



Chromatography Focus webinar 5

TLC and HPLC for Vitamin analysis

Wattanapong Sittisaree

MERCK

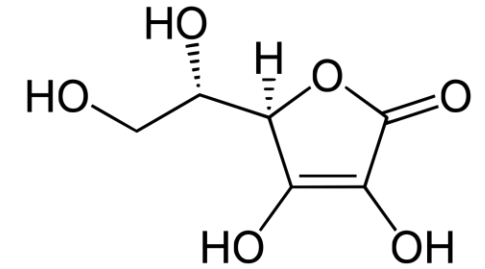
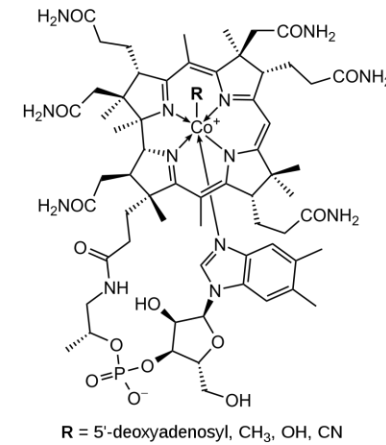
Agenda

- ☐ Vitamin overview
- ☐ Reference Material for Vitamin analysis
- ☐ TLC application for vitamin analysis
- ☐ HPLC application for vitamin analysis

Vitamin types

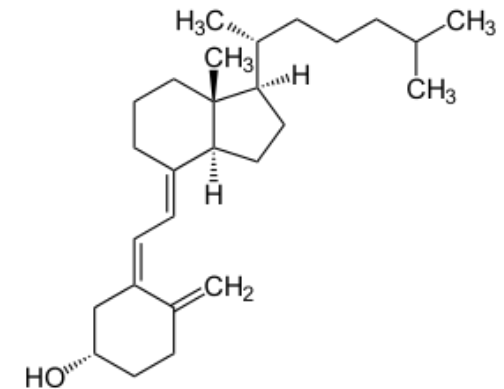
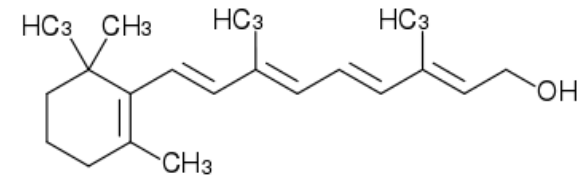
Water soluble

B and C



Fat soluble

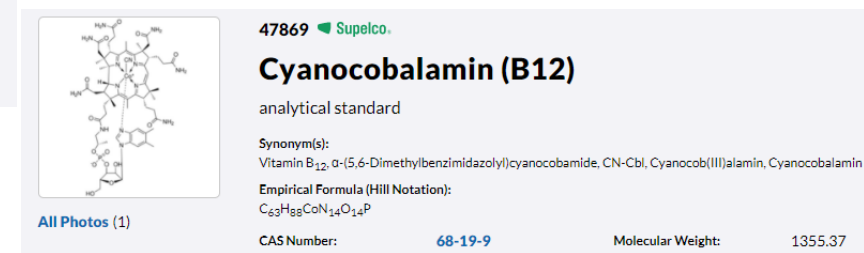
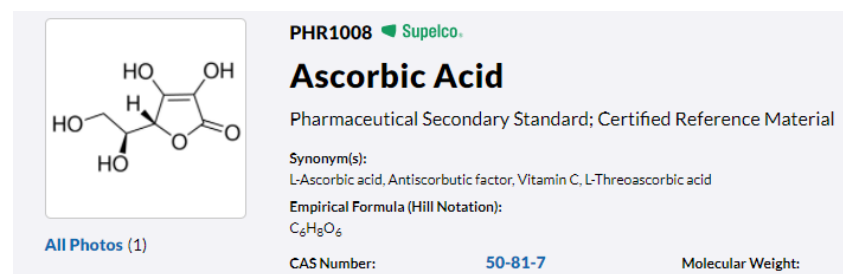
A, D, E, K



Vitamin Standards

- ❑ Vitamins are organic molecules and essential micronutrients required in small quantities for the proper functioning of body metabolism.
- ❑ We offer a full range of fat- and water-soluble vitamins for use in various chromatography, mass spectrometry, and other analytical applications.
- ❑ Our standards can be used in quality control analysis of vitamin-based dietary supplements, fortified foods, cosmetics, pharmaceutical preparations, or the diagnostic testing of vitamin deficiencies. O
- ❑ ur line of vitamin reference standards includes solution-based certified reference materials (CRMs), specially designed to minimize degradation and promote increased shelf life.

- Vitamin A
- Vitamin B
- Vitamin C
- Vitamin D
- Vitamin E
- Vitamin K



Vitamin analysis

TLC

Determination of Vitamin C derivatives from cosmetic formulation on HPTLC Silica gel 60 F254s

Chromatographic Conditions

Plate: HPTLC Silica gel 60 F254s 20x10 cm (1.15696.0001)

Mobile Phase (v/v): n-Heptane (1.04390) / THF (1.08107) 8:2 +0.1 % TFA (1.08262)

Migration distance: 5 cm

Chamber: Normal chamber without chamber saturation

Derivatisation: Anisaldehyde-sulfuric acid-reagent

Detection: Visible light

Sample preparation: 1 g cosmetic formulation (1 % and 2 % active) was stirred in 10 ml THF for 15 min and filtered over a 0.45 µm syringe filter. Application by CAMAG ATS4 6 mm bandwise.

LA014-10 0-1-0 21d RT

LA014-10 0-1-1 21d RT

LA014-10 0-1-2 21d RT

LA014-10 0-1-3 21d RT

LA014-10 0-1-4 21d RT

PMCA 0,2µl

PMCA 0,5µl

PMCA 1,0µl

0 21d RT

1 21d RT

2 21d RT

Rf: 0,58

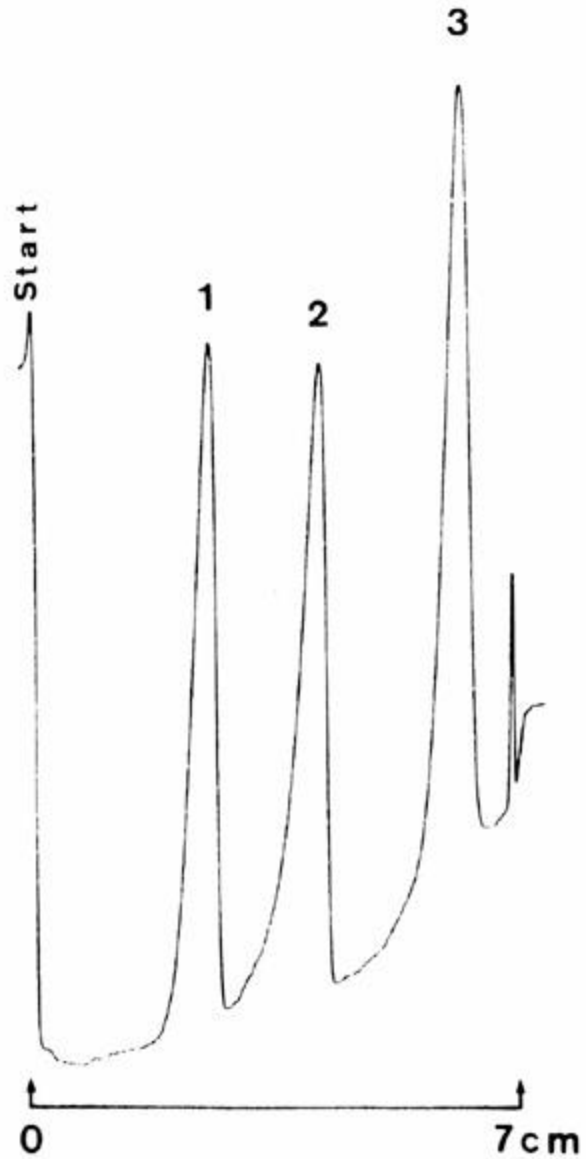
Rf: 0,58

Rf: 0,58

Chromatographic Data

Track No.	Compounds	Concentration (mg/ml)	Solvent	Application volume µl	hRf
Formulation 1	1 Placebo	~ 10	THF	2	
	2 1 % Vit C derivat	~ 10	THF	2	58
	3 1 % Active 2	~ 10	THF	2	58
	4 2 % Vit C Derivat	~ 10	THF	2	58
	5 2 % Active2	~ 10	THF	2	58
6, 7, 8, 9	Reference Vitamin C Derivat	1	Heptan/THF 1:1	0.2, 0.5, 1, 5	58
Formulation 2	10 Placebo	~ 10	THF	2	
	11 1 % Vit C derivat	~ 10	THF	2	58
	12 1 % Active 2	~ 10	THF	2	
	13 2 % Vit C derivat	~ 10	THF	2	58
	14 2 % Active 2	~ 10	THF	2	
15, 16, 17, 18	Reference Degradation product	1	THF	0.2, 0.5, 1, 5	77

water soluble vitamins 60 RP-18 F254s



TLC pre-coated plate RP-18 F 254s with concentrating zone (1.15498)

etonitrile/dichlormethane/water 90/10/10 (v/v)

th addition of 0,1 mol/l lithium chloride

:m

rmal chamber without chamber saturation

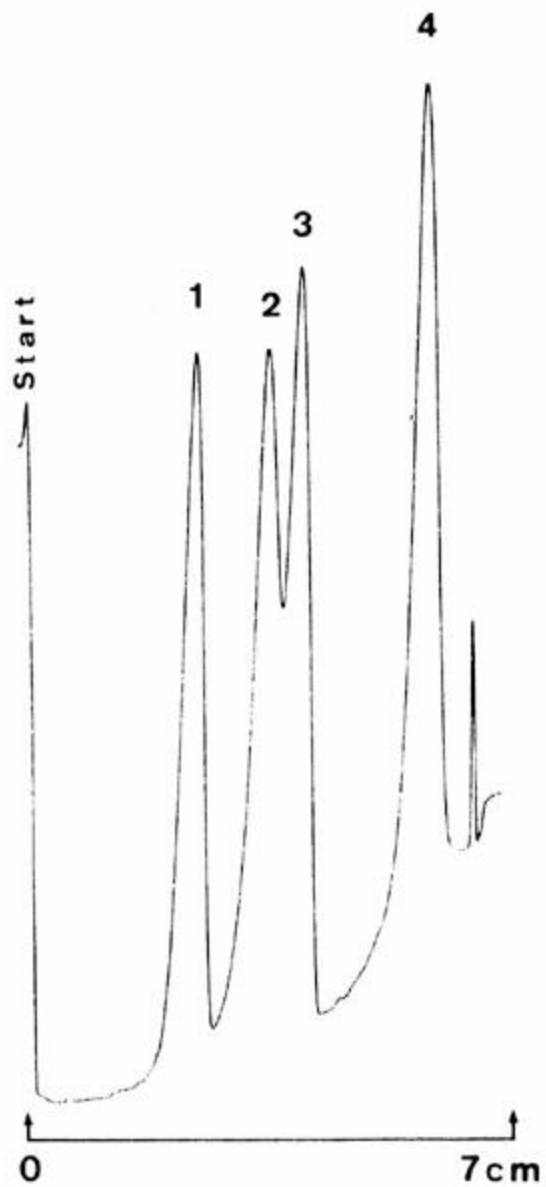
Nicotin amide

Nicotinic acid

L-Ascorbic acid (all 0,05%)

il

situ evaluation with TLC/HPTLC scanner (Camag) UV 270 nm



water soluble vitamins on 60 RP-18 F254s

HPTLC pre-coated plate RP-18 F 254s with concentrating zone (1.15498)

Acetonitrile/dichlormethane/water 90/10/10 (v/v)

with addition of 0,1 mol/l lithium chloride

7 cm

Normal chamber without chamber saturation

1. Nicotin amide

2. Iso-Nicotinic acid

3. Nicotinic acid

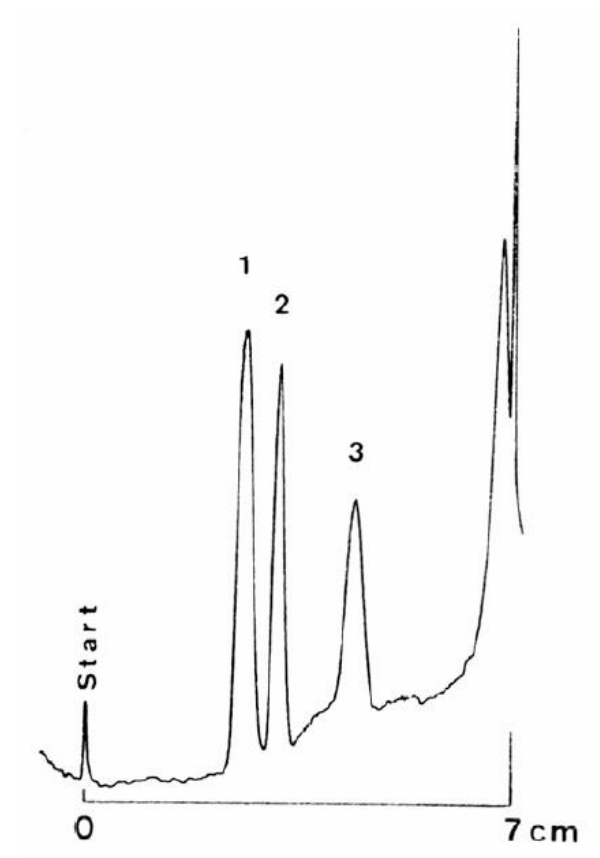
4. L-Ascorbic acid (all 0,05%)

1 μ l

In-situ evaluation with TLC/HPTLC scanner (Camag) UV 270 nm

Separation of vitamin HPTLC NH2 F254s

Plate	HPTLC pre-coated plate NH ₂ F 254s (P/N: 1.13192, Alternative P/N: 1.15647)
Eluent	Acetonitrile/water 70/30 (v/v)
Migration Distance	7 cm
Chamber	Normal chamber without chamber saturation
Compounds	1. Vitamin B6 2. Vitamin B12 3. Vitamin B1
Application volume	300 nl
Detection	In-situ evaluation with TLC/HPTLC scanner (Camag) UV 280 nm



1

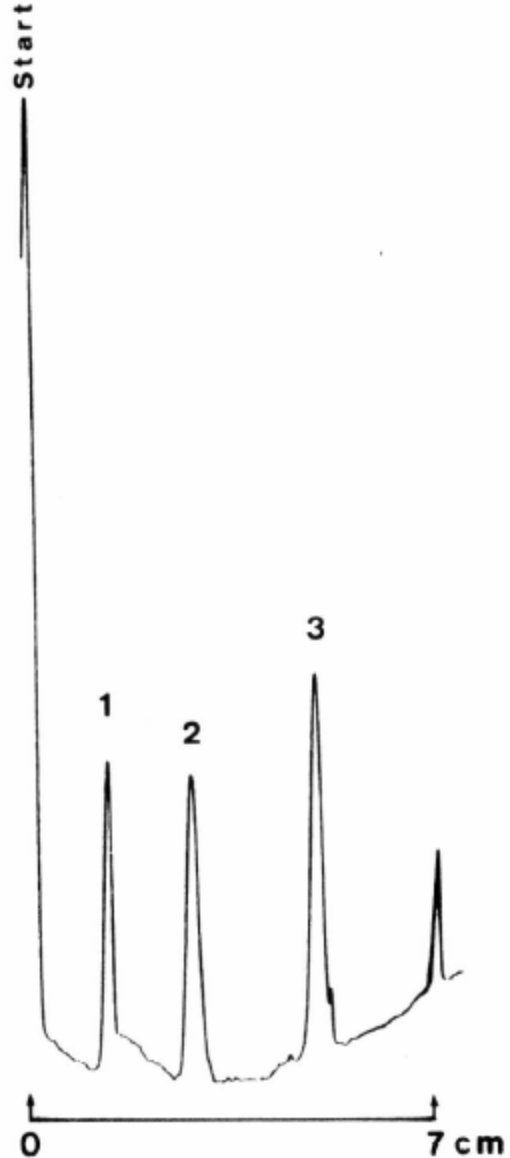
150 F254

rt

2

3

MERCK



of water soluble vitamins) F254

	TLC pre-coated plate gel 60 F254 with concentrating zone (P/N 1. 13728, alt P/N 1. 13727)
	Chloroform/methanol 60/40 (v/v) 80% saturated with water with addition of 0,01 mol/l lithium chloride
	7 cm
	Normal chamber without chamber saturation
	1. L-Ascorbic acid 2. Nicotinic acid 3. Nicotin anide (all 0,05%)
	1 µl
	In-situ evaluation with TLC/HPTLC scanner (Camag) UV 270 nm

Vitamin analysis

HPLC-RP

Separating Fat-Soluble Vitamins by Reversed Phase HPLC, Using Discovery Columns

Figure A. Vitamins A and E on a Discovery C18 Column

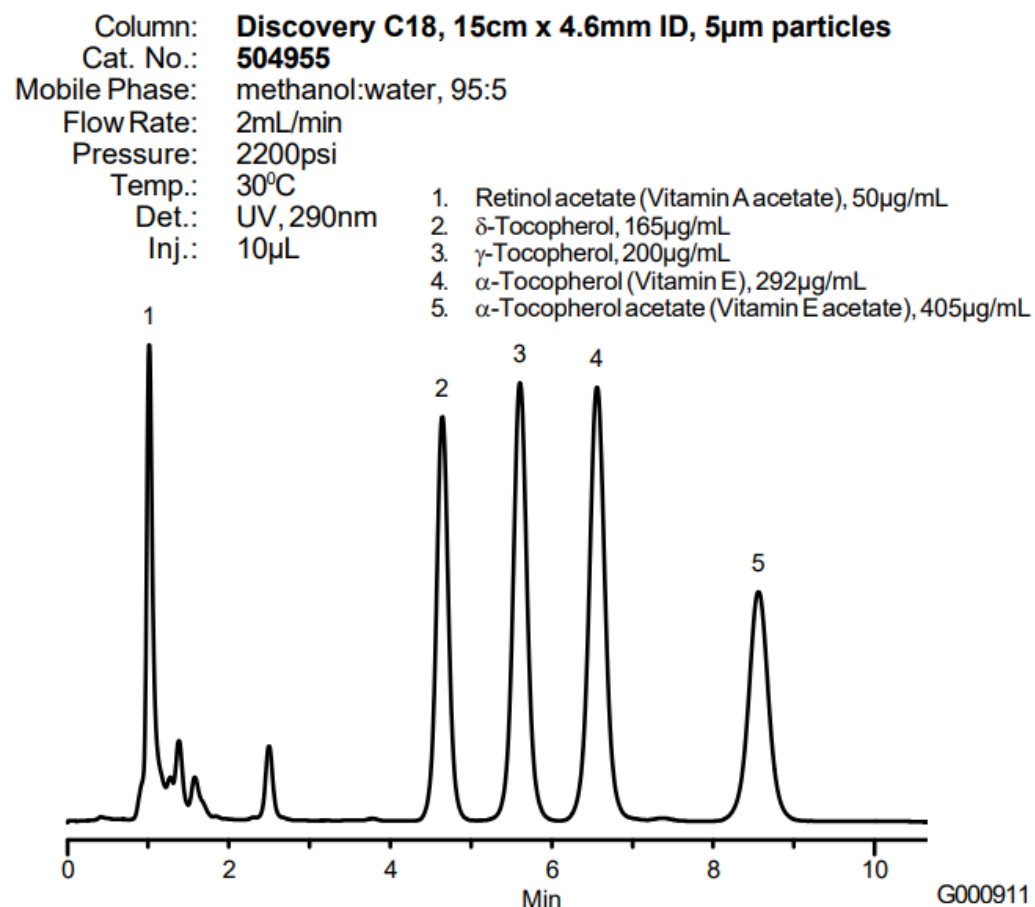


Figure F. Vitamins D₂ and D₃ on a Discovery C18 Column

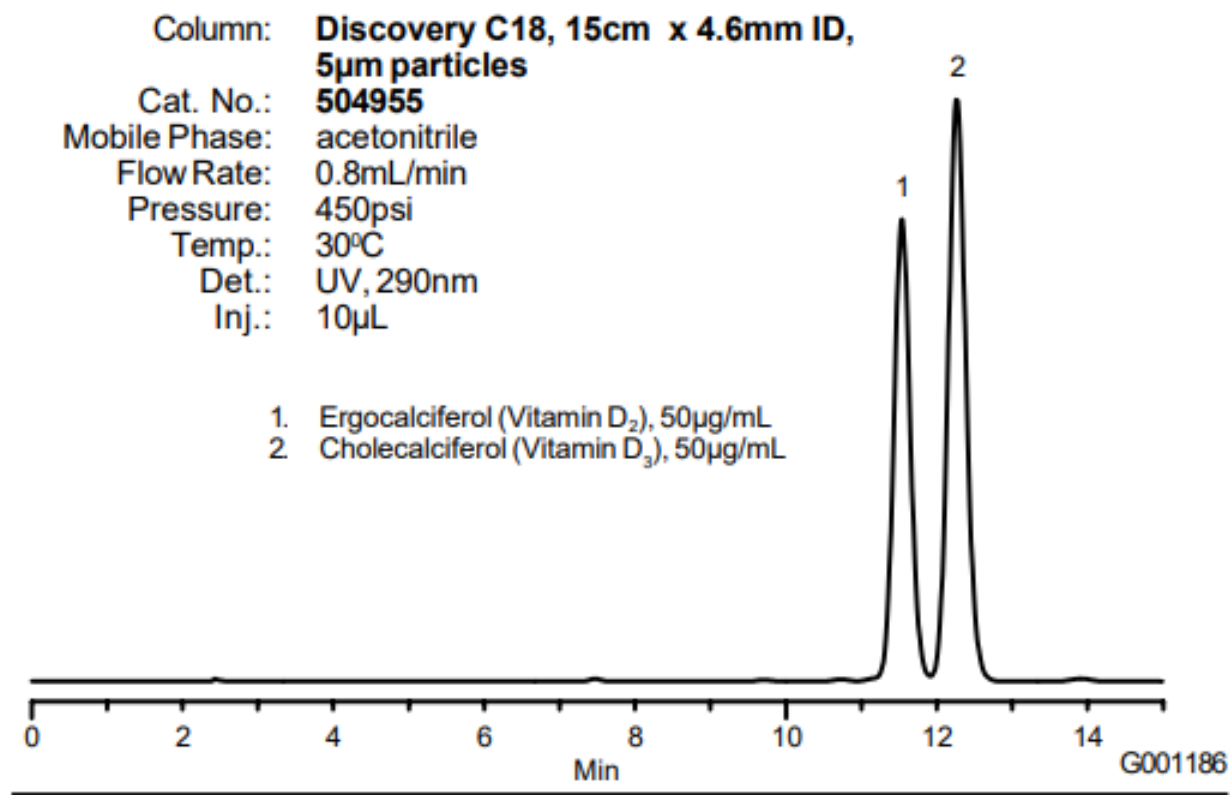


Figure B. Vitamins A and E on a Discovery C8 Column

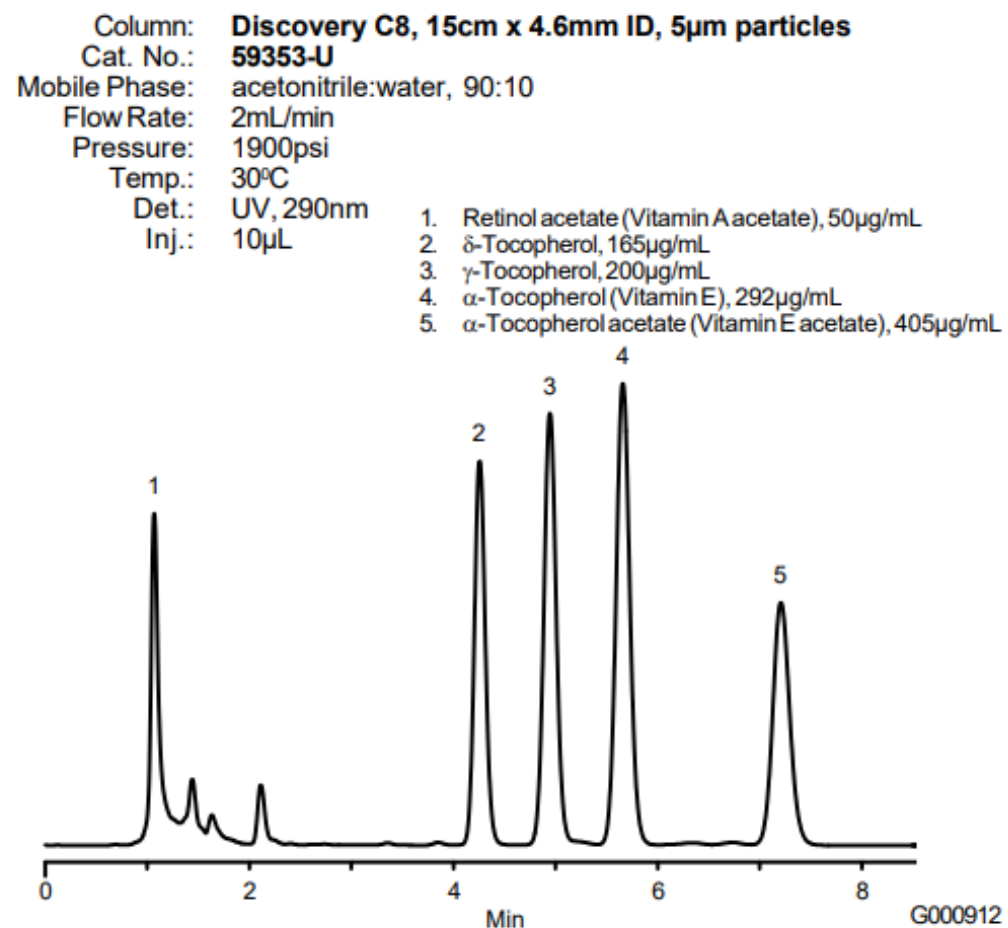


Figure D. Fat-Soluble Vitamins in Centrum Multivitamin Liquid

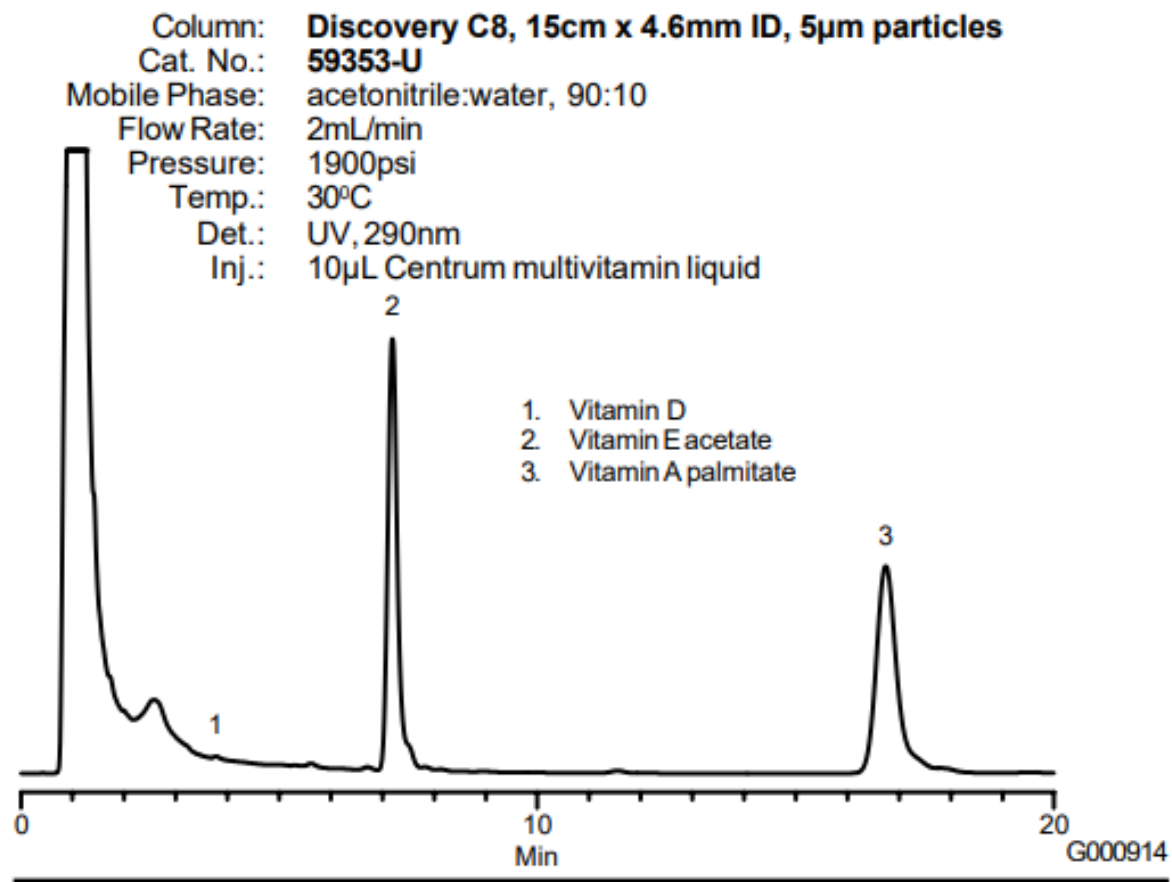


Figure C. Vitamins A, D, and E on a Discovery C8 Column

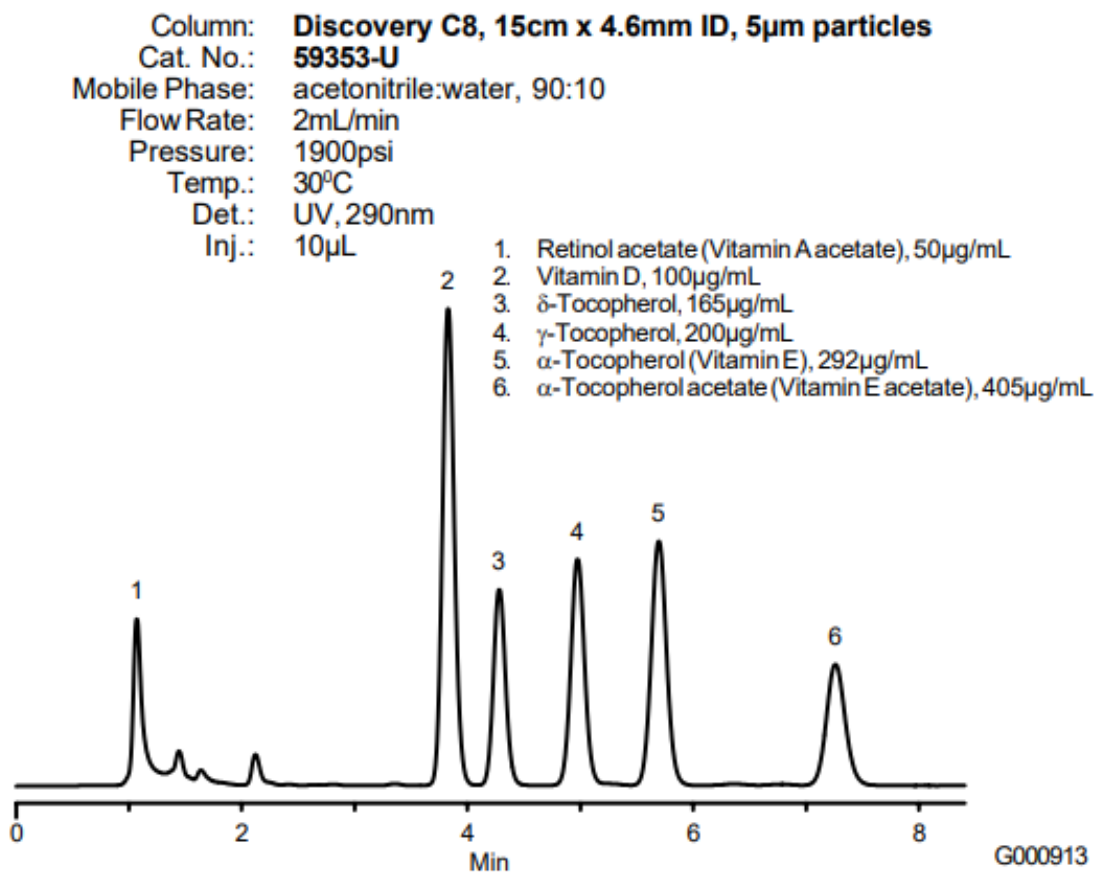
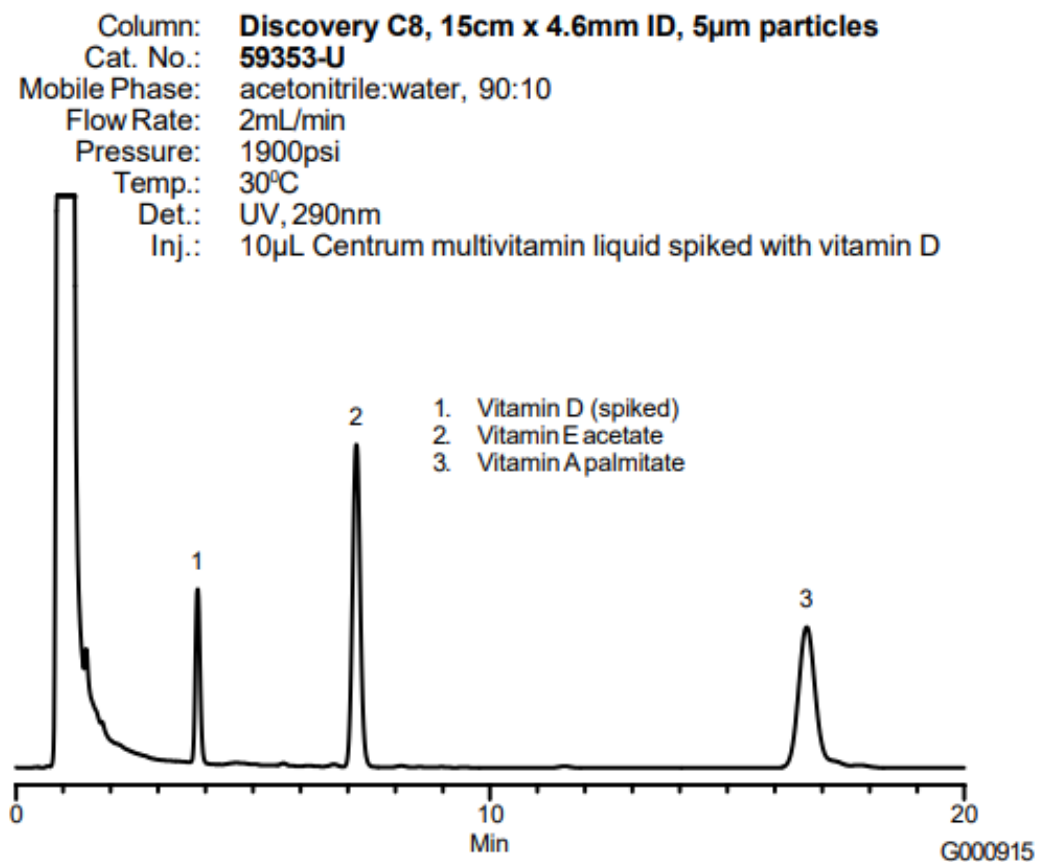
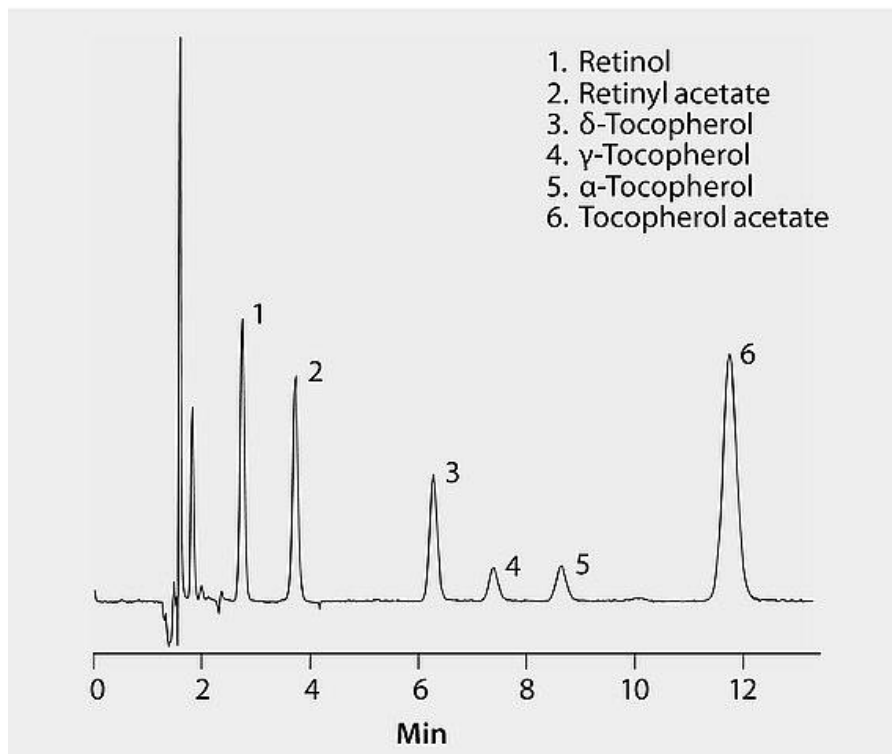


Figure E. Fat-Soluble Vitamins in Centrum Multivitamin Liquid, Spiked with Vitamin D



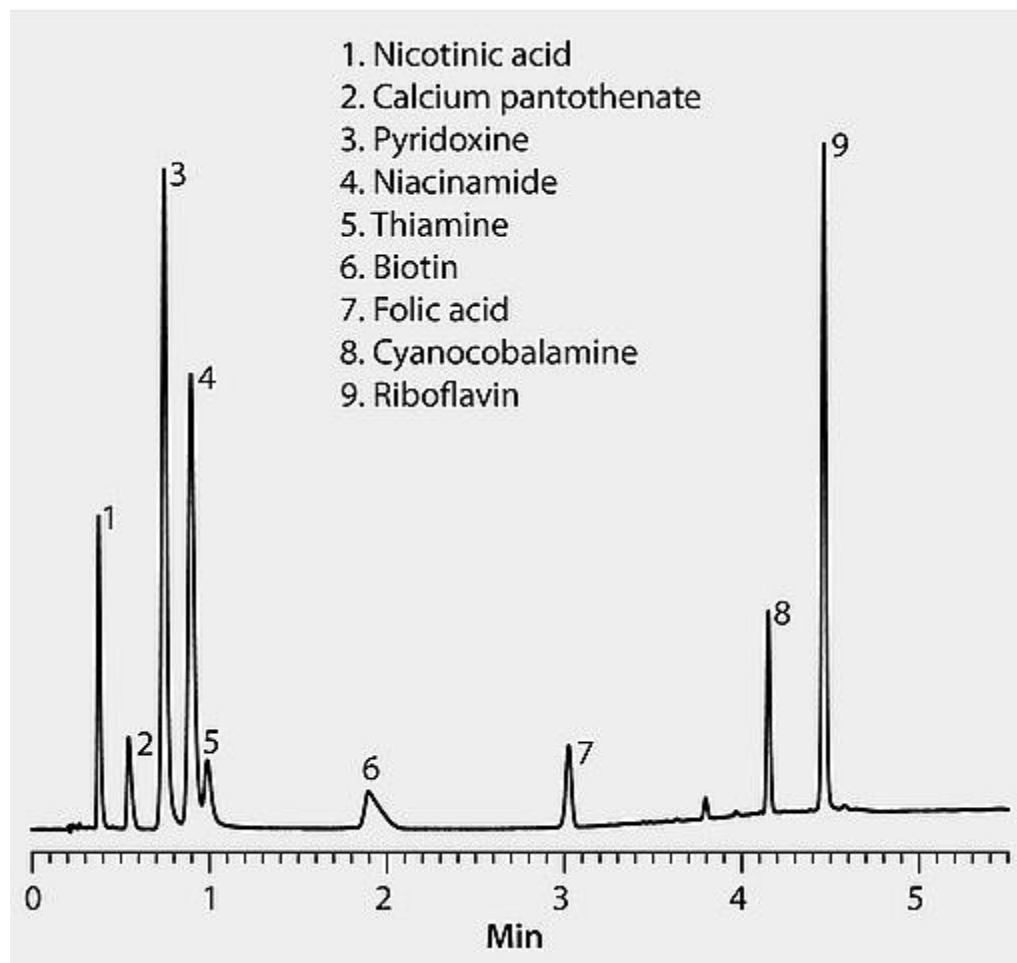
HPLC Analysis of Vitamins, Fat Soluble (A and E), on SUPELCOSIL™ LC-18



CONDITIONS

column	SUPELCOSIL™ LC-18, 25 cm × 4.6 mm I.D., 5 µm particles (58298)
mobile phase	[A] methanol: [B] deionized water (98:2, A:B)
flow rate	2 mL/min
sample	0.2-1 mg/mL each analyte in methanol
injection	20 µL
detector	UV, 325 nm (retinol, retinyl acetate) or 290 nm (tocopherols, tocopherol acetate)

HPLC Analysis of Water-Soluble B-Vitamins on Ascentis® Express C18

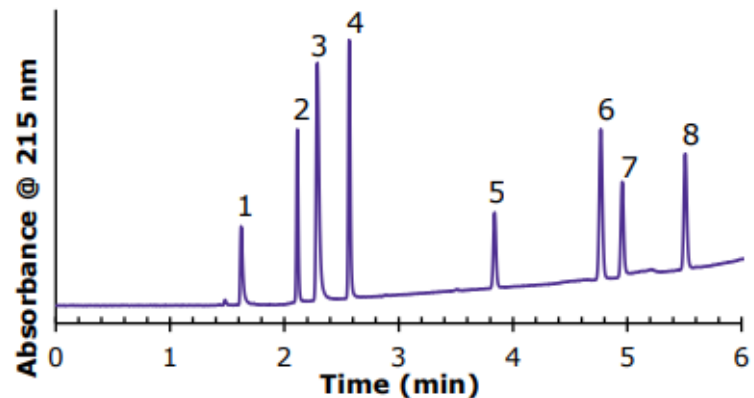


CONDITIONS

column	Ascentis® Express C18, 5 cm x 3.0 mm I.D., 2.7 µm particles (53811-U)
column temp.	35 °C
mobile phase	[A] 20 mM potassium phosphate, dibasic, pH 7; [B] methanol
gradient	0.5% B for 1.3 min, 0.5-30%B over 1.7 min; to 30% B in 1.4 min, 2 min equilibration at 0.5% B
flow rate	1.0 mL/min
pressure	4130 psi (285 bar)
sample	9 vitamins in water, 10 µg/mL for nicotinic acid and folic acid, 12 µg/mL for thiamine hydrochloride and cyanocobalamin, (20 µg/mL for pyridoxine, 30 µg/mL for riboflavin, 40 µg/mL for niacinamide, 100 µg/mL for calcium pantothenate, 120 µg/mL for biotin)
detector	UV, 210 nm

HPLC Analysis of Water Soluble Vitamins on Ascentis® Express AQ-C18, 5 µm

- ❑ Water soluble vitamins are important for various functions in the human body.
- ❑ They are naturally found in fruits, vegetables, and some animal products, and can also be taken as supplements.
- ❑ They are also commonly added to processed and fortified foods.
- ❑ The Ascentis® Express AQ-C18 is ideal for the separation of water soluble molecules such as these due to its resistance to dewetting when using aqueous mobile phases.



Peak Number	Compound
1	Thiamine (B1)
2	Ascorbic Acid (C)
3	Nicotinamide (B3)
4	Pyridoxine (B6)
5	Pantothenic Acid (B5)
6	Cyanocobalamin (B12)
7	Folic Acid (B9)
8	Riboflavin (B2)

Conditions:

column: Ascentis® Express AQ-C18, 25 cm x 4.6 mm I.D., 5 µm

mobile phase: [A] 0.025 M potassium phosphate in water, pH 2.4; [B] Methanol

gradient: 0% B to 65% B in 6 min; hold at 65% B for 2 min.

flow rate: 1.5 mL/min

column temp.: 30 °C

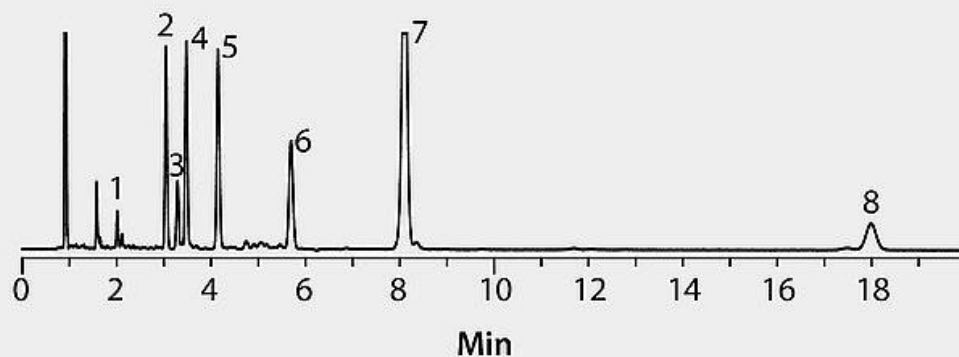
detector: UV, 215 nm

injection: 1 µL

sample: Water soluble vitamins, varied concentration, water

HPLC Analysis of Fat-Soluble Vitamins on Ascentis® Express C18, Methanol Mobile Phase

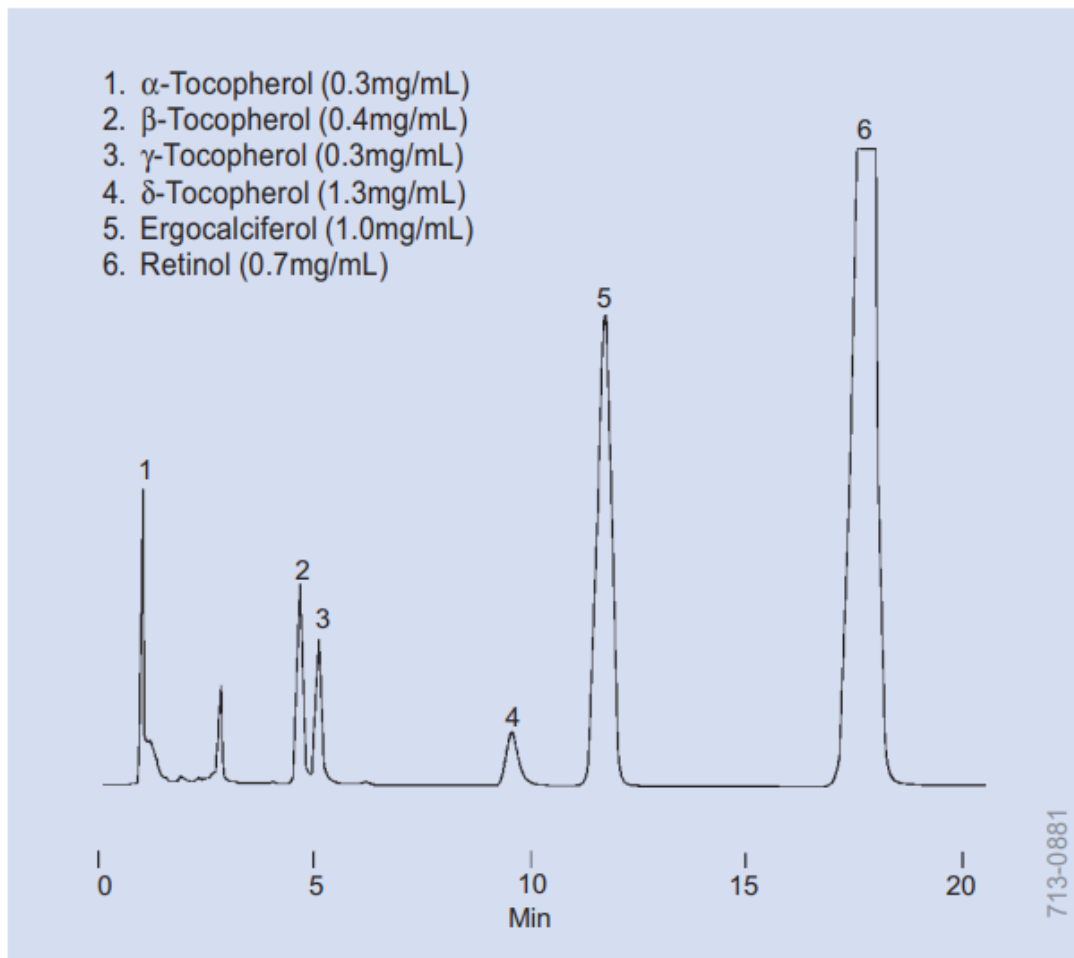
1. Retinol acetate (A)
2. Delta-tocopherol (E)
3. Ergocalciferol (D2)
4. Cholecalciferol (D3)
5. Alpha-tocopherol (E)
6. DL-alpha-tocopherol acetate (E)
7. Phylloquinone (K1)
8. Retinol palmitate (A)



Column	Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U)
Column Temp.	30 °C
Mobile Phase	Methanol
Flow Rate	1.5 mL/min
Pressure	3249 psi (224 bar)
Sample	0.5 mg/mL tocopherol acetate, alpha-tocopherol, delta-tocopherol and K1, 0.05 mg/mL D2, D3 and retinol acetate, 1.5 mg/mL retinol palmitate, all in ethanol.
Injection	5 µL
Detector	UV, 280 nm

Vitamin analysis

HPLC-NP



Vitamins, Fat Soluble (A and E) (HPLC)

Column: SUPELCOSIL LC-Si, 15cm x 4.6mm ID, 5 μ m particles

Cat. No.: 58200-U

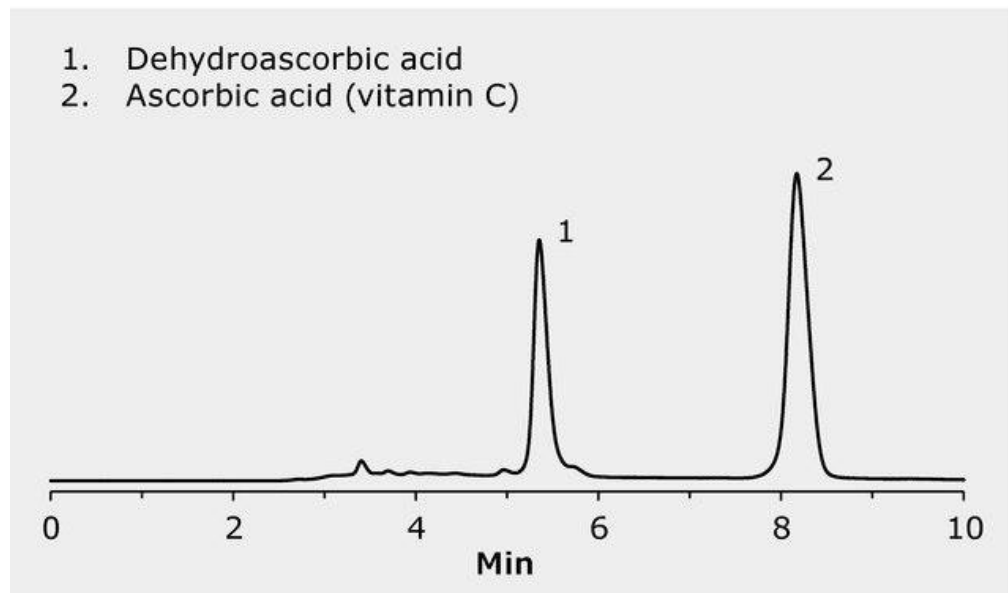
Mobile Phase: hexane:amyl alcohol (99.65:0.35)

Flow Rate: 2mL/min

Det.: UV, 280nm

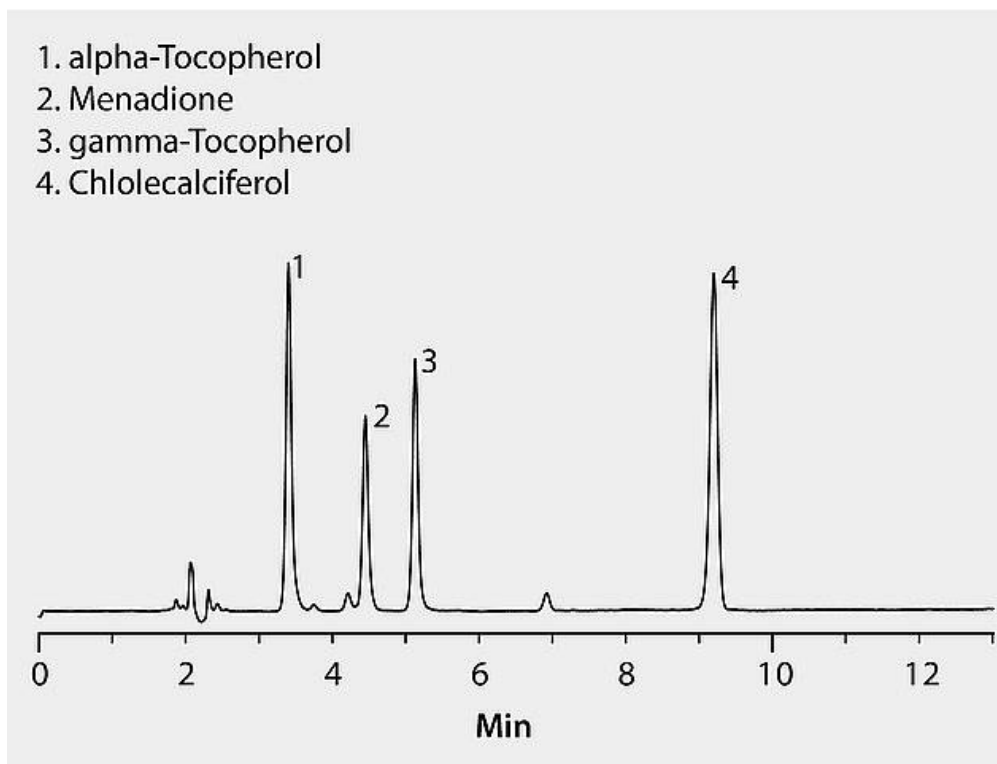
Inj.: 20 μ L

HPLC Analysis of Dehydroascorbic Acid and Ascorbic Acid (Vitamin C) on SeQuant ZIC-HILIC



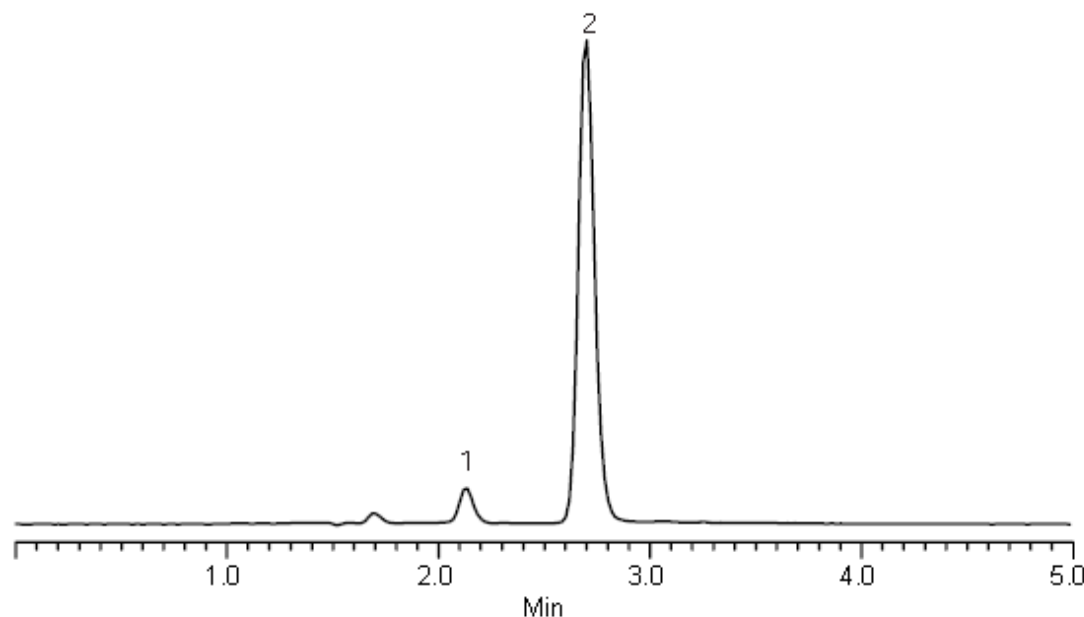
column	SeQuant ZIC-HILIC, PEEK, 150 x 4.6 mm, 5 µm, 200 Å... (1.50455.0001)
mobile phase	[A] acetonitrile; [B] ammonium acetate; (70:30, A,B)
flow rate	0.5 mL/min
pressure	375 psi (2.6 MPa)
injection	5 µL
detector	240 nm

HPLC Analysis of Fat Soluble Vitamins by Normal Phase Chromatography on Ascentis® Si



Column	Ascentis Si, 15 cm x 4.6 mm I.D., 5 µM particles (581512-U)
Column Temp.	30 °C
Mobile Phase	[A] hexane; [B] ethyl acetate
Gradient	10 to 30% B in 10 min; held at 30% B for 2 min
Flow Rate	1.0 mL/min
Injection	10 µL
Detector	UV, 290 nm
Sample	each compound in hexane:isopropanol (96:4)

Analysis of Ascorbic Acid and Dehydroascorbic Acid Using Ascentis RP-Amide



Conditions

column: Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 μ m particles (565324-U)
mobile phase: 25 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid)
flow rate: 1 mL/min.
temp.: 35 °C
det.: UV, 230 nm
injection: 10 μ L
sample: as listed each in mobile phase

Peak IDs

1. Dehydroascorbic acid (100 μ g/mL)
2. Ascorbic acid (20 μ g/mL)

Application note for vitamin analysis

<https://www.sigmaaldrich.com/TH/en/technical-documents/protocol/analytical-chemistry/small-molecule-hplc/dissolution-testing-folic-acid-tablets>

<https://www.sigmaaldrich.com/TH/en/technical-documents/protocol/analytical-chemistry/small-molecule-hplc/determination-vitamin-a-e-using-hplc-uv-detection-gb-method>

<https://www.sigmaaldrich.com/TH/en/technical-documents/protocol/food-and-beverage-testing-and-manufacturing/water-soluble-vitamins-titan-c18>

<https://www.sigmaaldrich.com/TH/en/technical-documents/technical-article/analytical-chemistry/solid-phase-extraction/fast-and-accurate-analysis-of-vitamin-d-metabolites>

Molnupiravir and Ritonavir

(the Co-administration of PAXLOVID)

Analytical standard for research and testing

EIDD-2801 analytical standard
for Molnupiravir

SCAN FOR MORE
INFORMATION



Ritonavir Certified
Reference Material

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special offer : Simple HPLC Method for Molnupiravir
and Ritonavir measurement

Column C18 HPLC column
(Purospher™ STAR HPLC Columns)



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SUPELCO FESTIVAL LIVE IN LOY KRATONG DAY

-How to deal with wastewater-

on Facebook Live
19th November 2021
Time: 17.09 – 17.39 pm

