



Determination of Vitamin B₂ and B₃ in Human Serum by LC-MS/MS using a Luna[®] Omega Polar C18 Column

Xianrong (Jenny) Wei, Simon Lomas and Sean Orlowicz
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

Riboflavin (vitamin B₂) functions as a coenzyme and has lower water solubility than other B vitamins. Nicotinic acid (vitamin B₃) on the other hand is a water-soluble organic compound and is convertible to nicotinamide, though not directly. In this application note, we utilize a simplified method for extraction of Riboflavin, Nicotinic Acid and Nicotinamide from human serum. A Luna Omega 3 μm Polar C18 was then chosen for the separation due its combined polar/non-polar selectivity and high HPLC efficiency level. As a result, excellent resolution, peak shape, and retention are seen for both the neat and extracted serum samples under LC-MS/MS conditions.

Mass Transitions:

ID	Q1	Q3	Dwell (msec)	CE	DP
Nicotinamide 1	123.1	80.0	75	30	90
Nicotinamide 2	123.1	95.8	75	30	90
Nicotinic acid 1	123.9	80.0	75	30	90
Nicotinic acid 2	123.9	95.8	75	30	90
Riboflavin 1	377.2	243.3	75	33	90
Riboflavin 2	377.2	172.3	75	50	90

Sample Extraction Procedure

1. Aliquot 50 μL of human serum to 1.8 mL amber microcentrifuge tube, add 1 mL DI water, mix
2. Add 20 μL of 70% HClO₄ to sample and mix well, centrifuge at 13,500 rpm for 10 min
3. Take 200 μL supernatant into amber autosampler vial
4. Inject 5 μL on the system

LC-MS/MS Analysis

Column: Luna Omega 3 μm Polar C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4760-E0
Mobile Phase: A. 10 mM Ammonium Formate with 0.1 % Formic Acid
B. 0.1 % Formic Acid in Acetonitrile

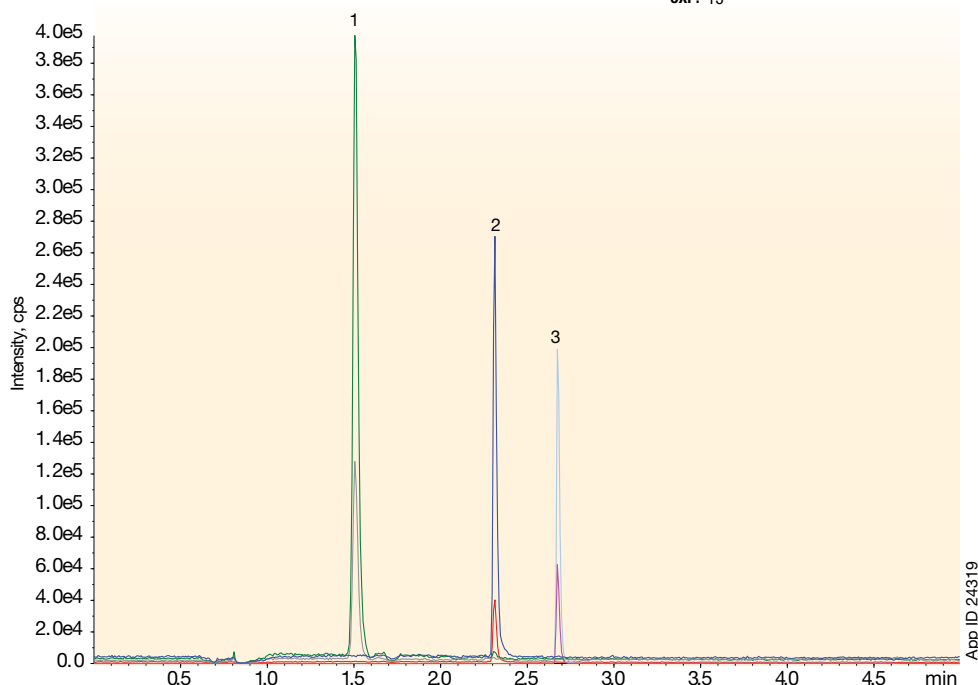
Gradient	Time (min)	B (%)
	0.01	2
	0.50	2
	3.0	90
	3.50	90
	3.51	2
	5.00	2

Temperature: Ambient
Flow Rate: 0.75 mL/min
Pressure: 70 bar
Injection Volume: 5 μL
Detector: SCIEX API 5000™ (ESI+)
Sample: 1. Nicotinic acid
2. Nicotinamide
3. Riboflavin

ESI Source Parameters:

CUR: 25
CAD: 10
TEM: 650
GS1: 50
GS2: 50
IS: 4000
EP: 10
CXP: 13

Vitamin B₂ and B₃ Extracted from Human Serum



App ID 24319



APPLICATION

Luna® Omega Ordering Information

5 µm Minibore and MidBore™ Columns (mm)								SecurityGuard™ Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*
Polar C18	00A-4754-AN	00B-4754-AN	00D-4754-AN	00F-4754-AN	00B-4754-YO	00D-4754-YO	00F-4754-YO	AJO-7600
								for ID: 2.0 - 3.0 mm

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJO-7601
					for ID: 3.2-8.0 mm

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: +86 400-606-8099
f: +86 (0)22 2532-1033
phen@agela.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

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

Mexico
t: 01-800-844-5226
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

3 µm Minibore Columns (mm)					SecurityGuard Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0*
Polar C18	00A-4760-AN	00B-4760-AN	00D-4760-AN	00F-4760-AN	AJO-7600
					for ID: 2.0-3.0 mm

3 µm MidBore Columns (mm)				SecurityGuard Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*
Polar C18	00B-4760-YO	00D-4760-YO	00F-4760-YO	AJO-7600
				for ID: 2.0-3.0 mm

3 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJO-7601
					for ID: 3.2-8.0 mm

1.6 µm Microbore Columns (mm)			
Phases	50 x 1.0	100 x 1.0	150 x 1.0
Polar C18	00B-4748-A0	00D-4748-A0	00F-4748-A0

1.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Polar C18	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJO-9505
					for ID: 2.1 mm

*SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000
†SecurityGuard Analytical Cartridges require holder, Part No.: KJO-4282



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.

Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions, which may be viewed at <http://www.phenomenex.com/TermsAndConditions>.

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Disclaimer

For RESEARCH USE ONLY. Not for use in diagnostic procedures.

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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