

Constituents and Additives

Food products are analyzed for a variety of reasons, e.g., compliance with legal and labeling requirements, assessment of product quality, determination of nutritive value, and detection of adulteration, etc. According to the Codex Alimentarius Commission – Food Additive means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value. The intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging transport or holding of such food results, or may be reasonably expected to result (directly or indirectly) in it or its by-products becoming a component or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving its nutritive value. Food additives do not include use of vitamins, minerals, herbs, salt, spices, yeast, hops, starter cultures, malt extract, etc. Food additives are intentionally added to food and must be safe for a lifetime of consumption based on current toxicological evaluation.

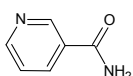
Food additives are classified on the basis of their functional use and are grouped as:

Colors	Preservatives	Acidity Regulators
Antioxidants	Ant caking agents	Antifoaming Agents
Artificial sweeteners	Enzymes	Emulsifiers
Emulsifying agents	Flavors	Flavor enhancers
Modified Starches	Phosphates	Stabilizers
Thickening and jellying agents.		

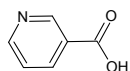
Examples provided so far in this compilation have been focused on adulteration and potential threats. The following applications illustrate analysis of sugars, vitamins and organic acids, ie, typical constituents and additives in food.

Vitamins

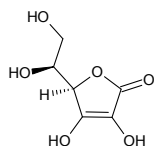
Nicotinamide
pKa 3.35 logP -0.37



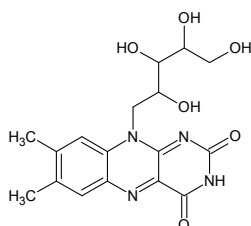
Nicotinic acid
pKa 3.0 4.7 logP 0.36



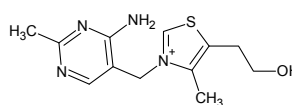
Ascorbic acid
pKa 4.1, 11.2 logP -1.85



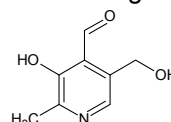
Riboflavin
pKa 10.2 logP -1.46



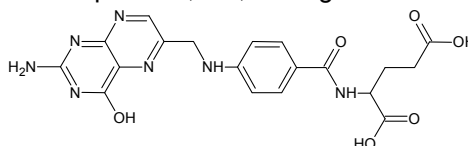
Thiamin
pKa 4.8, 9.0 logP -4.6



Pyridoxine
pKa 5.6 8.6 logP -0.77



Folic acid
pKa 2.7, 4.1, 8.9 logP -0.02



The term vitamin is derived from "vitamine," a combination of vital and amine, i.e. amine of life. 100 years ago it was believed that organic micronutrient food factors that prevent dietary-deficiency diseases might be chemical amines. Later this proved incorrect. Today it is well established that a vitamin is an organic compound being a vital nutrient in tiny amounts, not endogenously synthesized in enough quantities by the organism, and hence must be obtained from the diet. There are thirteen vitamins, classified by their biological and chemical activity, not their structure,, four are fat-soluble (A, D, E, and K) and the other nine are water-soluble (B1, B2, B3, B5, B6, B7, B9, B12, C). Ascorbic acid (vitamin C) is a vitamin for human, but not for most other animals. The largest number of vitamins (e.g., B complex vitamins) function as precursors for enzyme cofactors, that help enzymes in their work as catalysts in metabolism.

This application compilation focus on the hydrophilic and polar compounds, e.g. the water-soluble vitamins, and two examples are presented.

Further reading:

<http://www.nlm.nih.gov/medlineplus/ency/article/002399.htm>

<http://en.wikipedia.org/wiki/Vitamin>

Determination of Water Soluble Vitamins

SeQuant® ZIC®-HILIC

Column: SeQuant® ZIC®-HILIC (3.5 µm, 100 Å) PEEK 150×2.1 mm (1.50442.0001)

Recommended solvents and reagents

Acetonitrile: Hypergrade for LC-MS LiChrosolv® (1.00029)

Water: Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q® water purification system

Formic acid: 98-100% for analysis EMSURE® ACS, Reag. Ph Eur (1.00264)

Ammonium formate: Use ACS grade or HPLC grade.

Recommended filtration tools

Mobile phase filtration:

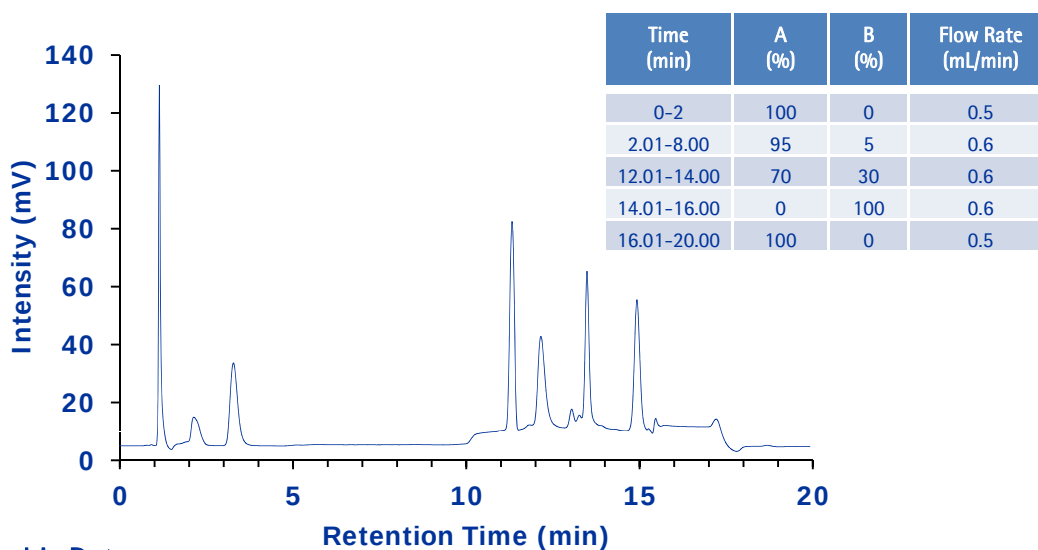
PTFE coated with funnel, base, stopper clamp (XX1004720)
Omnipore PTFE membrane filter 0.45µm (JHWP04700)

Determination of Water Soluble Vitamins

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (3.5 µm, 100Å) PEEK 150x2.1 mm	(1.50442.0001)
Injection:	5 µL	
Detection:	UV at 254 nm. Shimadzu LC-20 equipped with 2.5µL semi-micro flow-cell	
Flow Rate:	See Table	
Mobile Phase (v/v):	A: Acetonitrile and 100 mM Ammonium formate (50 mM), pH 3.5; 90:10 (v/v) B: Acetonitrile and 100 mM Ammonium formate (50 mM), pH 3.5; 50:50 (v/v)	
Gradient:	See Table.	
Temperature:	30 °C	
Diluent	Initial mobile phase	
Sample:	40 ppm of each vitamin in initial mobile phase composition	



Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (USP)
	Void volume (t ₀)	0.6	-
1	Nicotinamide	1.1	1.6
2	Pyridoxal	2.1	1.6
3	Nicotinic Acid	3.3	1.2
4	Ascorbic Acid	11.3	1.0
5	Thiamine	12.2	1.3
6	Riboflavin	13.5	1.1
7	Folic Acid	14.9	1.1

Determination of Ascorbic acid

SeQuant® ZIC®-HILIC

Column: SeQuant® ZIC®-HILIC (5 µm, 200 Å) PEEK 150×4.6 mm (1.50455.0001)

Recommended solvents and reagents

Acetonitrile: Isocratic grade for HPLC LiChrosolv® (1.14291)

Water: Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q® water purification system

Ammonium acetate: for analysis EMSURE® ACS, Reag. Ph Eur (1.01116)

Recommended filtration tools

Mobile phase filtration:

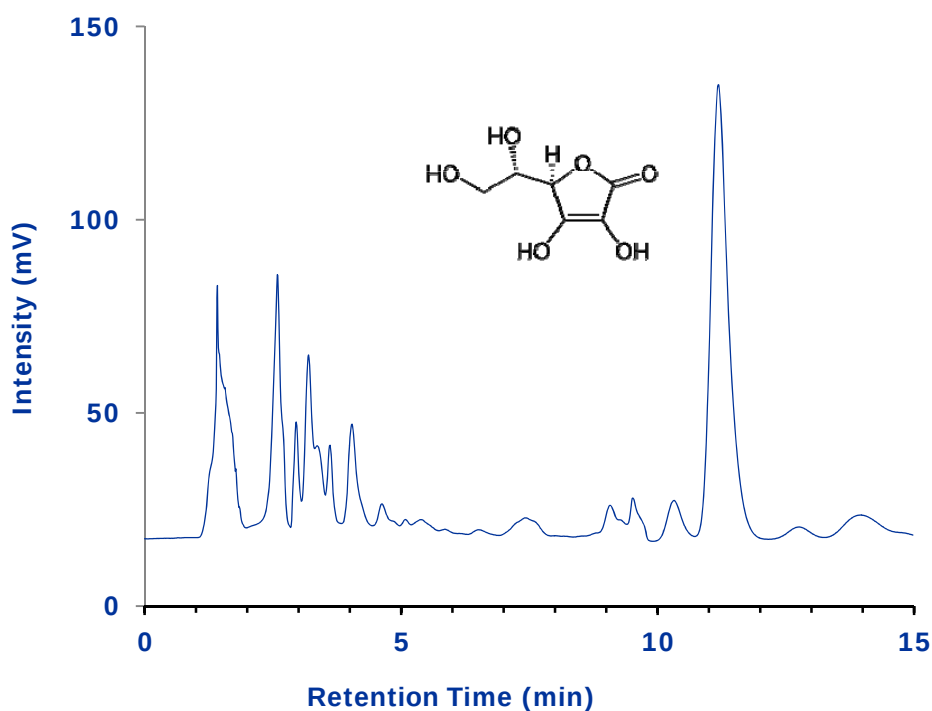
PTFE coated with funnel, base, stopper clamp (XX1004720)
Omnipore PTFE membrane filter 0.45µm (JHWP04700)

Determination of Ascorbic Acid in Wine

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5µm, 200Å) PEEK 150x4.6 mm	(1.50455.0001)
Injection:	250 µL	
Detection:	UV at 240 nm. Shimadzu LC-10Vp equipped with 2.5µL semi-micro flow-cell	
Flow Rate:	1.0 mL/min.	
Mobile Phase (v/v):	Acetonitrile and 200mM Ammonium acetate buffer, pH 6.8 (80:20)	
Pressure drop:	50 Bar (720 psi)	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	Riesling wine diluted 1:3 (v/v) with acetonitrile	



Chromatographic Data

No.	Compound	Time (min)	Retention Factor	Tailing Factor (USP)
	Void volume (t ₀)	1.5		
2	Ascorbic Acid	11.3	6.5	1.0