

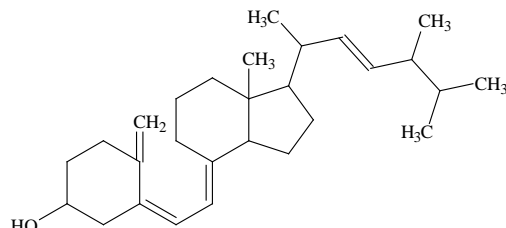
Vitamin D is found in foods, these include vitamin D2 (calciferol), which is found in mushrooms, and vitamin D3 (cholecalciferol), which is found in meat, egg, and fish. Other active compounds include the metabolites 25-hydroxyvitamin D3 and 1 α ,25-dihydroxyvitamin D3.

Vitamin D in foods is typically present at trace levels and it is difficult to separate from contaminants. Pretreatment prior to analysis involves hydrolysis to remove lipids, followed by solvent extraction. The resulting extract is separated by preparative LC for further purification and then analyzed by HPLC. Automated preparative purification was performed using a PLC561 preparative HPLC system.

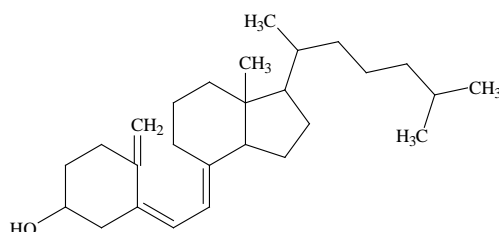
In this analysis, a two step separation was made to isolate vitamins D2 and D3, the first step used preparative LC normal-phase adsorption mode, and the second step HPLC reversed-phase partition mode. By combining these two techniques it was possible to isolate and detect both vitamin D2 and vitamin D3 with excellent results.

(K.Suzuki)

Structural formula



Vitamin D 2



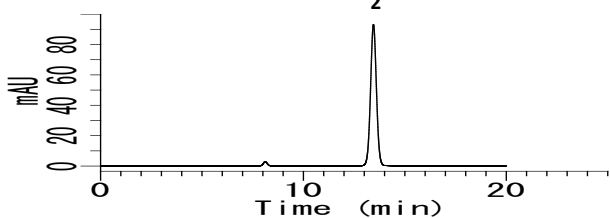
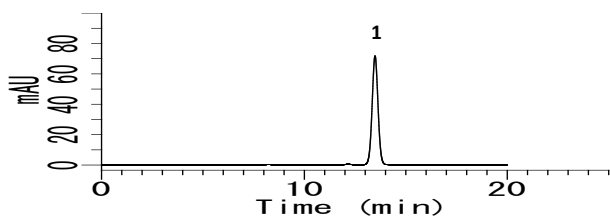
Vitamin D 3

Structures are created using Chemistry 4-D Draw which is provided By ChemInnovation Software, Inc.

Example: Analysis of vitamin D standards

Step One - preparative LC

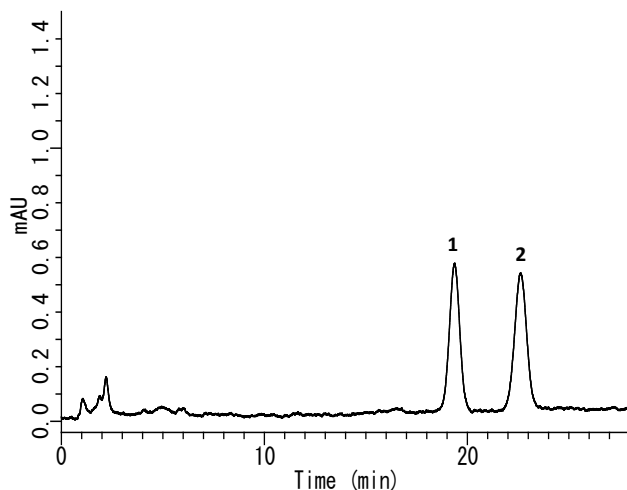
1. D2 (Calciferol) (10 mg/L)
2. D3 (Cholecalciferol) (10 mg/L)



In a one-step HPLC, no separations of vitamins D2 and D3 are performed.

Step Two - HPLC

1. D2 (Calciferol) (0.1 mg/L)
2. D3 (Cholecalciferol) (0.1 mg/L)



Step One - Conditions

- Column** : Inertsil NH2 (5 μ m, 250 x 10 mm I.D.)
Temperature : 40 $^{\circ}$ C
Detector : UV 265 nm
Injection volume : 500 μ L
Eluent : A) 2-propanol
B) n-hexane
A/B = 1/99, v/v
Flow rate : 7 mL/min

Step Two - Conditions

- Column** : Inertsil ODS-P (5 μ m, 250 x 4.6 mm I.D.)
Temperature : 40 $^{\circ}$ C
Detector : UV 265 nm
Injection volume : 200 μ L
Eluent : CH₃CN
Flow rate : 1.5 mL/min

Examples of vitamin D pretreatment in foods

Sample

- 0.2 g of dried shiitake mushroom, 2 g of beech, 2 g of cheese, and 1 g of powdered milk

Saponification

- 3 mL of 1 wt% sodium chloride solution.
 - 10 mL of 1 wt% pyrogallol-ethanol solution
 - 5 mL of 60 wt% potassium hydroxide solution
 - 70 °C, 60 min
 - Cool

Extraction

- 19 mL of 1 wt% sodium chloride solution.
 - A mixture of ethyl acetate-n-hexane (1:9 v/v) 15 mL
 - Shake

Two liquid Separation

- Aqueous layer } (a)
 - Organic layer }
 - Repeat (a)
 Combine organic layers

Concentration

- Concentration in vacuo, not more than 40 °C

Constant volume

2 mL of a mixture of 2-propanol-n-hexane (1:99 v/v)

Step One preparative LC

- 500 µL injection
 - Fractionation of vitamin D

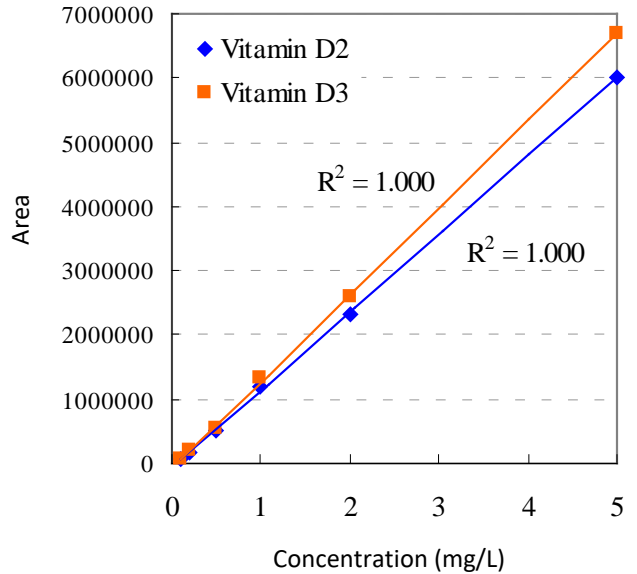
Concentration

- Dry, not more than 40 °C

Dissolution

- 250µL of a mixture of acetonitrile and methanol (9:1 v/v)

Step Two HPLC

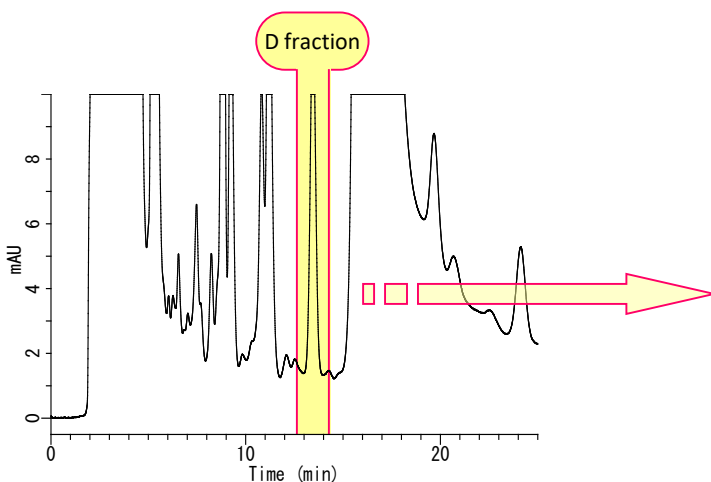


Calibration curve

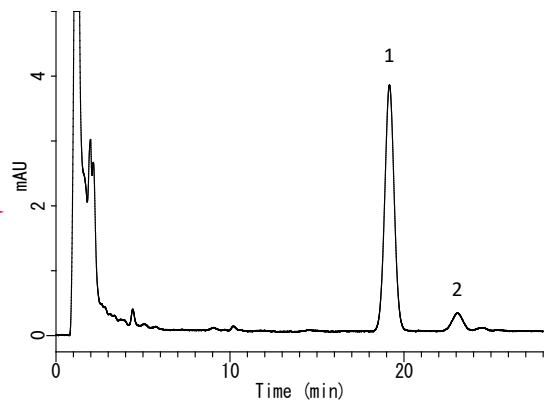
Dried shiitake mushroom extract

- 1. D2 (Calciferol)
- 2. D3 (Cholecalciferol)

Step One preparative LC



Step Two HPLC

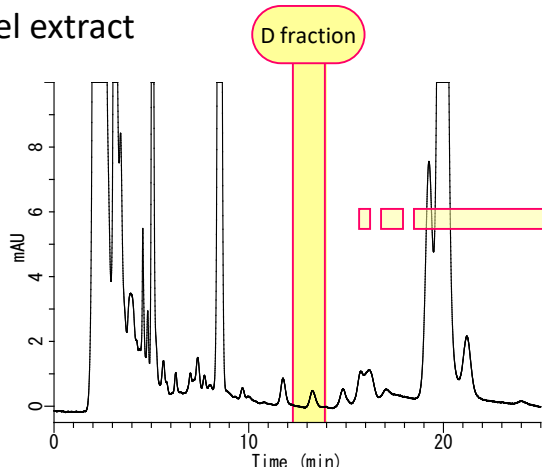


Examples of vitamin D pretreatment in foods

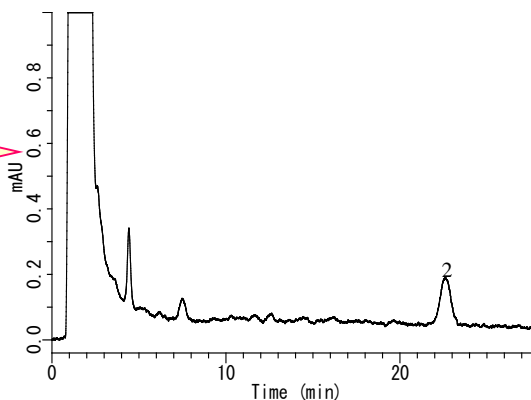
Step One preparative LC

Step two HPLC

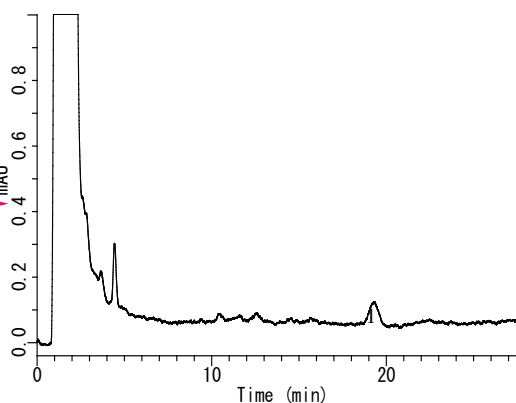
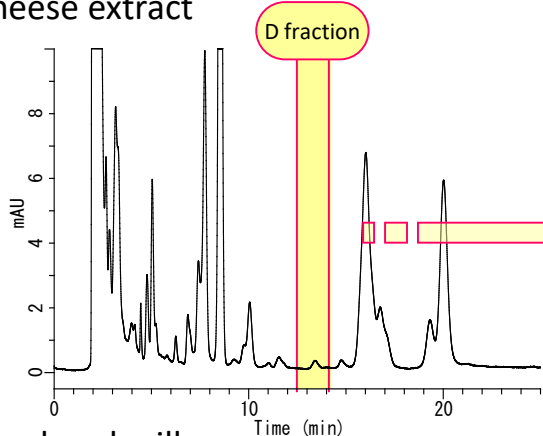
Eel extract



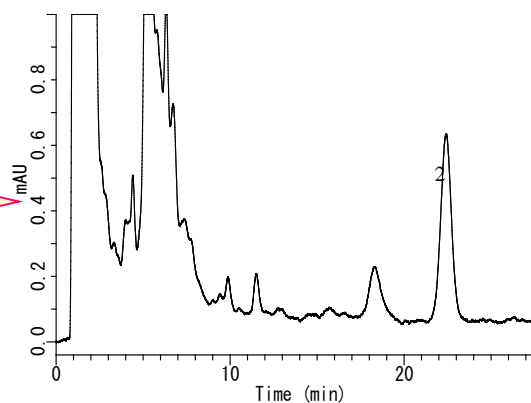
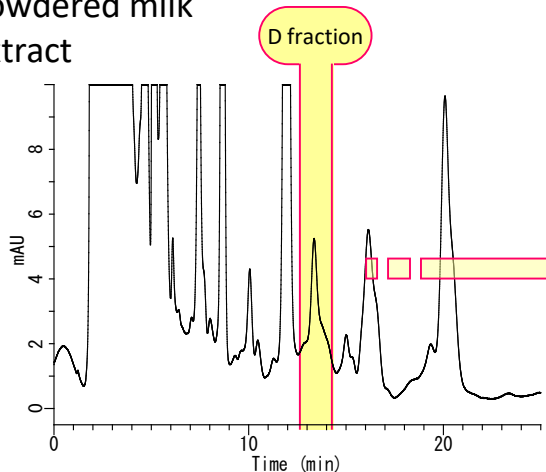
1. D2 (Calciferol)
2. D3 (Cholecalciferol)



Cheese extract



Powdered milk extract



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