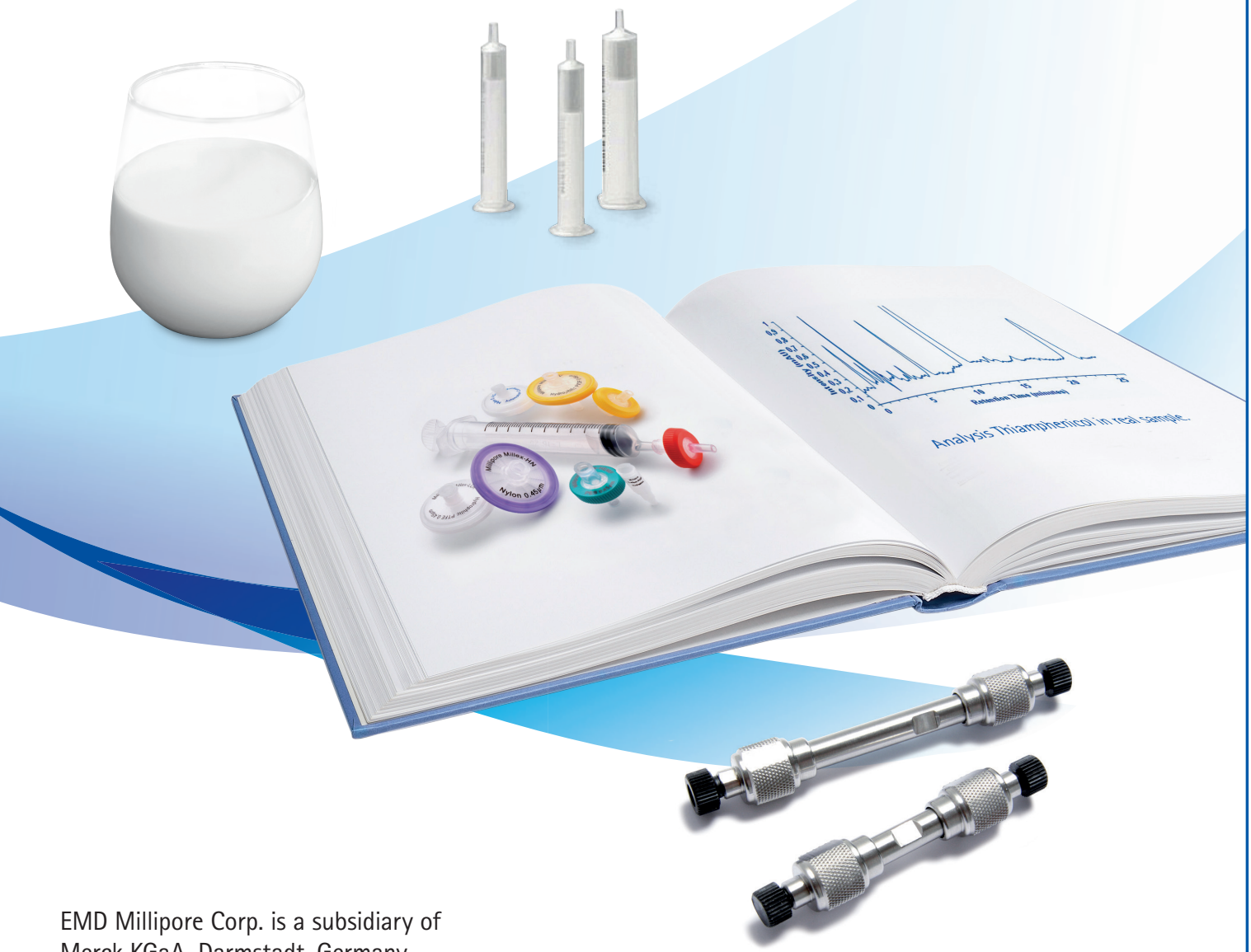
Solutions for Milk Testing

Chinese National Standard test methods

2015-3



EMD Millipore Corp. is a subsidiary of
Merck KGaA, Darmstadt, Germany

Content

Introduction

With the growing focus on food issues and food quality there is an increasing need for new analytical methods able to cope with large number of analytes in complex matrices. New analytical assays must provide sensitivity, robustness and high resolution within an acceptable analysis time…but they also need to be approved by local and global authorities.

In 2012 and 2013, we published two extensive application compilations with focus on food and beverage analysis. A lot of information can be found in those with relevance to difference global regulations in food testing and how to use official methods in food analysis.

The current application compilation focus specifically on milk and milk powder testing according to the Chinese National Standard, or Guobiao (simplified Chinese: 国标; traditional Chinese: 國標; pinyin: guó biāo). The GB standards are the Chinese national standards issued by the Standardization Administration of China (SAC); the Chinese National Committee of the International Organization for Standardization (ISO). A number of complete methods, including sample preparation protocols, are provided where we can support you with up-to-date knowledge about the regulations as well as our products and handling thereof.

Guobiao standards (GB and GB/T)

(simplified Chinese: 国标; traditional Chinese: 國標; pinyin: guó biāo),

Knowledge of Chinese standards is the key to understand technical market access and surveillance rules, testing procedures, and certification in China. The Chinese standardization system sets out quality and safety requirements for products circulated on the Chinese market. Any product entering the Chinese market therefore has the legal responsibility to conform to Chinese standards. China has a comprehensive system of standards covering almost all sectors of industrial activities

GB standards are the national standards issued by the Standardization Administration of China (SAC), the Chinese National Committee of the International Organization for Standardization (ISO). GB stands for Guobiao, which means national standard. GB standards are the basis for product testing according to the China Compulsory Certificate (CCC) certification for products. If there is no corresponding GB Standard, CCC is not required. SAC maintains a database of all Chinese National GB Standards, which is searchable in English or Mandarin. The database lists all mandatory and voluntary national standards.

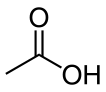
http://www.ccsa.org.cn/english/list_std.php?tbname=gb

Mandatory standards are prefixed "GB" and recommended (or voluntary) standards are prefixed "GB/T" where the T is derived from Chinese language meaning *推荐 tuījiàn*. The mandatory standards are enforced by laws and administrative regulations and concern the protection of human health, personal property and safety. All standards that fall outside of these characteristics are considered voluntary standards. The homepage of Chinese Food and Drug administration can be found via the following link:

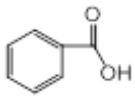
http://eng.sfda.gov.cn/WS03/CL0755/

Many Chinese national GB standards are adoptions from ISO, or other international standards. In 2006, for example, nearly half of all Chinese national GB standards were adoptions of international standards. Changes are made frequently within the regulatory system of the GB Standards, new standards are released, existing standards are changed or updated. In addition, China has also expressed a goal of significantly increasing the number of standards that are adoptions of international or advanced foreign standards. Useful links for further reading:

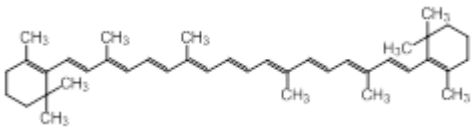
http://www.sac.gov.cn/SACSearch/outlinetemplet/gjbzcx.jsp
http://eng.sfda.gov.cn/WS03/CL0755/
http://www.standardsportal.org/usa_en/prc_standards_system/database_of_GB_standards.aspx



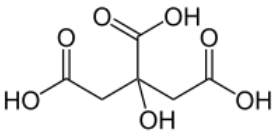
Acetic acid



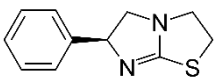
Benzoic Acid



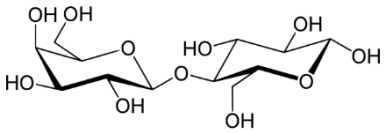
β-Carotene



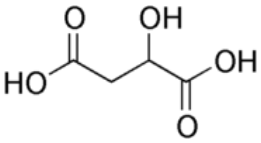
Citric acid



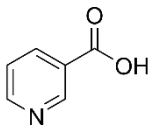
Levamisole



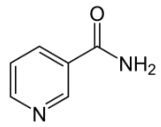
Lactose



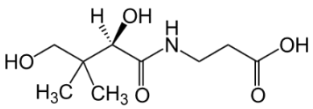
Malic acid



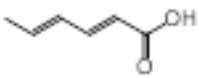
Niacin
(vitamin B3 or nicotinic acid)



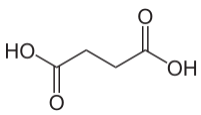
Nicotinamide
(Niacinamide)



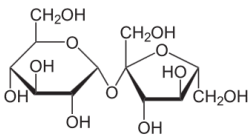
Pantothenic acid
(pantothenate or vitamin B5)



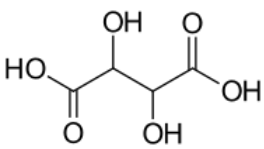
Sorbic Acid



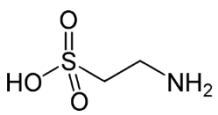
Succinic acid



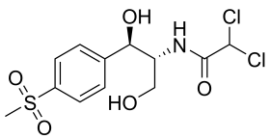
Sucrose



Tartaric acid



Taurine



Thiamphenicol

Carbohydrates

Carbohydrates (saccharides) are a major source of nutrition and a key form of energy for most organisms as well as being structural components of plants. Carbohydrates is one of the major classes of biomolecules and consist of carbon, hydrogen and oxygen atoms with the general formula Cn(H2O)n. Saccharides contain two carbonyl groups that are either of aldehyde or ketone type. Carbohydrates are furthermore divided into four chemical groups:

1) Monosaccharides (examples: Glucose and Fructose)
2) Disaccharides (examples: Lactose, Isomaltose and Trehalose)
3) Oligosaccharides
4) Polysaccharides

In general, the monosaccharides and disaccharides, lower molecular weight carbohydrates, are commonly referred to as sugars. Beside monosaccharides and disaccharides there are also neutral sugars, acidic sugars, amino sugars, sugar alcohols, and their various isomers. Low molecular weight saccharides are common in food, such as fruits, honey and sweets. The separation and identification of saccharides is challenging, especially for compounds having the same chemical formula and only small differences in their molecular structure, i.e. disaccharides maltose and isomaltose. In addition, carbohydrates from simple sugars to oligo- and polysaccharides represent a detection challenge in that they are lacking chromophores. RI and UV (195 - 205nm), are problematic to use due to issues of poor sensitivity, long detector equilibration times and their inability to handle gradient elution. Evaporative light scattering detection (ELSD) is a viable alternative, but just as with RI detectors, ELSD is sensitive to changes in the mobile phase composition making gradient elution difficult.

Various separation techniques have been used for carbohydrate analysis; anion chromatography, size exclusion chromatography, reversed phase chromatography, hydrophilic interaction liquid chromatography (HILIC), GC and TLC. HPLC using aminopropyl functionalized columns is one of the more common techniques for analysis of saccharides. In this technique, a mixture of acetonitrile and water is used as the mobile phase and the retention increases with reduction of water content just as in HILIC. In addition, the higher the molecular weight of the sugar, the longer it takes to elute. The aldehyde radicals in sugars can react with the amino radicals in the stationary phase to create Schiff bases, which can sometimes cause significant tailing on peaks for pentasaccharides (such as arabinose and ribose). This can be inhibited by adding a salt to the mobile phase. An advantage with an amino column is that it catalyze the mutarotation of reducing sugars effectively causing the retention time of the sugar to be the average of its two anomers, showing as only one peak in the chromatogram . This compilation illustrates how either particulate Purospher® or monolithic Chromolith® NH2 columns can be used for determination of sucrose and lactose (GB 5413.5-2010).

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.

Colorants

Food colorant, or color additive, is any dye, pigment or substance that imparts color when it is added to food or drink. Natural colors are not required to be certified by a number of regulatory bodies throughout the world. Colorants are used in foods for many reasons including:

a) offset color loss due to exposure to light, air, temperature extremes, moisture and storage conditions
b) correct natural variations in color
c) enhance colors that occur naturally
d) provide color to colorless and "fun" foods

Food colorings are tested for safety by various regulatory bodies. In the United States, FDC numbers are given to approved synthetic food, while E numbers are used within the European union for all additives, both synthetic and natural, that are approved in food applications.

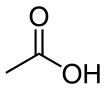
Natural food dyes
Because of consumer concerns about synthetic dyes a growing number of natural colorants are being commercially produced, exemplified herein with an application example of Annatto (E160b), a reddish-orange dye made from the seed of the achiote (Bixa orellana).

Permitted artificial colorants:
FDC Blue No. 1 – Brilliant Blue FCF, E133 (blue shade)
FDC Blue No. 2 – Indigotine, E132 (indigo shade)
FDC Green No. 3 – Fast Green FCF, E143 (turquoise shade)
FDC Red No. 40 – Allura Red AC, E129 (red shade)
FDC Red No. 3 – Erythrosine, E127 (pink shade)
FDC Yellow No. 5 – Tartrazine, E102 (yellow shade)
FDC Yellow No. 6 – Sunset Yellow FCF, E110 (orange shade)

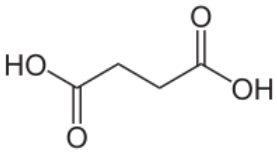
Some artificial colorants are only allowed to be used for limited use and others are banned or delisted. For instance Orange B (red shade) is allowed only for use in hot dog and sausage casings, whereas FDC Red No. 2 (Amaranth) is banned for use in food in US.

In this compilation, we have included a method for determination of β-Carotene (GB 5413.35-2010) which has the E number E160a when used as a food coloring agent, as well as some additional examples to illustrate the potential with monolithic columns for analysis of colorants in food and beverage samples.

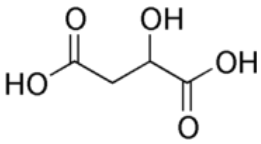
Organic Acids



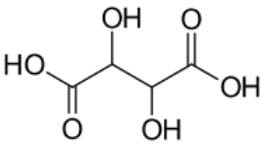
Acetic acid



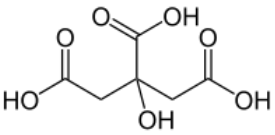
Succinic acid



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 Taurine (GB 5413.26-2010) as well as a method for tartaric acid, malic acid, citric acid and succinic acid following the GB/T 5009.157-2003, which is a voluntary method.

Preservatives

A preservative is a naturally occurring or synthetically produced substance that is added to food and beverages to prevent decomposition (either by microbial growth or chemical changes). Preservatives in food can be compounds used alone or combined with other methods of food preservation. Rosemary extract, hops, salt, sugar, vinegar, alcohol, diatomaceous earth and castor oil are used as food preservatives. They are examples of natural food preservatives.

Preservatives can be classified in two groups, Class I and II, where the former are represented by common household products such as vinegar, salt, sugar, honey, and vegetable oil. Class II preservatives refers to those preservatives which are chemically manufactured.

There are antimicrobial preservatives (inhibit growth of bacteria or fungi) such as sorbic acid and its salts, benzoic acid and its salts, calcium propionate, sodium nitrite/sodium nitrate, sulfites (sulfur dioxide, sodium bisulfite, potassium hydrogen sulfite, etc.) and disodium EDTA. There are also antioxidants (inhibit oxidation) such as BHA (Butylated hydroxyanisole), BHT (Butylated hydroxytoluene), TBHQ (tert-Butylhydroquinone) and propyl gallate. Other preservatives include ethanol and methylchloroisothiazolinone. Freezing, pickling, smoking and salting techniques can also be used to preserve food.

This application compilation include a method for determination of Benzoic and Sorbic acid (GB 21703-2010) as well as a few additional methods to illustrate the potential with monolithic columns for analysis of preservatives (Potassium Sorbate) together with caffeine in different beverage samples.

Vitamins

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 include tests for:

- Niacin (Vitamin B3) and Niacinamide (GB 5413.15-2010)
- Panthotenic acid (GB 5413.17-2010)

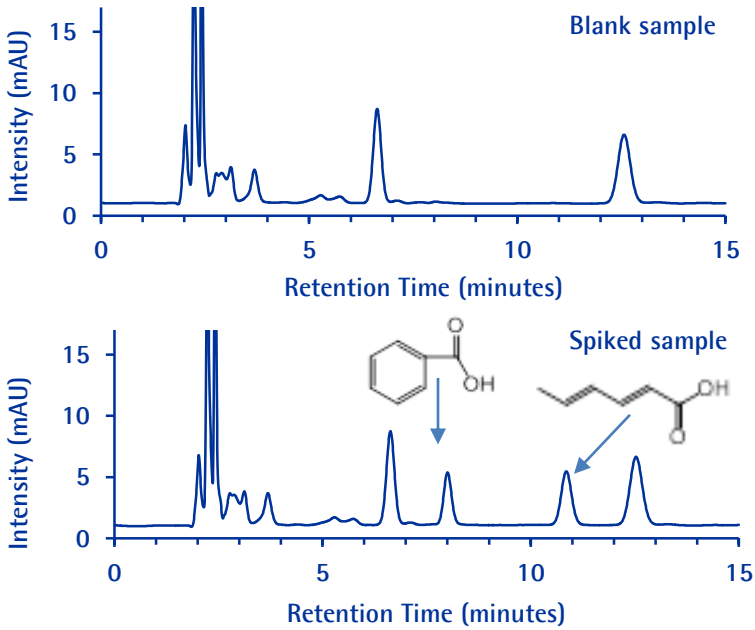
Further reading:
http://www.nlm.nih.gov/medlineplus/ency/article/002399.htm
http://en.wikipedia.org/wiki/Vitamin

Benzoic and Sorbic Acid in Milk Powder

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star LP RP-18 endcapped (5 µm) 250x4.6 mm	1.56200.0008
Injection:	10 µL	
Detection:	UV@ 227 nm	
Cell:	1ul/10mm	
Flow Rate:	1.2 mL/min	
Mobile Phase:	Phosphate buffer solution (pH6.7).Dissolve 2.5g KH_2PO_4 and 1.91g K_2HPO_4 in 1L H_2O. Mix Methanol and phosphate buffer solution 10:90 (v/v).	
Temperature:	30 °C	
Diluent	MeOH	
Sample:	Weigh 3.00 g of milk powder into a 100 mL volumetric flask. Add 10 mL water, 25 mL of 0.1 mol/L NaOH solution, sonicate for 15 minutes. After the solution is cooled down to room temperature, adjust pH value to 8.0 with 0.5 mol/L of H_2SO_4 solution. Add 2 mL of 92 g/L of $K_4Fe(CN)_6$ solution, 2 mL of 183g/L of ZnAC solution. Shakes vigorously the flask, cool the solution to room temperature. Add methanol to volume. Filtrate the solution with filter paper and 0.45 µm filter membrane respectively before analysis.	
Pressure Drop:	192 Bar (2784 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Benzoic acid	8.0	8323	1.05
2	Sorbic acid	10.9	8528	1.03

Benzoic and Sorbic Acid in Milk Powder

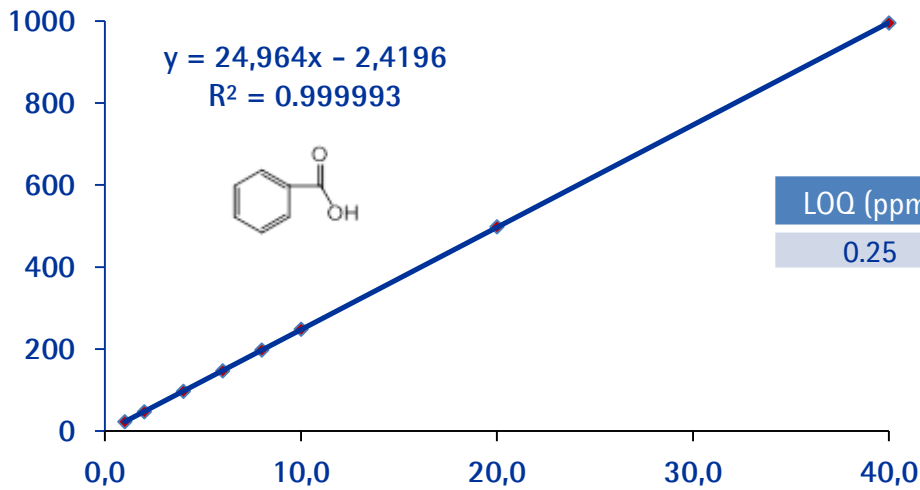
Purospher® STAR RP-18 endcapped

Repeatability: Analysis of repeated injections of 4 ppm standard solution (n=5).

Standard	Benzoic Acid	Sorbic Acid
1	97.9	141.0
2	97.3	139.5
3	97.3	139.1
4	97.4	139.6
5	97.6	139.8
Average	97.5	139.8
STDEV	0.219	0.655
RSD (%)	0.2	0.5

Linearity – Benzoic Acid: Analysis of repeated injections (n=5) of standard solutions in the calibration range 1-40 ppm (µg/mL).

Concentration (ppm)	Intensity (mV)
1.0	22.911
2.0	46.666
4.0	97.139
6.0	146.50
8.0	197.38
10.0	248.13
20.0	498.24
40.0	995.40



LOQ (ppm)	LOD (ppm)
0.25	0.08

Benzoic and Sorbic Acid in Milk Powder

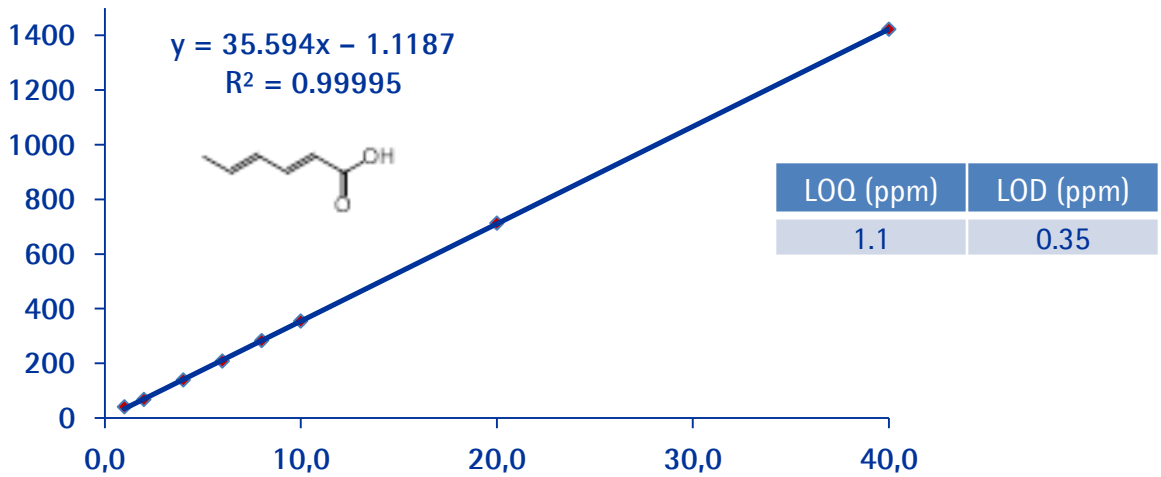
Purospher® STAR RP-18 endcapped

Repeatability: Analysis of repeated injections of 4 ppm standard solution (n=5).

Standard	Benzoic Acid	Sorbic Acid
1	97.9	141.0
2	97.3	139.5
3	97.3	139.1
4	97.4	139.6
5	97.6	139.8
Average	97.5	139.8
STDEV	0.219	0.655
RSD (%)	0.2	0.5

Linearity – Sorbic Acid: Analysis of repeated injections (n=5) of standard solutions in the calibration range 1-40 ppm (µg/mL).

Concentration (ppm)	Intensity (mV)
1.0	41.180
2.0	67.673
4.0	139.50
6.0	208.53
8.0	283.83
10.0	354.38
20.0	712.40
40.0	1422.6



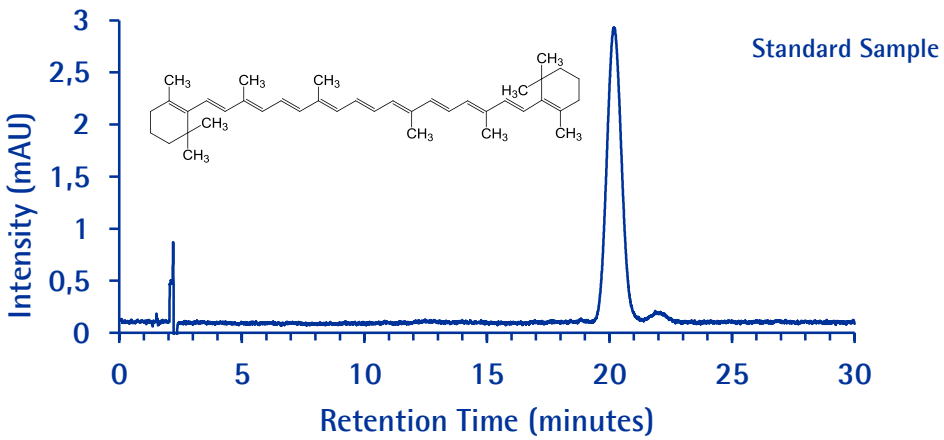
LOQ (ppm)	LOD (ppm)
1.1	0.35

β-carotene in Milk Powder

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	20 µL	
Detection:	UV@ 450 nm	
Cell:	1ul/10mm	
Flow Rate:	2.0 mL/min	
Mobile Phase:	Trichloromethane:acetonitrile:methanol 30:120:850 (v/v). Add 0.4 g ascorbic acid in 1L mixture.	
Temperature:	35 °C	
Diluent	n-hexane	
Sample:	Weigh 10.0 g milk powder into a 250 mL triangular flask. Add 1.0g ascorbic acid, 50 mL of 45°C~50°C water, blend the mixture. Add 100mL of 95% ethanol aqueous solution and 25mL of 55.6 % KOH aqueous solution to the flask, purge nitrogen gas to remove air out of the flask. Maintain temperature to 53 ± 2 °C under water bath, stir the solution with a magnetic stirrer for 45 minutes. Transfer the solution into a 500 mL separating funnel, add 100 mL petroleum benzene (boilng point range:30°C-60°C), vibrate gently, discharge gas, cover its cap. Vibrate the mixture for 10 minutes. transfer the water layer to another separating funnel for second extraction with 100mL petroleum benzene. Dry all organic phase layer with anhydrous Na2SO4. Filter the solution.. Evaporate the solution until nearly dry with a rotary evaporator at 40°C, in presence of N2, dissolve the residue with an appropriate volume. Transfer solution to 10 mL volumetric flask, add petroleum benzene to volume. Take 2.0mL solution mentioned above to a tube, dry it with N2, then dissolve the residue with 1.0mL n-Hexane. Filter solution (0.45 µm PTFE Millex filter) prior analysis.	
Pressure Drop:	162 Bar (2349 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	β-carotene	20.3	5084	1.19

β-carotene in Milk Powder

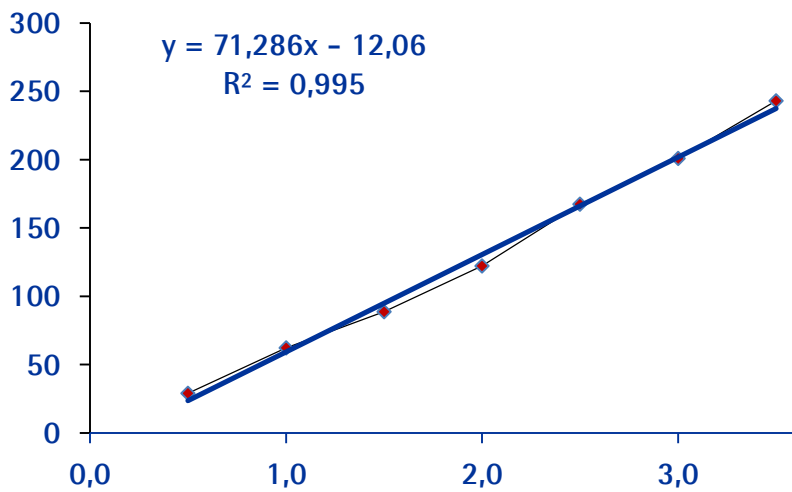
Purospher® STAR RP-18 endcapped

Repeatability: Analysis of repeated injections of a β-carotene standard solution (n=5).

Standard	Intensity (mV)
1	110.55
2	109.80
3	109.35
4	109.68
5	109.34
Average	109.74
STDEV	0.495
RSD (%)	0.5

Linearity: Analysis of repeated injections (n=5) of β-carotene standard solutions in the calibration range 0.5-3.5 ppm (µg/mL).

Concentration (ppm)	Intensity (mV)
0.5	28.998
1.0	62.216
1.5	88.850
2.0	122.18
2.5	167.42
3.0	200.90
3.5	243.02



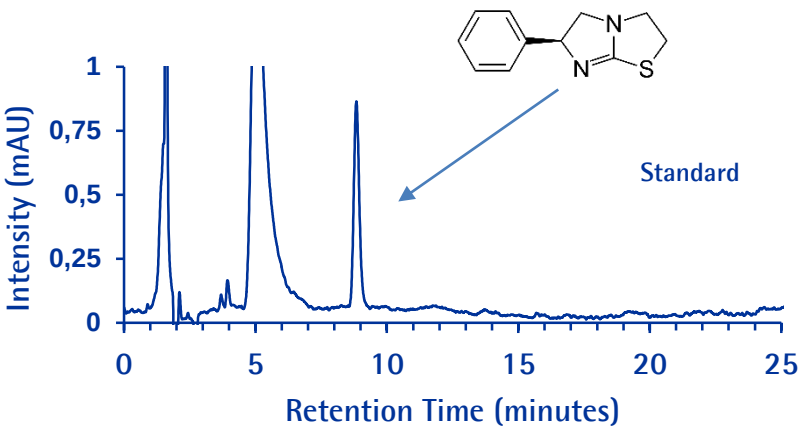
LOQ (ppm)	LOD (ppm)
0.84	0.28

Levamisole in Milk Powder

Purospher® STAR RP-18 endcapped

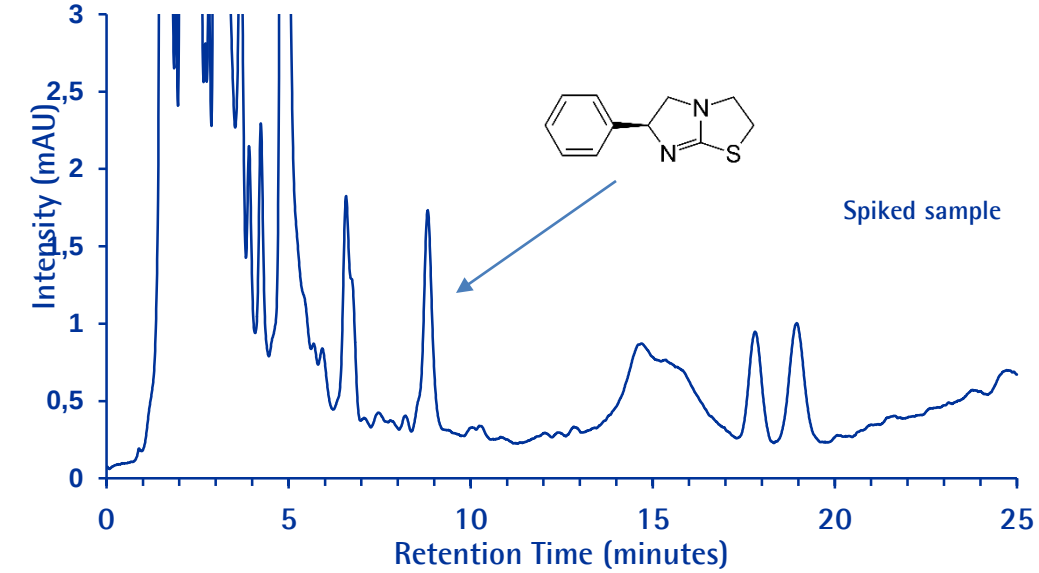
Chromatographic Conditions

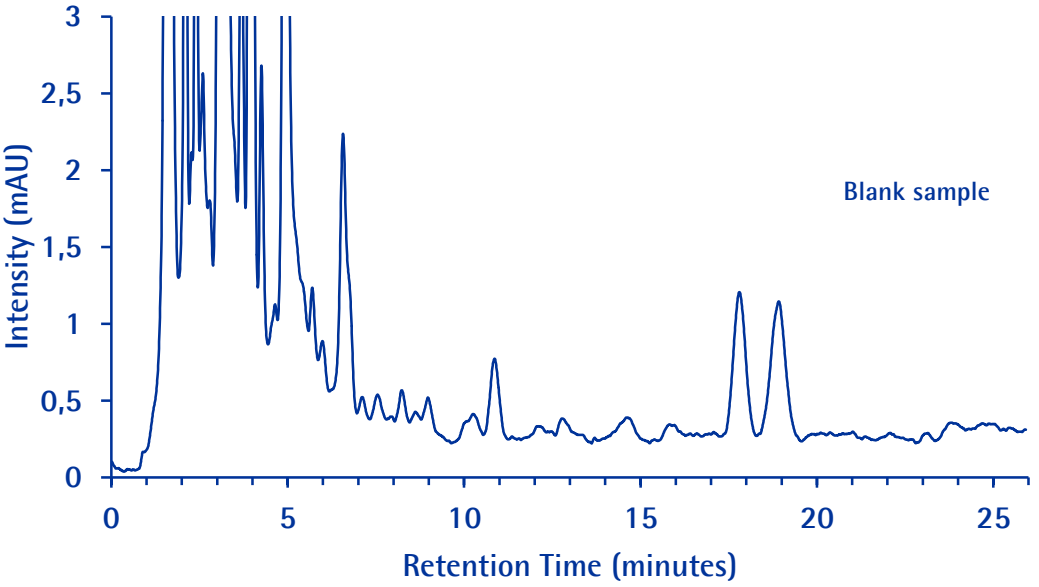
HPLC Column:	Purospher® Star RP-18 endcapped (5 µm) 150x4.6 mm	1.51455.0001
SPE Column	LiChrolut® RP-18 40-63um 500mg 3mL	1.02023.0001
Injection:	50 µL	
Detection:	UV@ 220 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Weigh 2.44g NaH_2PO_4 into a 1L volulmetric flask, add 850mL H_2O and 3 mLdiethyl amine. Adjust pH to7.5 with 10% H_3PO_4 solution, add H_2O to volume. Mix Methanol and NaH_2PO_4-Diethylamine aquoues solution 50:50 (v/v).	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	Weigh 5.0g milk powder into a 50 mL centrifuge tube. Add 5mL carbonate buffer solution (mix saturated $NaHCO_3$ aqueous solution and saturated Na_2CO_3 aqueous solution 900:100 (v/v) and 10 mL ethyl acetate into the tube. Vortex for 10min. Centrifuge at 6000 r/min for 10min. Take supernatant to a heart-shaped bottle. Repeat the extraction with another 10mL ethyl acetate, transfer the supernatant to the same bottle. Evaporate the solution to dryness with a rotary evaporator at 50°C. Dissolve the residue with carbonate buffer and follow SPE procedure.	
SPE procedure:	1. Condition LiChrolut® RP-18 SPE column - 3mL methanol and then 3mL carbonate buffer 2. Load the carbonate buffer dissolved sample, 3. Wash the SPE column with 3mL H_2O, 4. Elute with 5mL ACN. 5. Dry the elution solution with N_2. 6. Dissolve the residue with 1.0mL mobile phase, filter solution (0.45 µm filter) prior analysis.	
Pressure Drop:	177 Bar (2567 psi)	



Levamisole in Milk Powder

Purospher® STAR RP-18 endcapped





Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Levamisole	8.8	7484	1.00

Levamisole in Milk Powder

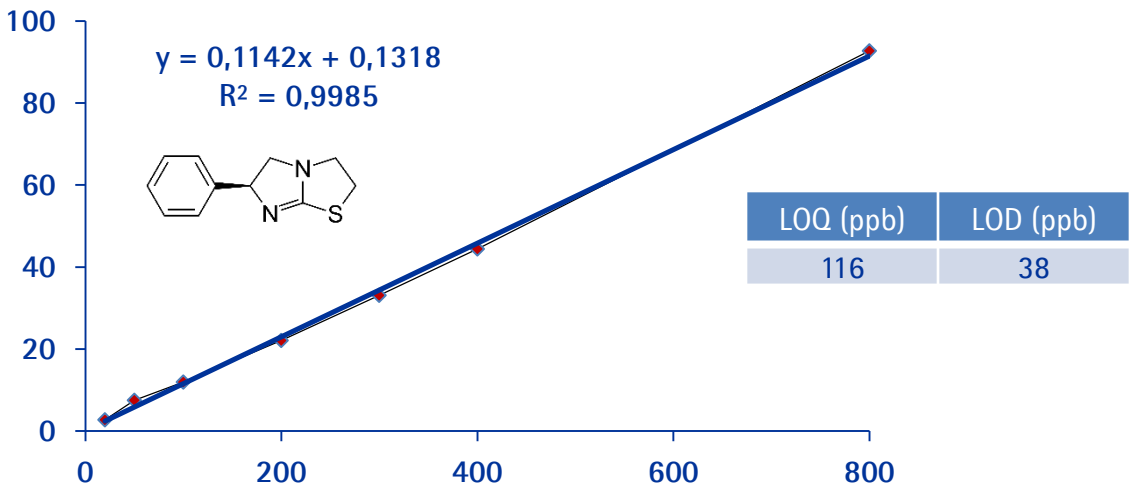
Purospher® STAR RP-18 endcapped

Repeatability: Analysis of repeated injections of 100 ppb (ng/mL) standard solution (n=5).

Standard	Intensity (mV)
1	11.966
2	11.599
3	12.549
4	12.571
5	12.161
Average	12.169
STDEV	0.419
RSD (%)	3.4

Linearity: Analysis of repeated injections (n=5) of standard solutions in the calibration range 20-800 ppb (ng/mL).

Concentration (ppb)	Intensity (mV)
20	2.75
50	7.46
100	11.9
200	22.1
300	33.1
400	44.4
800	92.7



LOQ (ppb)	LOD (ppb)
116	38

Levamisole in Milk Powder

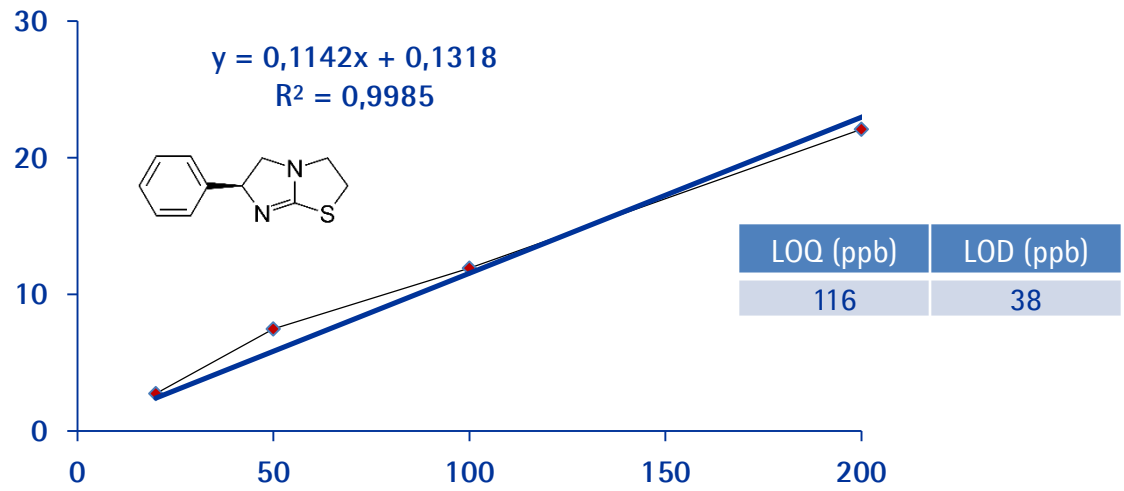
Purospher® STAR RP-18 endcapped

SPE Recovery: Analysis of samples spiked with 100 ppb (µg/kg) of Levamisole in milk powder

Spiked sample	Recovery (%)
1	95.33
2	92.78
3	94.98
Average	94.36
STDEV	1.38
RSD (%)	1.5

SPE Recovery: Analysis of samples spiked with 50 ppb (µg/kg) of Levamisole in milk powder

Spiked sample	Recovery (%)
1	89.13
2	87.67
3	89.54
Average	88.78
STDEV	0.98
RSD (%)	1.1



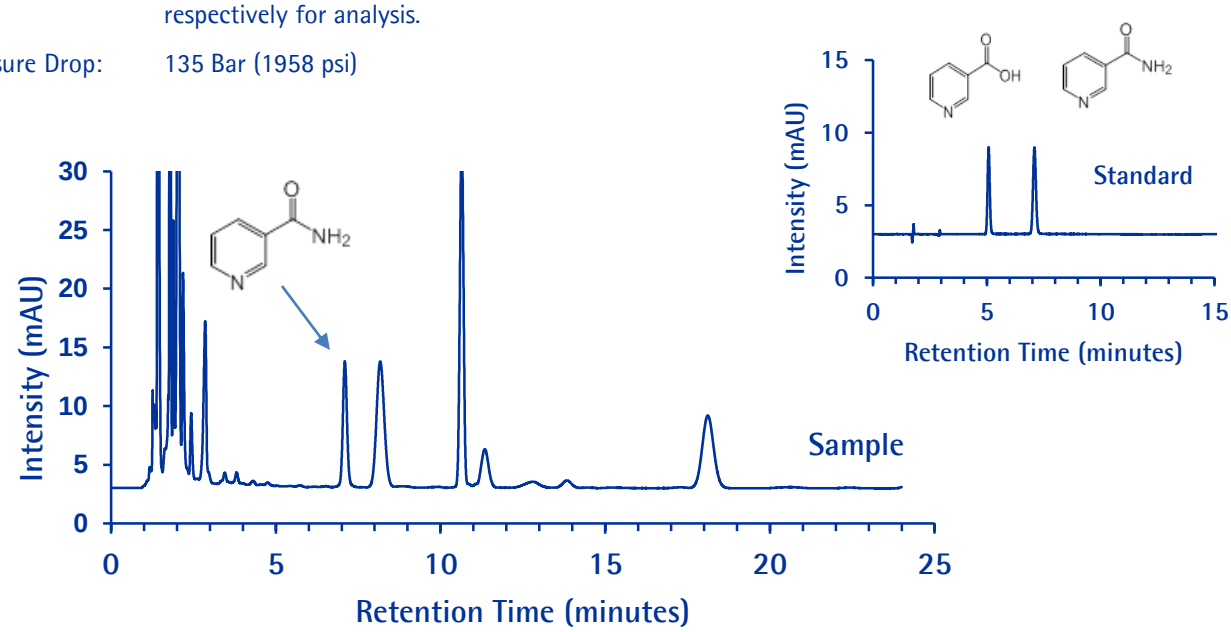
LOQ (ppb)	LOD (ppb)
116	38

Niacin and Niacinamide in Milk Powder

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

HPLC Column:	Purospher® Star RP-18 endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV@ 261 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Add 70 mL methanol, 20 mL isopropanol, 9.1 g sodium heptanesufonate into 910 mL H2O. Adjust pH value to 2.1 ± 0.1 with perchloric acid.	
Temperature:	25 °C	
Diluent	water	
Sample:	Weigh 5.00g milk powder into a 150 mL Erlenmeyer flask. Add 25 mL of 45°C~50°C water, sonicate for 10 minutes. Cool the solution to room temperature. Adjust pH value of the mixture to 1.7 ± 0.1 with 2.4 mol/L of HCl aqueous solution, stand the solution for 2 minutes. Adjust the pH value to 4.5 ± 0.1 with 2.5 mol/L of NaOH aqueous solution. Transfer the mixture to a 50-mL volumetric flask, add H2O to volume. Filtrate the suspension with filter paper and 0.45 µm filter membrane respectively for analysis.	
Pressure Drop:	135 Bar (1958 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Niacin	5.1	12700	1.09
2	Niacinamide	7.1	13802	1.09

Niacin and Niacinamide in Milk Powder

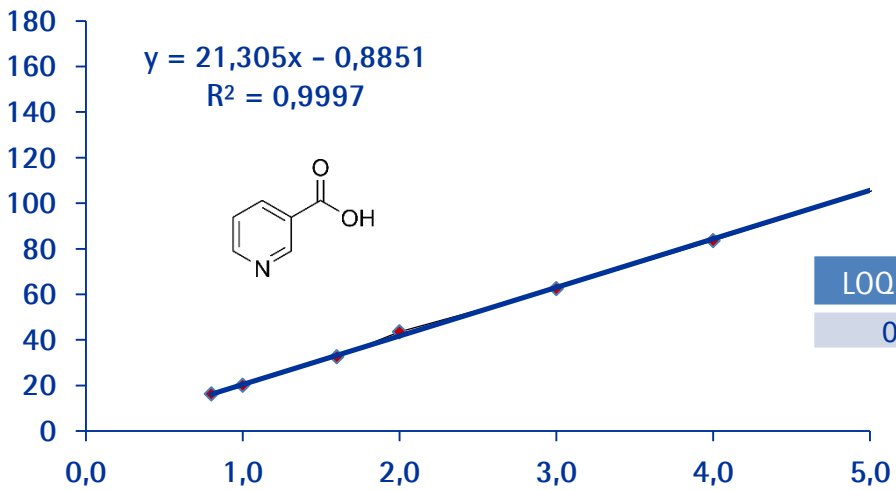
Purospher® STAR RP-18 endcapped

Repeatability: Analysis of repeated injections of 2 ppm (µg/mL) Niacin solution (n=5).

Niacin	Intensity (mV)
1	43.536
2	43.130
3	43.244
4	43.787
5	43.096
Average	43.359
STDEV	0.30
RSD (%)	0.7

Linearity: Analysis of repeated injections (n=5) of Niacin standard solutions in the calibration range 0.8-8.0 ppm (µg/mL).

Concentration (ppm)	Intensity (mV)
0.8	16,29
1.0	20,11
1.6	32,59
2.0	43,54
3.0	62,46
4.0	83,61
8.0	169,8



LOQ (ppm)	LOD (ppm)
0.45	0.15

Niacin and Niacinamide in Milk Powder

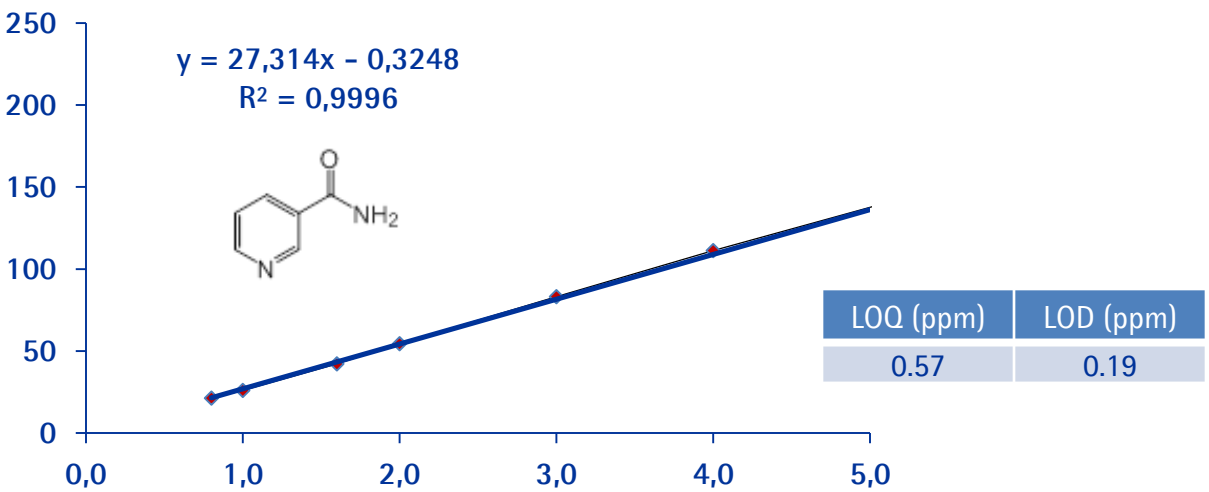
Purospher® STAR RP-18 endcapped

Repeatability: Analysis of repeated injections of 2 ppm (µg/mL) Niacinamide solution (n=5).

Niacinamide	Intensity (mV)
1	54.45
2	54.29
3	54.42
4	54.78
5	54.85
Average	54.56
STDEV	024
RSD (%)	0.4

Linearity: Analysis of repeated injections (n=5) of Niacinamide standard solutions in the calibration range 0.8-8.0 ppm (µg/mL).

Concentration (ppm)	Intensity (mV)
0.8	21,18
1.0	26,00
1.6	42,07
2.0	54,45
3.0	83,20
4.0	111,2
8.0	216,9



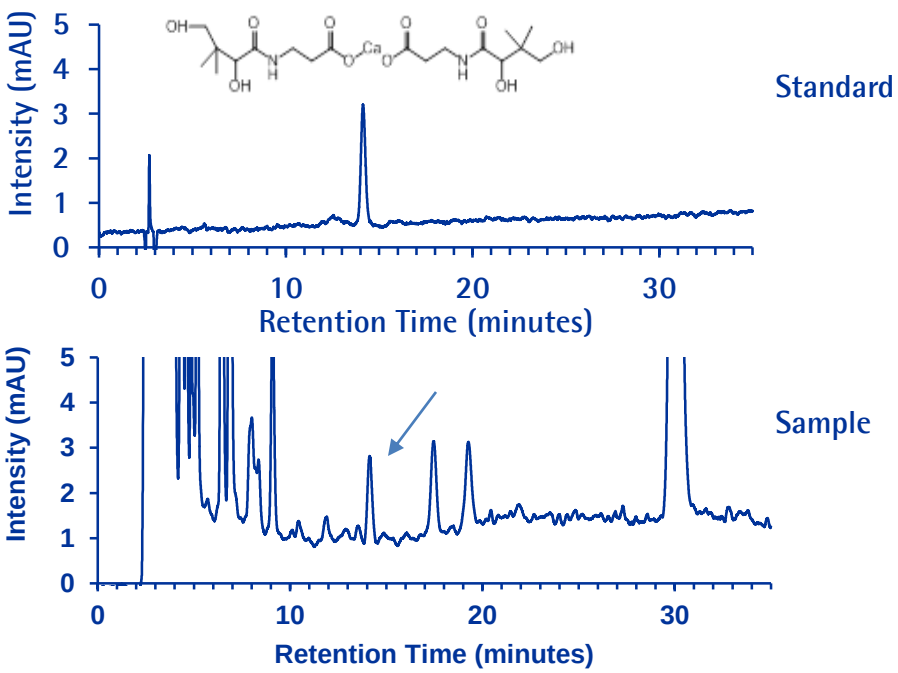
LOQ (ppm)	LOD (ppm)
0.57	0.19

Pantothenic acid in Milk Powder

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® Star RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	UV@ 450 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	50mM KH2PO4 aqueous solution. Dissolve 6.8g KH2PO4 in 1L H2O. Adjust pH to 3.0 with H3PO4. Mix Methanol and KH2PO4 aqueous solution 10:90 (v/v).	
Temperature:	30 °C	
Diluent	water	
Sample:	Weigh 5.0g milk powder into a 150-mL triangular flask. Add 30 mL of 40°C~50°C water, sonicate for 20 minutes. After the solution is cool down to room temperature, adjust pH value to 4.5 ± 0.1 with 0.1 mol/L of HCl solution, Add 5 mL of ZnSO4 solution(containing ZnSO4,15g/100mL). Transfer solution to 50-mL volumetric flask, add H2O to volume. Filtrate the solution with filter paper and 0.45 µm filter membrane respectively before analysis.	
Pressure Drop:	169 Bar (2450 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Calcium pantothenate	14.1	14450	1.16

Pantothenic acid in Milk Powder

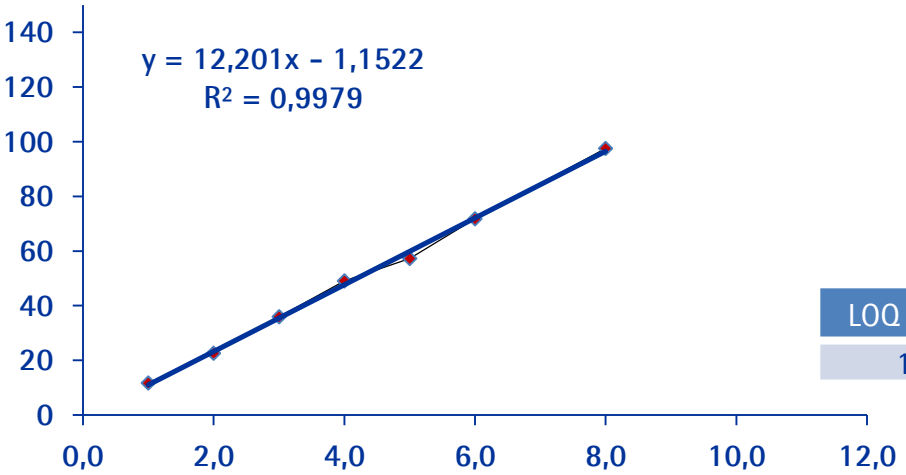
Purospher® STAR RP-18 endcapped

Repeatability: Analysis of repeated injections of 2 ppm (µg/mL) Pantothenate solution (n=5).

Niacin	Intensity (mV)
1	50.231
2	49.981
3	49.994
4	49.553
5	49.993
Average	49.951
STDEV	0.246
RSD (%)	0.5

Linearity: Analysis of repeated injections (n=5) of Pantothenate standard solutions in the calibration range 1-12 ppm (µg/mL).

Concentration (ppm)	Intensity (mV)
1.0	11.685
2.0	22.581
3.0	35.944
4.0	49.033
5.0	57.269
6.0	71.717
8.0	97.537
12.0	138.44



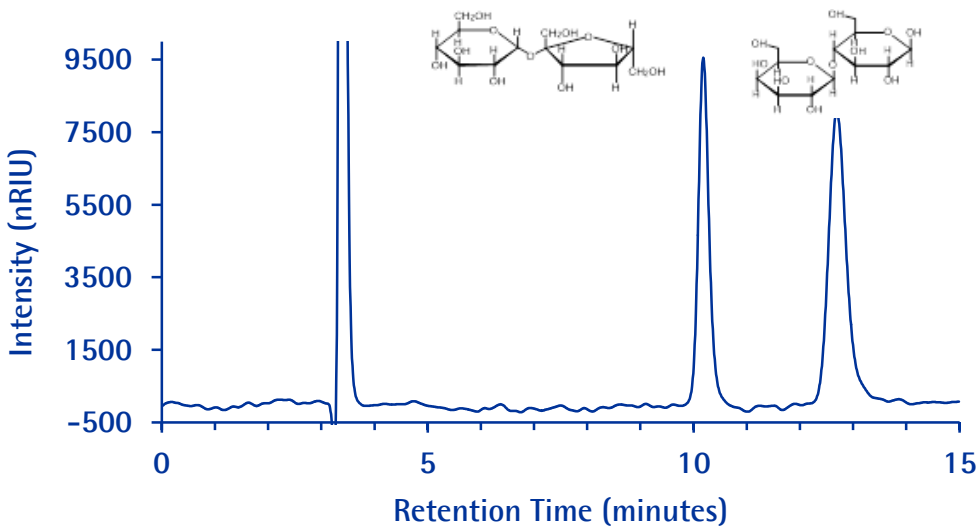
LOQ (ppm)	LOD (ppm)
1.7	0.57

Sucrose and Lactose in Milk Powder

Purospher® Star NH2

Chromatographic Conditions

Column:	Purospher® Star NH2 (5 µm) 250x4.6 mm	1.51913.0001
Injection:	10 µL	
Detection:	RI	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Mix Acetonitrile and Milli-Q water 70:30 (v/v)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	0.5mg/mL of Sucrose and 1.0mg/mL of Lactose in mobile phase. Unknown sample: Weigh 1.0g milk powder into a 50 mL volumetric flask. Add 15 mL H2O. Sonicate for 10 minutes. Add ACN to volume. After a few minutes' standing, filtrate the solution, take 5.0 mL supernatant to a10 mL volumetric flask, add ACN to volume, filter solution (0.45 µm filter) prior analysis.	
Pressure Drop:	63 Bar (920 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Sucrose	10.2	12891	0.94
2	Lactose	12.7	6294	1.24

Sucrose and Lactose in Milk Powder

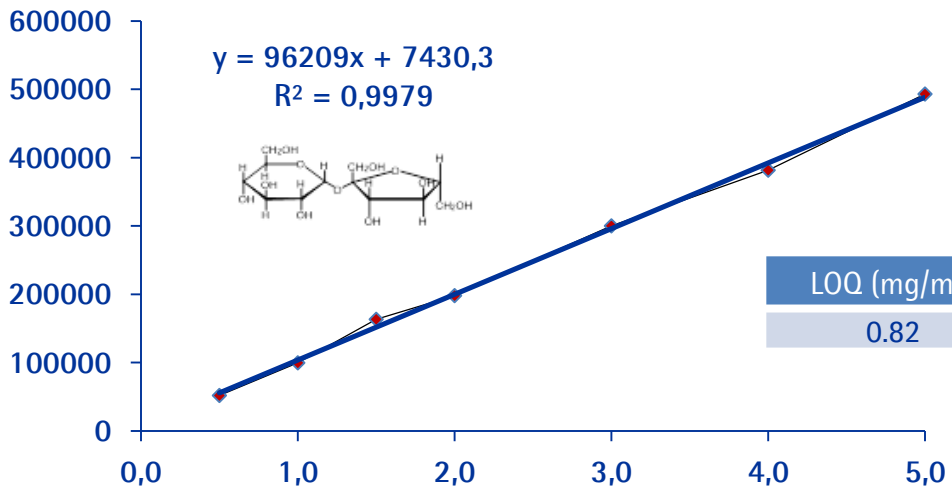
Purospher® Star NH2

Repeatability: Analysis of repeated injections of 3 mg/mL standard solution (n=5).

Standard	Sucrose	Lactose
1	293406	402551
2	291380	408137
3	290066	408708
4	293609	411025
5	293971	408205
Average	292486	407725
STDEV	1687	3124
RSD (%)	0.6	0.8

Linearity – Sucrose: Analysis of repeated injections (n=5) of standard solutions in the calibration range 0.5-5.0 mg/mL.

Concentration (mg/mL)	Intensity (mV)
0.5	51938
1.0	99566
1.5	163224
2.0	198164
3.0	299971
4.0	381782
5.0	492916



LOQ (mg/mL)	LOD (mg/mL)
0.82	0.27

Sucrose and Lactose in Milk Powder

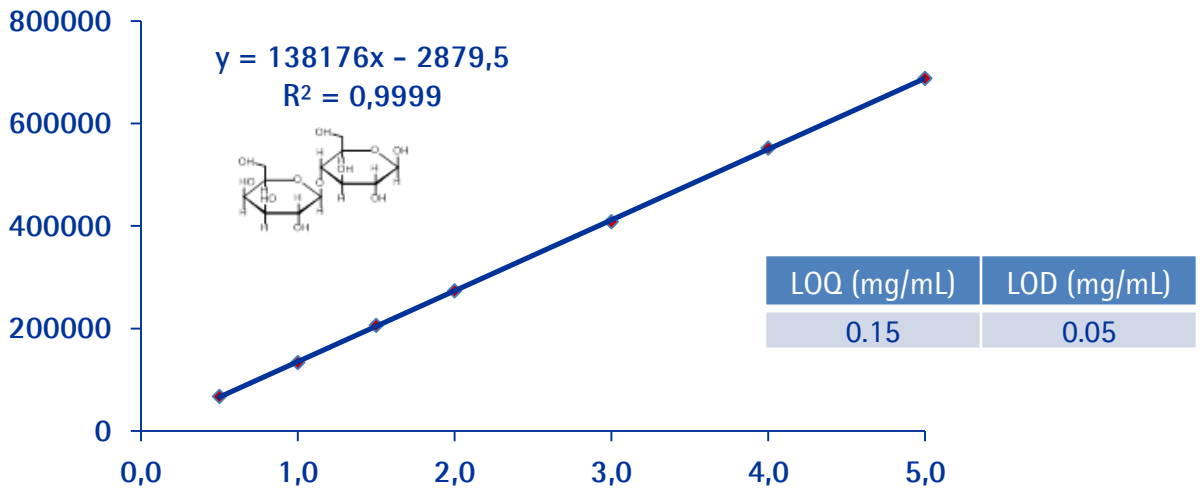
Purospher® Star NH2

Repeatability: Analysis of repeated injections of 3 mg/mL standard solution (n=5).

Standard	Sucrose	Lactose
1	293406	402551
2	291380	408137
3	290066	408708
4	293609	411025
5	293971	408205
Average	292486	407725
STDEV	1687	3124
RSD (%)	0.6	0.8

Linearity – Lactose: Analysis of repeated injections (n=5) of standard solutions in the calibration range 0.5-5.0 mg/mL.

Concentration (mg/mL)	Intensity (mV)
0.5	67284
1.0	133593
1.5	206165
2.0	273730
3.0	408205
4.0	551681
5.0	688177



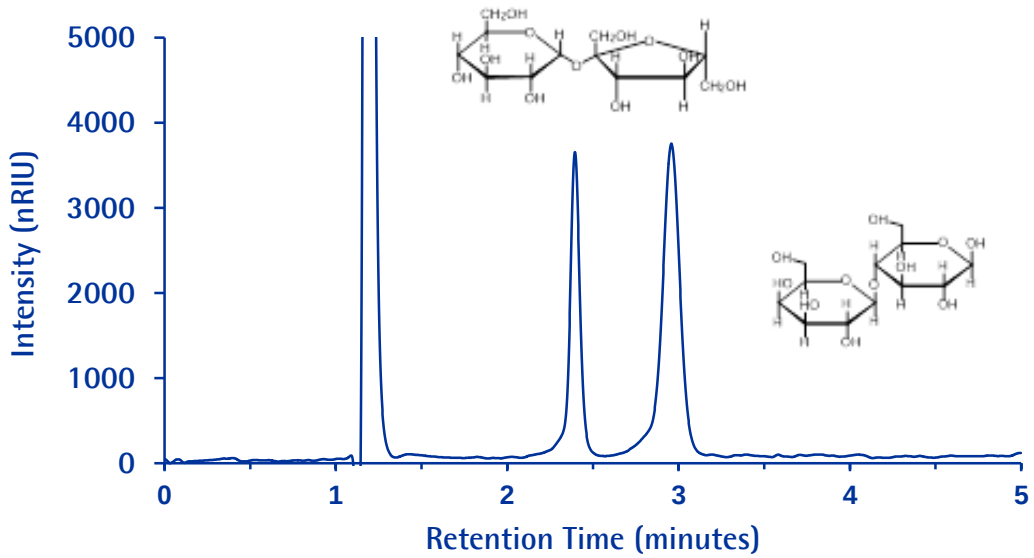
LOQ (mg/mL)	LOD (mg/mL)
0.15	0.05

Sucrose and Lactose in Milk Powder

Chromolith® Performance NH2

Chromatographic Conditions

Column:	Chromolith® Performance NH2, 100x4.6 mm	1.52028.0001
Injection:	5 µL	
Detection:	RI	
Cell:	1ul/10mm	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Mix Acetonitrile and Milli-Q water 70:30 (v/v)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	0.5mg/mL of Sucrose and 1.0mg/mL of Lactose in mobile phase. Unknown sample: Weigh 1.0g milk powder into a 50 mL volumetric flask. Add 15 mL H2O. Sonicate for 10 minutes. Add ACN to volume. After a few minutes' standing, filtrate the solution, take 5.0 mL supernatant to a10 mL volumetric flask, add ACN to volume, filter solution (0.45 µm filter) prior analysis.	
Pressure Drop:	33 Bar (479 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Sucrose	2.4	8732	0.93
2	Lactose	3.0	4019	0.87

Sucrose and Lactose in Milk Powder

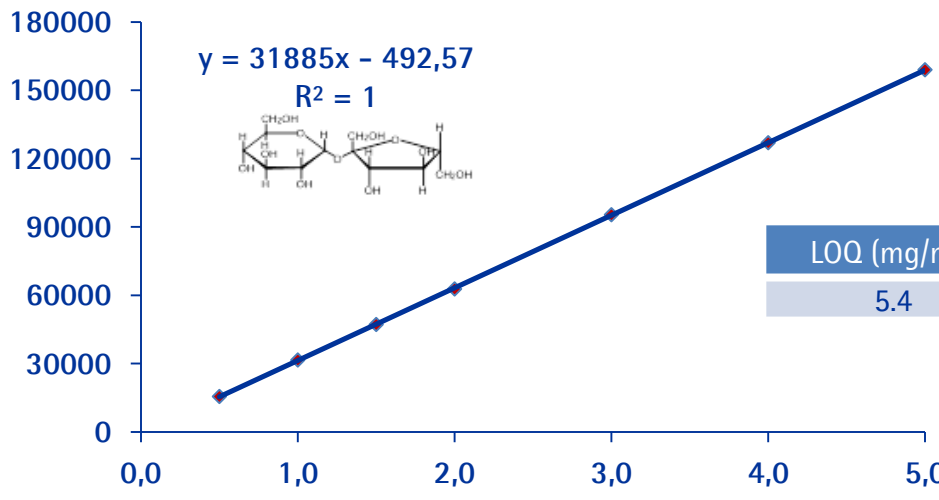
Chromolith® Performance NH2

Repeatability: Analysis of repeated injections of 3 mg/mL standard solution of sucrose and a 6 mg/mL standard solution of sucrose(n=5).

Standard	Sucrose	Lactose
1	95582	176824
2	95212	175893
3	96269	177697
4	95519	174698
5	95864	176520
Average	95689	176327
STDEV	398,5	1118
RSD (%)	0.4	0.6

Linearity – Sucrose: Analysis of repeated injections (n=5) of standard solutions in the calibration range 0.5-5.0 mg/mL.

Concentration (mg/mL)	Intensity (mV)
0.5	15611
1.0	31642
1.5	47135
2.0	62787
3.0	95382
4.0	127048
5.0	158987



LOQ (mg/mL)	LOD (mg/mL)
5.4	1.8

Sucrose and Lactose in Milk Powder

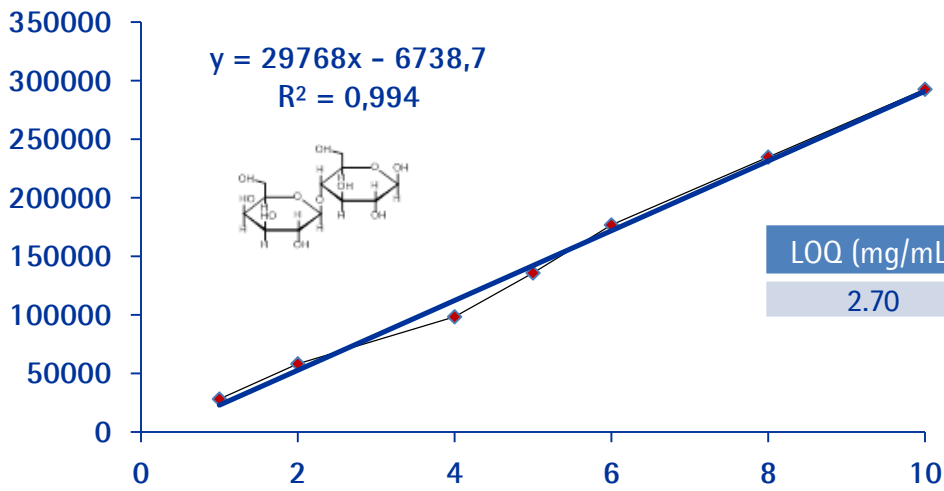
Chromolith® Performance NH2

Repeatability: Analysis of repeated injections of 3 mg/mL standard solution of sucrose and a 6 mg/mL standard solution of lactose (n=5).

Standard	Sucrose	Lactose
1	95582	176824
2	95212	175893
3	96269	177697
4	95519	174698
5	95864	176520
Average	95689	176327
STDEV	398,5	1118
RSD (%)	0.4	0.6

Linearity – Lactose: Analysis of repeated injections (n=5) of standard solutions in the calibration range 1.0-10.0 mg/mL.

Concentration (mg/mL)	Intensity (mV)
1.0	28243
2.0	58123
4.0	98308
5.0	135866
6.0	176673
8.0	234709
10.0	292561



LOQ (mg/mL)	LOD (mg/mL)
2.70	0.90

Taurine in Milk Powder

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

HPLC Column:	Purospher® Star RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	20 µL	
Detection:	UV@ 254 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	10mM sodium acetate (NaAC) buffer solution (pH4.2).Dissolve 0.82g NaAC in 800 mL H2O, adjust pH to 4.2 with glacial acetic acid, add H2O to 1L. Mix ACN and NaAC buffer solution 27:73 (v/v).	
Temperature:	30 °C	
Diluent	water	

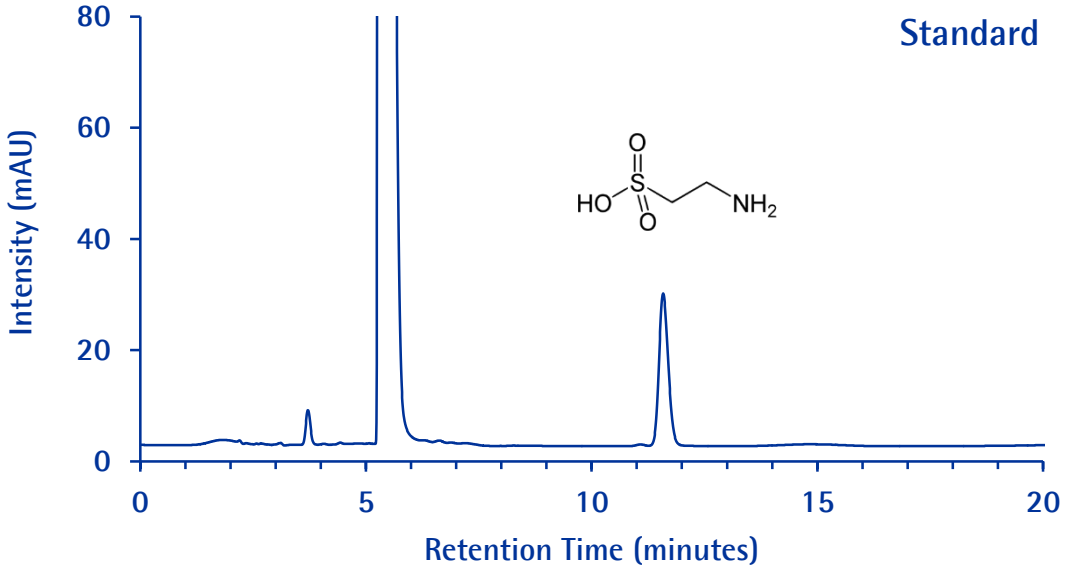
Sample:

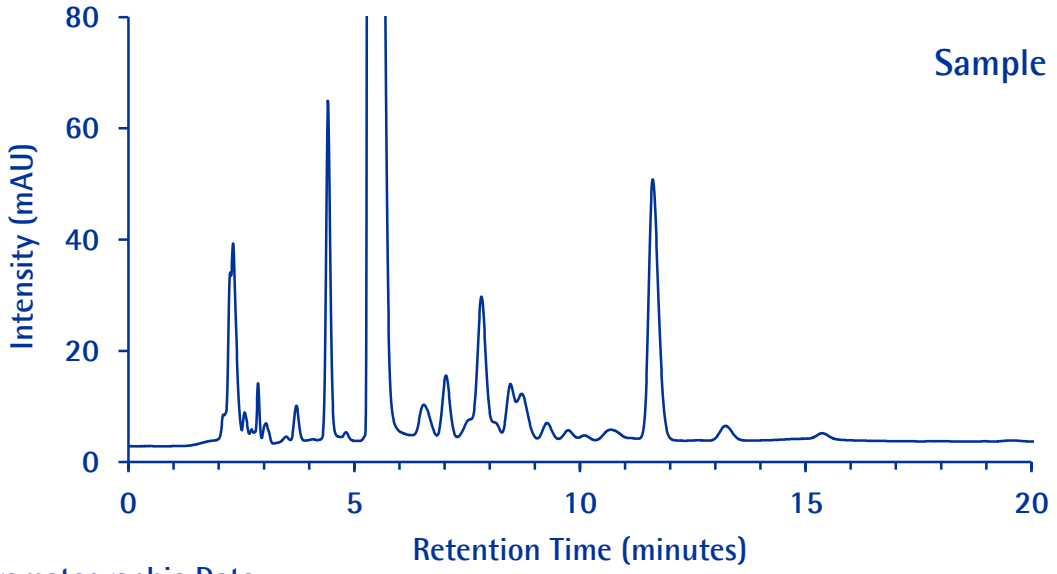
1. Weigh 5.00g milk powder into a 100 mL volumetric flask. Add 80 mL of 50~60°C hot water and sonicate for 10 minutes. Cool the mixture to room temperature, add into 1.0 mL of 0.15 g/mL $K4Fe(CN)_6$ solution, Vortex for 1 min. Add into 1.0 mL of 0.3 g/mL ZnAC solution, Vortex for 1 min. Add H2O to volume.
2. Centrifuge the mixture at 5000 rpm for 10 minutes. Store the supernatant in darkness at 4 °C if not used immediately .
3. Take 1.0 mL of the supernatant mentioned above or standard solution into a 10 mL glass-stopper test tube. Add 1.0mL of 80 mM Na_2CO_3 buffer solution.
   (0.424g Na_2CO_3 in 40 mL H2O, pH adjusted to 9.5 with 1M HCl. Add H2O to total 50 mL volume)
4. Add 1.0 mL of dansylchloride solution and blend the mixture well.
   (0.15g of dansylchloride is dissolved in 100 mL ACN.)
5. Store the mixture in darkness for 2h (shake the mixture one time every 1h).
6. Add 0.10 mL of 20mg/mL of methylamine hydrochloride aqueous solution to stop reaction.
7. Filtrate supernatant with 0.45 µm filter membrane for analysis.

Pressure Drop: 172 Bar (2494 psi)

Taurine in Milk Powder

Purospher® STAR RP-18 endcapped





Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Taurine	11.62	13476	1.08

Taurine in Milk Powder

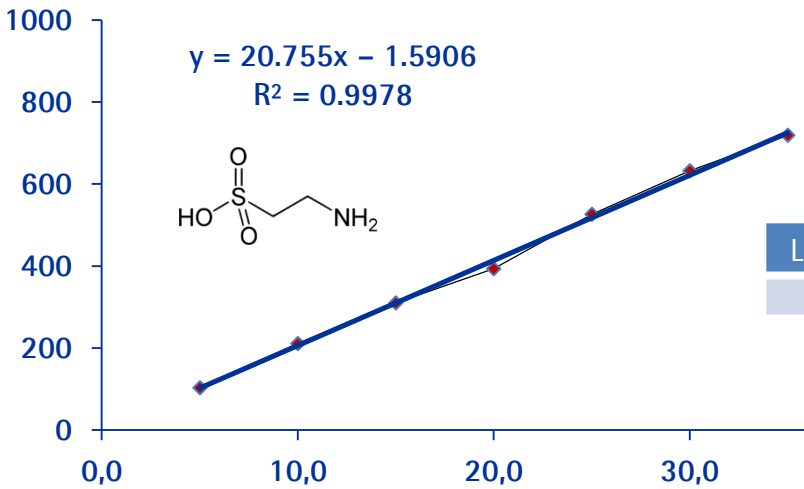
Purospher® STAR RP-18 endcapped

Repeatability: Analysis of repeated injections of 20 ppm µg/mL standard solution of sucrose and a 6 mg/mL standard solution of lactose (n=5).

Standard	Taurine
1	393.20
2	393.43
3	393.85
4	393.24
5	393.60
Average	393.47
STDEV	0.241
RSD (%)	0.1

Linearity – Lactose: Analysis of repeated injections (n=5) of standard solutions in the calibration range 5-35 mg/mL.

Concentration (mg/mL)	Intensity (mV)
5.0	103.32
10.0	211.08
15.0	309.89
20.0	393.13
25.0	525.71
30.0	632.32
35.0	719.12



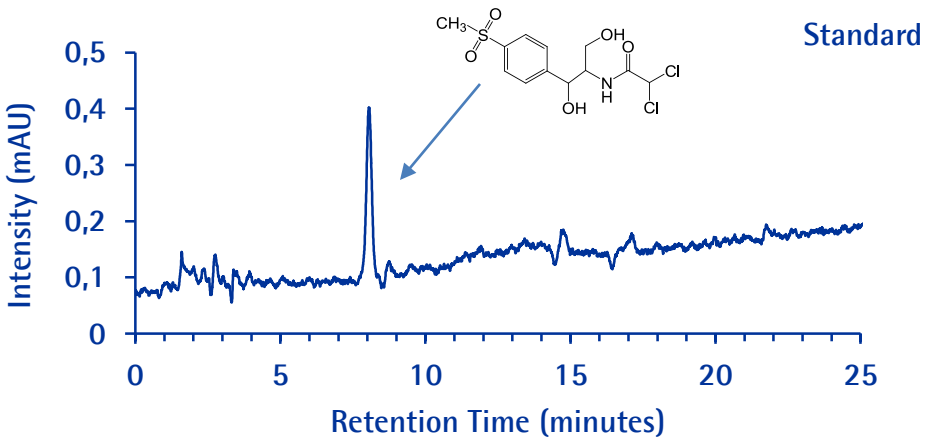
LOQ (mg/mL)	LOD (mg/mL)
5.6	1.8

Thiamphenicol in Milk Powder

Purospher® STAR RP-18 endcapped

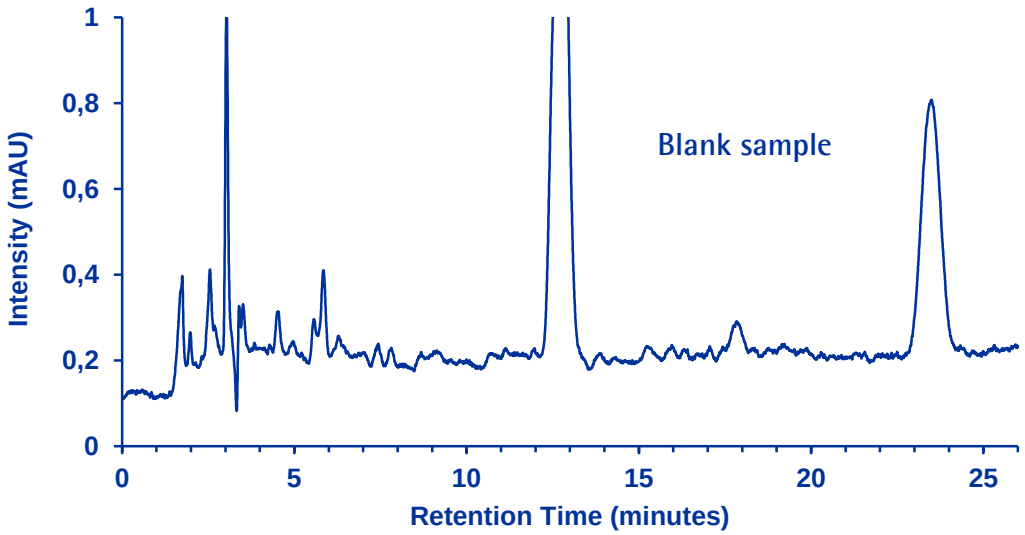
Chromatographic Conditions

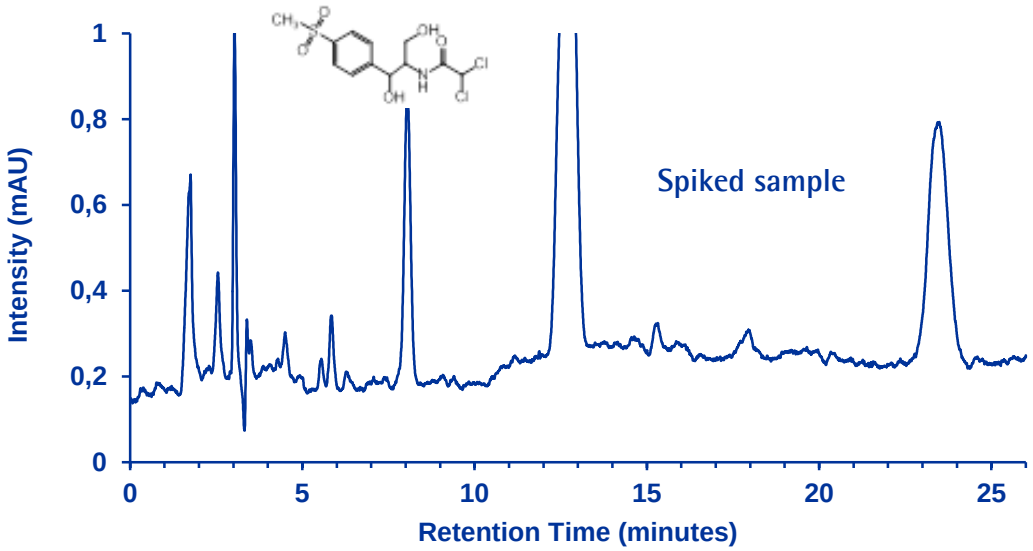
HPLC Column:	Purospher® Star RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
SPE Column	LiChrolut® RP-18 40-63um 500mg 3mL	1.02023.0001
Injection:	20 µL	
Detection:	UV@ 225 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Mix Acetonitrile and H2O 20:80 (v/v)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	Weigh 5.0g milk powder into a 50 mL centrifuge tube. Add 20 mL ethyl acetate. Vortex for 10min. Centrifuge at 4000 r/min for 5min. Take supernatant to a heart-shaped bottle. Repeat the extraction with another 20mL ethyl acetate, transfer the supernatant to the same bottle. Evaporate the solution to dryness with a rotary evaporator at 45°C. After a few minutes' standing, filtrate the solution. Dissolve the residue with total 10 mL H2O by sonicating. Transfer the water solution to a 50 mL centrifuge tube, add 20 mL n-hexane, vortex and centrifuge, and finally take the supernatant for SPE extraction.	
SPE procedure:	1. Condition LiChrolut® RP-18 SPE column - 5 mL acetonitrile and then 5 mL water 2. Load the sample, 3. Elute with 5mL ACN. 4. Dry the elution solution with N2. 5. Dissolve the residue with 1.0 mL mobile phase, filter solution (0.45 µm filter) prior analysis.	
Pressure Drop:	133 Bar (1928 psi)	



Thiamphenicol in Milk Powder

Purospher® STAR RP-18 endcapped





Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Thiamphenicol	8.1	8825	0.97

Thiamphenicol in Milk Powder

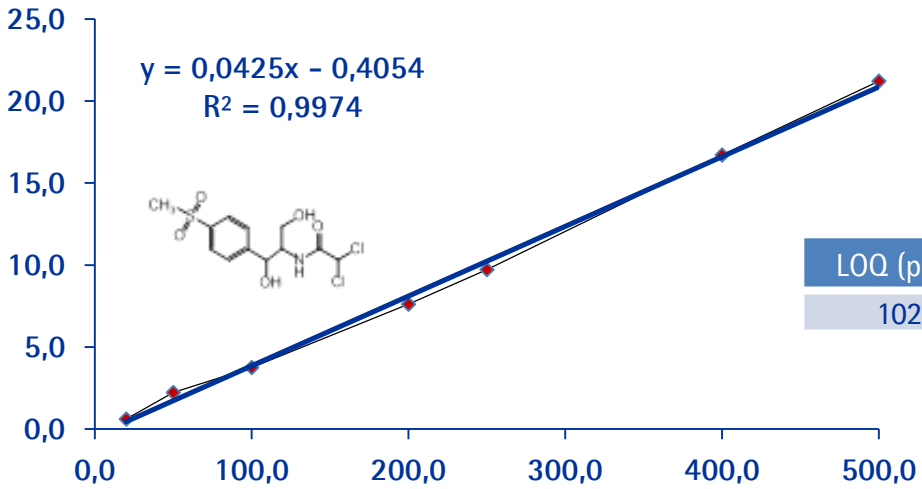
Purospher® STAR RP-18 endcapped

Repeatability: Analysis of repeated injections of 100 ppb (ng/mL) standard solution (n=5).

Standard	Intensity (mV)
1	3.573
2	3.997
3	4.064
4	3.721
5	4.107
Average	3.892
STDEV	0.233
RSD (%)	6.0

Linearity: Analysis of repeated injections (n=5) of standard solutions in the calibration range 20-500 ppb (ng/mL).

Concentration (ppb)	Intensity (mV)
20	0.6146
50	2.2253
100	3.7523
200	7.6017
250	9.7091
400	16.703
500	21.205



LOQ (ppb)	LOD (ppb)
102	34

Thiamphenicol in Milk Powder

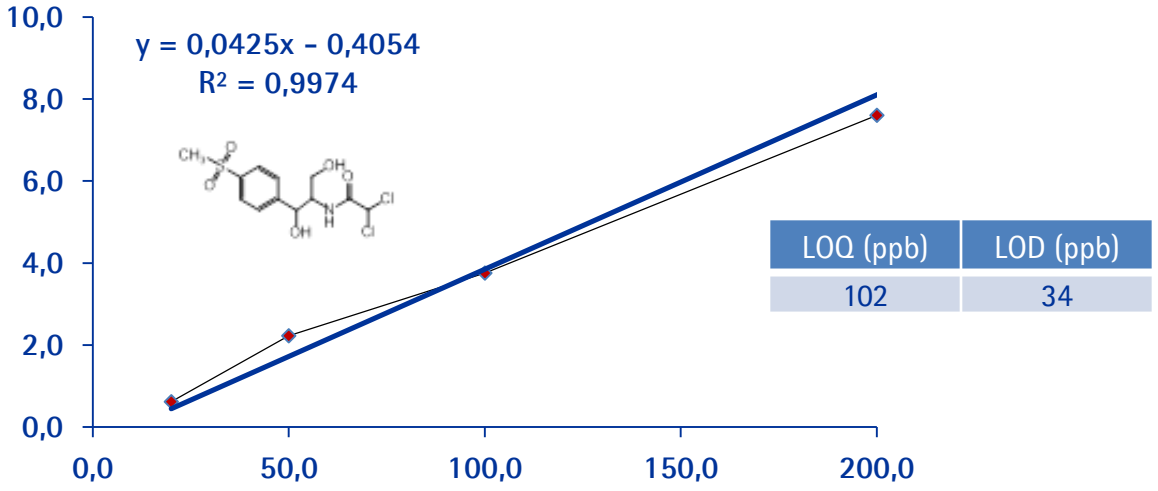
Purospher® STAR RP-18 endcapped

SPE Recovery: Analysis of samples spiked with 100 ppb (µg/kg) of Thiamphenicol (n=3).

Spiked sample	Recovery (%)
1	85.01
2	89.32
3	88.10
Average	87.5
STDEV	2.22
RSD (%)	2.5

SPE Recovery: Analysis of samples spiked with 50 ppb (µg/kg) of Thiamphenicol (n=3).

Spiked sample	Recovery (%)
1	79.13
2	77.93
3	72.31
Average	76.5
STDEV	3.64
RSD (%)	4.8



LOQ (ppb)	LOD (ppb)
102	34

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)

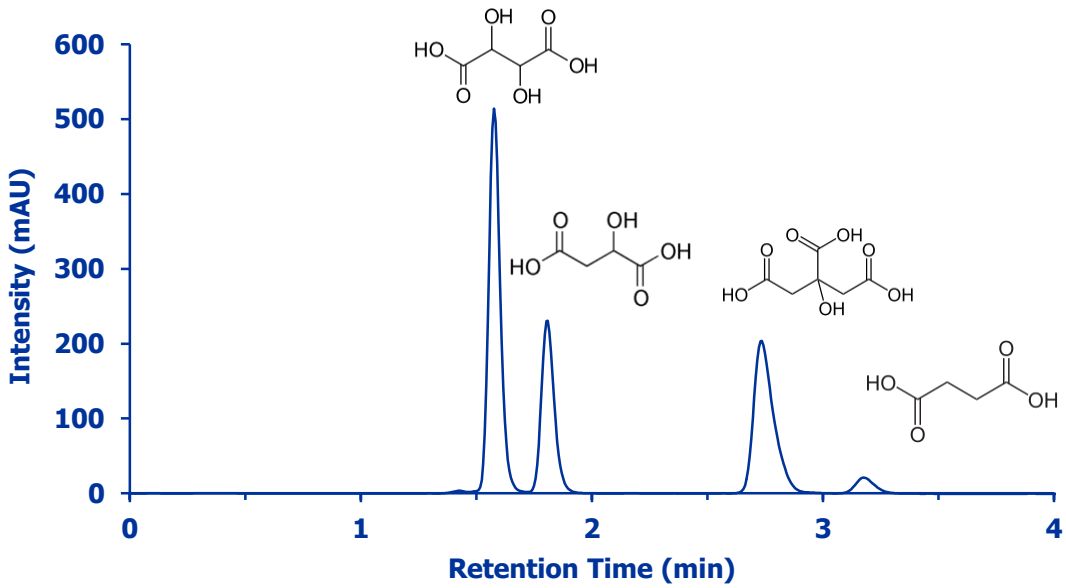
Sample Preparation
Sample: Commercial orangeade (soft drink)
Take 5 mL of orangeade and sonicate for 5 minutes, thereafter add 0.2 mL of phosphoric acid solution (1M) and make up to a final volume of 10mL by adding water. This gives a dilution factor of 2 for sample.

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)	



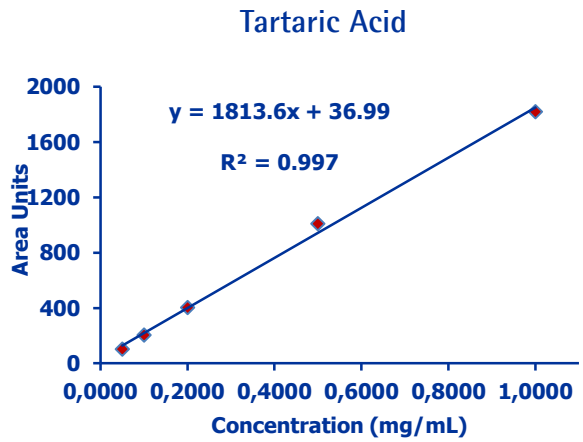
Chromatographic Data

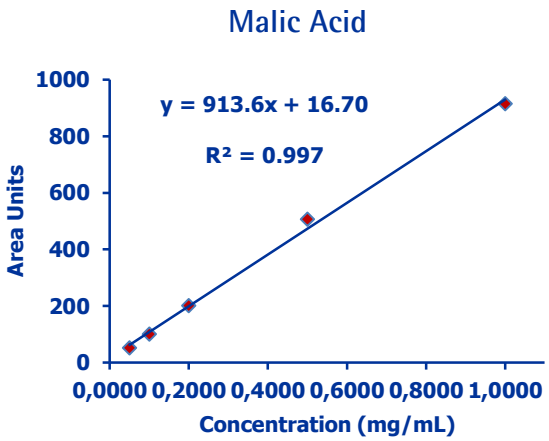
No.	Compound	Retention Time (min)	Retention factor	Asymmetry
1	Tartaric Acid	1.6	0.6	1.2
2	Malic Acid	1.8	0.8	1.2
3	Citric Acid	2.7	1.7	1.4
4	Succinic Acid	3.2	2.2	1.2

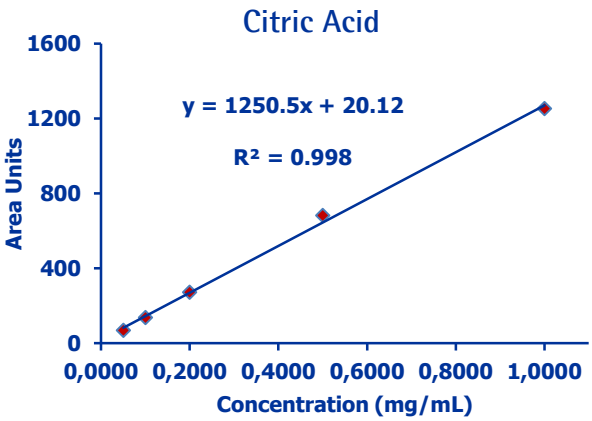
Analysis of Organic Acids in Beverages

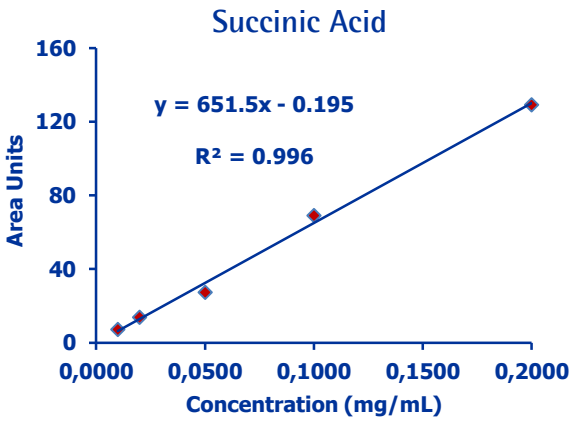
Chromolith® HighResolution RP-18 endcapped

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.









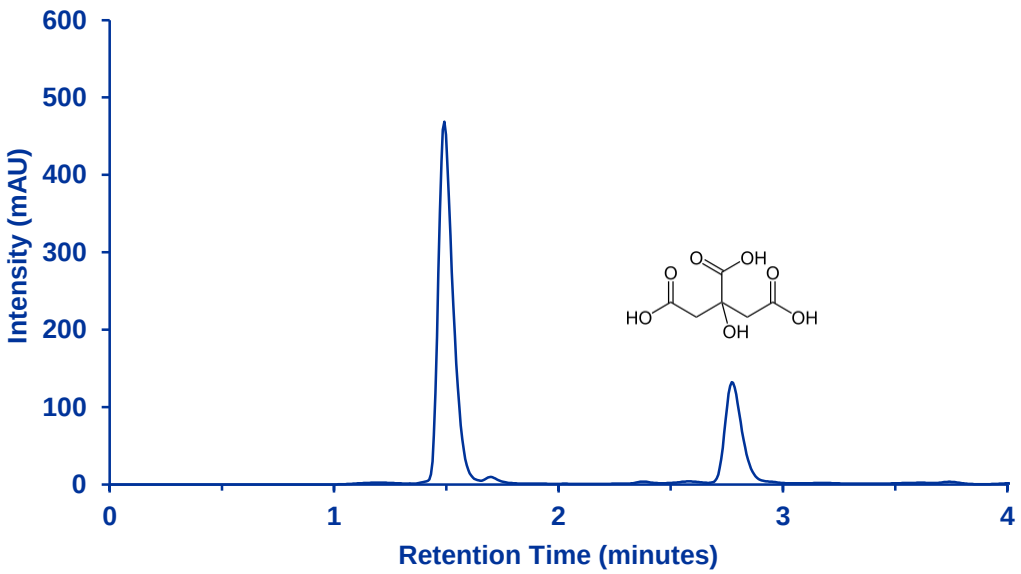
As a final test of the method, a commercial orangeade was analysed and as can be seen on next page only citric acid was found in the beverage. The citric acid concentration was determined to 1.1 mg/mL

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