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 Alimentarious 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:

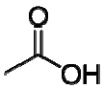
Colors	Preservatives	Acidity Regulators
Antioxidants	Ant caking agents	Antifoaming Agents
Artificial sweeteners	Enzymes	Emulsifiers
Emulsifying agents	Flavors	Flavor enhancers
Modified Starches	Phosphates	Stabilizers

Thickening and jellying agents.

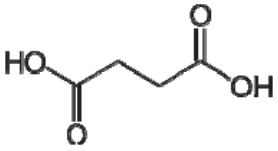
Examples provided so far in this compilation have been focused on adulteration and potential threats. The following applications illustrate analysis of sugars, vitamins and organic acids, ie, typical constituents and additives in food.

Organic acids

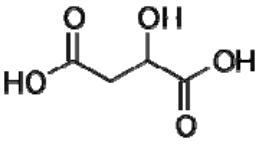
ZIC®-cHILIC coming soon



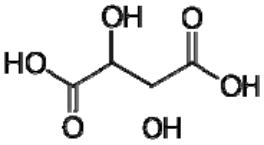
Acetic acid



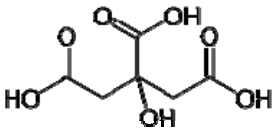
Succinic acid



Malic acid



Tartaric acid



Citric acid

Preserved by addition of Benzoic acid, lactic acid or pickled in acetic acid. Sushi, sauerkraut, balsamic vinegar and of course beer, vodka and it is crucial to get the right balance of organic acids in wine. Organic acids are present in every meal we eat. Separating organic acids with HILIC is orthogonal to using Reversed Phase Chromatography, the difficult hydrophilic acids citric and tartaric acid will be well retained in and well separated, there will be no co-elution of malic acid and succinic acid as is often the case in ion chromatography.

In grapes the predominant organic acids are tartaric and malic acid while succinic and citric acids are present in minor proportions. In winemaking a common differentiation is made between acids which come directly from the grape (tartaric, malic and citric acids) and those that are produced in the fermentation process (succinic, lactic and acetic acids).

HILIC has so far mainly been used as an MS friendly technique using volatile acetate or formate buffers. For QC applications with UV detectors RP columns and phosphate buffer has so far been predominant. Using HILIC with low UV cut of buffers like phosphate is possible despite the limited solubility of potassium phosphate in high acetonitrile eluents. There are some rules to using phosphate buffer in HILIC, the same rules apply to RP when using a high proportion of acetonitrile in the eluent.

1. Always use premixed eluents, never use pure Acetonitrile as one mobile phase constituent.
2. Never use over 80% Acetonitrile, at low buffer strengths 85% is the absolute maximum.
3. If using gradients make the difference between mobile phase A and B as small as possible.
4. HILIC gradients should be shallower than in RP since changes in mobile phase has a larger effect in HILIC than in RP.

Determination of Organic Acids in wine

SeQuant® ZIC®-cHILIC

ZIC®-cHILIC coming soon

Column:	SeQuant® ZIC®-cHILIC (3 µm, 100Å) PEEK 150×2.1 mm	(1.50658.0001*)

Recommended solvents and reagents

Acetonitrile	Isocratic grade for HPLC LiChrosolv®	(1.14291)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q® water purification system	(1.15333)
Potassium phosphate	for analysis EMSURE® ISO	(1.04873)

Recommended filtration tools

Mobile phase filtration:

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

*For more information on specifications and launch of SeQuant® ZIC®-cHILIC, please contact your Merck Millipore sales representative or visit www.merckmillipore.com/chromatography or www.sequant.com/zicchilic.

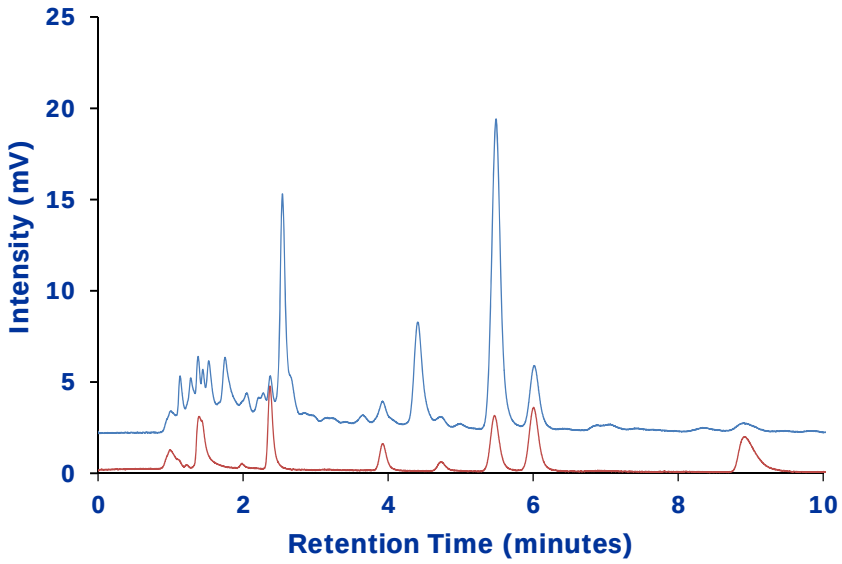
Determination of Organic Acids in wine

SeQuant® ZIC®-cHILIC

ZIC®-cHILIC coming soon

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3 µm, 100Å) PEEK 150x2.1 mm	(1.50658.0001*)
Injection:	5 µL	
Detection:	UV at 200 nm. Shimadzu LC-10Vp equipped with 2.5µL semi-micro flow-cell	
Flow Rate:	0.3 mL/min.	
Mobile Phase (v/v):	Acetonitrile and 25mM Potassium Phosphate buffer pH 6.0 (75:25)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	Riesling wine (blue), 10ppm mix of standards (red)	



Chromatographic Data

No.	Compound	Time (min)	Retention Factor
	Void volume (t0)	1	-
1	Acetic acid	2.4	1.4
2	Succinic acid	3.9	2.9
3	Malic acid	5.5	4.5
4	Tartaric acid	6.0	5.0
5	Citric acid	8.9	7.9

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
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