

# Separation of Vitamins D<sub>2</sub> and D<sub>3</sub> for Clinical Application

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## Introduction

In this poster we discuss the separation of Vitamin D<sub>2</sub> and D<sub>3</sub>, two crucial vitamins either ingested as supplements or synthesised naturally by the skin. They allow the adsorption of several minerals calcium, phosphate, iron, magnesium and zinc in the human body, which help prevent the disease osteomalacia and rickets, a weakening of the bones due to defective bone mineralization.

The separation chromatographically is important before detection of these compounds as they have the same molecular weight, meaning that MS detection cannot be relied on to separate them alone.

We highlight a rapid highly sensitive method in which a simple polar-endcapped column and mobile phase combination separates the two forms of Vitamin D, allowing high qualitative and quantitative results to be obtained.

## HPLC Analysis

Analysis of the vitamins is made challenging by the similarity of the two analytes involved:

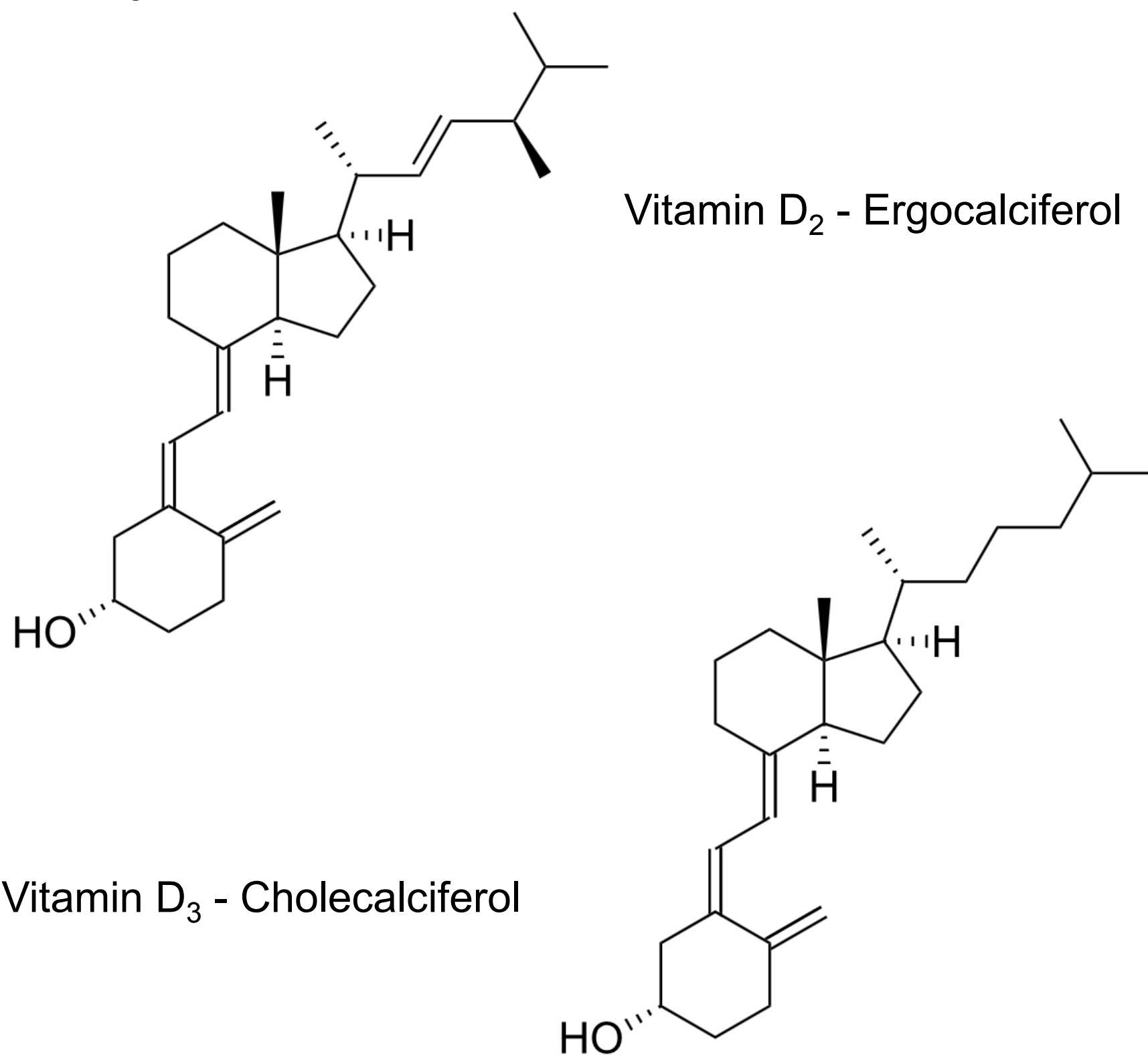
### Conditions

Column: 5µm Fortis® H2o 100x2.1mm

Mobile Phase: ACN: MeOH 85:15

Flow Rate: 1ml/min

Wavelength : 265nm



## Advantages of polar endcapped stationary phases

- Enhanced Resolution
- 100% Aqueous Compatible
- Fully Scaleable: UHPLC → Prep

## Stationary Phase Characteristics

### Fortis® H2o

- Silica Template, Monofunctional C18 bonding
- Unique polar endcapped
- Sharp Peak Shapes
- Highly Selective

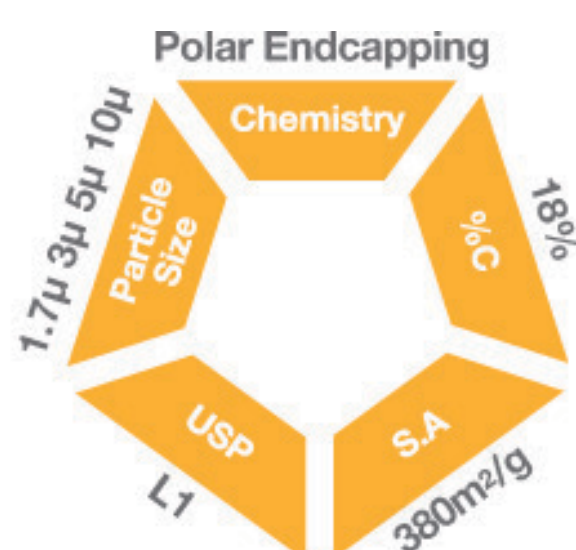
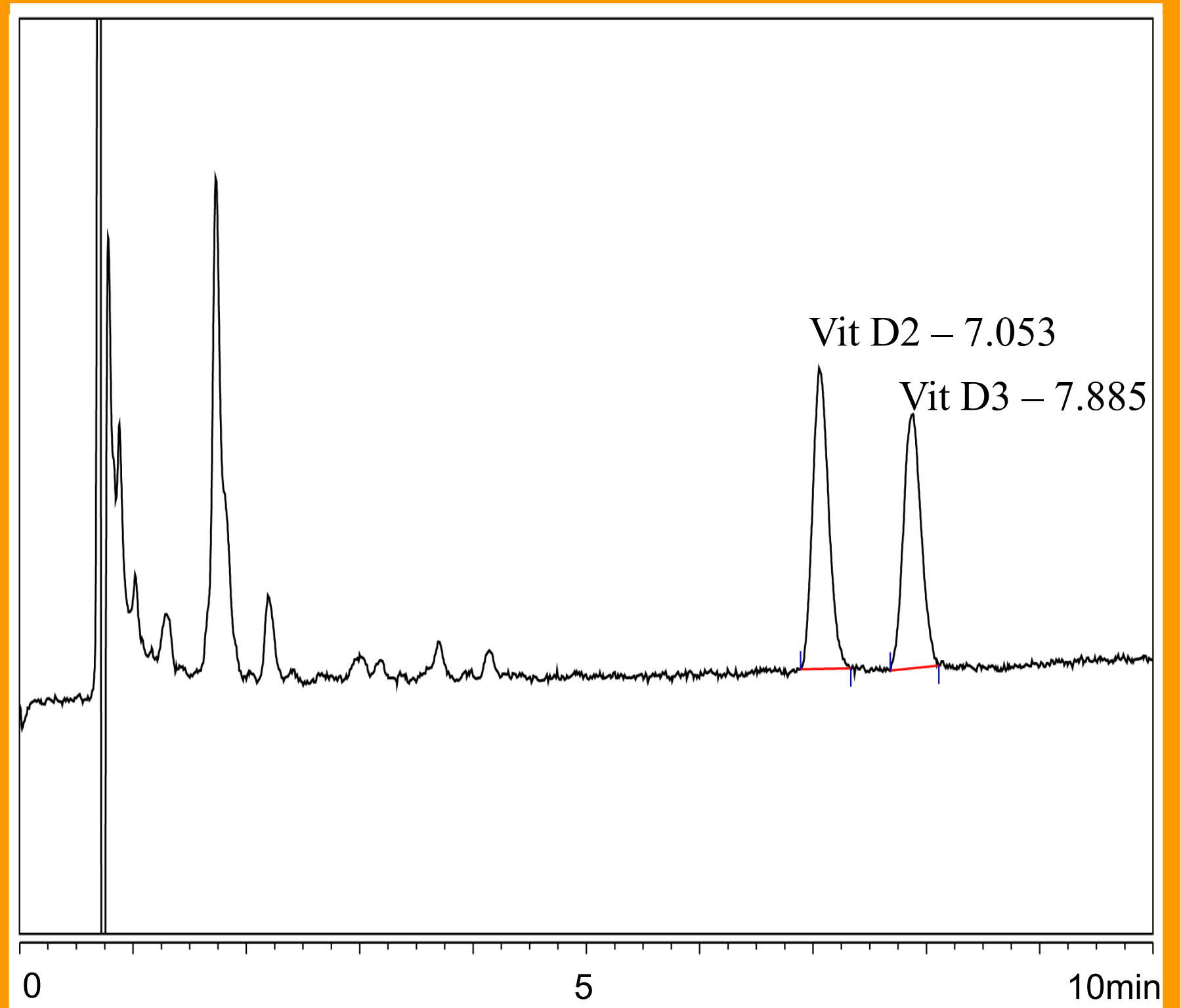


FIGURE 2. Vitamin D<sub>2</sub> & Vitamin D<sub>3</sub>



## Clinical Considerations

Vitamin D from the skin and diet is metabolized first in the liver to 25-hydroxyvitamin D which is used to determine the patients vitamin D status.

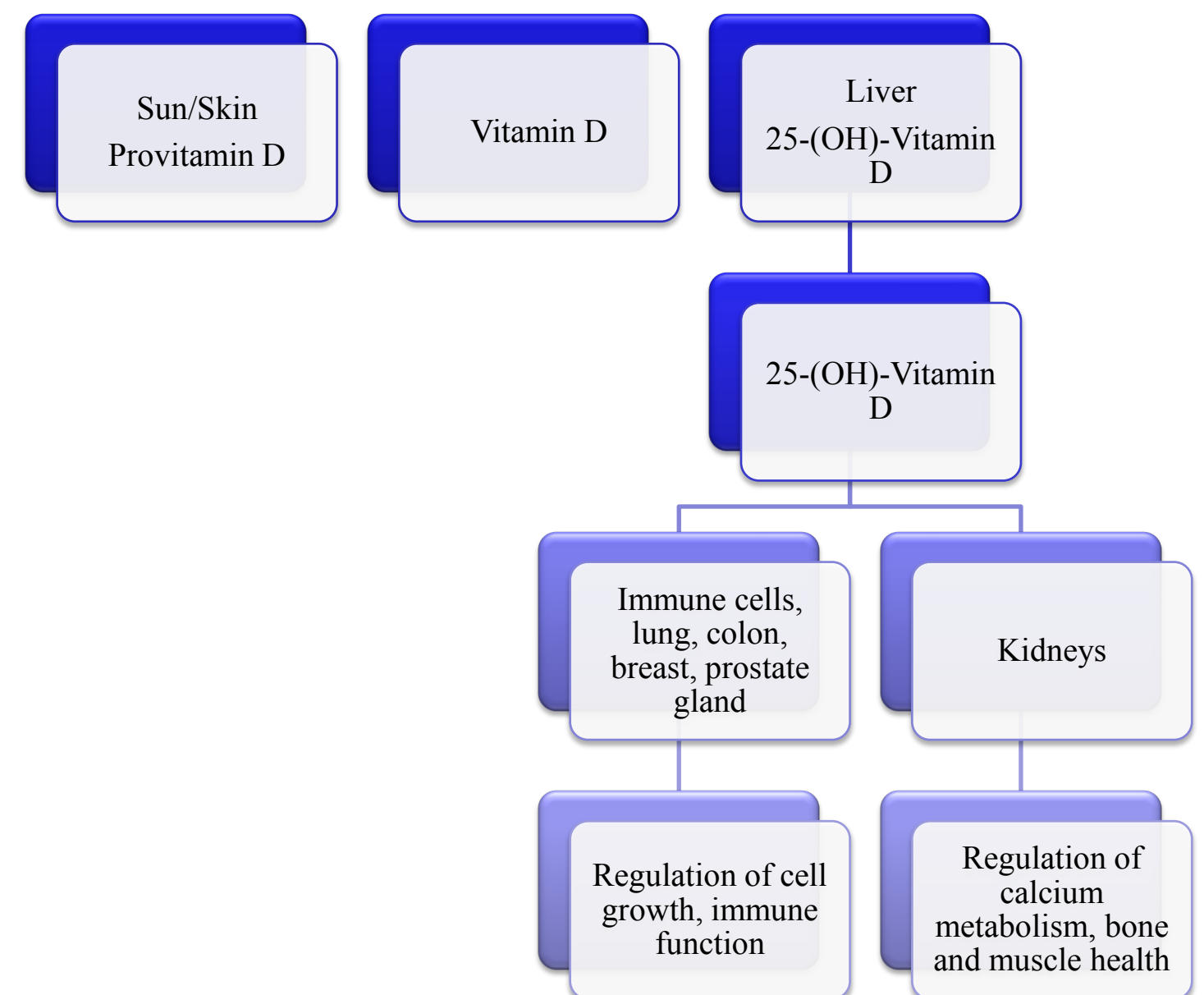
25-hydroxyvitamin D can then be metabolized in the kidney in the presence of enzyme 25-Hydroxyvitamin-D-1α-hydroxylase, and converted into 1,25-dihydroxyvitamin D or 1,25-(OH)<sub>2</sub>-Vitamin D.

Vitamin D<sub>2</sub> and D<sub>3</sub> are both widely utilized in food as supplements and are interchangeably used in the milk supply in the US.

There is a need to measure the Vitamin D concentration in order to adjust the supplemental dose and determine toxicity levels in certain clinical settings.

Determining just the 25(OH)<sub>2</sub>-Vitamin D level is inappropriate since this form does not reflect the general circulating portion of vitamin D.

Laboratories which measure a single component (D<sub>2</sub> or D<sub>3</sub>) render patients prone to dosage errors because the other component is ignored. Hence it is very important to select an analytical method that will accurately estimate the total circulating vitamin D forms.



## Conclusion

The analysis of Vitamin D<sub>2</sub> and D<sub>3</sub> is complicated by the structural similarity of the two analytes. However analysis of both forms and full chromatographic separation is necessary. By using a polar-endcapped stationary phase the analysis is completed with a simple mobile phase combination in a rapid time, allowing for the high throughput screening of samples.

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