

# APPLICATIONS

## Vitamin A and E Analysis From Infant Formula Using Luna<sup>®</sup> 3 μm NH<sub>2</sub> per AOAC OMA 2012.10

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**Food Industry  
Marketing Manager**

*Allen Misa is a downhill mountain biker who spends his days and weekends either riding off a face of a mountain or bouncing his daughters on his lap.*

### Introduction

Millions of parents globally rely on infant formula to provide valuable nutrition for their infant children. Infant formula is supplemented with important nutrients to aid in the healthy development of the infant. Two of the most important nutrients include Vitamins A and E. To increase shelf-life, Vitamin A is typically added as either in the palmitate or acetate form. Vitamin E is typically added unmodified and in the acetate form. As part of the Quality Assurance (QA) process, the FDA requires infant formula manufacturers to quantify Vitamin A and Vitamin E levels in the final product. These compounds are effectively analyzed using HPLC with both FLD and UV detection, preceded by a Liquid-Liquid Extraction (LLE) sample preparation step. Presented is an optimized HPLC method using Luna 3 μm NH<sub>2</sub>.

### Materials and Methods

#### Reagents and Chemicals

Vitamin A palmitate—Reference standard, Sigma (St. Louis, MO) Cat. No. R3375.

Vitamin A acetate—Reference standard, Sigma Cat. No.46958.

dl-α-Tocopherol acetate—Reference standard, Sigma Cat.No. T3376.

dl-α-Tocopherol—Reference standard, Sigma Cat. No.95240.

tert-butyl methyl ether —Mobile phase, Sigma Cat. 306975-1L.

Hexane —Mobile phase, Burdick & Jackson Cat. AH212-4.

### Experimental Conditions

#### Sample Preparation (Per AOAC OMA 2012.10)

#### Powder Samples:

- Transfer 25 g accurately weighed into a 250 mL volumetric flask. Dissolve using distilled water (approximately 40 °C) cool and make up to 250 mL with distilled water. Transfer 5 mL reconstituted sample to a 50 mL screw top centrifuge tube.

#### Ready-To-Feed Samples or Concentrated Liquid Products:

- Transfer 5.0 mL thoroughly agitated sample directly to a 50 mL screw top centrifuge tube. Liquid samples should be analyzed from a freshly opened container stored/refrigerated for no more than 48h, and never analyzed from a frozen sample.

#### Directly Following Either Powder or Ready-To-Feed Samples:

- Add 5 mL 2 % papain solution into the 50 mL screw top centrifuge tube
- Mix to disperse each sample, cap, and place the tubes in a 37 ± 2 °C water bath for 20–25 min. Remove the samples from the bath and cool. Place in a freezer for approximately 5 min or refrigerate for approximately 20 min.
- Add approximately 20 mL acidified methanol to each sample tube and mix.
- Accurately pipet 10.0 mL iso-octane into each sample tube.
- Close tightly to avoid leakage and shake the tube for 10 min preferably with a mechanical shaker.
- Centrifuge for 10 min at 4000 rpm to obtain a clear iso-octane layer. Remove enough iso-octane from the centrifuge tube to fill an injection vial. This extract is ready for LC analysis.

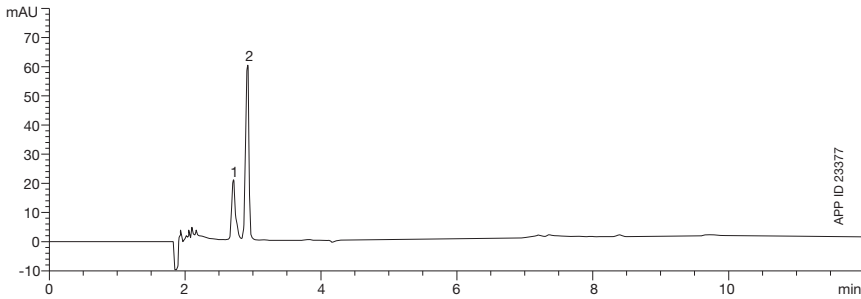
Note: Typically, a 50 μL injection volume is used for the standards and sample extracts, but this can be varied (20–100 μL) to suit sensitivity.

### LC-UV/LC-FLD Conditions

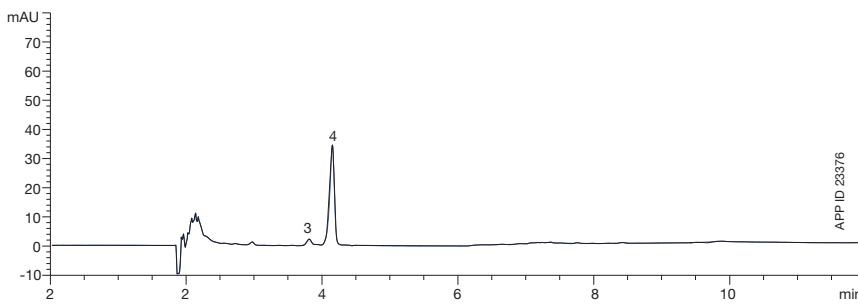
<b>Column:</b>	Luna 3 μm NH <sub>2</sub>										
<b>Dimensions:</b>	150 x 4.6 mm										
<b>Part No.:</b>	00F-4377-EO										
<b>Mobile Phase:</b>	A: Hexane B: Hexane with 50 % MTBE										
<b>Gradient:</b>	<table border="0"> <thead> <tr> <th>Time (min)</th> <th>% B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>0</td> </tr> <tr> <td>3</td> <td>0</td> </tr> <tr> <td>8</td> <td>100</td> </tr> <tr> <td>12</td> <td>100</td> </tr> </tbody> </table>	Time (min)	% B	0	0	3	0	8	100	12	100
Time (min)	% B										
0	0										
3	0										
8	100										
12	100										
<b>Flow Rate:</b>	1.0 mL/min										
<b>Injection:</b>	50 μL										
<b>Temperature:</b>	Ambient										
<b>Detection UV:</b>	325 nm										
<b>Detection FLD:</b>	280/310 nm (emission/excitation)										
<b>Sample:</b>	1. Vitamin A Palmitate isomer 2. Vitamin A Palmitate 3. Vitamin A Acetate isomer 4. Vitamin A Acetate 5. Vitamin E Acetate 6. Vitamin E										



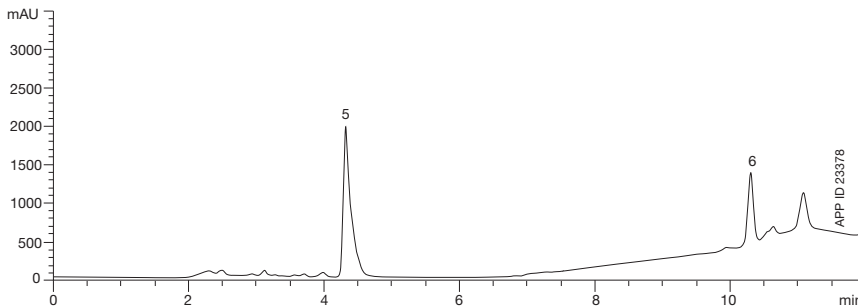
**Figure 1.**  
Enfamil<sup>®</sup> infant formula (liquid) UV chromatogram - Vitamin A forms



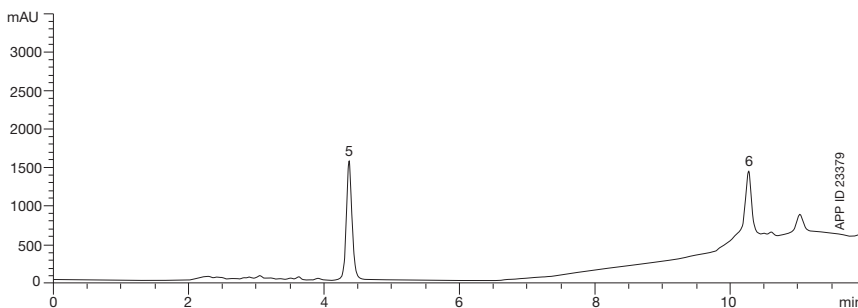
**Figure 2.**  
Gerber<sup>®</sup> infant formula (powder) UV chromatogram - Vitamin A forms



**Figure 3.**  
Enfamil infant formula (liquid) FLD chromatogram - Vitamin E forms



**Figure 4.**  
Gerber infant formula (powder) FLD chromatogram - Vitamin E forms



**LC-UV/LC-FLD Conditions**

<b>Column:</b>	Luna <sup>®</sup> 3 μm NH <sub>2</sub>
<b>Dimensions:</b>	150 x 4.6 mm
<b>Part No.:</b>	00F-4377-E0
<b>Mobile Phase:</b>	A: Hexane B: Hexane with 50% MTBE
<b>Gradient:</b>	<b>Time (min) % B</b>
	0 0
	3 0
	8 100
	12 100
<b>Flow Rate:</b>	1.0 mL/min
<b>Injection:</b>	50 μL
<b>Temperature:</b>	Ambient
<b>Detection UV:</b>	325 nm
<b>Detection FLD:</b>	280/310 nm (emission/excitation)
<b>Sample:</b>	1. Vitamin A Palmitate isomer 2. Vitamin A Palmitate 3. Vitamin A Acetate isomer 4. Vitamin A Acetate 5. Vitamin E Acetate 6. Vitamin E

## Results and Discussion

Manufacturers of infant formula add Vitamin A either as Vitamin A Palmitate or Vitamin A Acetate. Both forms of Vitamin A are quantified by monitoring the 325 nm wavelength.

**Figure 1.** shows the chromatographic data for an infant formula extract containing Vitamin A Palmitate. Per the AOAC Official Method of Analysis (OMA) 2012.10, the total Vitamin A reported is the sum of the main Trans-Vitamin A Palmitate peak and all Cis-Vitamin A Palmitate isomers.

**Figure 2.** shows an extracted infant formula containing Vitamin A Acetate. Both the Trans-Vitamin A Acetate and the smaller Cis-Vitamin A Acetate isomer peak are combined to obtain the reported total Vitamin A Acetate value.

The total Vitamin E reported values are quantified from the same sample using FLD detection. **Figures 3** and **4.** show the corresponding FLD chromatograms. The sum of both forms of Vitamin E is reported as the total Vitamin E value.

An important aspect of infant formula analysis is the relative instability of the Vitamin A and Vitamin E compounds. It is important to analyze samples from a freshly opened container and to avoid

analyzing a previously frozen sample. Also, infant formula that is closer to its expiration date will most likely obtain lower Vitamin A and Vitamin E values due to degradation.

## Conclusion

Vitamin A and Vitamin E analysis following the AOAC protocol can be effectively analyzed using Luna 3  $\mu$ m NH<sub>2</sub>. The data obtained showed adequate retention and resolution as well as a rapid analysis time. The smaller, high-efficiency 3  $\mu$ m particle resulted in narrow peak-shape allowing for more accurate quantitation and lower detection limits.

## Luna Ordering Information

3 $\mu$ m MidBore™ and Analytical Columns (mm)									SecurityGuard™ Cartridges (mm)	
Phases	30 x 3.0	50 x 3.0	150 x 3.0	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	4 x 2.0*	4 x 3.0*
									/10pk	/10pk
Silica(2)	—	00B-4162-Y0	00F-4162-Y0	00A-4162-E0	00B-4162-E0	00C-4162-E0	00D-4162-E0	00F-4162-E0	AJO-4347	AJO-4348
C8(2)	00A-4248-Y0	00B-4248-Y0	00F-4248-Y0	00A-4248-E0	00B-4248-E0	00C-4248-E0	00D-4248-E0	00F-4248-E0	AJO-4289	AJO-4290
C18(2)	00A-4251-Y0	00B-4251-Y0	00F-4251-Y0	00A-4251-E0	00B-4251-E0	00C-4251-E0	00D-4251-E0	00F-4251-E0	AJO-4286	AJO-4287
CN	—	00B-4254-Y0	00F-4254-Y0	00A-4254-E0	00B-4254-E0	—	00D-4254-E0	00F-4254-E0	AJO-4304	AJO-4305
Phenyl-Hexyl	—	00B-4256-Y0	00F-4256-Y0	—	00B-4256-E0	00C-4256-E0	00D-4256-E0	00F-4256-E0	AJO-4350	AJO-4351
NH <sub>2</sub>	—	00B-4377-Y0	00F-4377-Y0	—	00B-4377-E0	—	00D-4377-E0	00F-4377-E0	AJO-4301	AJO-4302
HILIC	—	00B-4449-Y0	00F-4449-Y0	—	—	—	00D-4449-E0	00F-4449-E0	AJO-8328	AJO-8329
PFP(2)	—	00B-4447-Y0	00F-4447-Y0	—	00B-4447-E0	—	00D-4447-E0	00F-4447-E0	AJO-8326	AJO-8327

for ID: 2.0-3.0 mm    3.2-8.0 mm



If Luna analytical columns do not provide at least an equivalent separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.



# APPLICATIONS

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## Disclaimer

Dimensions and chromatographic conditions are the same for all columns unless otherwise noted. Comparative separations may not be representative of all applications.

SecurityGuard is patented by Phenomenex. U.S. Patent No. 6, 162, 362

**CAUTION:** this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP or ULTRA holders, or to any cartridges.

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