Fully Automated Sample Preparation for the Determination of Vitamin D on the Biomek® i7 Hybrid Using Advanced Sample Cleanup with Phree™ Phospholipid Removal Cartridges

Kerstin Thurow[1], Heidi Fleischer[1], Anna Bach[1], Bhagya Wijayawardena[2], Miranda Kheradmand[2], Jenny Lewis[3], and Dr. Bryan Tackett[3]

[1]Center for Life Science Automation, University Rostock, Friedrich-Barnewitz-Str. 8, 18119 Rostock, Germany
[2]Beckman Coulter Life Sciences, 5350 Lakeview Pkwy S Dr., Indianapolis, IN 46268 USA
[3]Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

Researchers often quantify the level of vitamin D using LC-MS based methods. The sample preparation for these analytical methods, however, are labor intensive and prone to human error. In this application note we evaluate the feasibility of automating the sample prep for vitamin D determination on the Biomek i7 hybrid workstation. We used Phree phospholipid removal cartridges (Part No. 8B-S133-TAK, Phenomenex, Torrance, US) for the protein precipitation prior to the sample cleanup by solid phase extraction.

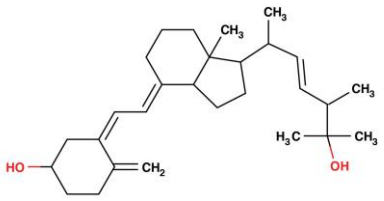
The quantitative determination of 25(OH)D_2 and 25(OH)D_3 is becoming increasingly important to understand the effects of overdosing and underdosing of vitamins. Due to the complicated matrix, the sample preparation for vitamin D analysis is extensive, including protein precipitation and extraction steps. The protein precipitation and the separation of the metabolites from the vitamin D-binding proteins is usually carried out by adding acetonitrile, methanol, mixtures of acetonitrile and methanol, or 2-propanol followed by a centrifugation step. In addition to the proteins, there are other substances in biological matrices, in particular phospholipids (components of the cell membrane), whose highly ionic nature interferes with the ionization in the mass spectrometer. In addition, phospholipids can cause reduced column lifetime as they build up on the stationary phase, which leads to changes in the separation properties and an increase in the baseline noise. Therefore, the removal of the phospholipids is an important step in the sample preparation. The main methods of removing phospholipids are solid phase extraction (SPE) with strong cation exchangers, and liquid-liquid extraction (LLE). However, performing these methods increases the time it takes to complete the analytical measurement process. To reduce sample preparation time, we automated a sample preparation method for vitamin D determination that uses Phree phospholipid removal cartridges and solid phase extraction.

The use of Phree phospholipid removal cartridges for combined protein precipitation and sample clean-up reduced the number of processing steps and the processing time. The high recovery rate is indicative of efficient removal of phospholipids (**Figure 2**). The low CV values (2-9 %) of the automated workflow indicates high repeatability.

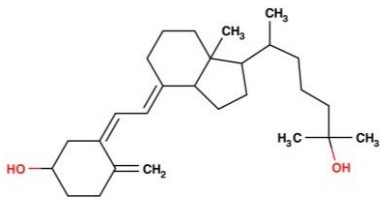
Sample Preparation

Pre-treatment:	Transfer 850 µL Acetonitrile
Add:	Transfer 50 µL internal standard followed by 100 µL serum sample
Mix:	Solution with multiple aspiration/dispensing
Incubate:	Samples at room temperature for 4 min
Apply:	Positive pressure for 100 sec (500 mbar)
Measure:	Samples using LC-MS or LC-MS/MS

Figure 1. Structures of 25(OH)D_2 and 25(OH)D_3



25-hydroxyvitamin-D2



25-hydroxyvitamin-D3

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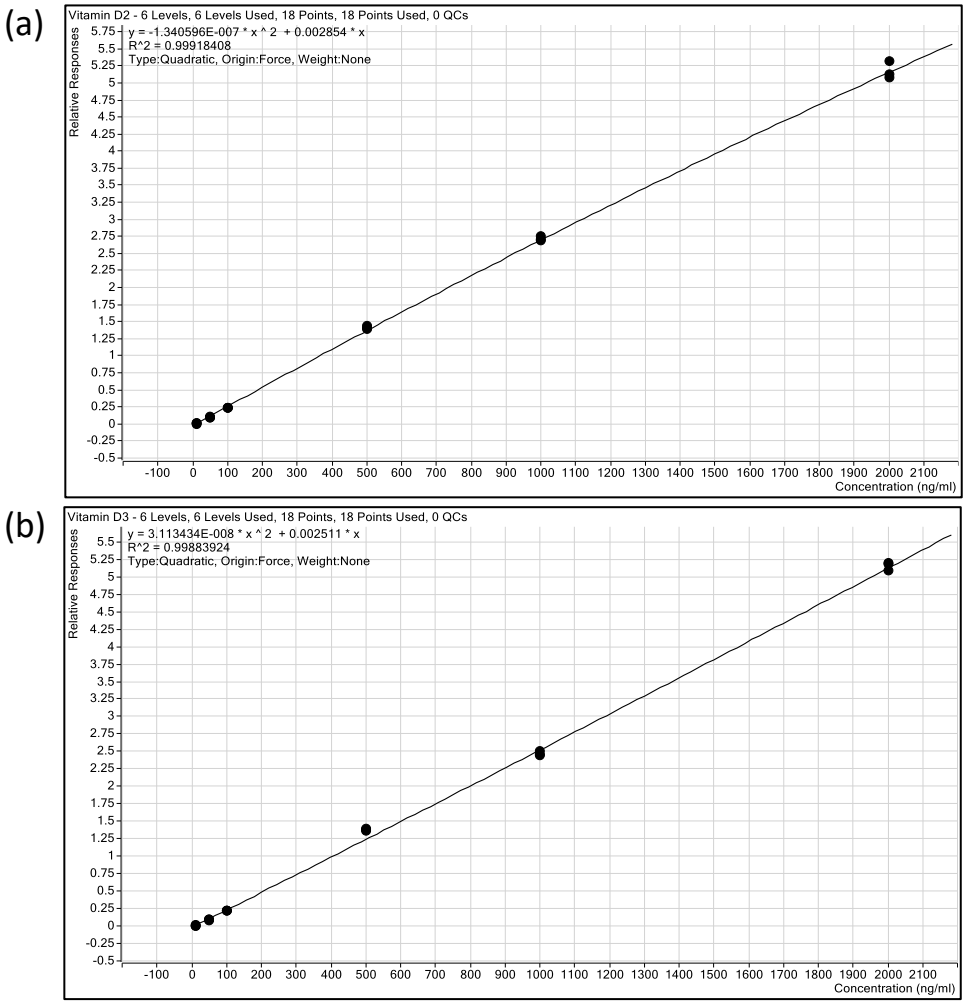
Figure 2. Calibration Curves for (a) 25(OH)D_2 and (b) 25(OH)D_3



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Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

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

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

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

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

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

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

Indonesia
t: +62 21 5010 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

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

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

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

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

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

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

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

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

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

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

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

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
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