Constituents and Additives

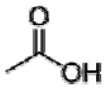
Food products are analyzed for a variety of reasons, e.g., compliance with legal and labeling requirements, assessment of product quality, determination of nutritive value, and detection of adulteration, etc. According to the Codex Alimentarious Commission – "Food Additive" means any substance not normally 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 term "Food additive" 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:

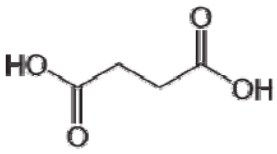
Colors	Preservatives	Acidity Regulators
Antioxidants	Anti-caking agents	Antifoaming Agents
Artificial sweeteners	Enzymes	Emulsifiers
Emulsifying agents	Flavors	Flavor enhancers
Modified Starches	Phosphates	Stabilizers

Thickening and jellying agents.

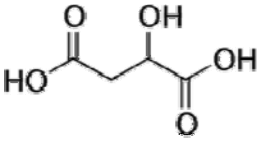
Organic Acids



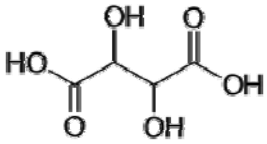
Acetic acid



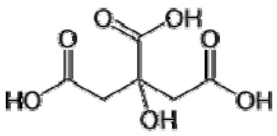
Succinic acid



Malic acid



Tartaric acid



Citric acid

Organic acids are organic compounds with acidic properties where the carboxylic acids are the most common; being weak acids that do not dissociate completely in water. The predominant organic acids in grapes 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). Organic acids are also used in food preservation because they can penetrate bacteria's cell wall and disrupt their normal physiology. Hence, organic acids are present in every meal we eat, and there is necessary to have analytical methods able of accurate determination (both quantitatively and qualitatively).

Organic acids are hydrophilic compounds, and to be retained in reversed phase mode it is a requirement to either add ion-pairing reagents, work at low pH, and or use completely aqueous mobile phases. In this compilation, we have included a method for determination of tartaric acid, malic acid, citric acid and succinic acid following the current Chinese Standard method: GB/T 5009.157-2003 Determination of organic acid in foods and applied said method for determination of citric acid.

Analysis of Organic Acids in Beverages

Recommended column:
Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm (1.52022.0001)

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

Di-ammonium hydrogenphosphate for analysis EMSURE® ACS,ISO,Reag. Ph Eur (1.01207)
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur (1.00573)

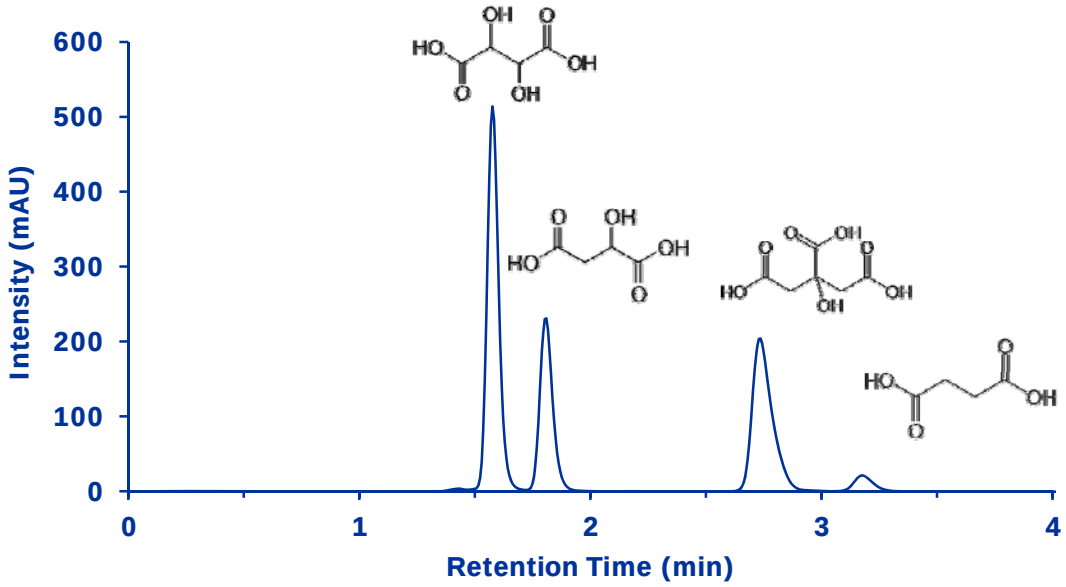
Sample Preparation
Sample: Commercial orangeade (soft drink)
Take 5 mL of orangeade and sonicate for 5 minutes, thereafter add 0.2 mL of phosphoric acid solution (1M) and make up to a final volume of 10mL by adding water. This gives a dilution factor of 2 for sample.

Analysis of Organic Acids - Standards

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	(1.52022.0001)
Injection:	20 µL	
Detection:	UV, 210 nm	
Cell:	1 µL/10 mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	10 mM Di-ammonium hydrogen phosphate solution (pH 2.7)	
Temperature:	30 °C	
Diluent	water	
Sample:	Standard solution with 1mg/ml of tartaric acid, malic acid and citric acid, and 0.2mg/ml of succinic acid diluted in water	
Pressure Drop:	32 Bar (464psi)	



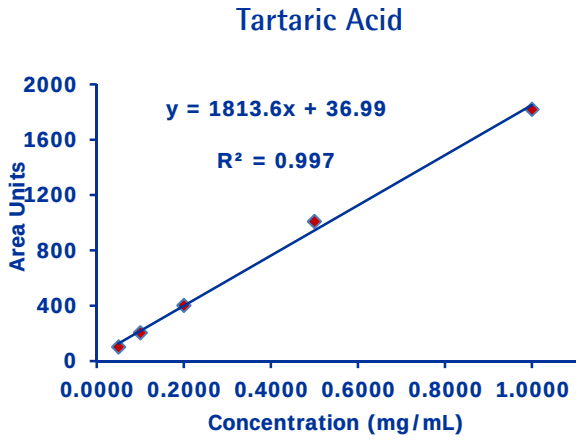
Chromatographic Data

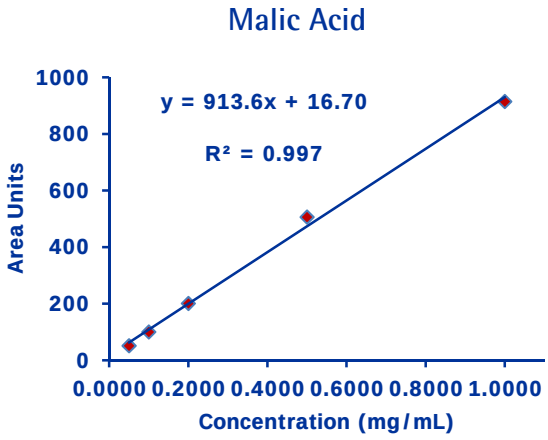
No.	Compound	Retention Time (min)	Retention factor	Asymmetry
1	Tartaric Acid	1.6	0.6	1.2
2	Malic Acid	1.8	0.8	1.2
3	Citric Acid	2.7	1.7	1.4
4	Succinic Acid	3.2	2.2	1.2

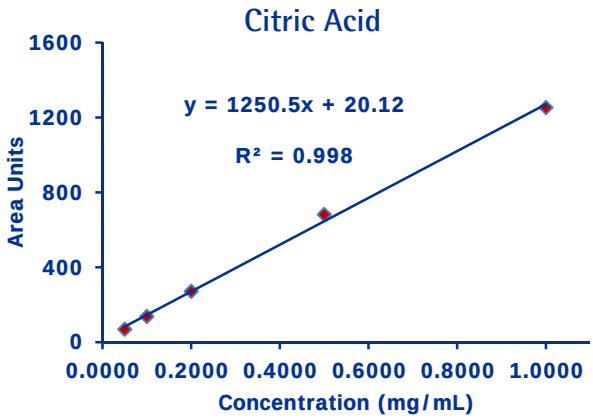
Analysis of Organic Acids in Beverages

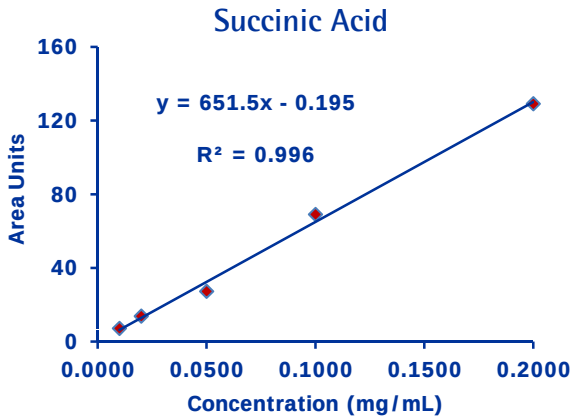
Chromolith® HighResolution RP-18 endcapped

Calibration curves were constructed in the range 0.005-1.0 mg/mL for tartaric, malic and citric acid, while the calibration range for succinic acid was 0.001-0.20 mg/mL. Five (n=5) replicate injections of standard solution were analyzed at the five different concentration levels to determine the method linearity. The relative standard deviation for replicate injections at all concentration levels was better or equal to 1% for all four compounds.









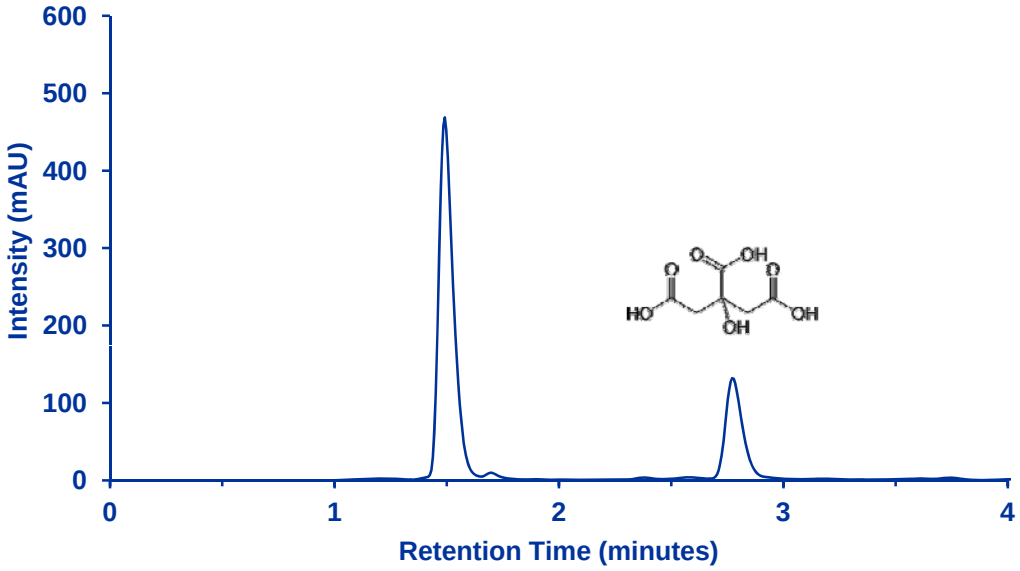
As a final test of the method, a commercial orangeade was analysed and as can be seen on next page only citric acid was found in the beverage. The citric acid concentration was determined to 1.1 mg/mL

Analysis of Organic Acids in Beverages

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	(1.52022.0001)
Injection:	20 µL	
Detection:	UV, 210 nm	
Cell:	1 µL/10 mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	10 mM Di-ammonium hydrogen phosphate solution (pH 2.7)	
Temperature:	30 °C	
Diluent	water	
Sample:	5mL of orangeade was sonicated for 5 minutes. Thereafter 0.2ml of phosphoric acid solution (1M) was added. Finally the solution was diluted to 10mL by water.	
Pressure Drop:	32 Bar (464psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical plates	Asymmetry
1	Citric Acid	2.8	6366	1.4