

# Comparison of Biotage® Extrahera™ vs. Manual Sample Processing Using a Vacuum Manifold

## Extraction of 25-OH Vitamin D from Plasma Using ISOLUTE® SLE+

Automated sample preparation using the Biotage® Extrahera™ was compared to an equivalent manual method utilizing a vacuum manifold. Analytes were extracted from pooled stripped plasma using a supported liquid extraction procedure. ISOLUTE® SLE+ 400 µL sample volume plates, part number 820-0400-P01 were used for extraction.

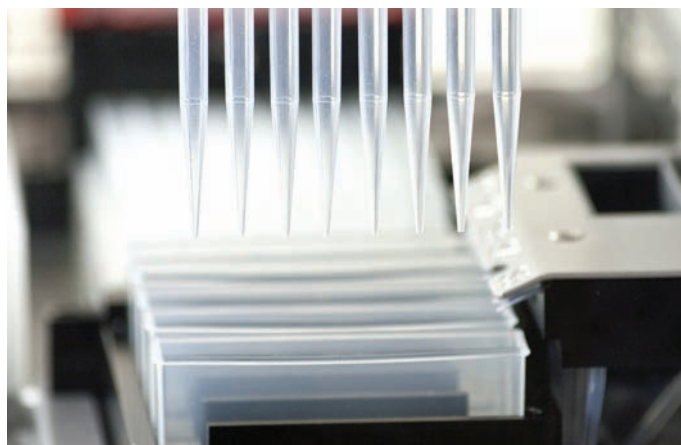
Resulting extracts from both sample preparation methods were subsequently analyzed by LC-MS/MS.



## Procedure

A pooled plasma sample was prepared in a sufficient quantity to run a full 96-well plate for each processing method. This pooled plasma sample was fortified with 25-OH Vitamin D2 and D3 at a concentration of 30 ng/mL respectively. 25-OH Vitamin D3-d6 was added as an internal standard at a concentration of 30 ng/mL.

From this pooled plasma sample 200 µL was transferred to all wells of two 96-well plates.



**All subsequent aspects of sample preparation were performed in duplicate on two separate plates utilizing either Extrahera or manual preparation using a calibrated air-displacement pipette.**

The pooled plasma sample was then pre-treated 1:1 (v/v) with Water:Propan-2-ol 1:1 (v/v) (200 µL).

After pre-wetting the pipette tips via aspirate/dispense cycling and to mix the samples, 300 µL of the pre-treated sample was loaded to each well of the ISOLUTE® SLE+ plates. Flows were initiated using a pulse of positive pressure (Extrahera) or vacuum (manual method).

After leaving for 5 minutes to allow the sample to completely absorb into the plates, elution was performed by the application of 2 x 750 µL of Heptane to the ISOLUTE® SLE+ plates.

The extracts were collected in 2 mL 96-well collection plates under gravity elution, and as a final step to recover all available solvent from the media, by applying a pulse of positive pressure (Extrahera) or vacuum (manual method).

The extracts were evaporated to dryness in a TurboVap® 96 at 37 °C or a SPE Dry at 40 °C and reconstituted in 100 µL of 30:70 (v/v) water/methanol solution.

The plates were mixed on an orbital shaker for 10 minutes.

## HPLC Conditions

<b>Instrument:</b>	Waters Aquity UHPLC
<b>Column:</b>	Restek Pinnacle DB BiPhenyl, 50 mm x 2.1 mm 1.9 µm
<b>Mobile Phase:</b>	80:20 (v/v) 2mM ammonium formate with 0.1 % formic acid (aq.)/ Methanol with 0.1 % formic acid at 0.4 mL/min
<b>Injection Volume:</b>	15 µL

## Experimental Precautions

The following precautions were performed to minimize differences between the manual and Extrahera extracted plates.

- » Both plates were evaporated side by side on the same evaporation instrument (TurboVap® 96).
- » During analysis on the LC-MS system samples were injected alternately from the two plates to reduce the effect of any sample stability issues.
- » The same batch/bottles of samples, reagents and solvents were used for both methods.

## Mass Spectrometry

<b>Instrument:</b>	Waters Quattro Premier XE
--------------------	---------------------------

### MRM Conditions

Analyte	Transition	RT (min)	Dwell (sec)	Cone (V)	Col Energy (V)
25-OH Vitamin D2	395.5 to 119.2	1.7	0.1	30	26
25-OH Vitamin D3	383.5 to 107.2	1.6	0.1	30	25
25-OH Vitamin D3-d6	389.6 to 263.5	1.5	0.1	30	16

## Results

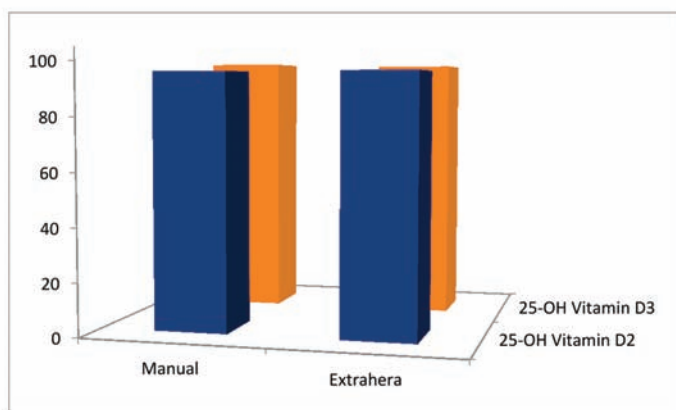
Average peak area data was calculated for all three compounds to compare any improvements in analyte recovery between Biotage® Extrahera™ and manually processed samples.

Peak area ratio data was also generated for all samples by referencing the analyte vs. 25-OH Vitamin D3-d6. This provides standardized data to allow a comparison of the % RSD of the Extrahera vs. manual data sets.

	Extrahera Peak Area Ratio Summary	Manual Peak Area Ratio Summary
Average 25-OH D2 Peak Area	1697	1577
Improvement (%) vs. Manual Method	7.6	-
Average 25-OH D3-d6 Peak Area	1441	1384
Improvement (%) vs. Manual Method	4.1	-
Average Peak Area Ratio	1.1771	1.1421
% RSD of Extrahera Extraction	7.7	7.5
Improvement (%) vs. Manual Method	-3.6	-

	Extrahera Peak Area Ratio Summary	Manual Peak Area Ratio Summary
Average 25-OH D3 Peak Area	4758	4528
Improvement (%) vs. Manual Method	5.1	-
Average 25-OH D3-d6 Peak Area	1441	1384
Improvement (%) vs. Manual Method	4.1	-
Average Peak Area Ratio	3.3051	3.2813
% RSD of Extrahera Extraction	5.9	6.7
Improvement (%) vs. Manual Method	11.8	-

Additional experiments were also completed using serum as the sample matrix. The average recovery comparison data is presented in **Figure 1** below.



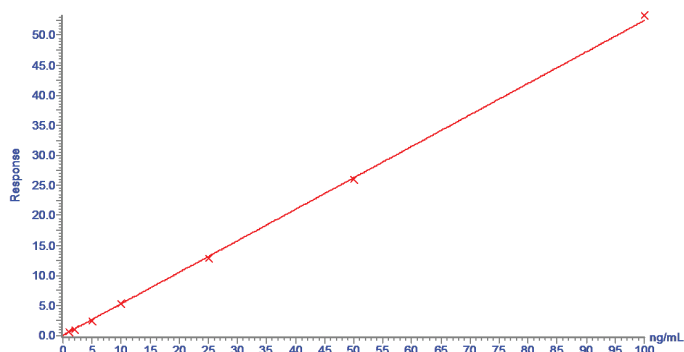
**Figure 1.** Average recovery (n=8) of 25-OH-Vitamin D2 and 25-OH-Vitamin D3 from serum

Calibration series samples prepared manually in Phosphate Buffered Saline with Bovine Serum Albumin (PBS-BSA) and those commercially available from Chromsystems were also processed and extracted using the procedure above with both Biotage® Extrahera™ and manual sample processing methods.

Calibration curves for both manual and Extrahera processed samples over concentration range 1–100 ng/mL for 25-OH Vitamin D2 and D3 in Phosphate Buffered Saline with Bovine Serum Albumin (PBS-BSA) are shown in **Figures 2 to 5**.

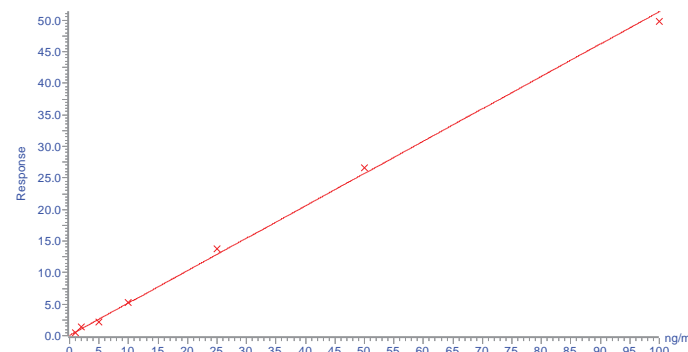
Chromsystems calibration curves for both manual and Extrahera processed samples for 25-OH Vitamin D2 (15.8–61.6 ng/mL) and D3 (4.5–66.7 ng/mL) are shown in **Figures 6 to 9**.

Compound name: 25 OH-Vitamin D2 (1)  
Correlation coefficient:  $r = 0.999446$ ,  $r^2 = 0.998892$   
Calibration curve:  $0.523968 \cdot x + 0.0587074$   
Response type: Internal Std (Ref 3), Area \* (IS Conc. / IS Area)  
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



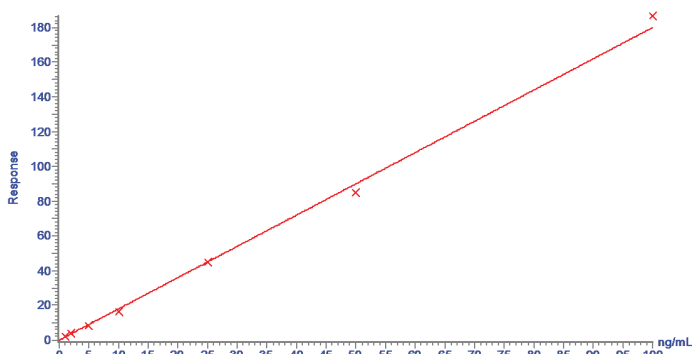
**Figure 2.** 25-OH Vitamin D2 in PBS-BSA – Biotage® Extrahera™

Compound name: 25 OH-Vitamin D2 (1)  
Correlation coefficient:  $r = 0.998431$ ,  $r^2 = 0.996865$   
Calibration curve:  $0.512922 \cdot x + 0.0533979$   
Response type: Internal Std (Ref 3), Area \* (IS Conc. / IS Area)  
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



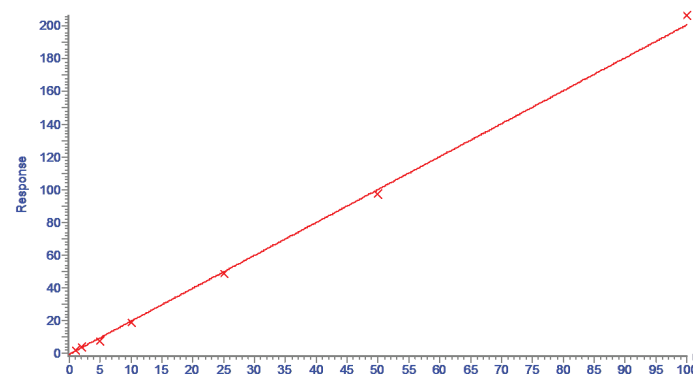
**Figure 3.** 25-OH Vitamin D2 in PBS-BSA – Manual

Compound name: 25 OH-Vitamin D3 (1)  
Correlation coefficient:  $r = 0.998842$ ,  $r^2 = 0.997685$   
Calibration curve:  $1.80001 \cdot x + -0.0531757$   
Response type: Internal Std (Ref 3), Area \* (IS Conc. / IS Area)  
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



**Figure 4.** 25-OH Vitamin D3 in PBS-BSA – Biotage® Extrahera™

Compound name: 25 OH-Vitamin D3 (1)  
Correlation coefficient:  $r = 0.999054$ ,  $r^2 = 0.998108$   
Calibration curve:  $2.00995 \cdot x + -0.403428$   
Response type: Internal Std (Ref 3), Area \* (IS Conc. / IS Area)  
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



**Figure 5.** 25-OH Vitamin D3 in PBS-BSA - Manual

Compound name: 25 OH-Vitamin D2 (1)  
 Correlation coefficient:  $r = 0.996517$ ,  $r^2 = 0.993045$   
 Calibration curve:  $0.893101 \cdot x + 2.43618$   
 Response type: Internal Std (Ref 3), Area \* (IS Conc. / IS Area)  
 Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

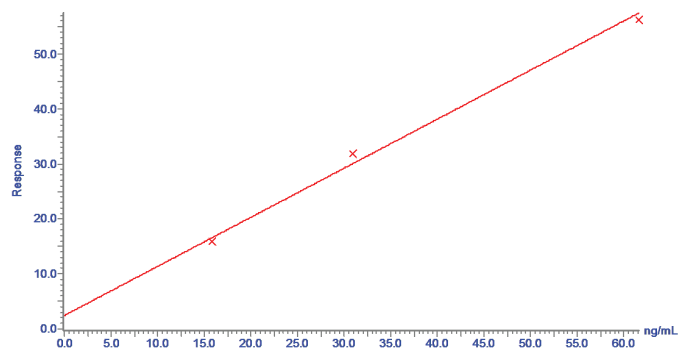


Figure 6. 25-OH Vitamin D2 Chromsystems – Biotage® Extrahera™

Compound name: 25 OH-Vitamin D2 (1)  
 Correlation coefficient:  $r = 0.996832$ ,  $r^2 = 0.993675$   
 Calibration curve:  $0.757448 \cdot x + 0.437403$   
 Response type: Internal Std (Ref 3), Area \* (IS Conc. / IS Area)  
 Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

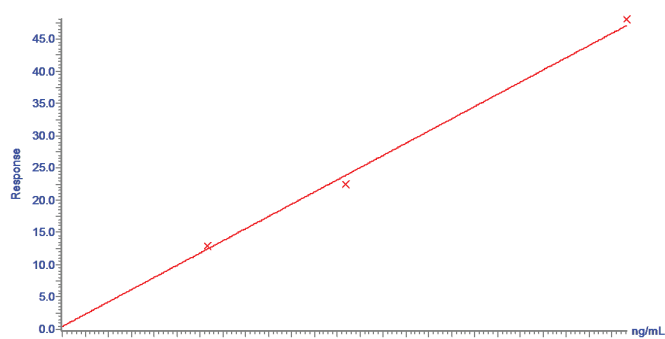


Figure 7. 25-OH Vitamin D2 Chromsystems – Manual

Compound name: 25 OH-Vitamin D3 (1)  
 Correlation coefficient:  $r = 0.999602$ ,  $r^2 = 0.999205$   
 Calibration curve:  $2.42281 \cdot x + -1.82497$   
 Response type: Internal Std (Ref 3), Area \* (IS Conc. / IS Area)  
 Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

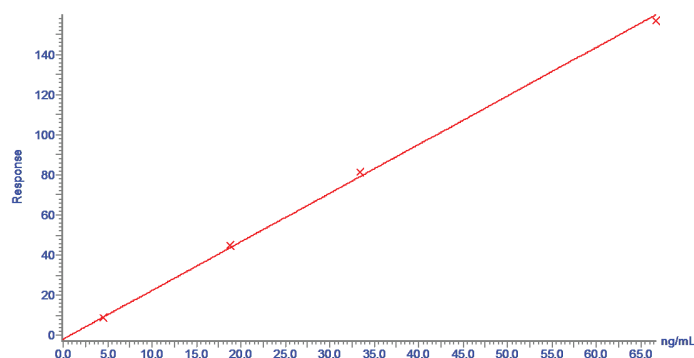


Figure 8. 25-OH Vitamin D3 Chromsystems – Biotage® Extrahera™

Compound name: 25 OH-Vitamin D3 (1)  
 Correlation coefficient:  $r = 0.998765$ ,  $r^2 = 0.997533$   
 Calibration curve:  $2.04345 \cdot x + 1.82945$   
 Response type: Internal Std (Ref 3), Area \* (IS Conc. / IS Area)  
 Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

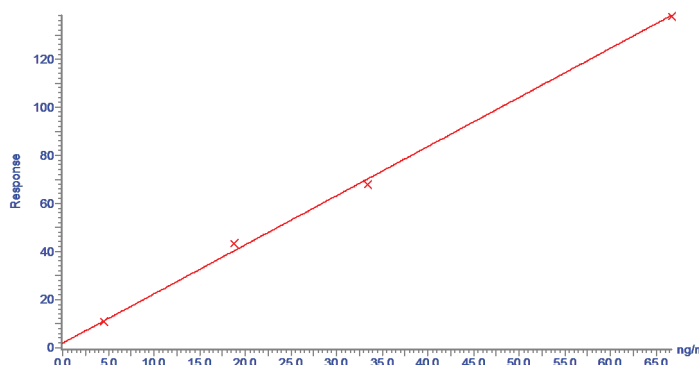


Figure 9. 25-OH Vitamin D3 Chromsystems - Manual

## Conclusion

A reduction to the % RSD was measured when using the Biotage® Extrahera™ for 25-OH Vitamin D3, the precision of the D2 data was slightly better when processed manually.

For 25-OH Vitamin D3 the % RSD improved by 11.8 %, for 25-OH Vitamin D2 there was a slight drop in the % RSD of 3.6 % to 7.74 vs. 7.47, this is not considered to be statistically significant.

The results suggest that methods performed on the Extrahera could give higher recoveries due to increases in the absolute average peak areas.

% RSD	Manual Procedure (n=93)	Extrahera (n=92)
25-OH Vitamin D2	7.47	7.74
25-OH Vitamin D3	6.70	5.91

Average Peak Area	Manual Procedure (n=93)	Extrahera (n=962)
25-OH Vitamin D2	1577	1697
25-OH Vitamin D3	4528	4758
25-OH VitaminD3-d6	1384	1441

All analytes returned average peak areas that were higher when sample extraction was performed using the Biotage® Extrahera™, than the manual method with an average peak area increase of 5 %. The greatest improvement was measured with 25-OH Vitamin D<sub>2</sub> where the average peak area was increased by 7 %.

The serum samples extracted and analyzed under the same conditions showed near identical recoveries between Extrahera and manual processing, 99 % versus 100 % for 25-OH Vitamin D<sub>3</sub> respectively and 97 % for 25-OH Vitamin D<sub>2</sub> for both methods.

The calibration series data for samples prepared in PBS-BSA and the Chromsystems calibrators both returned good correlation coefficients with  $r^2$  greater than 0.99 for samples prepared using Extrahera and processed manually.



#### EUROPE

Main Office: +46 18 565900  
Toll Free: +800 18 565710  
Fax: +46 18 591922  
Order Tel: +46 18 565710  
Order Fax: +46 18 565705  
order@biotage.com  
Support Tel: +46 18 56 59 11  
Support Fax: + 46 18 56 57 11  
eu-1-pointsupport@biotage.com

#### NORTH & LATIN AMERICA

Main Office: +1 704 654 4900  
Toll Free: +1 800 446 4752  
Fax: +1 704 654 4917  
Order Tel: +1 704 654 4900  
Order Fax: +1 434 296 8217  
ordermailbox@biotage.com  
Support Tel: +1 800 446 4752  
Outside US: +1 704 654 4900  
us-1-pointsupport@biotage.com

#### JAPAN

Tel: +81 3 5627 3123  
Fax: +81 3 5627 3121  
jp\_order@biotage.com  
jp-1-pointsupport@biotage.com

#### CHINA

Tel: +86 21 2898 6655  
Fax: +86 21 2898 6153  
cn\_order@biotage.com  
cn-1-pointsupport@biotage.com

To locate a distributor,  
please visit our website at  
[www.biotage.com](http://www.biotage.com)

Part Number: PPS366

© 2014 Biotage. All rights reserved. No material may be reproduced or published without the written permission of Biotage. Information in this document is subject to change without notice and does not represent any commitment from Biotage. E&OE. A list of all trademarks owned by Biotage AB is available at [www.biotage.com/legal](http://www.biotage.com/legal). Other product and company names mentioned herein may be trademarks or registered trademarks and/or service marks of their respective owners, and are used only for explanation and to the owners' benefit, without intent to infringe.