Lipid Analysis

by Evaporative Light Scattering Detection

GRACE

Brochure #521A

High Performance Analysis of Polar and Non-Polar Lipid

Introduction

The composition of the lipid fraction in fats and oils influences the nutritional value of the food and is important for health related issues such as digestion, heart disease, and more.

Triglycerides are a major component of the non-polar lipid fraction. This is usually very complex due to various chain lengths and different degrees of saturation. Phospholipids and sphingolipids have a positive influence on cholesterol levels and are also used for their emulsifying qualities.

Combine the benefits of Evaporative Light Scattering Detection with Alltech's HPLC columns for sensitive, efficient, gradient-compatible separations of both polar and non-polar lipid fractions.

References

Analysis of Phospho- and Sphingolipids in Dairy Products by a New HPLC Method, R. Rombaut, J. V. Camp and K. Dewettinck - Ghent University, Belgium, published in J. Dairy Sci. 88:482-488.

Triglyceride Analysis of Edible Oils by HPLC-ELSD, R. Rombaut, I. Foubert, V. Van Hoed, K. Degrave, R. Verhé and K. Dewettinck - Ghent University, Belgium, to be published.

Benefits of Evaporative Light Scattering Detection (ELSD) for Lipid Analysis

- More sensitive than RI and low-wavelength UV
- Gradient compatible
- Eliminates need for derivatization
- Solvent choice not limited by UV-cutoff

The Evaporative Light Scattering Detector (ELSD) is a universal HPLC detector that detects any compound less volatile than the mobile phase to low nanogram levels. It has many advantages over RI and low-wavelength UV detection for lipid analyses.

Since most lipid compounds are not good UV absorbers, they must be monitored at low wavelengths. This can be hindered by the mobile phase solvent's UV cutoff and poor baseline stability. With ELSD, the mobile phase evaporates before detection, so it is blind to the mobile phase's spectroscopic properties and compositional changes.

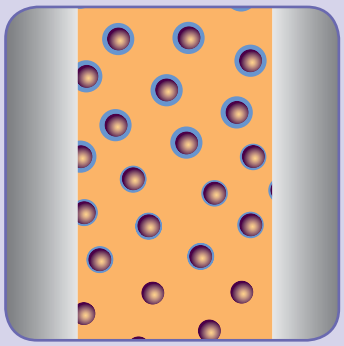
Although refractive index (RI) detection is universal in nature, its lack of sensitivity and gradient incompatibility make it less than ideal for lipid analysis. Evaporation of the mobile phase before detection makes the ELSD gradient compatible for improved resolution and faster separations. In addition, ELSDs can be as much as 10 to 1000 times more sensitive than RI detectors.

How does Evaporative Light Scattering Detection work?

ELSDs detect any compound less volatile than the mobile phase using a simple three-step process:

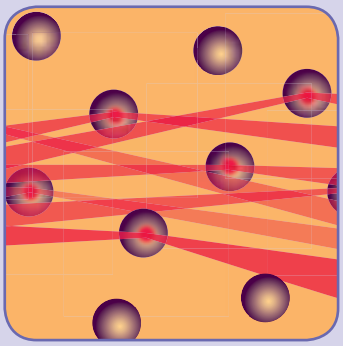
1. Nebulization

Column effluent passes through a needle and mixes with nitrogen gas to form a dispersion of droplets.



2. Evaporation

Droplets pass through a heated "drift tube" where the mobile phase evaporates, leaving a fine mist of dried sample particles in solvent vapor.



3. Detection

The sample particles pass through a cell and scatter light from a laser beam. The scattered light is detected, generating a signal.

Alltima™ HP C18 Hi-Load Columns for Triglycerides

- Excellent peak symmetry
- Superior retention and loading capacity
- Stable from pH 1-10
- Extremely low bleed

Alltima HP C18 Hi-Load columns use high purity, low metal, pH stable silica for high resolution with no tailing and little to no bleed. The high carbon load, combined with strong organic mobile phases, enhance triglyceride solubility and performance with ELSD or MS detection. Alltima HP C18 Hi-Load columns are ideal for analysis of complex hydrophobic mixtures.

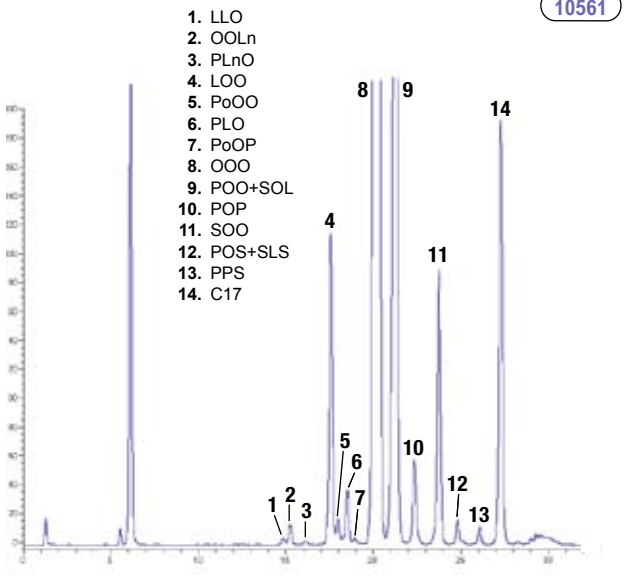
Prevail™ Silica for Polar Lipids

- High capacity and resolution of polar lipids
- Long column life
- Stable from pH 1-8

Prevail silica's high surface area and high purity increase capacity and resolution of polar lipids while maintaining excellent peak shape. Choose 3µm particles for high efficiencies, and a variety of column formats for speed and ELSD and MS compatibility. Prevail Silica is ideal for analysis of polar lipids in dairy products.

Olive Oil

CHROM 10561



Column:	Alltima HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)
Mobile Phase:	**A:** Acetonitrile **B:** Dichloromethane

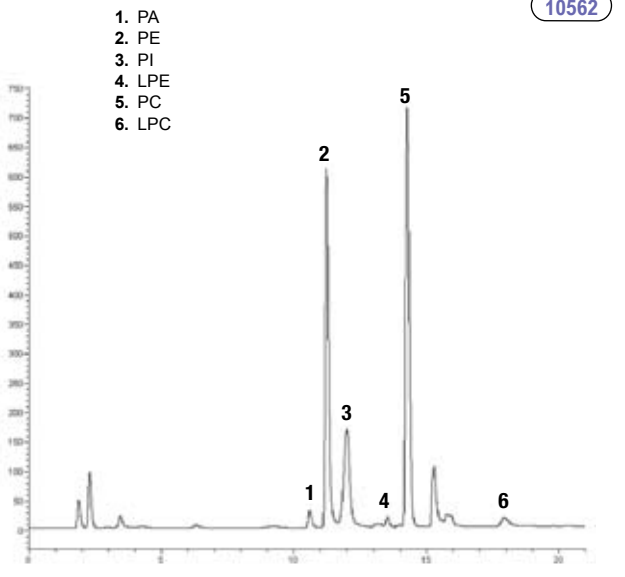
Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate:	0.72mL/min
Detector:	ELSD
Column Temp:	20°C

Lecithin, 0.5mg/mL

CHROM 10562



Column:	Prevail Silica, 3µm, 150 x 3.0mm (**Part No. 99341**)
Mobile Phase:	**A:** Chloroform **B:** Methanol **C:** Triethylamine buffer pH3

Gradient:

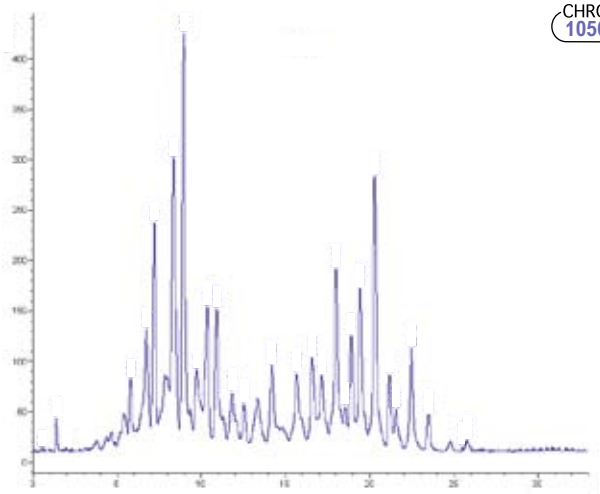
Time:	0	16	17
%B:	12	60	12
%C:	0.5	12	0.5

Flow Rate:	0.5mL/min
Detector:	ELSD
Column Temp:	40°C

Chromatograms courtesy of Prof. Dr. Ir. K. Dewettinck and Ir. R. Rombaut - Ghent University (Belgium) Laboratory of Food Technology & Engineering.

Butter

CHROM 10563



Column: Alltima™ HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)
Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

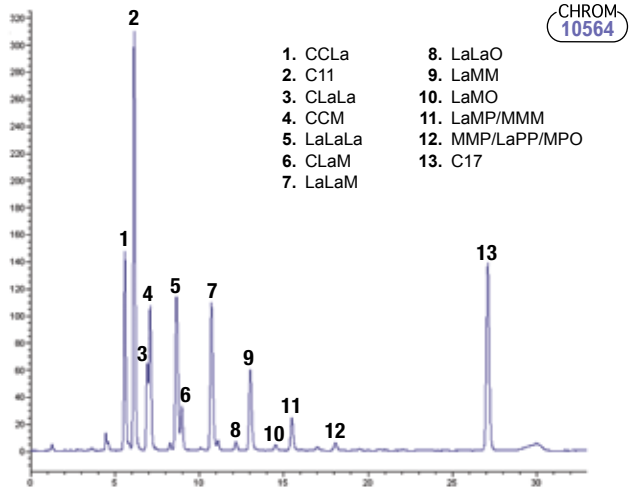
Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate: 0.72mL/min
Detector: ELSD
Column Temp: 20°C

Coconut Oil

CHROM 10564



Column: Alltima HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)
Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

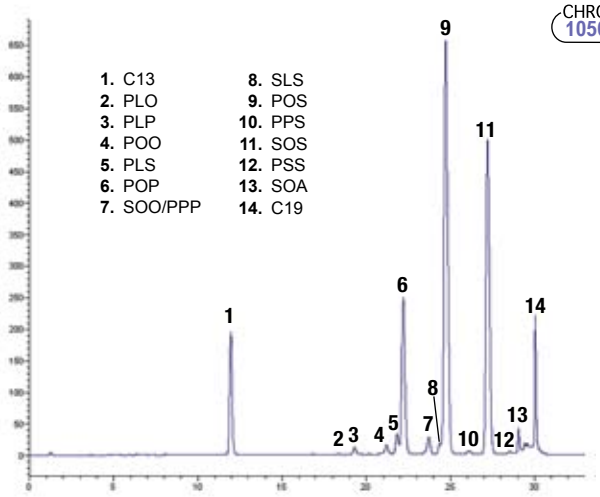
Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate: 0.72mL/min
Detector: ELSD
Column Temp: 20°C

Cocoa Butter

CHROM 10565



Column: Alltima HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)
Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

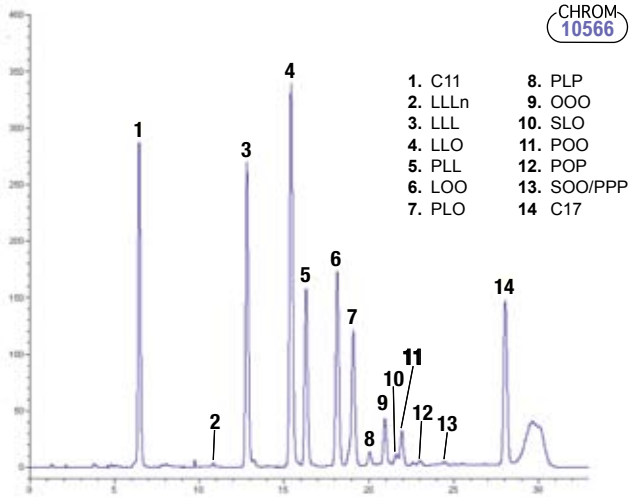
Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate: 0.72mL/min
Detector: ELSD
Column Temp: 20°C

Corn Oil

CHROM 10566



Column: Alltima HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)
Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

Gradient:

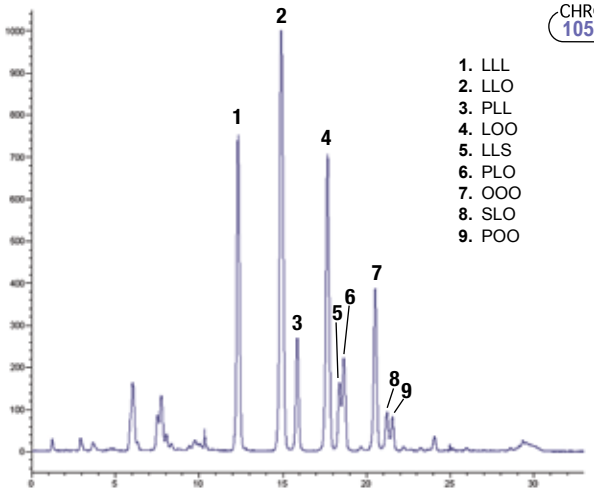
Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate: 0.72mL/min
Detector: ELSD
Column Temp: 20°C

Chromatograms courtesy of Prof. Dr. Ir. K. Dewettinck and Ir. R. Rombaut - Ghent University (Belgium) Laboratory of Food Technology & Engineering.

Sunflower Oil

CHROM 10567



Column: Alltima™ HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)

Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

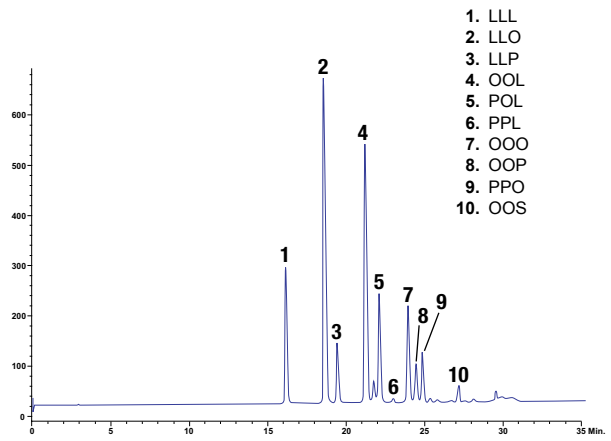
Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate: 0.72mL/min
Detector: ELSD
Column Temp: 20°C

Sesame Seed Oil

CHROM 10581



Column: Alltima HP C18 Hi-Load, 5µm, 250 x 4.6mm (**Part No. 87698**)

Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

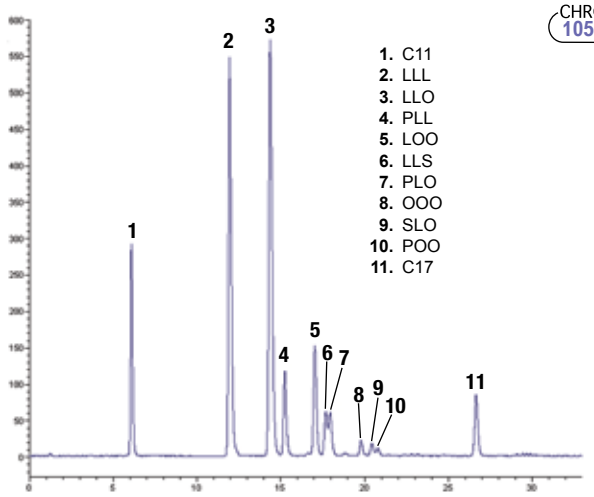
Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate: 1.0mL/min
Detector: ELSD
Column Temp: 20°C

Refined Sunflower Oil

CHROM 10569



Column: Alltima HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)

Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

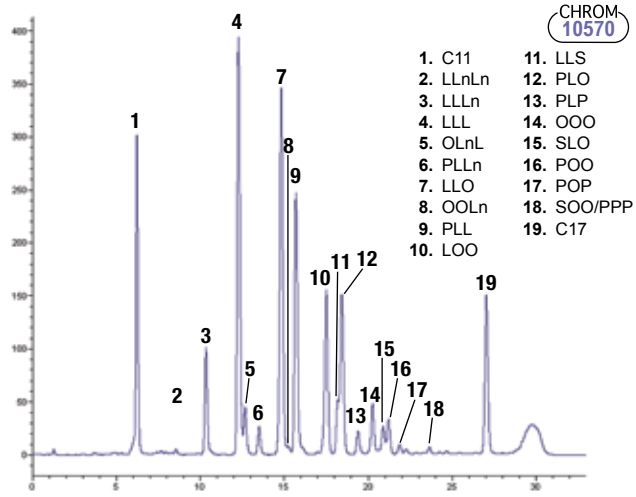
Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate: 0.72mL/min
Detector: ELSD
Column Temp: 20°C

Soybean Oil

CHROM 10570



Column: Alltima HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)

Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

Gradient:

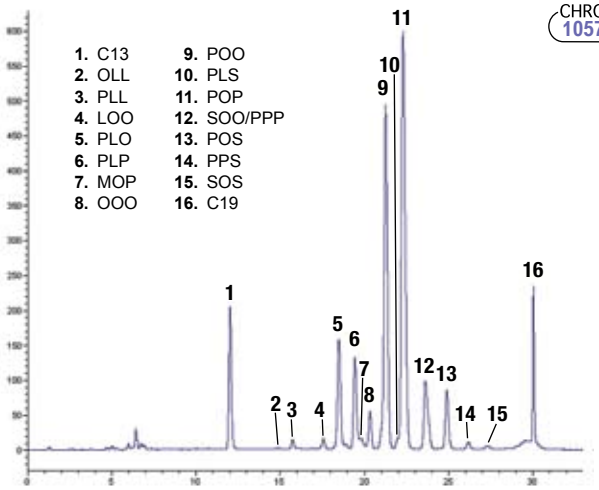
Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate: 0.72mL/min
Detector: ELSD
Column Temp: 20°C

Chromatograms courtesy of Prof. Dr. Ir. K. Dewettinck and Ir. R. Rombaut - Ghent University (Belgium) Laboratory of Food Technology & Engineering.

Palm Oil

CHROM 10571



Column: Alltima™ HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)

Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

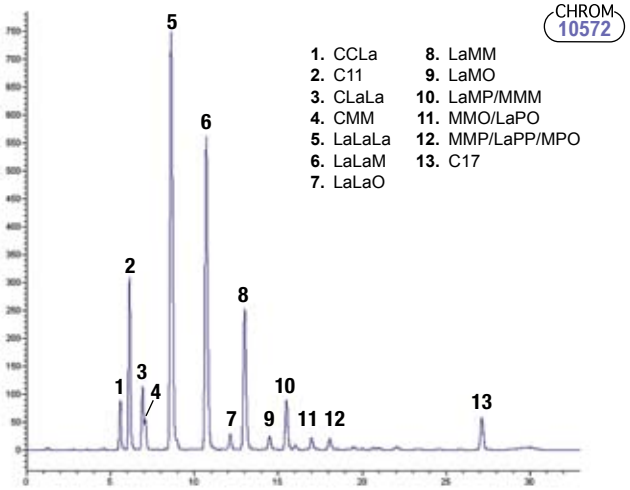
Flow Rate: 0.72mL/min

Detector: ELSD

Column Temp: 20°C

Palm Kernel Stearin Fraction

CHROM 10572



Column: Alltima HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)

Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

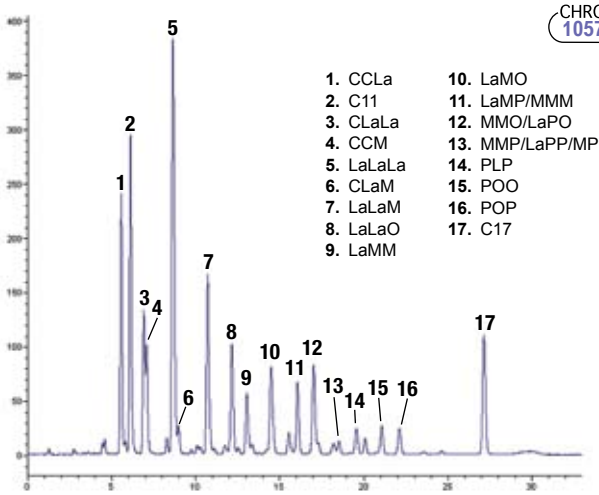
Flow Rate: 0.72mL/min

Detector: ELSD

Column Temp: 20°C

Palm Kernel Olein Fraction

CHROM 10573



Column: Alltima HP C18 Hi-Load, 3µm, 150 x 3.0mm (**Part No. 87610**)

Mobile Phase: **A:** Acetonitrile
B: Dichloromethane

Gradient:

Time:	0	25	26	27	28
%B:	30	51	70	70	30

Flow Rate: 0.72mL/min

Detector: ELSD

Column Temp: 20°C

Compound Translation Key

Triglycerides

A	Arachidic Acid
C	Caproic Acid
La	Lauric Acid
L	Linoleic Acid
Ln	Linolenic Acid
M	Myristic Acid
O	Oleic Acid
P	Palmitic Acid
S	Stearic Acid

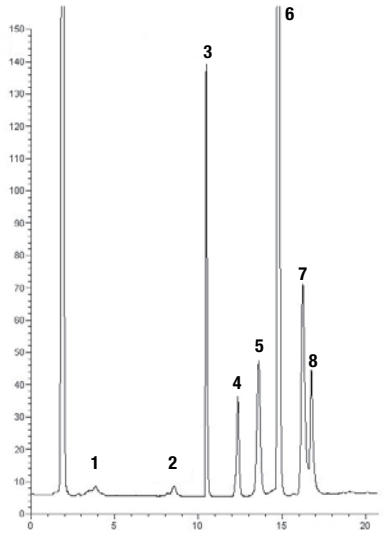
Polar Lipids

PS	Phosphatidylserine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PC	Phosphatidylcholine
PA	Phosphatidic Acid
LPE	lyso-Phosphatidylethanolamine
LPC	lyso-Phosphatidylcholine
GLUCCER	Glucosylceramide
LACCER	Lactosylceramide
SM1	Sphingomyelin 1
SM2	Sphingomyelin 2
U	Unknown

Chromatograms courtesy of Prof. Dr. Ir. K. Dewettinck and Ir. R. Rombaut - Ghent University (Belgium) Laboratory of Food Technology & Engineering.

Polar Lipid Standards

CHROM 10575



1. GLUCCER
2. LACCER
3. PE
4. PI
5. PS
6. PC
7. SM1
8. SM2

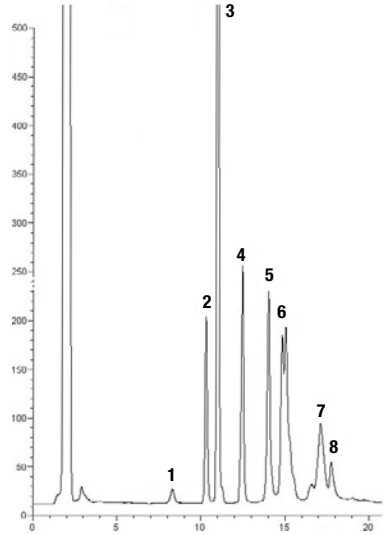
Column: Prevail™ Silica, 3µm, 150 x 3.0mm (**Part No. 99341**)
Mobile Phase: **A:** Chloroform; **B:** Methanol; **C:** Triethylamine buffer pH3
Gradient:

Time:	0	16	17
%B:	12	60	12
%C:	0.5	12	0.5

Flow Rate: 0.5mL/min
Detector: ELSD
Column Temp: 40°C

Acid Whey

CHROM 10576



1. LACCER
2. PA
3. PE
4. PI
5. PS
6. PC
7. SM1
8. SM2

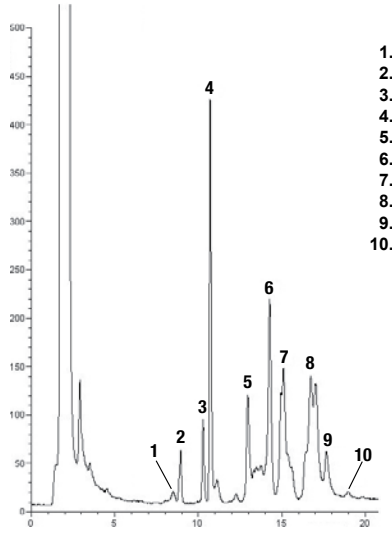
Column: Prevail Silica, 3µm, 150 x 3.0mm (**Part No. 99341**)
Mobile Phase: **A:** Chloroform; **B:** Methanol; **C:** Triethylamine buffer pH3
Gradient:

Time:	0	16	17
%B:	12	60	12
%C:	0.5	12	0.5

Flow Rate: 0.5mL/min
Detector: ELSD
Column Temp: 40°C

Gouda Cheese

CHROM 10577



1. LACCER
2. U
3. PA
4. PE
5. PI
6. PS
7. PC
8. SM1
9. SM2
10. LPC

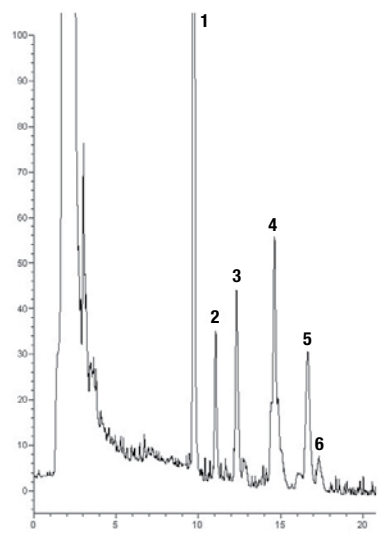
Column: Prevail Silica, 3µm, 150 x 3.0mm (**Part No. 99341**)
Mobile Phase: **A:** Chloroform; **B:** Methanol; **C:** Triethylamine buffer pH3
Gradient:

Time:	0	16	17
%B:	12	60	12
%C:	0.5	12	0.5

Flow Rate: 0.5mL/min
Detector: ELSD
Column Temp: 40°C

Butter

CHROM 10578



1. PE
2. PI
3. PS
4. PC
5. SM1
6. SM2

Column: Prevail Silica, 3µm, 150 x 3.0mm (**Part No. 99341**)
Mobile Phase: **A:** Chloroform; **B:** Methanol; **C:** Triethylamine buffer pH3
Gradient:

Time:	0	16	17
%B:	12	60	12
%C:	0.5	12	0.5

Flow Rate: 0.5mL/min
Detector: ELSD
Column Temp: 40°C

Chromatograms courtesy of Prof. Dr. Ir. K. Dewettinck and Ir. R. Rombaut - Ghent University (Belgium) Laboratory of Food Technology & Engineering.

Ordering Information

Triglycerides – Alltima™ HP Columns

Alltima HP HPLC Columns			
Phase	**Format**	**I.D. x Length**	**Part No.**
C18 Hi-Load, 3µm	Expedite™ MS	2.1 x 10mm	**87691**
	Expedite MS	2.1 x 20mm	**87692**
	LC/MS	2.1 x 50mm	**87506**
	LC/MS	2.1 x 100mm	**87687**
	LC/MS	2.1 x 150mm	**87688**
	Solvent-Reducer	3.0 x 150mm	**87610**
	Expedite MS	4.6 x 10mm	**87693**
	Expedite MS	4.6 x 20mm	**87694**
	Analytical	4.6 x 50mm	**87827**
	Analytical	4.6 x 100mm	**87685**
	Analytical	4.6 x 150mm	**87686**
	Rocket™	7.0 x 33mm	**87689**
	Rocket	7.0 x 53mm	**87690**
C18 Hi-Load, 5µm	LC/MS	2.1 x 150mm	**87699**
	LC/MS	2.1 x 250mm	**87700**
	Solvent-Reducer	3.0 x 150mm	**87611**
	Solvent-Reducer	3.0 x 250mm	**87612**
	Analytical	4.6 x 150mm	**87697**
	Analytical	4.6 x 250mm	**87698**

Polar Lipids – Prevail™ Columns

Prevail HPLC Columns			
Phase	**Format**	**I.D. x Length**	**Part No.**
Silica, 3µm	Expedite MS	2.1 x 10mm	**43841**
	Expedite MS	2.1 x 20mm	**43826**
	LC/MS	2.1 x 50mm	**43868**
	LC/MS	2.1 x 100mm	**43805**
	LC/MS	2.1 x 150mm	**99267**
	Solvent-Reducer	3.0 x 150mm	**99341**
	Expedite MS	4.6 x 10mm	**43858**
	Expedite MS	4.6 x 20mm	**43816**
	Analytical	4.6 x 50mm	**43842**
	Analytical	4.6 x 100mm	**99269**
	Analytical	4.6 x 150mm	**99271**
	Rocket	7.0 x 33mm	**99284**
	Rocket	7.0 x 53mm	**99283**
Silica, 5µm	Analytical	2.1 x 150mm	**99273**
	Solvent-Reducer	3.0 x 150mm	**99339**
	Solvent-Reducer	3.0 x 250mm	**99340**
	Analytical	4.6 x 150mm	**99275**
	Analytical	4.6 x 250mm	**99277**

Alltima HP All-Guard™ Cartridges*			
Phase	**I.D. x Length**	**Qty**	**Part No.**
C18 Hi-Load	2.1 x 7.5mm	3	**87701**
	3.0 x 7.5mm	3	**87624**
	4.6 x 7.5mm	3	**87702**
All-Guard Cartridge Holder (Includes Direct-Connect™ Column Coupler)		ea	**80101**

*All-Guard Holder required

Prevail™ All-Guard Cartridges*			
Phase	**I.D. x Length**	**Qty**	**Part No.**
Silica	2.1 x 7.5mm	3	**99133**
	3.0 x 7.5mm	3	**99354**
	4.6 x 7.5mm	3	**99291**
All-Guard Cartridge Holder (Includes Direct-Connect Column Coupler)		ea	**80101**

*All-Guard Holder required

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