

# APPLICATIONS

## A Fast and Effective Quantitation Method for Vitamin A and E from Human Serum Using Novum™ SLE in Conjunction with a Kinetex® EVO C18 Column

Shahana Wahab Huq, Seyed Sadjadi, and Sean Orłowicz  
Phenomenex, Inc., 411 Madrid Avenue, Torrance, CA 90501, USA

Nutritional supplements, like vitamins and minerals, are a critical measure of a patient's overall health condition that has undergone a gastric bypass and other types of bariatric surgery. In order to quantify the nutritional intake of a patient, we have developed a simple and reliable method to extract vitamin A and E from human serum, using Novum Simplified Liquid Extraction (SLE). A Kinetex 5 µm, EVO C18, 100 x 2.1 mm HPLC column was utilized to obtain the best selectivity of the two vitamers of vitamin E, alpha and gamma tocopherol along with vitamin A, while a polarity switching technique in mass spectrometric ionization was employed.

### Introduction

Nutritional supplements, like vitamins and minerals, are highly recommended for patients that have undergone any type of bariatric surgery. Certain procedures put the patients at a much greater risk for deficiency, namely the malabsorptive procedures like the duodenal switch and gastric bypass. The dramatic alteration of a body's ability to absorb these important supplements and resulting deficiency may have serious medical implications and therefore should be monitored closely. In order to quantify the nutritional intake of a patient, we have developed a simple and reliable method to extract vitamin A and E from human serum, using Novum SLE in conjunction with Kinetex 5 µm, EVO C18, 100 x 2.1 mm HPLC column. A fast and effective LC/MS/MS method was developed to obtain the best selectivity of the vitamin A and E along with its two vitamers alpha and gamma Tocopherol. A cleaner background was observed for both alpha and gamma under negative ionization mode. By taking advantage of the polarity switching technique (from positive to negative ionization) after vitamin A elutes from the column, a much cleaner background, resolved in a higher signal for both vitamers of vitamin E under APCI mode.

### Experimental Conditions

#### Sample Preparation

- Sample Pre-treatment**  
Dilute 200 µL Human serum with 100 µL isopropanol (IPA) and 150 µL of water. Vortex 30 seconds.
- Load**  
Onto the **Novum MAX 96-Well plate** (P/N: 8E-S138-5GA).  
Apply a short pulse of vacuum for 10-15 seconds.
- Wait**  
5 minutes
- Elution**  
Dispense ethyl acetate/acetone (90:10), 2 x 900 µL (2 aliquots) to elute by gravity (~ 5 minutes) and collect the eluent. Apply vacuum at 5" of Hg for 20-30 secs to complete the extraction.
- Dry down**  
Evaporate the final extract to complete dryness under slow stream of nitrogen at 45 °C
- Reconstitute**  
Dried residue in 200 µL of initial mobile phase



**Sean Orłowicz**

**Manager,  
PhenoLogix**

When not in the lab, Sean enjoys just about anything involving the outdoors; hiking, climbing, surfing, etc. He is especially at home in the mountains, being an avid skier and motorcyclist.



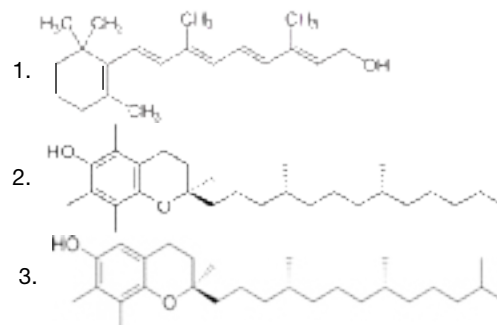
### HPLC

<b>Column:</b>	Kinetex 5 µm EVO C18	
<b>Dimensions:</b>	100 x 2.1 mm	
<b>Part No.:</b>	00D-4633-AN	
<b>Mobile Phase:</b>	A: Water B: Isopropanol/acetonitrile (1:1)	
<b>Gradient:</b>	<b>Time (min)</b>	<b>B (%)</b>
	0.0	65
	3.5	95
	4.0	95
	4.1	65
	5.05	65
<b>Flow Rate:</b>	0.6 mL/min	
<b>Injection Volume:</b>	5 µL	
<b>Temperature:</b>	Ambient	
<b>Instrument:</b>	Agilent® 1260	

### MS/MS Instrument

SCIEX Triple Quad™ 5000  
APCI (Dual polarity)

### Structures and LogP Values of Vitamin A and E



### Results and Discussion

The organo silica chemistry of Kinetex EVO C18 enhanced the interaction between the stationary phase and liposoluble vitamins, resulting in greater selectivity for the two vitamers α and γ tocopherol (**Fig. 1**).

The recovery (analyte recovery more than 92 % with CV between 2-9 %) of the three vitamins were best optimized under a neutral load condition (**Fig. 2**) followed by elution with a mixture of ethyl acetate and acetone in the ratio of 90 to 10 (**Fig. 3**).

Extraction of the human serum samples in the method development, required use of a larger bed mass of SLE Novum media. The extent of cleanliness was much improved switching from Novum MINI to MAX (**Fig. 4**)<sup>1</sup>.

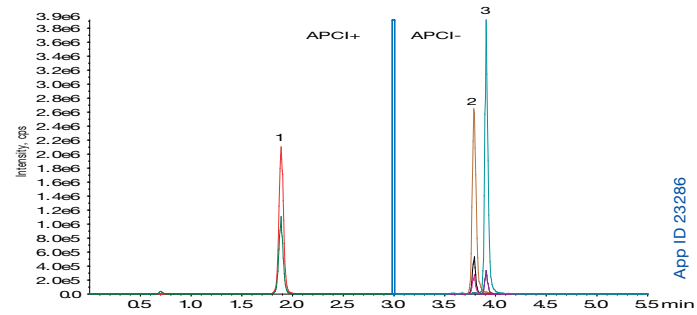
The extraction efficiency of Novum was improved by increasing the surface area (larger bed mass of Novum MAX). The partitioning of target analytes from aqueous into the organic extraction solvent is highly dependant on mass transfer rate. More the surface area, better the aqueous can interact with the extraction solvent and higher the analyte transfer rate.

Larger elution volume applicability of Novum™ MAX media, ensured effective partitioning of target analytes from aqueous into the organic.

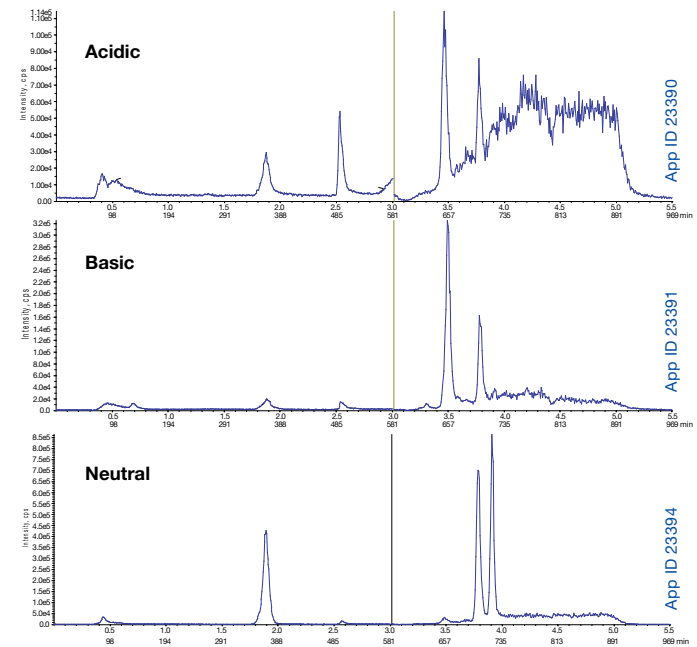
The linearity curve generated for vitamin A extraction from human serum, extended 1,000 fold dynamic range, covering the expected range (Fig 5). The linear regression value of 0.999 or greater reflects the robustness of the method.

Presence of endogenous level vitamin E ( $\alpha$  and  $\gamma$  tocopherol) observed in serum samples obtained from three different suppliers (Fig. 6). A food grade, albumin enriched, egg white was utilized to generate the linearity curve, that did not show any endogenous level (Fig. 6). The extracted curve for  $\alpha$  and  $\gamma$  tocopherol shows good linearity and correlation over 1,000 fold conc. range (Fig. 7 & 8).

**Figure 1.** LC/MS/MS Analysis of Vitamin A and E Using Dual Polarity Technique in MS



**Figure 2.** Optimize Load Condition (Novum MINI, P/N: 8E-S138-FGA)



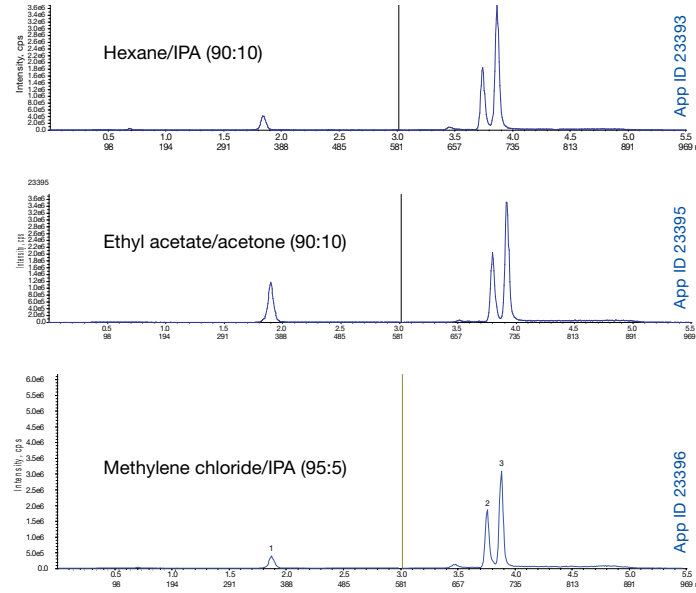
**Conclusion**

In this work we present a simple and yet efficient sample extraction method for the analysis of vitamin A and E, using supported liquid extraction product, Novum MAX. The robustness of the method along with its simplicity and ease of use, showcases Novum as a viable alternative to the time consuming liquid liquid extraction (LLE) for clinical sample preparation.

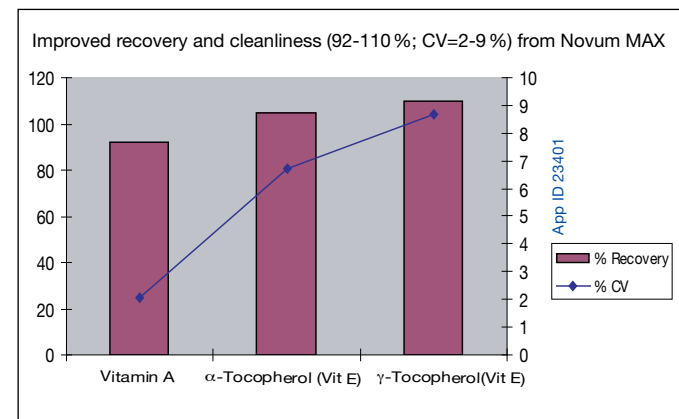
**References**

1. S Huq, S Sadjadi, and S Orlowicz, "A Fast and Effective Quantitation Method for Vitamin A and E from Human Serum Using Novum™ SLE in Conjunction with a Kinetex® EVO C18 Column." Mass Spec Application for Clinical Laboratory Conference, US, 2016

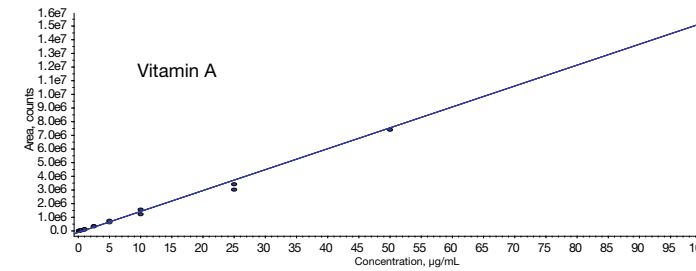
**Figure 3.** Optimize (Novum MINI) Elution Condition Under Neutral Load)



**Figure 4.** Recovery and Extent of Cleanliness Using the Optimized Large Load and Large Elution on Novum MAX

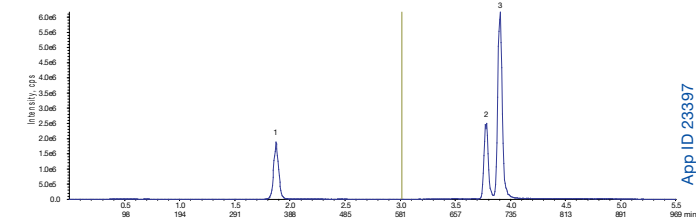


**Figure 5.** Linearity Curve of Vitamin A Extracted Samples on Novum MAX (Matrix: doubly-charcoal stripped serum)

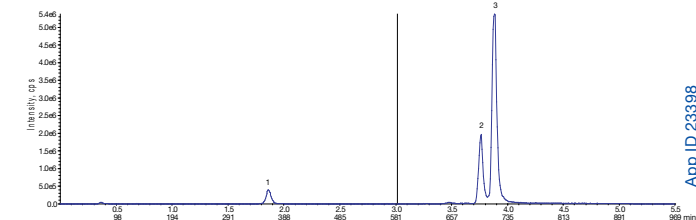


**Figure 6.** Presence of Endogenous Level of Vitamin E ( $\alpha$  and  $\gamma$ ) from Serum (Three Different Sources)

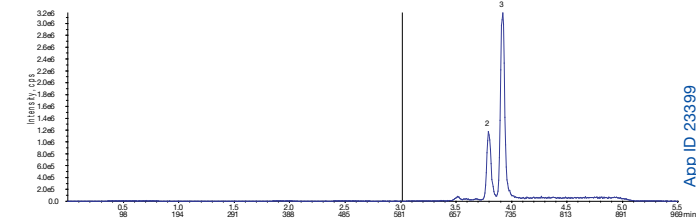
1. Vitamin A (retinol); 2.  $\gamma$ -Tocopherol (vitamin E); 3.  $\alpha$ -Tocopherol (vitamin E)



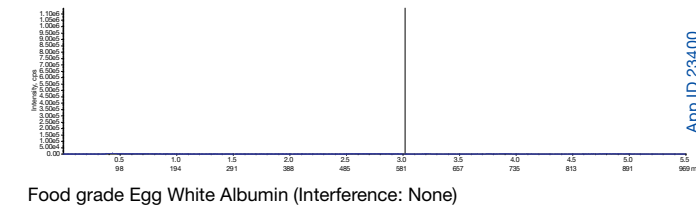
Unadulterated Serum (Interference: Vit. A,  $\alpha$  and  $\gamma$ )



Charcoal-Stripped Serum (Interference: Vit. A,  $\alpha$  and  $\gamma$ )

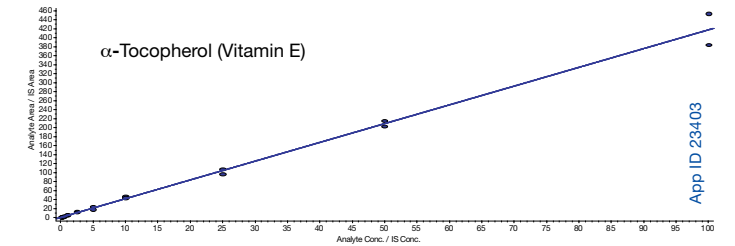


Doubly Charcoal-Stripped Serum (Interference:  $\alpha$  and  $\gamma$ )

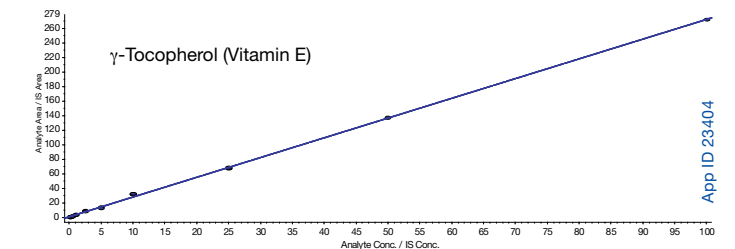


Food grade Egg White Albumin (Interference: None)

**Figure 7.** Linearity Curve of  $\alpha$ -Tocopherol Extracted Samples on Novum™ MAX (Matrix: Egg White Albumin)



**Figure 8.** Linearity Curve of  $\gamma$ -Tocopherol Extracted Samples on Novum MAX (Matrix: Egg White Albumin)



# APPLICATIONS

## Ordering Information

### Kinetex<sup>®</sup> EVO C18 Core-Shell LC Columns

5 µm Minibore Columns (mm)				SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>‡</sup>
30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
00A-4633-AN	00B-4633-AN	00D-4633-AN	00F-4633-AN	AJO-9298
for 2.1 mm ID				

5 µm MidBore <sup>™</sup> Columns (mm)			SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>‡</sup>
50 x 3.0	100 x 3.0	150 x 3.0	3/pk
00B-4633-YO	00D-4633-YO	00F-4633-YO	AJO-9297
for 3.0 mm ID			

5 µm Analytical Columns (mm)				SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>‡</sup>
50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJO-9296
for 4.6 mm ID				

**Australia**  
t: +61 (0)2-9428-6444  
f: +61 (0)2-9428-6445  
auiinfo@phenomenex.com

**Austria**  
t: +43 (0)1-319-1301  
f: +43 (0)1-319-1300  
anfrage@phenomenex.com

**Belgium**  
t: +32 (0)2 503 4015 (French)  
t: +32 (0)2 511 8666 (Dutch)  
f: +31 (0)30-2383749  
beinfo@phenomenex.com

**Canada**  
t: +1 (800) 543-3681  
f: +1 (310) 328-7768  
info@phenomenex.com

**China**  
t: +86 (0)20 2282-6668  
f: +86 (0)20 2809-8130  
chinainfo@phenomenex.com

**Denmark**  
t: +45 4824 8048  
f: +45 4810 6265  
nordicinfo@phenomenex.com

**Finland**  
t: +358 (0)9 4789 0063  
f: +45 4810 6265  
nordicinfo@phenomenex.com

**France**  
t: +33 (0)1 30 09 21 10  
f: +33 (0)1 30 09 21 11  
franceinfo@phenomenex.com

**Germany**  
t: +49 (0)6021-58830-0  
f: +49 (0)6021-58830-11  
anfrage@phenomenex.com

**India**  
t: +91 (0)40-3012 2400  
f: +91 (0)40-3012 2411  
indiainfo@phenomenex.com

**Ireland**  
t: +353 (0)1 247 5405  
f: +44 1625-501796  
eireinfo@phenomenex.com

**Italy**  
t: +39 051 6327511  
f: +39 051 6327555  
italiainfo@phenomenex.com

### www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

**Luxembourg**  
t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
nlinfo@phenomenex.com

**Mexico**  
t: 001-800-844-5226  
f: 001-310-328-7768  
tecnicomx@phenomenex.com

**The Netherlands**  
t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
nlinfo@phenomenex.com

**New Zealand**  
t: +64 (0)9-4780951  
f: +64 (0)9-4780952  
nzinfo@phenomenex.com

**Norway**  
t: +47 810 02 005  
f: +45 4810 6265  
nordicinfo@phenomenex.com

**Puerto Rico**  
t: +1 (800) 541-HPLC  
f: +1 (310) 328-7768  
info@phenomenex.com

**Spain**  
t: +34 91-413-8613  
f: +34 91-413-2290  
espinfo@phenomenex.com

**Sweden**  
t: +46 (0)8 611 6950  
f: +45 4810 6265  
nordicinfo@phenomenex.com

**United Kingdom**  
t: +44 (0)1625-501367  
f: +44 (0)1625-501796  
ukinfo@phenomenex.com

**USA**  
t: +1 (310) 212-0555  
f: +1 (310) 328-7768  
info@phenomenex.com

**All other countries**  
**Corporate Office USA**   
t: +1 (310) 212-0555  
f: +1 (310) 328-7768  
info@phenomenex.com

2.6 µm Minibore Columns (mm)				SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>‡</sup>
30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
00A-4725-AN	00B-4725-AN	00D-4725-AN	00F-4725-AN	AJO-9298
for 2.1 mm ID				

2.6 µm MidBore <sup>™</sup> Columns (mm)			SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>‡</sup>
50 x 3.0	100 x 3.0	150 x 3.0	3/pk
00B-4725-YO	00D-4725-YO	00F-4725-YO	AJO-9297
for 3.0 mm ID			

2.6 µm Analytical Columns (mm)				SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>‡</sup>
50 x 4.6	100 x 4.6	150 x 4.6	3/pk	
00B-4725-E0	00D-4725-E0	00F-4725-E0	AJO-9296	
for 4.6 mm ID				

1.7 µm Minibore Columns (mm)				SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>‡</sup>
50 x 2.1	100 x 2.1	150 x 2.1	3/pk	
00B-4726-AN	00D-4726-AN	00F-4726-AN	AJO-9298	
for 2.1 mm ID				

<sup>‡</sup> SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000

## Ordering Information

### Novum<sup>™</sup> Simplified Liquid Extraction (SLE)

Part No.	Description	Unit/Box
8E-S138-FGA	Novum SLE MINI 96-Well Plate	1/Box
8E-S138-5GA	Novum SLE MAX 96-Well Plate	1/Box

## Accessories

Collection Plates (deep well, polypropylene)		Unit
AHO-7192	96-Well Collection Plate, 350 µL/well	50/pk
AHO-7193	96-Well Collection Plate, 1 mL/well	50/pk
AHO-7194	96-Well Collection Plate, 2 mL/well	50/pk
AHO-8635	96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AHO-8636	96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AHO-7279	96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk
Sealing Mats		Unit
AHO-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AHO-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AHO-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AHO-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AHO-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AHO-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AHO-7362	Sealing Tape Pad	10/pk
Vacuum Manifold		Unit
AHO-8950	96-Well Plate Manifold, Universal with Vacuum Gauge	ea



If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

### Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions which may be viewed at [www.phenomenex.com/TermsAndConditions](http://www.phenomenex.com/TermsAndConditions).

### Trademarks

Kinetex is a registered trademark, Novum, SecurityGuard, and MidBore are trademarks of Phenomenex. Agilent is a registered trademark of Agilent Technologies, Inc. Triple Quad is a trademark of AB Sciex Pte. Ltd. AB Sciex<sup>™</sup> is being used under license.

Kinetex EVO is patented by Phenomenex. U.S. Patent Nos. 7,563,367 and 8,658,038 and foreign counterparts.

© 2016 Phenomenex, Inc. All rights reserved.