

APPLICATIONS

Extraction of 25-OH Vitamin D2/D3 From Plasma Using Strata[®] DE Supported Liquid Extraction (SLE)

Matthew Brusius
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

In this application we provide a supported liquid extraction (SLE) method for the cleanup of 25-OH Vitamin D2/D3 metabolites from plasma. This method provides recoveries of greater than 80 % and RSD values less than 10 % for both 25-OH Vitamin D2 and D3. The results presented in this application note show an acceptable method for extracting Vitamin D metabolites from plasma by utilizing Strata DE, a diatomaceous earth SLE product.

Pre-treatment

Dilute 100 µL of spiked plasma (100 ng/mL) with 100 µL of 1:1 (IPA/Water).

SLE Protocol

| | |
|-----------------------|---|
| 96-Well Plate: | Strata DE 200 µL 96-Well Plate |
| Part No.: | 8E-S325-FGB |
| Load: | 200 µL Pre-treated sample onto plate (apply vacuum or positive pressure to pull/push sample into sorbent) |
| Wait: | 5 minutes |
| Elute: | 2x 600 µL Heptane |
| Apply: | Vacuum or apply positive pressure at 5-10" Hg for 10 seconds |
| Dry: | Sample under slow stream of Nitrogen at 30 °C |
| Reconstitute: | 100 µL Methanol/Water (80:20) with internal standard |

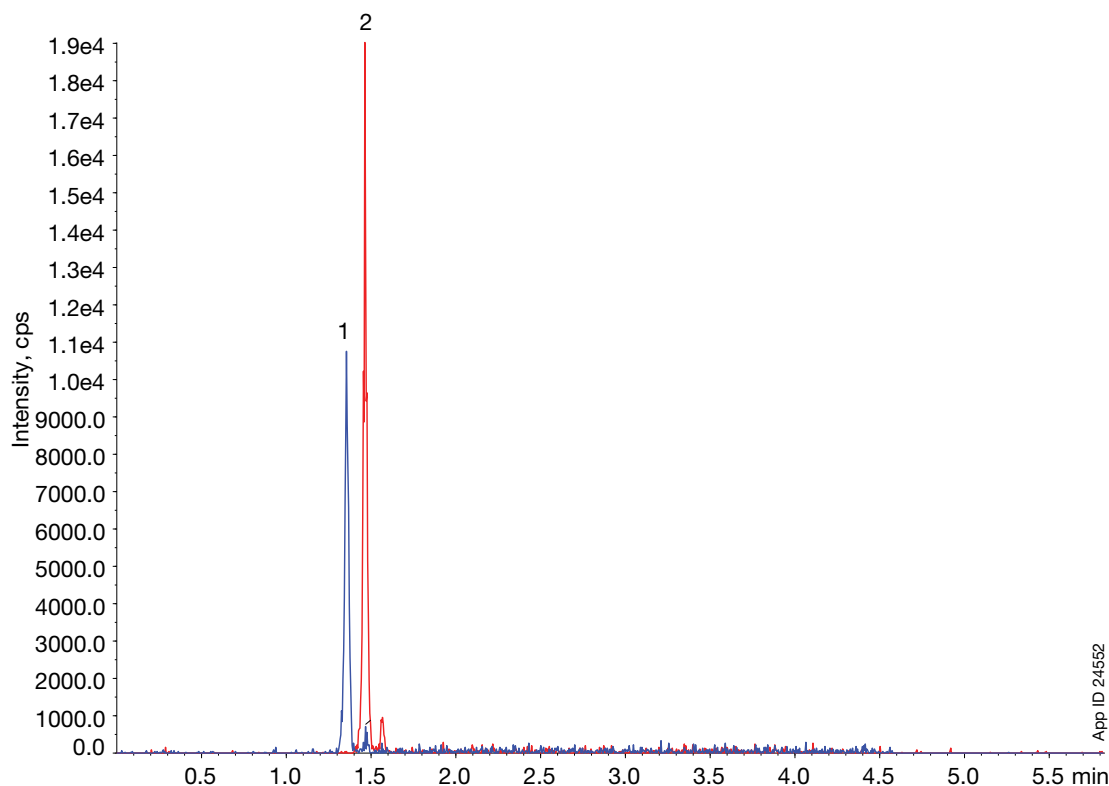
LC-MS/MS Conditions

Column: Kinetex[®] 2.6 µm EVO C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4725-AN
Guard Cartridge: SecurityGuard[™] ULTRA EVO C18
Guard Part No.: AJ0-9298
Mobile Phase: A: 0.1 % Formic acid in Water
 B: 0.1 % Formic acid in Acetonitrile

| Gradient: Time (min) | % B |
|----------------------|-----|
| 0 | 60 |
| 0.83 | 95 |
| 3.33 | 95 |
| 3.34 | 60 |
| 5.83 | 60 |

Injection Volume: 20 µL
Flow Rate: 0.5 mL/min
Detection: MS/MS (SCIEX API 4000[™]), ESI+
Sample: 1. 25-OH-Vitamin D3
 2. 25-OH-Vitamin D2

| | 25-OH Vitamin D2 | 25-OH Vitamin D3 |
|------------------|------------------|------------------|
| Recovery | 96 % | 84 % |
| RSD (n=8) | 9 % | 9 % |



APPLICATIONS

Ordering Information

Kinetex[®] EVO C18 Core-Shell LC Columns

| 2.6 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 |
| EVO C18 | 00A-4725-AN | 00B-4725-AN | 00D-4725-AN | 00F-4725-AN | AJ0-9298 for 2.1 mm ID |

| 2.6 µm MidBore [™] Columns (mm) | | | | SecurityGuard [™] ULTRA Cartridges [†] |
|--|-------------|-------------|-------------|---|
| Phases | 50 x 3.0 | 100 x 3.0 | 150 x 3.0 | 3/pk |
| EVO C18 | 00B-4725-YO | 00D-4725-YO | 00F-4725-YO | AJ0-9297 for 3.0 mm ID |

| 2.6 µm Analytical Columns (mm) | | | | SecurityGuard [™] ULTRA Cartridges [†] |
|--------------------------------|-------------|-------------|-------------|---|
| Phases | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 3/pk |
| EVO C18 | 00B-4725-E0 | 00D-4725-E0 | 00F-4725-E0 | AJ0-9296 for 4.6 mm ID |

[†] SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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

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: 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

Strata[®] DE Supported Liquid Extraction

| Part No. | Description | Unit |
|-------------|------------------------------------|-------|
| 8E-S325-FGB | Strata DE SLE 200 µL 96-Well Plate | 2/pk |
| 8E-S325-5GB | Strata DE SLE 400 µL 96-Well Plate | 2/pk |
| 8B-S325-KDG | Strata DE SLE 12 cc Tube | 20/pk |
| 8B-S325-VFF | Strata DE SLE 60 cc Tube | 16/pk |

Presston[™] 100 Positive Pressure Manifold

| Part No. | Description |
|----------|--|
| AH0-9334 | Presston 100 Positive Pressure Manifold, 96-Well Plate |
| AH0-9342 | Presston 100 Positive Pressure Manifold, 1 mL Tube Complete Assembly |
| AH0-9347 | Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly |
| AH0-9343 | Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly |

Presston 100 Tube Adapter Kits (for AH0-9334)

| Part No. | Description |
|----------|-----------------------|
| AH0-9344 | 1 mL Tube Adapter Kit |
| AH0-9345 | 3 mL Tube Adapter Kit |
| AH0-9346 | 6 mL Tube Adapter Kit |



WARRANTY Phenomenex warrants that for a period of 12 months following delivery, the Presston 100 Positive Pressure Manifold you have purchased will perform in accordance with the published specifications and will be free from defects in materials or workmanship. In the event that the Presston 100 Positive Pressure Manifold does not meet this warranty, Phenomenex will repair or replace defective parts. Please visit www.phenomenex.com/Presston for complete warranty information.



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 <http://www.phenomenex.com/TermsAndConditions>.

Trademarks

Strata and Kinetex are registered trademarks and Presston, MidBore, and SecurityGuard are trademarks of Phenomenex. API 4000 is a trademark of AB SCIEX Ptd. Ltd. AB SCIEX is being used under license.

Disclaimer

FOR RESEARCH PURPOSES ONLY. Not for use in diagnostic procedures.

© 2017 Phenomenex, Inc. All rights reserved.