

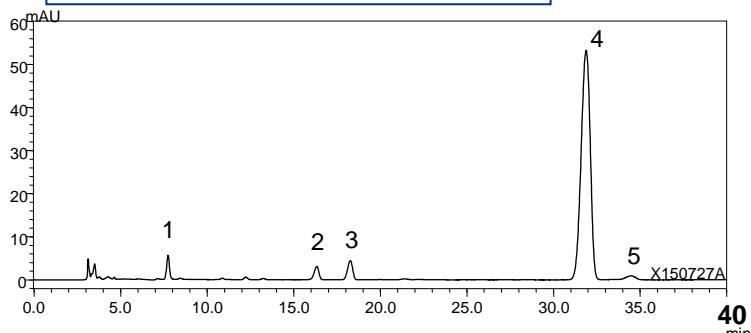
## Method Development for Rapid SFC Analysis of Vitamin D<sub>3</sub> and Related Compounds

T150727AE

By utilizing the advantages of Supercritical Fluid Chromatography (SFC) such as high permeability and diffusibility, we could generally achieve higher resolution by SFC analysis in a shorter run time than by HPLC. Base line separation of Vitamin D<sub>3</sub> (Cholecalciferol), three related compounds (Pre-cholecalciferol, 5,6-trans-Cholecalciferol, Tachysterol<sub>3</sub>) and the antioxidant ( $\alpha$ -Tocopherol) is achieved using an Alcyon SFC Triart Diol column under SFC conditions with shorter analysis time, which is one quarter the time for the normal phase HPLC method.

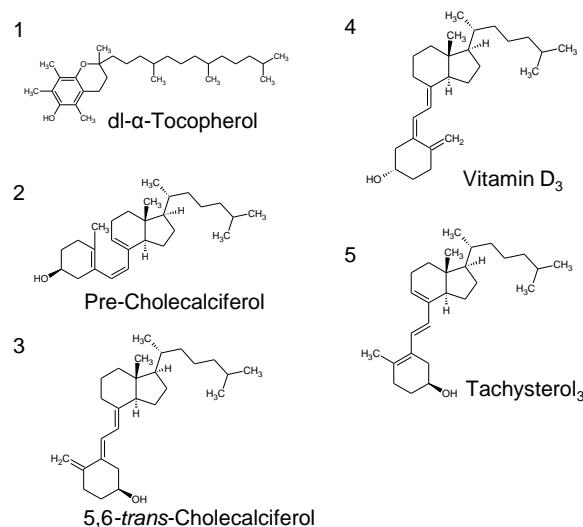
Alcyon SFC Triart Diol is based on organic/inorganic hybrid particle and has excellent chemical stability.

### Normal Phase HPLC Method



#### Conditions

Column	: YMC-Pack SIL (3 $\mu$ m, 12 nm) 250 X 4.6 mmI.D.
Eluent	: <i>n</i> -hexane/1-pentanol (1000/6)
Flow rate	: 1.0 mL/min
Temperature	: 25 °C
Detection	: UV at 254 nm
Injection	: 50 $\mu$ L



Sample :  
Concentrated cholecalciferol powder, thermally stressed (25  $\mu$ g/mL)  
Supplied by DSM Nutritional Products  
Intentionally prepared to cholecalciferol related compounds  
Not commercially available.



### Development of SFC separation conditions

#### Procedure for Developing Separation Condition

#### 1<sup>st</sup> STEP <Method Scouting>

[Column]

##### Alcyon SFC

- ① Triart Diol
  - ② Triart PFP
  - ③ Triart C18
  - ④ SIL
  - ⑤ CN
- 5  $\mu$ m, 150 X 4.6 mmI.D.

[Mobile phase]

- ① A) CO<sub>2</sub>  
B) methanol
- ② A) CO<sub>2</sub>  
B) ethanol
- ③ A) CO<sub>2</sub>  
B) 2-propanol

Composition : A/B (98/2)

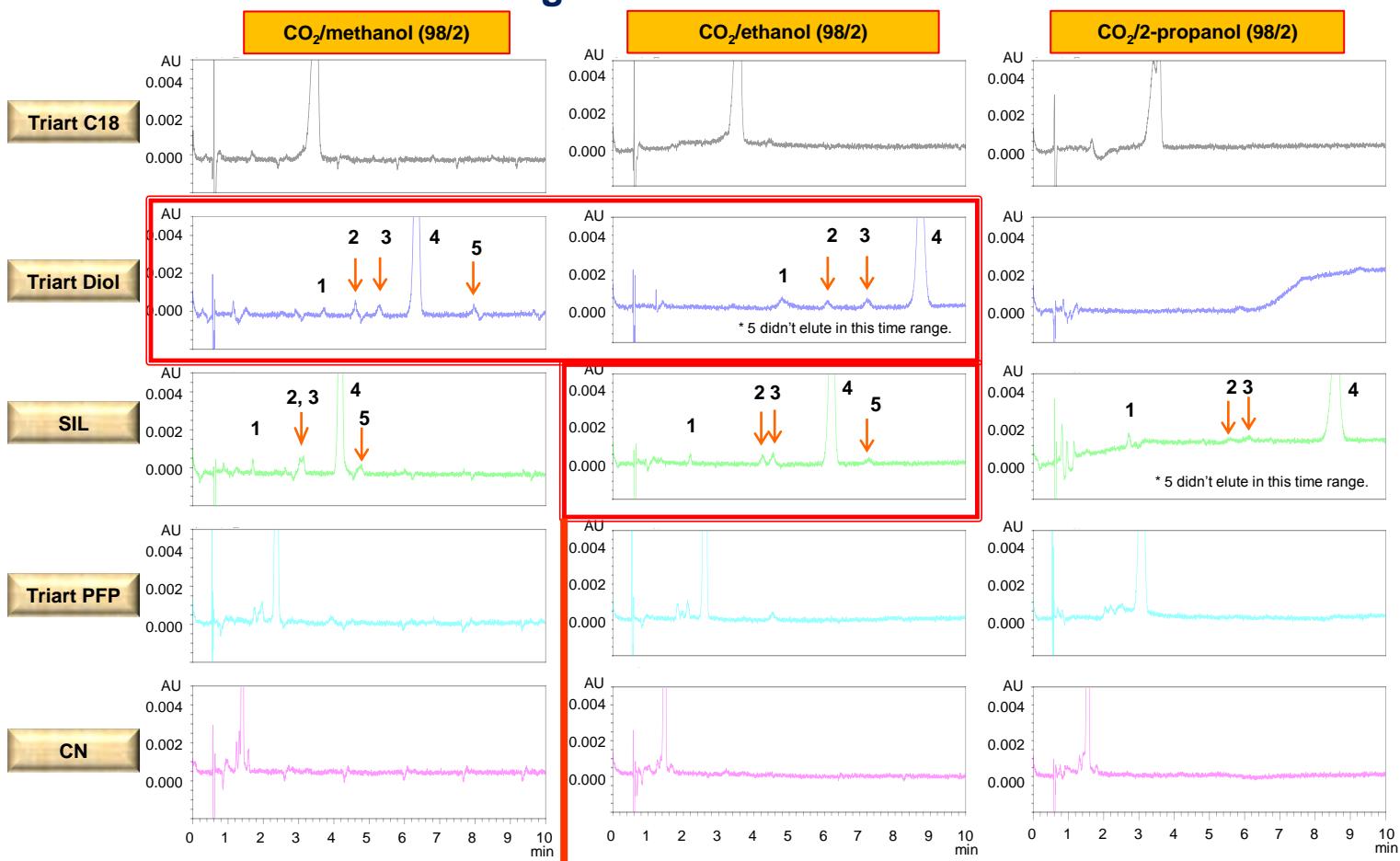
#### 2<sup>nd</sup> STEP <Optimization>

[Items to be optimized]

- Modifier ratio
- Particle size of packing material (5  $\mu$ m  $\rightarrow$  3  $\mu$ m)
- Column dimensions (150 X 4.6 mmI.D.  $\rightarrow$  250 X 4.6 mmI.D.)
- Column temperature

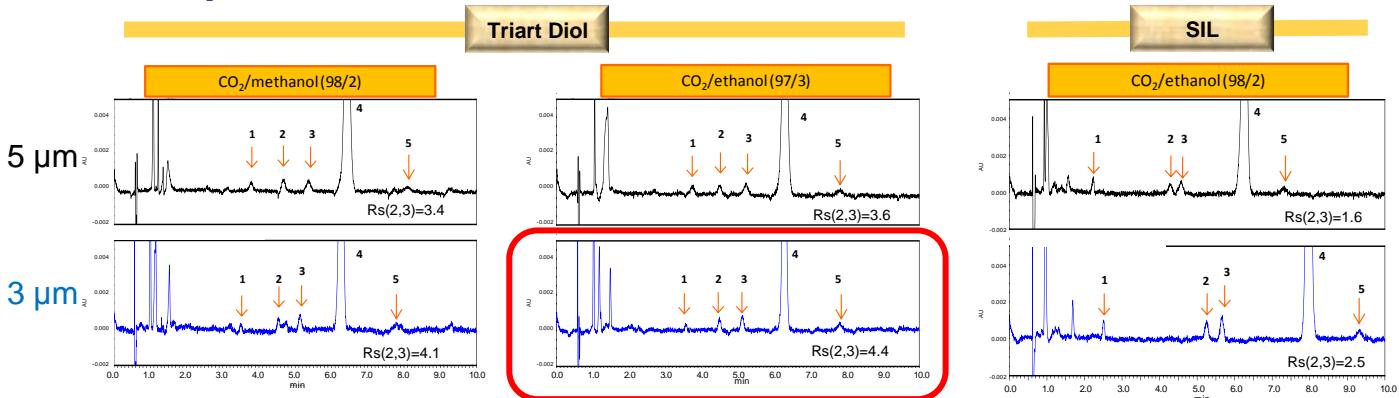
Flow rate	: 3.0 mL/min
Temperature	: 35°C
Detection	: UV (254 nm)
Back pressure	: 13.8 MPa (2000 psi)
Injection	: 10 $\mu$ L

# 1<sup>st</sup> STEP <Method Scouting>

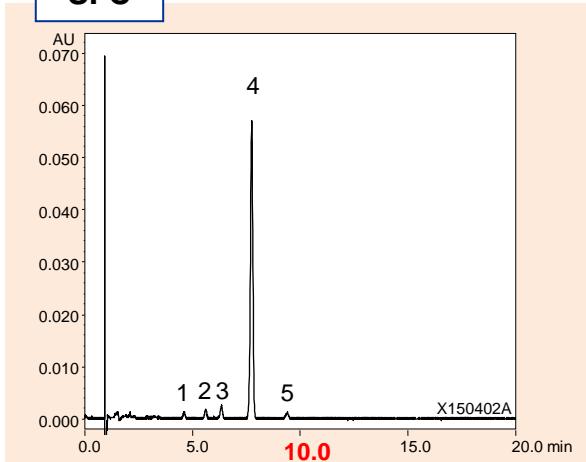


Modifier ratio and particle size optimization

## 2<sup>nd</sup> STEP <Optimization>



### SFC



Fine tuning of the separation method from the condition using combination of Triart Diol 3 µm and CO<sub>2</sub>/ethanol as a mobile phase was conducted. Column dimension, mobile phase composition and column temperature were optimized. The final condition and SFC chromatogram obtained with this new method are shown.

#### Condition

Column	: Alcyon SFC Triart Diol (3 µm, 12 nm) 250 X 4.6 mmL.D.
Eluent	: CO <sub>2</sub> /ethanol (96/4)
Flow rate	: 3.0 mL/min
Temperature	: 40°C
Detection	: UV at 254 nm
Back pressure	: 13.8 MPa (2000 psi)
Injection	: 20 µL