

Isolation of Vitamin A, E, D, and Cholesterol by ANKOM<sup>FLEX</sup> (Method: VC R7)

**Definition**

This method is used to isolate Vitamin A, E, D, and Cholesterol within a given sample using filter aids.

**Scope**

This method is applicable to (i) foods that do not filter well on the standard VC R4 method and (ii) compound feeds, where 10g sample max yield acceptable precision.

**A. Apparatus**

1. ANKOM<sup>FLEX</sup> 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. Filter Aid (FLEX-FA2) - *for use in digestion vessels*
9. Diffuser (FLEX-DF) - *for use in digestion vessels*
10. Filter (Filter 50/40) - *for use in digestion vessels*

**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, grind, or thoroughly mix a representative sample prior to sampling for analysis on the ANKOM<sup>FLEX</sup>. The **maximum sample size is 10 g**. If more than 10 g of sample is required, contact ANKOM Technology for assistance.

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

1. Assemble the digestion vessel as follows: Place the vitamin filter in the vessel bottom assembly, and then the **diffuser** on top of the vitamin filter. Then complete assembly of the digestion vessel with the vessel bottom.
2. Add a stir-bar into the digestion vessel.
3. Add **1g Filter Aid** into the vessel.
4. Weigh sample, up to 10 g, into the digestion vessel, on top of the stir bar and the filter aid.  
**Note:** Exceeding the sample size could result in digestion filters plugging or SPE columns overloading.
5. Install digestion vessels on the FLEX and follow the instructions in the Operator's Manual on how to: Start an Assay
6. Select Method: **VC R7**
7. After the ANKOM<sup>FLEX</sup> 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.
8. Reconstitute the isolated vitamins with the appropriate solvent for further quantitation on HPLC.

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