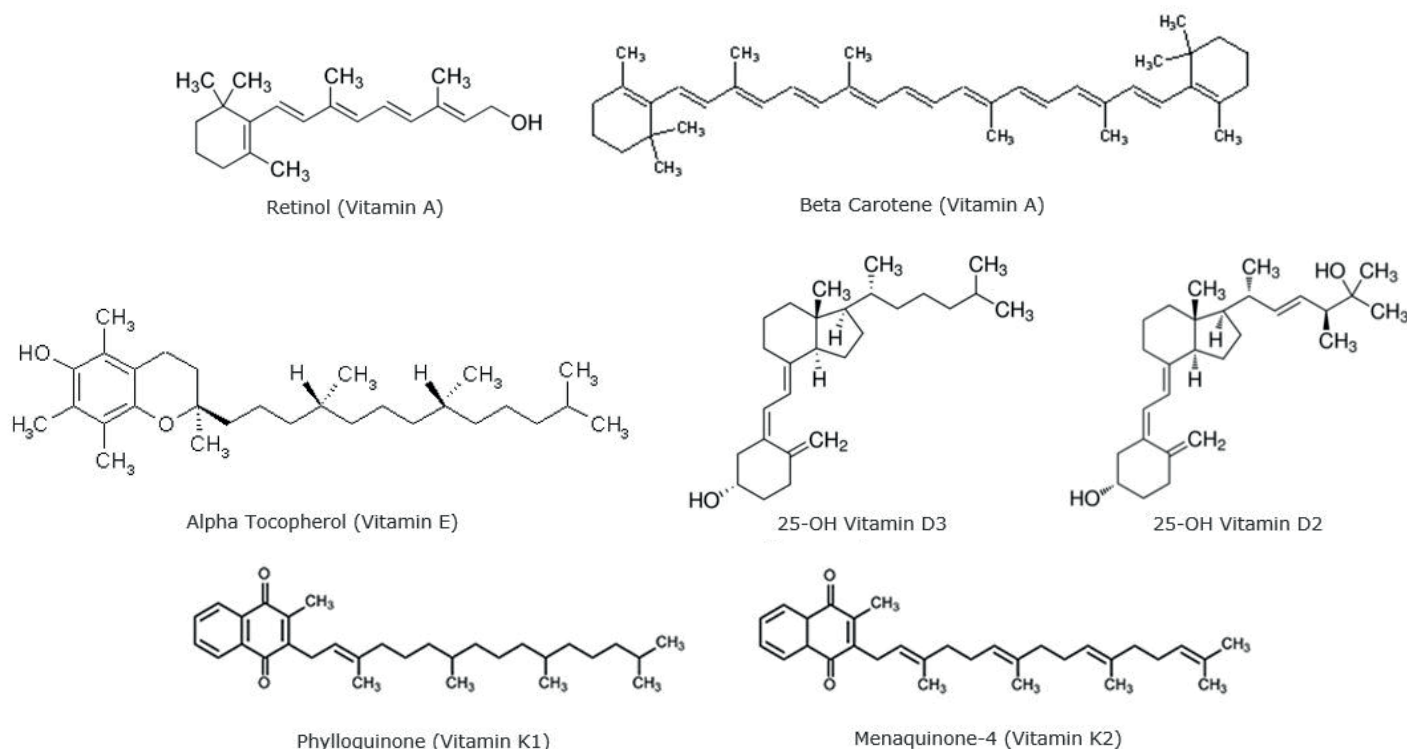


# Extraction of Fat-Soluble Vitamins from Human Serum Using ISOLUTE® SLE+ Prior to UHPLC/MS-MS Analysis



**Figure 1.** Structures of Retinol and Beta Carotene (Vitamin A), 25-OH-Vitamins D2 and D3 (Vitamin D), Alpha Tocopherol (Vitamin E) and Phylloquinone and Menaquinone-4 (Vitamin K).

This application note describes the extraction of a panel of fat-soluble vitamins (including those representing Vitamins A, D, E & K) from human serum using ISOLUTE® SLE+ Supported Liquid Extraction plates prior to LC/MS analysis.

The simple sample preparation procedure delivers clean extracts and analyte recoveries approximately or above 90% with RSDs lower than 10% for all analytes.

## Analytes

Retinol, Beta Carotene, 25-OH Vitamin D2, 25-OH Vitamin D3, Alpha Tocopherol, Phylloquinone, Menaquinone-4.

## Internal Standards

D<sub>6</sub> 25-OH Vitamin D<sub>3</sub> was used as an internal standard for 25-OH Vitamin D<sub>2</sub>, 25 OH Vitamin D<sub>3</sub> and Retinol. It is recommended that an additional internal standard is used for Vitamin K.

**Table 1.** Concentration ranges for fat-soluble vitamins.

Analyte name and Vitamin reference	Lower limit of Quantification (ng/mL)	Upper limit of Quantification (ng/mL)
Alpha Tocopherol (Vitamin E)	800	40000
Retinol and Beta Carotene (Vitamin A)	80	4000
25-OH Vitamins D2 & D3 (Vitamin D)	4	200
Phylloquinone and Menaquinone-4 (Vitamin K)	0.4	20

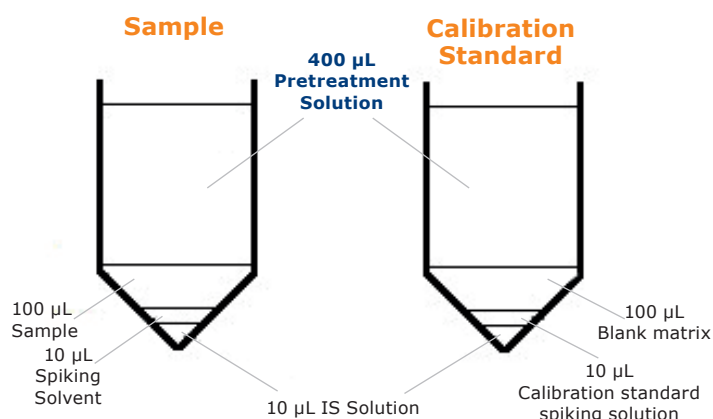
## Sample Preparation Procedure

### Format

ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-P01.

### Sample Pre-treatment

Internal standard solution (10 µL) and 10 µL of either spiking solvent or calibration standard spiking solution (see 'Chemicals and Reagents' section for preparation details) were transferred to a 2 mL collection plate. 100 µL of sample or blank matrix was added, capped, briefly mixed and then left to stand in the dark for 1 hour to equilibrate. The sample was then combined with 400 µL of pre-treatment solvent, briefly mixed and then left to stand for a further 5 minutes.



**Figure 2.** Demonstrating the different preparation procedure for samples and Calibration Standards.

### Sample Loading

Approximately 500 µL pre-treated serum (or as much of the sample as possible) was transferred to an ISOLUTE® SLE+ plate. The sample was vigorously drawn up and down into the pipette tip with additional air (by setting a larger volume than the sample e.g. 650 µL) a number of times immediately prior to transfer to create a temporary suspension. If necessary, a low positive pressure was used to push the sample into the SLE material. The ISOLUTE SLE+ plate was then left to equilibrate for 5 minutes.

### Elution

Analytes were eluted with 2 x 500 µL heptane.

### Post Elution and Reconstitution

The extract was dried in a stream of air or nitrogen using a Biotage® SPE Dry 96 at room temperature, 20 to 40 L/min.

Evaporated samples were reconstituted with propan-2-ol (IPA, 150 µL) and mixed thoroughly.

## UPLC Conditions

### Instrument

Waters Acquity UPLC

### Column

Restek Raptor Biphenyl (100 mm x 2.1 mm, 2.7 µm) with a Restek EXP holder and guard.

### Mobile Phase

A: 5 mM ammonium acetate 0.1% formic acid (v/v) in water

B: Methanol : Propan-2-ol (3:1 v/v) containing 5 mM ammonium acetate and 0.1% formic acid

### Flow Rate

0.4 mL/min

### Column Temperature

40 °C

### Autosampler Temperature

10 °C

### Injection Volume

10 µL (Partial Loop with Needle Overfill)

**Table 2.** UHPLC Gradient.

Time (min)	%A	%B
0	60	40
3	0	100
5.1	0	100
5.2	60	40
7	60	40



Biotage® SPE Dry Sample Concentrator System.

## MS Conditions

### Instrument

Waters Quattro Premier XE

### Desolvation Gas Flow

1200 L/hr

### Cone Gas Flow

50 L/r

### Source Temp

150 °C

### Desolvation Temp

450 °C

### Capillary Voltage

4 kV

### Extractor Voltage

3 V

## Results

Extraction recoveries were first measured using a manual processing method (using a Biotage® PRESSURE+ 96 manifold). The method was then transferred to a Biotage® Extrahera™ for automated processing. The Extrahera™ recoveries were slightly lower in line with the slower mixing of this compared to the manual method. Extraction recoveries (manual and automated methods), and associated RSDs are shown in table 4.



Biotage® PRESSURE+ 96 Positive Pressure Manifold.

**Table 3.** MS conditions and retention times for target analytes.

Analytes	MRM Transition	Collision Energy, V	Cone, V	Period
Alpha Tocopherol (E)	433.3 > 165.9	22	25	2
Retinol (A)	269.3 > 92.9	20	18	1
Beta Carotene (A)	536.3 > 444.4	15	30	2
25-OH Vitamin D2	395.5 > 269.5	30	30	1
25-OH Vitamin D3	383.5 > 257.5	17	30	1
Phylloquinone (K1)	445.3 > 186.9	20	22	2
Menaquinone-4 (K2)	451.4 > 187.0	23	30	2
D <sub>6</sub> 25-OH Vitamin D3 (IS)	389.6 > 263.5	16	30	1

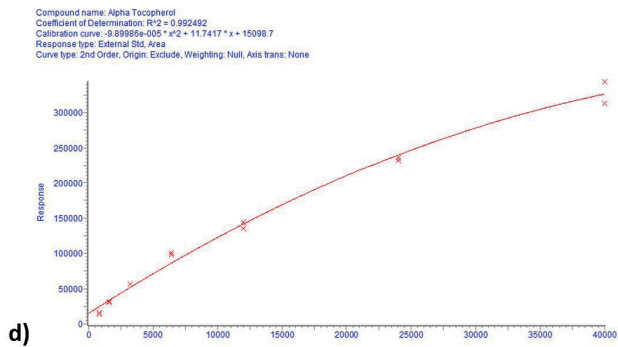
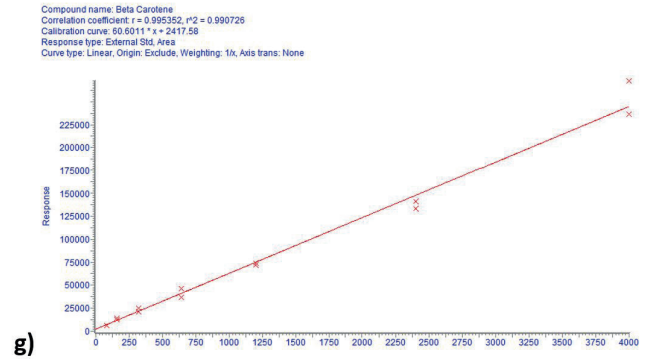
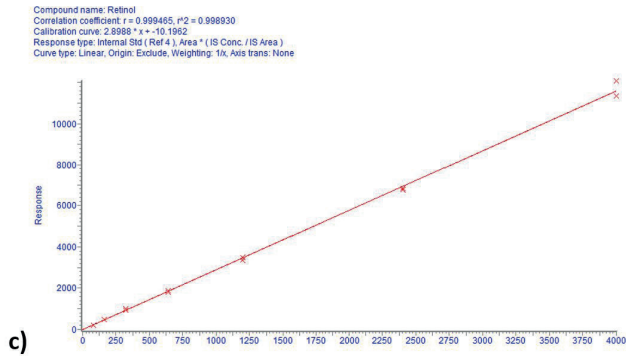
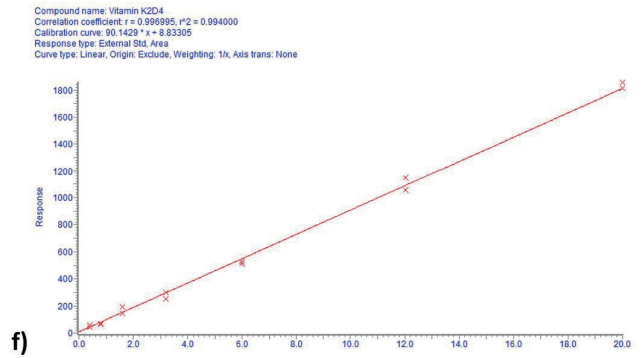
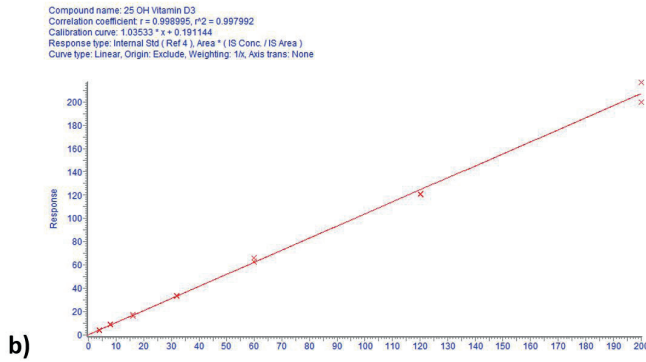
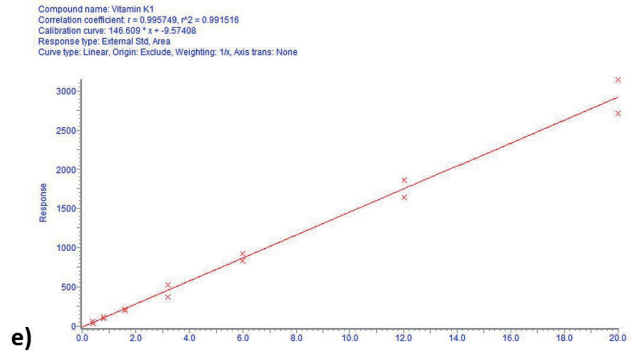
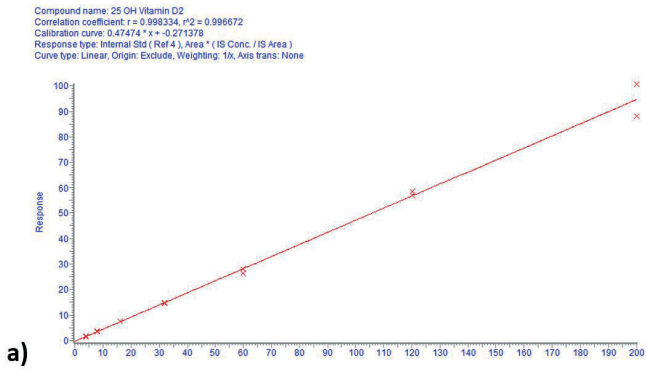
All analytes were measured in positive mode using Electrospray ionization.

**Note:** The transition for Alpha Tocopherol was significantly different from the optimum settings. Due to the high MS sensitivity of the analyte and the high concentrations expected this was intentionally de-tuned on the instrument.

**Table 4.** Analyte calibration curve  $r^2$  and LOQ performance.

Analytes	Manual		Biotage® Extrahera™	
	Recovery	% RSD	Recovery	% RSD
Retinol (A)	94.9	3.3	75.9	5.3
Beta Carotene (A)	89.0	4.1	71.3	10.5
25 OH Vitamin D2 (D)	101.0	5.1	79.2	5.4
25 H Vitamin D3 (D)	95.6	4.6	81.9	5.5
Alpha Tocopherol (E)	99.1	4.6	84.0	4.6
Phylloquinone (K)	95.7	10.5	71.0	6.3
Menaquinone-4 (K)	95.7	9.6	73.4	4.5

D<sub>6</sub> 25-OH Vitamin D3 was used as an internal standard for 25-OH Vitamin D2, 25-OH Vitamin D3 and Retinol.

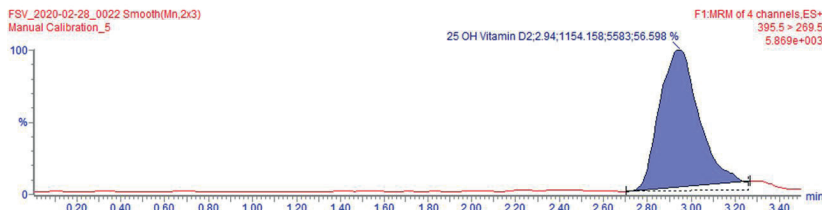


**Figure 3.** Calibration curves for 25-OH Vitamin D2 (a), 25-OH Vitamin D3 (b), Retinol (c), Alpha Tocopherol (d), Vitamin K1 (Phylloquinone) (e), Vitamin K2d4 (Menaquinone) (f) and Beta Carotene (g).

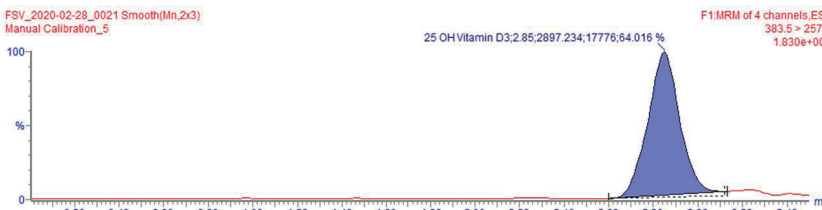
Analyte

Plasma Extract (Std 5)

25-OH Vitamin D2



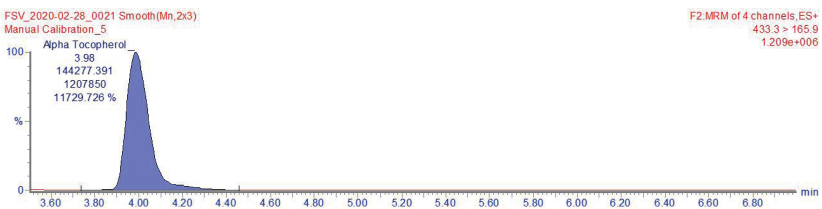
25-OH Vitamin D3



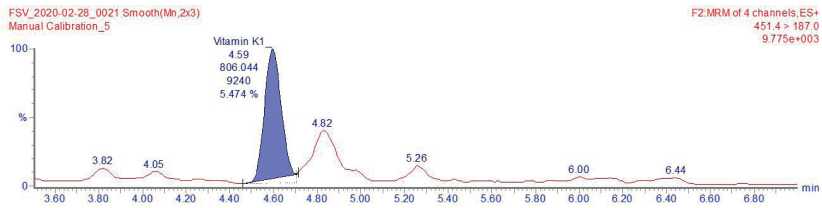
Retinol



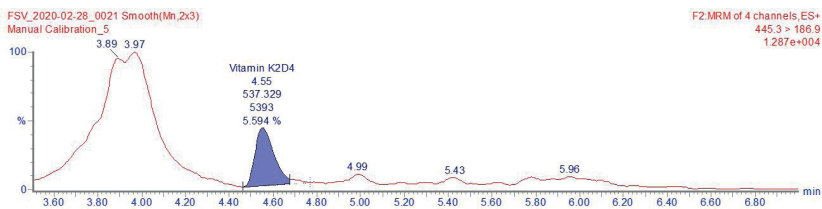
Alpha Tocopherol



Vitamin K1



Vitamin K2



Beta Carotene

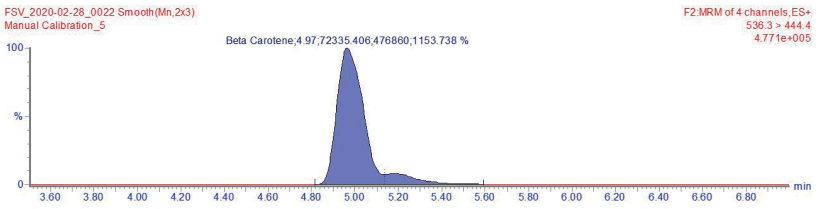


Figure 4. Representative chromatography for stripped serum spiked at a mid-calibration range (6 ng/mL Vitamin K, 60 ng/mL Vitamin D, 1.2 µg/mL Vitamin A and 12 µg/mL Vitamin E.



## Discussion and Conclusion

This method provides high, reproducible recoveries of a range of fat-soluble vitamins in human serum, in clinically appropriate concentration ranges.

Due to the extremely non-polar (hydrophobic) nature of the analytes, and the wide difference in biological concentration range, some non-standard modifications to the standard supported liquid extraction process were adopted in this application. See the 'Additional Method Notes' section for a comprehensive description of the steps taken to ensure successful extraction of these analytes.

## Chemicals and Reagents

### Stock and Sub Stock Solvent

Where solids were provided these were diluted in a stock solution solvent of MTBE + 1 mg/mL BHT.

Stock solutions were prepared at 1 mg/mL (Retinol, Phylloquinone, Menaquinone). Due to the challenges of precisely weighing an oil Alpha Tocopherol was prepared in stock solution solvent at known concentrations between 1 and 5 mg/mL. Due to limited solubility beta carotene was prepared in stock solution solvent at a concentration of 100 µg/mL. 25-OH Vitamin D<sub>2</sub> and D<sub>3</sub> were purchased as solutions. All stock solutions were stored protected from light at approximately -20 °C.

### Spiking solvent

Spiking solvent was prepared by combining BHT in propan-2-ol (IPA) at a level of 0.1% w/v or 1 mg/mL. As an example: 100 mL of propan-2-ol would be added 100 mg of BHT.

### Internal standard solution

Internal standard (D<sup>6</sup> 25-OH Vitamin D<sub>3</sub>) was diluted in spiking solvent to a concentration of 1 µg/mL. A 10 µL aliquot of this is equivalent to 100 µL of sample containing internal standard at a level of 100 ng/mL.



## Combined Fat-Soluble Vitamin Spiking Solution

A spiking solvent was prepared by combining fat-soluble vitamin solutions and diluting with spiking solvent such that the following concentrations were met: Vitamin K<sub>1</sub> and K<sub>2</sub>D<sub>4</sub> = 200 ng/mL, 25-OH Vitamin D<sub>2</sub> and D<sub>3</sub> = 2 µg/mL, Retinol and Beta Carotene = 40 µg/mL and Alpha Tocopherol = 400 µg/mL. It is recommended that at least 0.5 mL of this solution is prepared on a daily basis. The "Additional Information" section contains an example spiking procedure to reach the required concentrations. It is recommended that this solution is prepared daily.

## Calibration standards

Calibration standard spiking solutions were prepared from the combined fat-soluble vitamin spiking solution. The "Additional Information" section contains an example spiking procedure to reach the required concentrations. It is recommended that this solution is prepared daily.

## Pretreatment solvent

BHT was combined with a solution of IPA/heptane (1:3, v/v) at a level of 1 mg/mL.

## Elution solvent

Heptane was used as the SLE elution solvent. Hexane is an acceptable alternative, but extract cleanliness may be slightly compromised (slightly higher levels of co-extracted phospholipids may be observed).

## Reconstitution Solvent

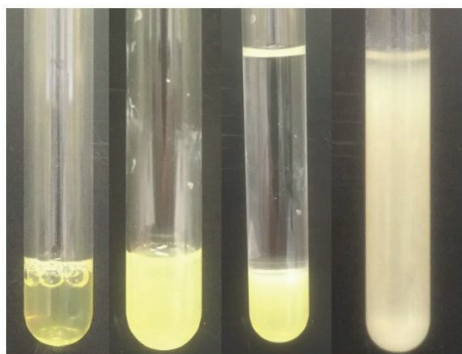
Propan-2-ol was used as the reconstitution solvent.

## Other Chemicals and Reagents

- » Method development was performed using vitamin stripped serum was purchased from Golden West.
- » Methanol (LC-MS grade), propan-2-ol (isopropanol) (99.9%), MTBE (99%) ethyl acetate and formic acid (98%) were purchased from Honeywell Research Chemicals (Bucharest, Romania).
- » All analyte standards and deuterated internal standards were purchased from Sigma- Aldrich Company Ltd. (Gillingham, UK).
- » Water used was 18.2 MOhm-cm, drawn from a Direct-Q5 water purifier.
- » Mobile phase A was prepared by accurately weighing approximately 385.4 mg of ammonium acetate. The ammonium acetate (385.4 mg assumed) was then combined with 1 L of water and 1 mL of formic acid. The solution was replaced after 48 hours.
- » Mobile phase B was prepared by accurately weighing approximately 385.4 mg of ammonium acetate. The ammonium acetate (385.4 mg assumed) was then combined with 750 mL of methanol, 250 mL of propan-2-ol and 1 mL of formic acid. The solution was replaced after 48 hours.
- » Mobile Phase B was partially prepared with propan-2-ol to give improved chromatographic retention times of the later eluting analytes, particularly Beta-Carotene.
- » Due to analyte instability the preservative BHT is included in all stock and pre-treatment solvents. BHT is also known as 2,6 Di-tert-butyl-4-methylphenol (Sigma Aldrich (B1378)).

## Additional Method Notes

- » In vivo most fat-soluble vitamins are highly protein bound so the serum samples must be treated to significantly reduce these interactions prior to any extraction.
- » Any sample incubations were performed in the dark. If using the Biotage® Extrahera™ the internal lights were switched off.
- » By adding heptane as a pre-treatment solvent, the solubility of protein freed fat-soluble vitamins in the pre-treated sample was increased. Rapidly aspirating and dispensing the sample in the tip prior to analysis formed a temporary milky colored suspension. Suspension formation was aided by the presence of propan-2-ol which is separately soluble in both serum and heptane.



**Figure 5.** Images representing sample consistency of sample alone, during sample incubation, after addition of pretreatment solvent and immediately after mixing.

- » A volume of 500  $\mu\text{L}$  could be loaded on to the ISOLUTE® SLE 400 because the sample only contained approximately 200  $\mu\text{L}$  of aqueous solvent.
- » Due to the consistency of the pre-treated serum it may not flow into the SLE bed under gravity and may require application of positive pressure or vacuum for loading.
- » Increased vitamin losses were seen when the extracted samples were evaporated at 40 °C and so this step is performed at room temperature.
- » Propan-2-ol was used as the reconstitution solvent in order to keep the beta carotene in solution over the period of the LC analysis.
- » The method detailed here involved the use of one internal standard that was used for the measurement of 25-OH Vitamin D<sub>2</sub>, 25-OH Vitamin D<sub>3</sub> and Retinol. Due to the suppression levels seen with Vitamin K measurement, the use of an isotope or structurally similar compound as an additional internal standard to Vitamin K<sub>1</sub> and K<sub>2</sub>D<sub>4</sub> is strongly recommended.
- » The calibration line for Alpha Tocopherol will show an element of non-linearity due to the high levels of analyte being injected. A similar non-linear plot may also be present for Beta Carotene.
- » The preparation method of quality control samples should be carefully considered due to the possible binding of some fat-soluble vitamins to polypropylene in a highly aqueous environment. Although this isn't an issue for the analytical procedure described here, as the analytes would come back into solution once heptane had been mixed with it, it could be an issue for externally prepared QCs.
- » All calibration ranges were set with the aim of quantifying the majority of normal samples. With the Vitamin K analytes these ranges could not be confirmed and so the lowest range that could be confidently measured was used. Due to the small Vitamin K peaks generated it is recommended that if the experiment is performed in an area where manual integration is not permitted, the extracts are analyzed on a more sensitive MS than the one used in this experiment.
- » Vitamin K<sub>1</sub> and K<sub>2</sub>D<sub>4</sub> were the only compounds that showed a significant level of suppression. This was shown to decrease significantly when less serum was extracted. If running the method on a more sensitive LC-MS than is documented here it is recommended that a reduction of sample volume from 100 to 50 or 20  $\mu\text{L}$  is considered. Note if this is performed the concentration of analytes in sub stock 2 will need to be reduced accordingly.
- » To optimize MS performance the analyte detection was separated into two periods. Vitamin Ds (D<sub>3</sub>, D<sub>2</sub> and D<sub>6</sub>–D<sub>3</sub> (IS)) and Retinol were measured in period 1 from 0 to 3.5 minutes. All other components were measured between 3.5 and 6 minutes.
- » For increased sensitivity:
  - » Decrease reconstitution solvent volume below 150  $\mu\text{L}$
  - » Consider using an LC-MS instrument with greater sensitivity
  - » Due to the variability of stock solution concentrations and expected levels of each component in serum, the preparation of appropriate calibration range standards can be challenging. Below is an example of the preparation of calibration standards in a quantity appropriate for up to n=4 analysis which can be used as a guide: Stock solution concentrations assumed.
    - » Retinol = 1 mg/mL
    - » Beta carotene = 100  $\mu\text{g/mL}$
    - » 25-OH Vitamin D<sub>2</sub> = 50  $\mu\text{g/mL}$
    - » 25-OH Vitamin D<sub>3</sub> = 100  $\mu\text{g/mL}$
    - » Alpha Tocopherol = 2 mg/mL
    - » Phylloquinone = 1 mg/mL
    - » Menaquinone-4 = 1 mg/mL
  - » Substock 1. Combine 10  $\mu\text{L}$  of Phylloquinone (K<sub>1</sub>) with 10  $\mu\text{L}$  Menaquinone-4 (K<sub>2</sub>D<sub>4</sub>) and then dilute this mixture to a total volume of 1 mL with the addition of stock and sub stock solvent (MTBE + 0.1% w/v BHT). This solution contains Phylloquinone and Menaquinone at concentrations of 10  $\mu\text{g/mL}$  each.
  - » Substock 2. Combine 10  $\mu\text{L}$  of substock 1, 10  $\mu\text{L}$  of 25-OH Vitamin D<sub>3</sub>, 20  $\mu\text{L}$  of retinol, 20  $\mu\text{L}$  of 25-OH Vitamin D<sub>2</sub>, 100  $\mu\text{L}$  of Alpha Tocopherol and 200  $\mu\text{L}$  of Beta Carotene. Dilute this mixture to a total volume of 0.5 mL with precipitation solvent (IPA + 0.1% BHT).
  - » This solution contains Alpha Tocopherol at 400  $\mu\text{g/mL}$ , Retinol and Beta Carotene at 40  $\mu\text{g/mL}$ , 25-OH Vitamin D<sub>2</sub> and D<sub>3</sub> at 2  $\mu\text{g/mL}$  and Vitamin K<sub>1</sub> and K<sub>2</sub> at 0.2  $\mu\text{g/mL}$ .

Calibration standards were prepared by combining substock 2 with spiking solvent (see Table 5).

**Table 5.** Spiking regimen of calibration standard spiking solutions.

Standard ID	Volume of Substock 2 (µL)	Volume of Spiking Solvent (µL)
1	10	490
2	10	240
3	20	230
4	40	210
5	30	70
6	60	40
7	100	0

## Ordering Information

Part Number	Description	Quantity
<b>820-0400-P01</b>	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Plate	1
<b>PPM-96</b>	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1
<b>SD-9600-DHS</b>	Biotage® SPE Dry Sample Concentrator system	1
<b>121-5203</b>	Collection Plate, 2 mL Square	50
<b>121-5204</b>	Pierceable Sealing Mat	50



# Appendix

## Biotage® Extrahera™ Settings

The method described in this application note was automated on the Biotage® Extrahera™ using ISOLUTE® SLE+ 400 µL capacity 96-well plates. This appendix contains the software settings required to configure Extrahera™ to run this method. Screenshots may or may not match those here depending upon the instrument software version.

<b>Sample Name:</b>	Fat Soluble Vitamins in Serum
<b>Sample Plate/Rack:</b>	2 mL 96 well FSV
<b>Extraction Media:</b>	ISOLUTE® SLE+ 400 µL 96



### Settings

#### “Sample” Tab

<b>Sample Type:</b>	FSV Sample Mix
<b>Starting Sample Volume (µL):</b>	300
<b>Method Comment:</b>	

Prior to running on the Extrahera, combine 100 µL serum, 10 µL IS solution and 10 µL Propan-2-ol or spiking solution then leave for 1hr to equilibrate. A larger than true starting sample volume has been set to maximise sample mixing prior to loading.

Method name: Fat Soluble Vitamins in Serum | Sample plate/rack: 2 mL 96 well FSV | Extraction media: ISOLUTE SLE+ 400 µL 96...

Pretreatment  
 Load  
 Elution

**Sample** | Pretreatment | Load | Elution (2)

Sample type: FSV Sample Mix  
 Starting sample volume in plate/rack (µL): 300  
 Reuse tips for pretreatment mix and sample load?: Yes  
 Sample pipette tip type: 1000 µL Biotage tip

Method comment: Prior to running on the Extrahera combine 100 µL serum, 10 µL IS solution and 10 µL Propan-2-ol or spiking solution. A larger than true starting sample volume has been set to maximise sample mixing prior to loading.

Method name: Fat Soluble Vitamins in Serum | Sample plate/rack: 2 mL Sample Plate, 96 | Extraction media: ISOLUTE SLE+ 400 µL 96...

Pretreatment  
 Load  
 Elution

**Pretreatment** | Sample | Load | Elution (2)

Number of steps: 1  
 Solvent: Heptane : IPA (3:1)  
 Volume (µL): 400  
 Mix number of times: 5 | Mix volume (µL): 700  
 Wait time (min): 5

Pause after last step?: No  
 Dispose solvent tips after each step?: No

Pretreatment	
<b>Steps</b>	2
<b>Solvent</b>	Heptane : IPA (3:1)
<b>Volume</b>	400
<b>Mix</b>	5
<b>Volume</b>	700
<b>Wait</b>	5
<b>Pause</b>	No
<b>Dispose tips</b>	No

Method name: Fat Soluble Vitamins in Serum | Sample plate/rack: 2 mL Sample Plate, 96 | Extraction media: ISOLUTE SLE+ 400 µL 9...

Sample | Pretreatment | Load | Elution (2)

Pretreatment:  On

Load:  On

Elution:  On

Sample volume (µL): 700

Air push time (s): 0 | Advanced pressure settings: Edit...

Premix?:  Yes | Number of times: 5

Wait time (min): 5

Pause after each load?:  No | Collect in position: D (Wa...)

**Load**

Volume	700
Premix	Yes
No of times	5
Wait time	5
Pause	No
Collect in	D
Pressure	Advanced
Steps	2
0 bar	120
2 bar	30

Method name: Fat Soluble Vitamins in Serum | Sample plate/rack: 2 mL Sample Plate, 96 | Extraction media: ISOLUTE SLE+ 400 µL 9...

Sample | Pretreatment | Load | Elution (2)

Pretreatment:  On

Load:  On

Elution:  On

Number of steps: 2

Air push after last elution?:  No | Air push time (s): 0

Dispose solvent tips after each step?:  No

Solvent 1: Heptane | Volume (µL): 500 | Collect in position: A

Solvent 2: Heptane | Volume (µL): 500 | Collect in position: A

Wait time (min): 0 | Advanced pressure settings: Edit...

Repeat (number of times): 1 | Pause after this step?:  No

**Elution****Activated**

Number of steps	2
Air push	No
Dispose tips	No
Air Push	No

**Solvent 1 - Heptane**

Volume	500
Collect position	A
Advanced settings	Yes
Number of Adv. steps	2
Pressure 1	0
Time 1	180
Pressure 2	2
Time 2	30
Air push	No
Repeat	1
Pause	No

**Solvent 2 - Heptane**

Volume	500
Collect position	A
Advanced settings	Yes
Number of Adv. steps	3
Pressure 1	0.5
Time 1	180
Pressure 2	2
Time 2	60
Pressure 3	5
Time 3	10
Air push	No
Repeat	1
Pause	No

Dispose tips

No

**Elution 1 Advanced settings**

Use advanced pressure settings?:  Yes | Number of steps: 2

1. Pressure (bar): 0.0 | Positive pressure time (s): 180

2. Pressure (bar): 2.0 | Positive pressure time (s): 30

Air Push?:  No | Air push time (s): 0

**Elution 2 Advanced settings**

Use advanced pressure settings?:  Yes | Number of steps: 3

1. Pressure (bar): 0.5 | Positive pressure time (s): 180

2. Pressure (bar): 2.0 | Positive pressure time (s): 60

3. Pressure (bar): 5.0 | Positive pressure time (s): 10

Air Push?:  No | Air push time (s): 0

## Solvent Properties

Solvent Description	
1	Heptane : Propan-2-ol
2	Heptane
3	
4	
5	
6	
7	
8	
9	
10	



Solvent	1	2	3	4	5	6	7	8	9	10
<b>Reservoir Type</b>	<b>Refillable</b>				<b>Non Refillable</b>					
Capacity										
Aspiration flow rate	10	10								
Dispense flow rate	10	10								
Aspiration post dispense?	No	No								
Lower air gap flow rate	10	10								
Lower air gap volume	5	5								
Upper air gap flow rate	120	20								
Upper air gap volume	100	100								
Upper air gap dispense pause	200	200								
Conditioning?	Yes	Yes								
Frequency	1 <sup>st</sup> Asp. only									
Cond. Times	4	4								
Cond. Flow rate	20	20								
Cond. volume	100	100								
Chlorinated	No	No								
Serial dispense	No	No								
Highly Volatile	No	No								

Sample	Air Gap
Sample name <b>FSV Sample Mix</b>	Lower air gap flow rate (mL/min) <b>20</b>
Sample description <b>Fast mix, zero pause</b>	Lower air gap volume (µL) <b>5</b>
Aspiration flow rate (mL/min) <b>100</b>	Upper air gap flow rate (mL/min) <b>100</b>
Dispense flow rate (mL/min) <b>220</b>	Upper air gap volume (µL) <b>700</b>
Aspirate post dispense? <b>Yes</b> <input checked="" type="checkbox"/>	Upper air gap dispense pause (ms) <b>0</b>
Aspirate post dispense flow rate (mL/min) <b>20</b>	
Aspirate post dispense volume (µL) <b>100</b>	

### "Sample" Screen

Sample name	Aqueous sample
Sample description	Fast mix, zero pause
Aspiration flow rate	100
Dispense flow rate	220
Aspirate post dispense?	Yes
Aspirate post dispense flow rate	20
Aspirate post dispense volume	100
Lower air gap flow rate	20
Lower air gap volume	5
Upper air gap flow rate	100
Upper air gap volume	700
Upper air gap dispense pause	0

### Extraction Media

Name

Manufacturer

Part number

Capacity volume (µL)

Format

Comment

### Pipetting Height

Solvent dispensation height (mm)

Sample dispensation height (mm)

Aspiration height (mm)

### "Extraction Media" Screen

Name	ISOLUTE® SLE+ 400 µL 96 Well Plate
Manufacturer	Biotage
Part number	820-0400-P01
Capacity volume	0
Format	96
Comment	
Solvent dispensation height	-125
Sample dispensation height	-126.5
Aspiration height	-139.5

### Sample Plate/Rack

Name

Capacity volume (µL)

Plate/rack height (mm)

Format

### Pipetting Height

Aspiration height (mm)

Pretreatment dispensation height (mm)

### "Sample Plate/Rack" Screen

Name	2 mL 96 well FSV
Capacity volume	1800
Format	96
Aspiration height	-161.5
Pretreatment dispensation height	-128

< Cancel		Edit Pipette Tip - 1000 µL Biotage tip		Save >	
<b>Pipette Tip</b>					
Name					
1000 µL Biotage tip					
Manufacturer					
Biotage					
Part number					
414141					
Capacity (µL)					
1000					
Length (mm)					
95					

**"Pipette tip" Screen**

Name	1000 µL Biotage Tip
Manufacturer	Biotage
Part number	414141
Capacity (µL)	1000
Length (mm)	95

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