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.

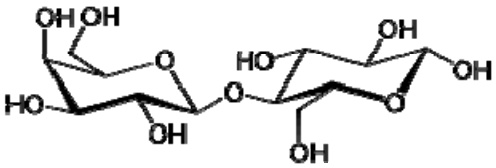
Food additives are classified on the basis of their functional use and are grouped as:

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.

Sugar analysis



Lactose

In chromatography the mutarotation of reducing sugars in solution causes these to elute as two peaks, one for each anomer. From 1975 before HILIC became known as HILIC it was extensively used for sugar separations using first silica but later aminopropyl silica columns. The amino columns catalyse the mutarotation of sugars effectively causing the retention time of the sugar to be the average of the two anomers, showing as only one peak in the chromatogram. These amino columns are however notoriously unstable as they catalyse their own degradation.

The use of an amine containing buffer component, like ammonium hydroxide (NH4OH) in the mobile phase also catalyzes the anomer interconversion. Silica based chromatography columns are not stable at this high pH but the polymeric ZIC®-pHILIC column can be used for several weeks and hundreds of injections using a 1% ammonium hydroxide eluent (pH~11). The chemical stability of the ZIC®-pHILIC columns allow for direct ESI-MS quantitation of simple and complex carbohydrates at basic pH and elevated temperatures. By using high pH mobile phase to collapse anomers of for example glucose and lactose into a single peak it is possible to, simplify identification of carbohydrates in different types of samples even with difficult matrices. Combined with simple sample preparation procedures like protein precipitation or liquid-liquid extraction, efficient and cost-efficient analytical work-schemes can be developed and used for monitoring of sugar, sugar alcohols and other carbohydrates in different type of formulations and matrices.

Lactose is a reducing sugar often needed to be quantified in milk products. Lactose free products are in most cases defined as those with a non measurable level of remaining lactose. In Scandinavia the measurement is performed by an enzymatic method having a LOQ of 100 ppm. This method is, however, not applicable if the lactose has been removed by enzymatic degradation. Then a chromatographic method has to be used.

On the following pages lactose determination using a simple sample preparation consisting of protein percipitation, centrifugation and filtration is presented. For more information on chromatography of carbohydrates please visit www.sequant.com/sugars

Determination of Lactose in Milk

SeQuant® ZIC®-pHILIC

Column:	SeQuant® ZIC®-pHILIC (5 µm, polymer) PEEK 100×2.1 mm	(1.50462.0001)

Recommended solvents and reagents

Acetonitrile :	Hypergrade for LC-MS LiChrosolv®	(1.00029)
Water:	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q® water purification system	(1.15333)
Ammonia:	25% solution for analysis EMSURE®	(1.05432)

Recommended filtration tools

Mobile phase filtration:

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

Sample filtration:

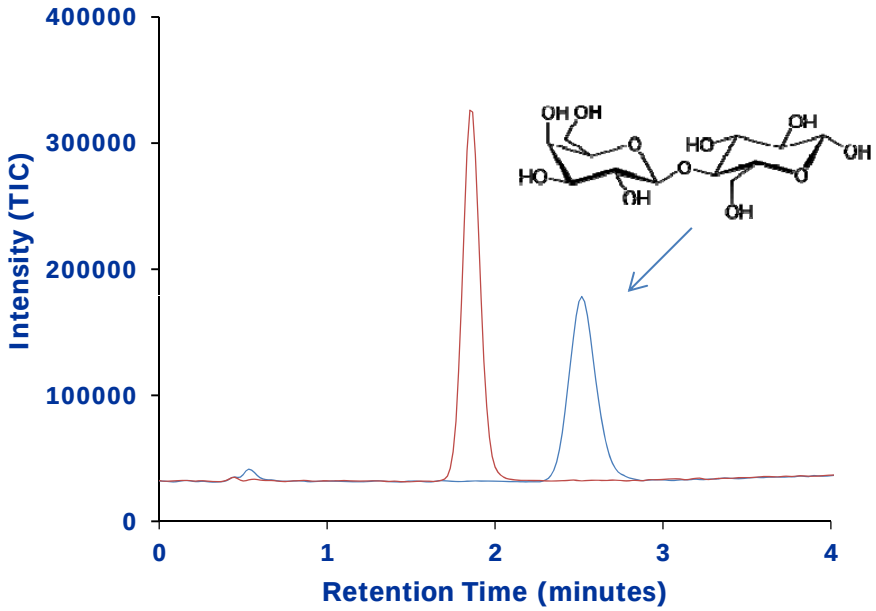
Millex-LCR Filter, 0.45 µm, PTFE, 13 mm, non-sterile	(SLCRT13NL)
Samplicity™ starter bundle with filter 0.45µm	(SAMPLCRBL)

Determination of Lactose in Milk

SeQuant® ZIC®-pHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-pHILIC (5 µm, polymer) PEEK 100x2.1 mm	(1.50462.0001)
Injection:	2 µL	
Detection:	ESI MS SIM, negative mode	
Flow Rate:	0.35 mL/min.	
Mobile Phase (v/v):	Acetonitrile and 1% Ammonia Gradient 73:27 to 60:40 in 0 to 3 min.	
Temperature:	55 °C	
Diluent:	Mobile phase	
Sample:	Milk samples Valio Lactose free milk, Glucose/Galactose (red), Reduced fat milk Norrmejerier, Lactose (blue), Milk samples were diluted in water 5 times. Preciptation in 4 parts basic acetonitrile (1% ammonium hydroxide). Centrifugation: 6400rpm 5 min. Filtration: 0.45mm PTFE syringe filter. Dilution 100x.	



Chromatographic Data

No.	Compound	Time (min)	Retention Factor
	Void volume (t0)	0.5	-
1	Glucose/Galactose	1.9	2.7
2	Lactose	2.5	4.1