

## Vitamin D in dry blood spot

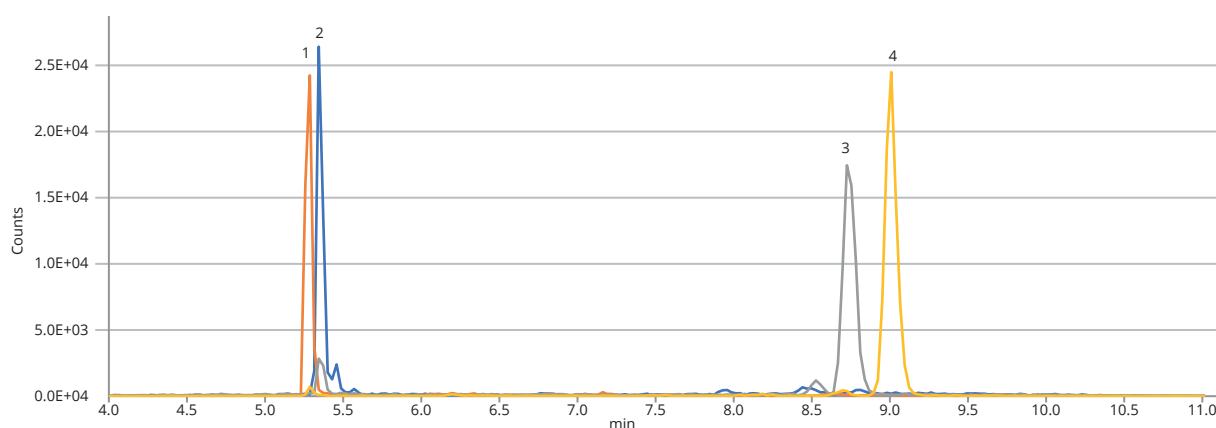
Vitamin D is a group of steroids that have various effects on the human body and support the immune system. This application shows LC/MS/MS separation of the hydroxylated and non-hydroxylated forms of vitamin D2 and vitamin D3. The amount of their hydroxylated forms determines the total vitamin D in a blood sample. Non-hydroxylated forms of vitamin D2 and vitamin D3 are important for food analysis. The LC/MS/MS chromatogram shows the separation of all the above mentioned analytes.

### Substance

1. 25-OH-Vitamin D2, 25-Hydroxycholecalciferol, Ercalcidiol, CAS Number 21343-40-8
2. 25-OH-Vitamin D3, 25-hydroxycholecalciferol, Calcifediol, CAS Number 19356-17-3
3. Vitamin D2, Ergocalciferol, CAS Number 50-14-6
4. Vitamin D3, Cholecalciferol, CAS Number 67-97-0

### Formula

1. (3S,5Z,7E,20R,22E)-9,10-Secoergosta-5,7,10,22-tetraene-3,25-diol
2. (3S,5Z,7E,20R)-9,10-Secocholesta-5,7,10-triene-3,25-diol
3. (3S,5Z,7E,20R,22E,24R)-9,10-Secoergosta-5,7,10,22-tetraen-3-ol
4. (3S,5Z,7E)-9,10-Secocholesta-5,7,10-trien-3-ol



Standard mixture on ARION® column



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### Chromatography method:

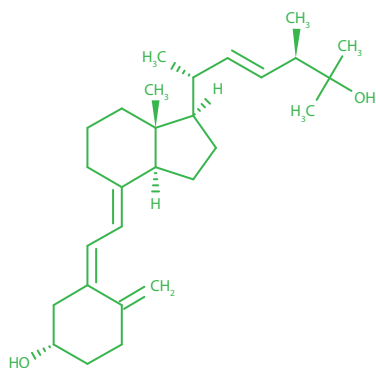
<b>Column</b>	ARION® Plus C18, 2.2 µm		
<b>Dimensions</b>	100 mm × 2.1 mm		
<b>Part number</b>	ARI-5720-EI21		
<b>Mobile phase</b>	A: H <sub>2</sub> O, 0,1% formic acid B: MeOH, 0,1% formic acid		
<b>Gradient elution</b>	<b>Time</b>	<b>A (%)</b>	<b>B (%)</b>
	0.0	70	30
	2.0	0	100
	7.0	0	100
	7.1	30	70
	12.0	30	70
<b>Flow rate</b>	0.4 ml/min		
<b>Temperature</b>	25 °C		
<b>Injection volume</b>	10 µl		
<b>Detection</b>	UV @280 nm		
<b>Analytes</b>	<b>1. 25-OH-Vitamin D3</b> <b>2. 25-OH-Vitamin D2</b> <b>3. Vitamin D2</b> <b>4. Vitamin D3</b>		

### MS method:

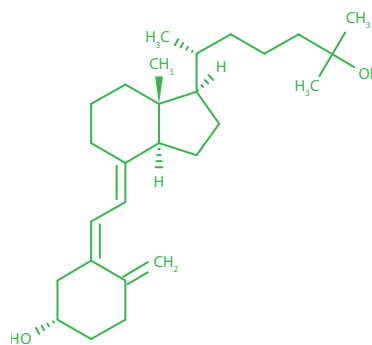
<b>Ionisation</b>	Positive APCI		
<b>Collision gas</b>	Nitrogen		
<b>MRM transition</b>	<b>Analyte</b>	<b>Q1 (Da)</b>	<b>Q3 (Da)</b>
	25-OH-Vitamin D2	413.32	395.30
	25-OH-Vitamin D3	401.22	365.40
	Vitamin D2	397.44	379.20
	Vitamin D3	385.32	259.40
<b>Dwell time</b>	150 ms		

This application was developed by Ján Šmoldas.

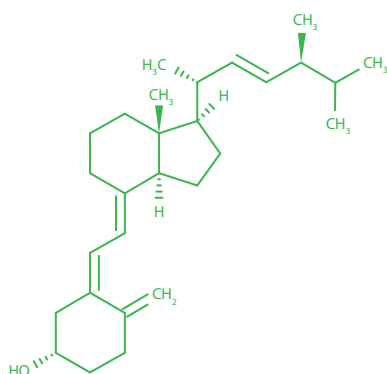
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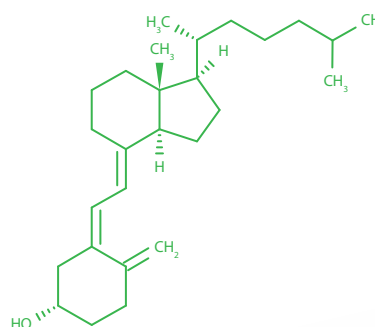
25-OH-Vitamin D2



25-OH-Vitamin D3



Vitamin D2



Vitamin D3

