Isolation of Vitamin A, E, and D by ANKOM^{FLEX} (Method: VitAED SPE R2)

Definition

This method is used to isolate Vitamin A, E, and D within a given sample, using ambient overnight saponification and SPE extraction and evaporation of the ANKOM^{FLEX}.

Scope

This method is applicable to food and feed samples.

Apparatus

1. ANKOM^{FLEX} Analyte Extractor
2. Ported Digestion Vessels
3. Magnetic cross stir-bars – *for use in digestion vessels*
4. Prepacked SPE Columns (FLEX-SPE)
5. Vitamin Filters (FLEX-VF)
6. Analytical Balance – capable of weighing 1mg.
7. 100-250ml containers with caps – *for saponification*
8. Stir plate, Shaker or equivalent – *for saponification agitation*
9. Stir plate – *for aliquoting agitation*
10. 100-250ml container with cap – *for infant formula slurry preparation*
11. Stir Plate and Stir Bar – *for infant formula slurry preparation*

Reagents

Use distilled water (DI) throughout.

(a) n-Hexane (reagent grade or higher)
(b) Ethanol (95% or higher)
(c) Pyrogallol (or equivalent)
(d) Potassium hydroxide (KOH)
(e) Butylated hydroxytoluene (BHT)
(f) 2% (w/v) pyrogallol in ethanol: Weigh 8g ± 0.1g pyrogallol into 500ml volumetric flask. Make up to mark with Ethanol. Mix well.
(g) 12.7N Potassium hydroxide (KOH) solution: Slowly add 500g KOH into 500g DI water, while continually mixing. Allow to cool to room temperature.
(h) 0.05g/L BHT in hexane: Weigh 0.05g ± 0.005g BHT into a 1L volumetric flask. Make up to the mark with hexane. Mix well.

Sample Type & Size

Sample Type	Size (g)
Oils e.g. melted margarine	4
High fat samples (>40% fat) e.g. butter, peanut butter, meat	4
Wet pet food	40
Dry pet food	20
Fresh fruits and vegetables	40
Beverages	40
Nutritional powders & supplements	1-4
Infant Formula	see page 2
Animal Feed	40
Other	20

Sample Preparation

1. Beverages & Oils: Samples must be at room temperature and mixed thoroughly prior to sampling.
2. Infant Formula: See page 2 of this document.
3. All other samples: Homogenize/grind/mix a portion (approximately 4 times that of the sample size in the table) as fine as possible prior to sampling.

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

1. Weigh an empty container (W1) including cap and stir bar.
2. Weigh sample or infant formula slurry (W2) into the container. It is very important to not exceed the specified sample size (Table 1). Exceeding the sample size could result in SPE columns overloading.
3. Add water until a total of 40g (sample + water = 40g). Exception: For animal feed samples, add 40ml water.
4. Add 100ml of 2% (w/v) pyrogallol in ethanol.
5. Add 40ml of 12.7N KOH solution.
6. Purge container with nitrogen before closing it tightly.
7. Saponify the mixture for 16hrs at ambient temperature, while continually mixing on stir plate or in shaker (170rpm).
8. Add 72ml DI water.
9. Weigh container (W3) including saponified sample and stir bar.
10. Assemble four FLEX digestion vessels (ported glass vessel + vessel base + vitamin filter) and add a magnetic cross stir-bar into each digestion vessel.
11. Install vessels in the FLEX digestion oven.
12. Close vessel tops by pressing the vessel tops toggle on the Home screen. This step is very important. If vessel tops are not closed, the saponified solution will leak through the vessels.
13. Select Method: VitAED SPE R2.
14. Transfer a 60ml aliquot of the saponified sample into a ported digestion vessel:
    - 14.1. While the saponified sample is stirring on a stir plate, use an empty 60ml syringe to draw a 60ml aliquot of the saponified solution into the syringe. You may wipe the outside of the syringe tip at this time.
    - 14.2. Weigh the full syringe (W4).
    - 14.3. Expel the saponified solution into the digestion vessel through the port and close the red cap when you are done. Do NOT wipe the tip of the syringe at this time and make sure to not lose any drips. This is a quantitative process.
    - 14.4. Weigh the emptied syringe (W5).
15. Repeat step 14 to transfer saponified sample into the other digestion vessels of the FLEX.
16. Close each vessel port with a red cap.
17. Follow instructions in the operating manual on how to: Start an Assay. Do NOT open vessel tops at any point during set-up as saponified solution will leak through the vessels.
18. 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.
19. Reconstitute the isolated vitamins with the appropriate solvent for further quantitation on HPLC.

Procedure continued on next page

Sample Preparation – Infant Formula Slurry

To evenly disperse vitamin supplements in infant formula samples, the sample must be slurried prior to subsampling for vitamin analysis on the ANKOM^{FLEX}. Use the following procedure to prepare slurries.

Infant Formula Slurry

1. Weigh 20g, to the nearest 0.1g of infant formula powder into a container (IF1).
2. Add 133g, to the nearest 0.5g warmed DI water (60C) to the container (IF2).
3. Add a magnetic stir bar and close the container.
4. Place on stir plate and mix the slurry for 15min.
5. The slurry now is ready to be used for subsampling.
6. Step 2 of SOP for Method: VitAED SPE:
   Weigh 40g, to the nearest 0.5g of the infant formula slurry into the container (IF3).
7. Step 3 of SOP for Method: VitAED SPE:
   Do not add additional water to the container.
8. Add water until a total of 40g (sample + water = 40g).
9. Continue to follow step 4 of the method SOP.
10. Calculate the ratio of infant formula powder (R) in the slurry.

$$R = \frac{IF1}{(IF1+IF2)}$$

Procedure *(continued)*

20. Calculate the weight (g) of sample or infant formula slurry (S) in the saponified aliquot (step 14).

$$S = \frac{W2(W4-W5)}{(W3-W1)}$$

21. Calculate the weight (g) of infant formula powder in the saponified aliquot (IF0).

$$IF0 = (S)(R)$$