with a few drops of 12.7 N KOH. Confirm with pH strip. Warm to 50C.

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. Pre-treat the feed sample with Bromelain to disperse vitamins within the sample.
 - a. Add 0.3 \pm 0.01 g Ascorbic Acid, 0.22 \pm 0.01 g Pyrogallol and 0.15 \pm 0.01 g BHT into a beaker.
 - b. Weigh (**W1**) 120 \pm 5 ml water (pH 8, 50C) into the beaker and mix well. BHT will not dissolve.
 - c. Weigh (**W2**) 40 \pm 2 g of the feed sample into the beaker and mix well.
 - d. Add 0.25 \pm 0.01 g Bromelain into the beaker and mix well - until all clumps are gone.
 - e. Fully cover the beaker with foil (to maintain heat) and allow to digest for 60min at room temperature.
2. Assemble digestion vessels (digestion vessel + vessel bottom assembly + vitamin filter) and add a stir-bar into each digestion vessel.
3. After digestion is complete, weigh a representative aliquot (20 \pm 3g) (**W3**) from the slurried sample into the FLEX digestion vessel and install the vessel on the FLEX. The aliquot contains approximately 5g of the feed sample.
4. Follow the instructions in the Operator's Manual on how to: Start an Assay
5. Select Method: VC R6
6. 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.
7. Reconstitute the isolated vitamins with the appropriate solvent for further quantitation on HPLC.

E. Calculation

Calculate the amount of sample per vessel in gram: $W2 / (W1+W2) * W3$.

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