

## Vitamins Profile

### Service Code: Vitamins

**Summary:** Extraction of fat- and water-soluble vitamins in plasma by LLE. After extraction, supernatants are dried and reconstituted, separated by RPLC and analyzed by ESI<sup>-</sup> by MRM methods. Values are reported as uM, while CV's are generally 10%.

Container:

Normal Volume: 100 uL blood serum or plasma

Minimal Volume:

**Special Handling:**

Sample Collection:

Reference:

Simultaneous quantification of water-soluble and fat-soluble vitamins in parenteral nutrition admixtures by HPLC-UV-MS/MS. Raphaël Vazquez, PharmD; My-Dung Le Hoang, PhD; Jean Martin, PharmD; Yasmine Ait Yahia, PharmD; Hervé Graffard, BSc; François Guyon, PharmD, PhD; Bernard Do, PharmD, PhD, EJHP Science, 15,2009, 28-35

**Table I: Analytes reported. Others such as glucosylceramides on special request:**

Analyte	Abbr.	Formula	MRM	LOQ(nM)
<b>trans-Retinal</b>	<b>Vit A</b>	<b>C<sub>20</sub>H<sub>28</sub>O</b>	<b>269→93</b>	<b>50</b>
Thiamine	Vit B1	C <sub>12</sub> H <sub>17</sub> CIN <sub>4</sub> OS	265->122	10
Riboflavin	Vit B2	C <sub>17</sub> H <sub>20</sub> N <sub>4</sub> O <sub>6</sub>	377.1->243.1	50
<b>Nicotinamide</b>	<b>Vit B3</b>	C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O	123->80	10
D-Pantothenate	Vit B5	C <sub>9</sub> H <sub>17</sub> NO <sub>5</sub>	220->90	50
Pyridoxine	Vit B6	C <sub>8</sub> H <sub>11</sub> NO <sub>3</sub>	170.1->152	50
<b>Cyanocobalamine</b>	<b>Vit B12</b>	C <sub>63</sub> H <sub>88</sub> CoN <sub>14</sub> O <sub>14</sub> P	1355.5->912.3	300
<b>Cholecalciferol</b>	<b>Vit D3</b>	C <sub>27</sub> H <sub>44</sub> O	385→107	10
(±)alpha-tocopherol	Vit E	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	431->165	10
Biotin	Vit H	C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> S	245.2->227.2	50
Phylloquinone	Vit K1	C <sub>31</sub> H <sub>46</sub> O <sub>2</sub>	451->187	10

**Table II: Internal standards and corresponding analytes**

Internal Standards	Source	Cat#	Analytes quantified	Stock
Biotin-d <sub>2</sub> (Vitamin H)	isosciences	5023	Vit-B12, Vit B7	100uM
Pyridoxine-d <sub>3</sub> (Vitamin B6)	isosciences	P2007098	Vit-B6	100uM
[ <sup>13</sup> C <sub>4</sub> , <sup>15</sup> N <sub>2</sub> ]-Riboflavin (Vitamin B2)	isosciences	7072	Vit-B2	100uM
[ <sup>13</sup> C <sub>4</sub> ]-Thiamine (Vitamin B1)	isosciences	9209	Vit-B1	100uM
[ <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N <sub>2</sub> ]-Pantothenate (Vitamin B5)	isosciences	5065	Vit-B3, Vit-B5	100uM
α-Tocopherol-d <sub>6</sub> (Vitamin E)	isosciences	10097	Vit-A, Vit-D3, Vit E	100uM
Phytonadione-d <sub>7</sub> (Vitamin K1)	isosciences	5072	Vit K1	100uM

## Materials

1. Agilent 6410 with 1260 LC unit, chilled autosampler, with standard 54-well autosampler plate
2. Vortexer
3. Refrigerated centrifuge, capable of 13,000g with eppendorf tube compatible rotor
4. Eppendorf Vacufuge
5. ice bucket, ice
6. Prepared stock solutions of authentic vitamin standards and isotope-labelled (internal standards (see Tables I & II).
7. eppendorf tubes (polypropylene)
8. LCMS grade water, acetonitrile, methanol
9. ACS reagent grade chloroform, ammonium acetate, acetic acid

## PROCEDURES:

### Preparation of standards

200uM stock solution in 50:50 Water: Methanol

### Extraction solvent preparation:

Methanol:Acetonitrile:Acetone (1:1:1) with 1uM of internal standard

### Plasma/Serum Sample Preparation

- 1 Remove samples to be extracted from -80 °C freezer and put on dry ice.
- 2 Pre-chill extraction solvent in -20 °C freezer.
- 3 Aliquot 100ul plasma/serum to 2mL labeled eppendorf tube
- 4 Add 400ul extraction solvent.
- 5 Vortex for 15 seconds, incubate on ice for 10 minutes, centrifuge 5 min at 13,000 rpm, transfer supernatant to autosampler vial (NO insert).
- 6 Dry samples using vacuum centrifuge at 30 °C and reconstitute in 100ul of reconstitution solvent (centrifuge 2min if sample is cloudy).
- 7 Store at 4°C for LC-MS analysis

### Tissue Sample Preparation

1. Weigh frozen tissue samples and transfer to labeled eppendorf tubes, record weight. Homogenize tissues using cooled Bullet Blender Gold or probe sonicator, as appropriate. Keep samples cool while homogenizing.
2. Add appropriate amount of extraction solution to all tubes, then vortex to mix.
3. Incubate 5 minutes on ice water, then vortex again. Incubate 5 more minutes, then vortex again.
4. Centrifuge 10 minutes at 15,000g, 4 °C.
5. Transfer supernatant into a clean, labeled autosampler vial for LC-MS analysis

6. Reserve remaining tissue sample/extract at -80°C until analysis is complete

Once analysis is complete, dry and lyophilized extracted tissue, weigh, measure protein content using the Bradford method.

### Cell Sample Preparation

1. Put samples in a box with dry ice. Put extraction solvent on dry ice.
2. Working one plate at a time, remove plate from the cooler and place on a surface of regular ice.
3. Clean cell scraper with MeOH and kimwipe.
4. Add appropriate amount of extraction solvent to the plate.
5. Scrape cells with cell scraper, then scrape solvent to one corner of the plate.
6. Transfer supernatant to a labeled 2mL eppendorf vial. Put vial on dry ice.
7. Repeat procedure with all additional eppendorf vials.
8. Centrifuge all vials at 15,000g for 10 minutes at 4 °C

Transfer 600 uL of supernatant to clean autosampler vials (no insert). Store samples in refrigerator; store remaining sample at -80 °C.

### LCMS Calibration

Mix the following standards in autosampler vials (no insert):

Standard name	Concentration	Volume IS1	Volume IS2	Volume IS3	Volume water
STD 0 (0 pg)	0	0	0	50	500
STD 50 (50 ng)	0.1 ng/uL	2.5	2.5	50	495
STD 200 (200 ng)	0.4 ng/uL	10	10	50	480
STD 500 (500 ng)	1 ng/uL	25	25	50	450

### LC-MS procedure

1. LC column: Waters C18 2mm x 150mm; ?? °C
2. Mobile phase A: 5 mM ammonium acetate in water
3. Mobile phase B: 5 mM ammonium acetate in methanol (MeOH)
4. Gradient: 0min, 0%B; 7min, 95%B; 28min, 95%B, 28.1min, 0%B; 35min, 0%B; flow rate: 250ul/min
5. Autosampler: 4°C, 15 uL injection
6. Agilent 6410 QQQ: ESI<sup>-</sup>, Method: **QM-???** or equivalent

Collect standard curve data first, then sample data if system is suitable. Analytes are Absolutely quantitated by: Cell: conc (ng/uL)\*100ul/0.5ml; Muscle: conc (ng/uL)\*100ul/mass

Relative quantitation: 0.05 ml IS to the sample, 0.1ug IS to the sample, so it is normalized: muscle: 0.1ug\* ratio/mass, cell: 0.1ug\*ratio/0.5ml

### Quantification:

Internal standard mixture is spiked in samples and calibration standards. External calibration curve is constructed from calibration standards and it is used to calculate metabolite concentrations in biological samples.