



COSMOSIL

Vitamin Analysis by HPLC

Technical Note

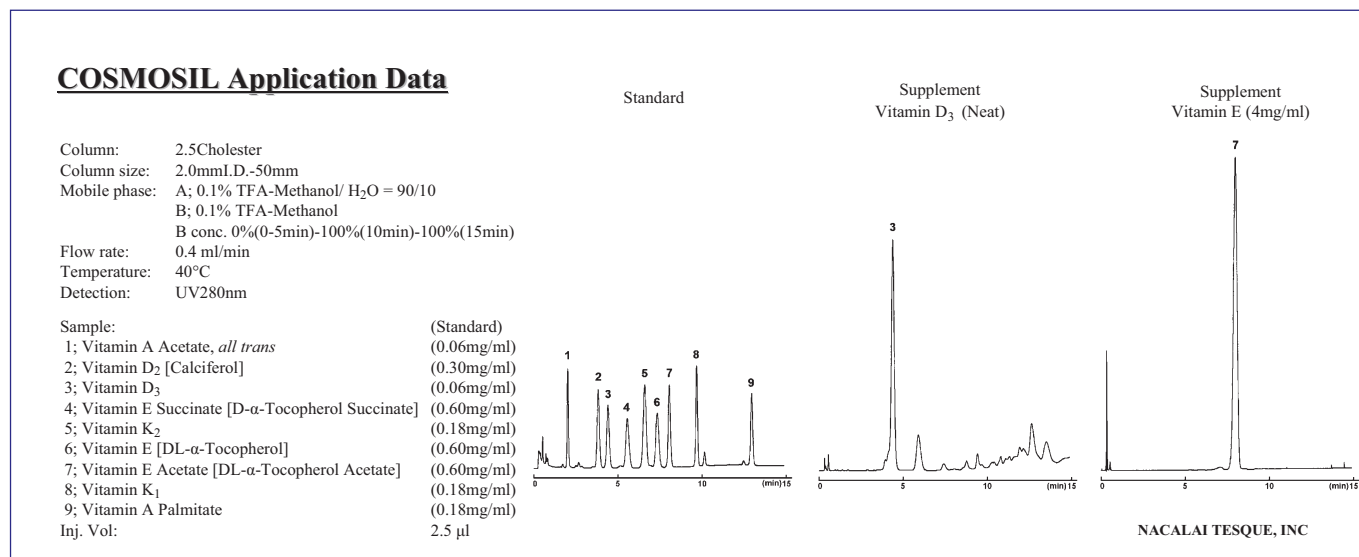
Vitamins are trace-amount organic compounds that regulate physiological functions of an organism. Vitamins are classified into two main groups, water-soluble and fat-soluble. Excellent High Performance Liquid Chromatography (HPLC) analyses of vitamins can be achieved by using COSMOSIL 5C₁₈-MS-II, PAQ, Cholester or πNAP columns.



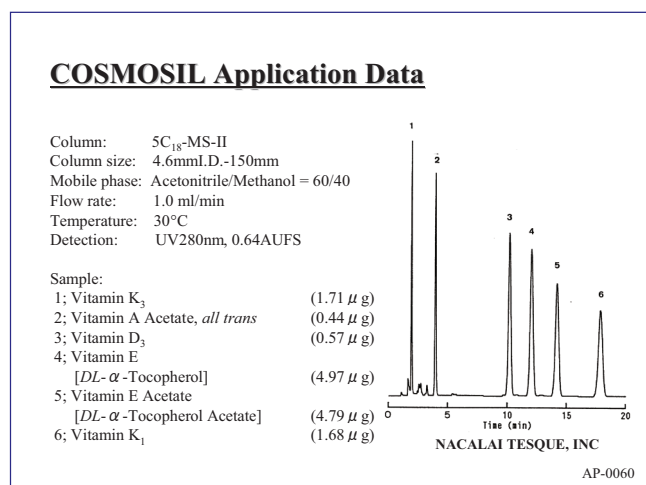
Fat-soluble Vitamins

(1) Fat-soluble Vitamins

- Supplement analysis by COSMOSIL 2.5 Cholester column

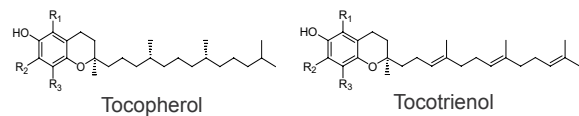


- Fat-soluble vitamins analysis by COSMOSIL C₁₈-MS-II column



(2) Vitamin E

COSMOSIL π NAP offers complete separation of Vitamin E structural isomers that are difficult to analyze by C_{18} columns due to similar hydrophobicity.

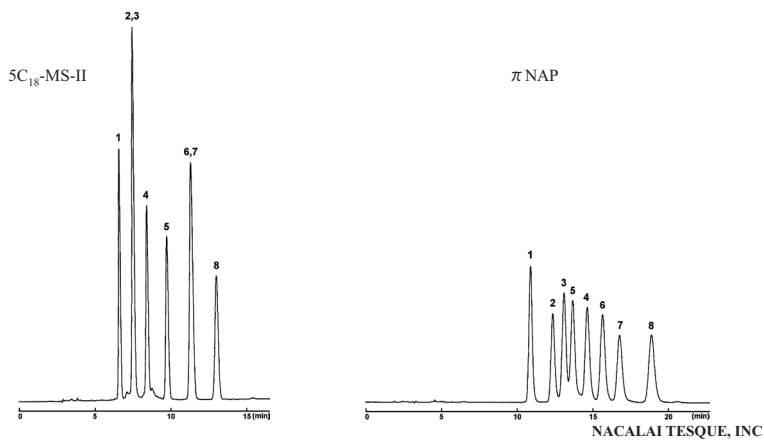


• Tocopherol / Tocotrienol Isomers

COSMOSIL Application Data

Column: $5C_{18}$ -MS-II
 Column size: 4.6mm I.D.-250mm
 Mobile phase: $5C_{18}$ -MS-II Methanol
 π NAP Methanol/ H_2O = 90/10
 Flow rate: 1.0 ml/min
 Temperature: 30°C
 Detection: UV295nm

Sample: 1; δ -Tocotrienol
 2; γ -Tocotrienol
 3; β -Tocotrienol
 4; α -Tocotrienol
 5; δ -Tocopherol
 6; γ -Tocopherol
 7; β -Tocopherol
 8; α -Tocopherol



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(3) Vitamin D

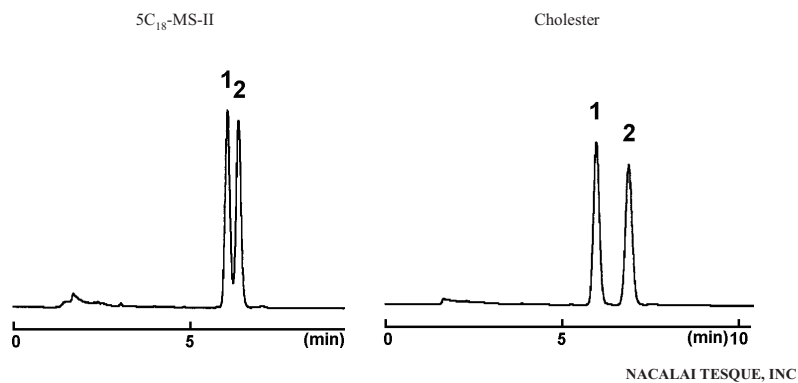
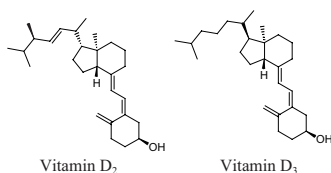
COSMOSIL Cholester offers complete separation of Vitamin D_2 and D_3 that are difficult to analyze by C_{18} columns.

• Vitamin D_2 and D_3

COSMOSIL Application Data

Column: $5C_{18}$ -MS-II
 Column size: 4.6mm I.D.-150mm
 Mobile phase: Methanol
 Flow rate: 1.0 ml/min
 Temperature: 30°C
 Detection: UV265nm

Sample: 1; Vitamin D_2 [Calciferol] (0.3 μ g)
 2; Vitamin D_3 (0.1 μ g)



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(4) Vitamin A

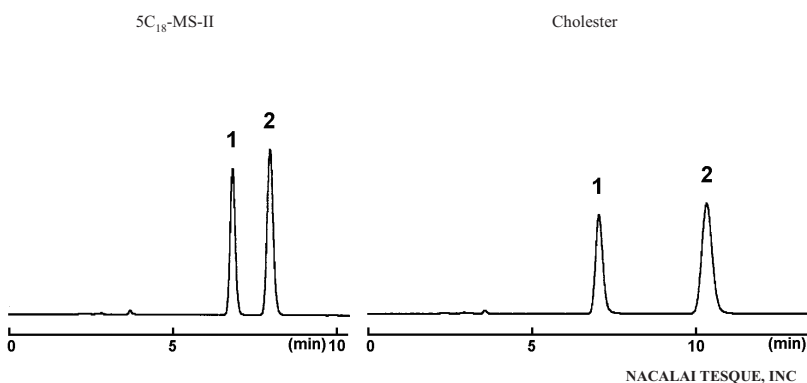
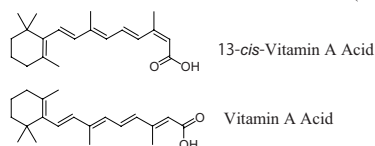
COSMOSIL Cholester offers improved separations for vitamin A_1 and A_2 in all-trans double bond forms and their naturally-occurring 13-*cis* isomers.

• 13-*cis*-vitamin A acid and vitamin A

COSMOSIL Application Data

Column: $5C_{18}$ -MS-II
 Column size: 4.6mm I.D.-150mm
 Mobile phase: Methanol / 20mmol/l Phosphate
 buffer(pH2.5) = 90/10
 Flow rate: 1.0 ml/min
 Temperature: 30°C
 Detection: UV350nm

Sample: 1; 13-*cis*-Retinoic Acid (0.04 μ g)
 2; Vitamin A Acid, all-*trans*
 [all-*trans*-Retinoic Acid] (0.04 μ g)

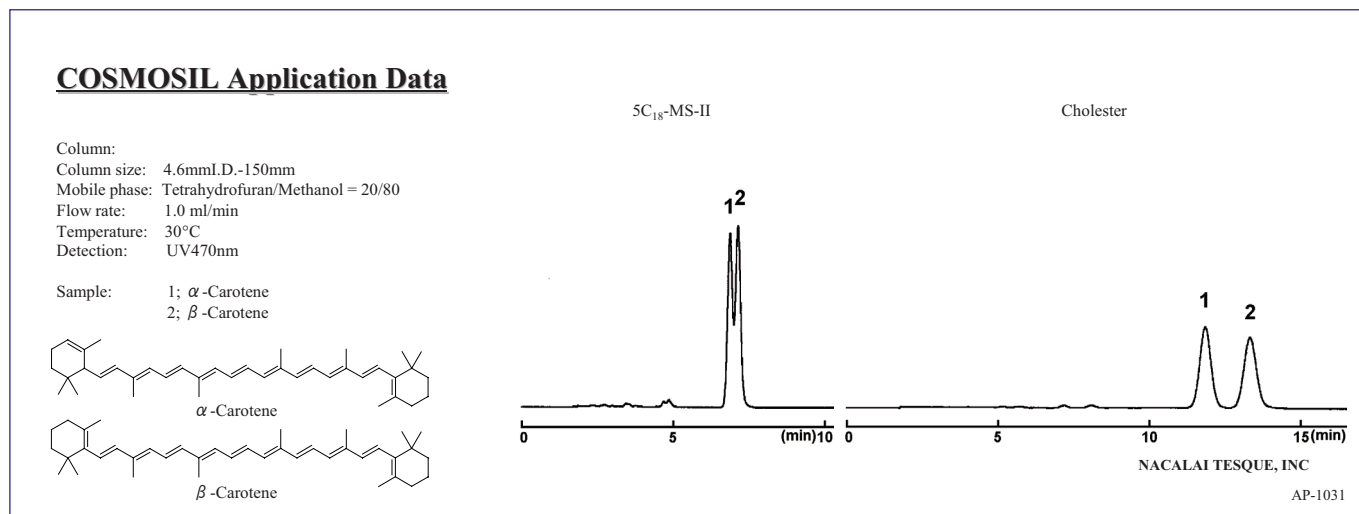


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(4) Vitamin A (continued)

Provitamin A is one of natural carotenoid pigments that changes to retinol in the body. COSMOSIL Cholester offers improved separation of Provitamin A, beta and alpha carotenes.

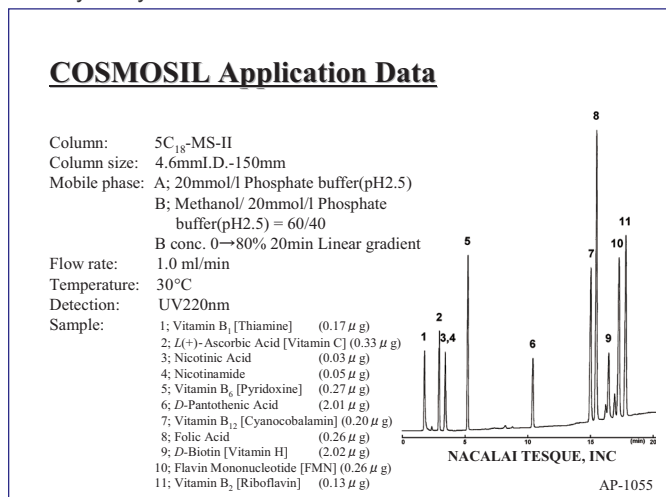
- Carotenes



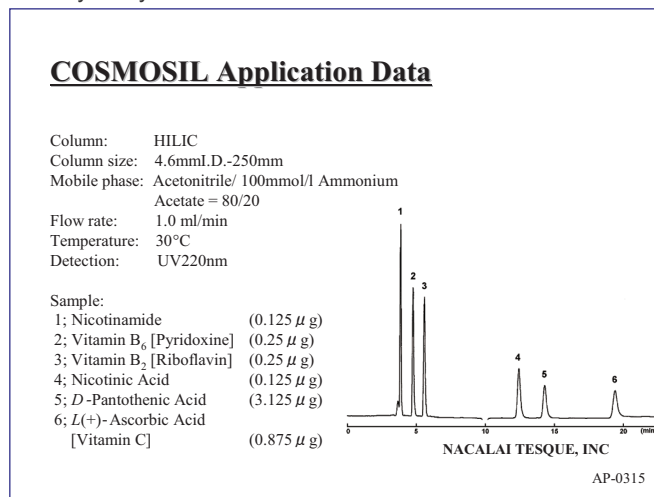
Water-soluble Vitamins

(1) Water-soluble Vitamins

- Analysis by C₁₈-MS-II



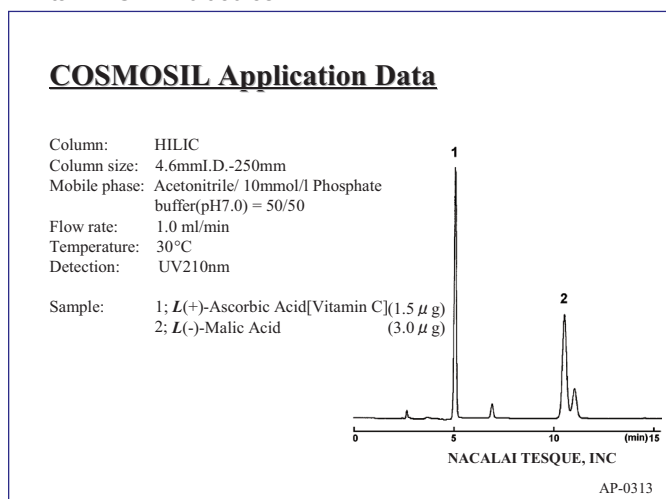
- Analysis by HILIC



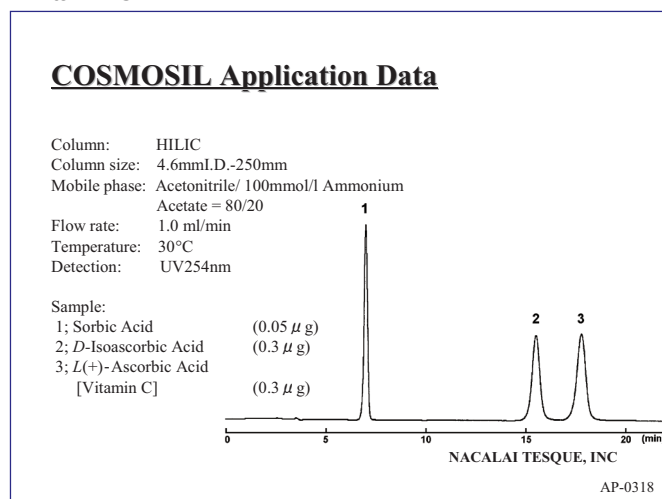
(2) Vitamin C

COSMOSIL HILIC offers improved separation of Vitamin C derivatives that are difficult to analyze by reverse phase columns.

- Vitamin C in Fruit Juice



- Vitamin C



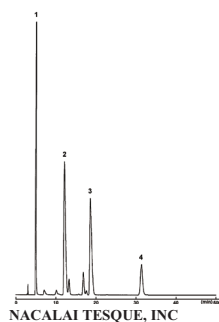
(3) Vitamin B

• Vitamin B₂

COSMOSIL Application Data

Column: 5C₁₈-MS-II
Column size: 4.6mmI.D.-250mm
Mobile phase: Methanol/ 0.2%KH₂PO₄ = 20/80
Flow rate: 1.0 ml/min
Temperature: 35°C
Detection: UV260nm

Sample:
1; Adenosine (1.0 μg)
2; Flavin Adenine Dinucleotide [FAD] (4.0 μg)
3; Flavin Mononucleotide [FMN] (4.0 μg)
4; Vitamin B₂ [Riboflavin] (1.0 μg)



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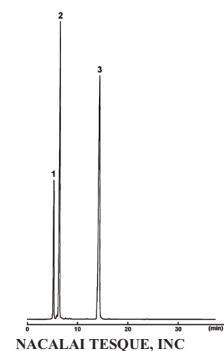
• Vitamin B₁₂

COSMOSIL Application Data

Column: 5C₁₈-MS-II
Column size: 4.6mmI.D.-250mm
Mobile phase: 100mmol/l Sodium *l*-Hexanesulfonate,
Acetonitrile/ 20mmol/l Phosphate
buffer(pH3.5) = 20/80

Flow rate: 0.5 ml/min
Temperature: 40°C
Detection: UV266nm

Sample:
1; Vitamin B₁₂ [Cyanocobalamin] (0.5 μg)
2; Hydroxocobalamin Acetate (0.5 μg)
3; Mecobalamin (1.0 μg)



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(4) Vitamins in Food

• Water-soluble Vitamins in Energy Drinks

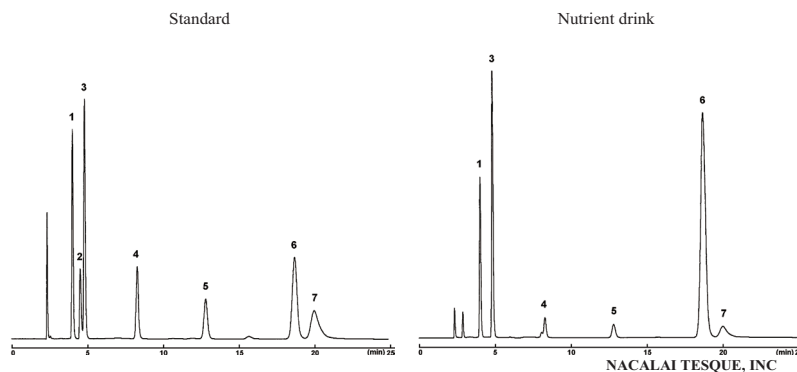
COSMOSIL Application Data

Column: 5C₁₈-PAQ
Column size: 4.6mmI.D.-250mm
Mobile phase: Methanol/ 5mmol/l Sodium *l*-Hexanesulfonate,
20mmol/l Phosphoric Acid = 15/85
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV220nm

Sample: 1; Citric Acid (10mg/ml)
2; *L*-Carnitine (20mg/ml)
3; Nicotinamide (0.2mg/ml)
4; Vitamin B₆ [Pyridoxine] (0.2mg/ml)
5; Vitamin B₁ [Thiamine] (0.2mg/ml)
6; Caffeine (0.2mg/ml)
7; Flavin Mononucleotide [FMN] (0.2mg/ml)

Injection Vol. 1.0 μl

Standard
(10mg/ml)
(20mg/ml)
(0.2mg/ml)
(0.2mg/ml)
(0.2mg/ml)
(0.2mg/ml)
(0.2mg/ml)



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