Analysis of 1α,25-dihydroxyvitamin D3 (calcitriol) in a biological fluid using SLE-SPE-LC-MS/MS

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Overview
1α,25-dihydroxyvitamin D3 (calcitriol) is the biological form of vitamin D3 and is used as an indicator for a number of disease states such as chronic renal failure and hypoparathyroidism. With the increased interest to monitor calcitriol in the clinical environment, the design of a robust assay is essential to ensure that appropriate quantitation is obtained. Here, a simple and sensitive method has been developed which uses a comprehensive sample preparation technique of supported assisted liquid liquid extraction (SLE) and solid phase extraction (SPE), coupled with LC-MS/MS analysis to give an accurate and precise measurement of calcitriol in plasma.

The assay developed has been shown to be precise, accurate and linear over the range 1 ng/mL to 100 ng/mL in accordance with the 2001 FDA guidleines [1].

Introduction
Vitamin D plays important roles in bone health and a variety of other pathophysiological conditions such as diabetes and cardiovascular disease. There are two common forms of vitamin D, cholecalciferol (D3) and ergocalciferol (D2). Vitamin D3 is produced in the skin after exposure to ultraviolet B light from the sun or artificial sources, and can also be obtained from diet, as it occurs naturally in a small range of foods. In some countries staples such as milk, flour and margarine are artificially fortified with vitamin D, and it is also available as a supplement in tablet form. In the body, vitamin D3 undergoes metabolism to 25-hydroxyvitamin D3 in the liver, and then is further metabolised to 1α,25-dihydroxyvitamin D3 in the kidney. 1α,25-dihydroxyvitamin D3 is the biological active form of vitamin D3 and is useful for evaluation of several diseases including chronic renal failure, sarcoidosis, hypoparathyroidism and rickets.

Measurement of 1α,25-dihydroxyvitamin D3 is very challenging due to the presence of interfering substances in plasma. It has been historically measured by radio receptor assay and radio immunoassay, which require extensive time consuming sample preparation to remove interfering substances and matrix effects.

Methods
Sample Preparation
Thermo Scientific HyperSep Retain PEP 30 mg/mL 96-well plate
Experimental SLE 200mg/mL 96-well plate
Sample pretreatment: Aliquot 475 µL of blank plasma or sample into a clean tube. Add 25 µL of standard spiking solution in methanol, for blanks add methanol. Add 20 µL of internal standard spiking solution in methanol, for blanks and samples add methanol. Add 20 µL of 10% ammonia solution in methanol, mix well.

Separation Conditions
Instrumentation: Thermo CTC Autosampler, Thermo Scientific Accela 600 pump
Thermo Scientific HyperSep Retain PEP 30 mg/mL 96-well plate
Thermo Scientific HyperSep Retain PEP
Bed Weight 200 mg/mL 96-well plate
Conditioning N/A
Load Load with a pulse of vacuum and wait 5 minutes
Wash N/A
Elute with 8 x 500 µL MTBE. Evaporate to dryness under nitrogen. Reconstitute in 1 mL 60:40 methanol: water. Gently vortex mix and sonicate for 5 minutes
2 x 500 µL methanol, elute very slowly over ~3 mins. Evaporate to dryness under nitrogen. Reconstitute in 1 mL 70:30 methanol: water. Gently vortex mix and sonicate for 5 minutes

Results
Figure 1 displays chromatograms of extracted calcitriol standard at 70 ng/mL in plasma and deuterated calcitriol internal standard at 125 ng/mL. These chromatograms exemplify the extraction procedure in action showing a strong signal for calcitriol and low background from the matrix interferences.

Conclusions
- Supported liquid extraction coupled with solid phase extraction offers an alternative to radio immunoassay as a sample preparation technique for the analysis of calcitriol in plasma.
- We have demonstrated that this assay gives good linearity, accuracy and precision and low levels of carryover for a simple and efficient analysis, that is required by the growing clinical interest in calcitriol.

References:

Additional Information
For additional information, please visit our Chromatography Resource Centre which can be found at: www.thermoscientific.com/CRC

Acknowledgement
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