Lipid Analysis

by Evaporative Light Scattering Detection
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.

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.

References


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.

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.

**Column:**

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>Time</th>
<th>%B</th>
<th>Flow Rate</th>
<th>Detector</th>
<th>Column Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Acetonitrile</td>
<td>0</td>
<td>30</td>
<td>0.72mL/min</td>
<td>ELSD</td>
<td>20°C</td>
</tr>
<tr>
<td>B: Dichloromethane</td>
<td>25</td>
<td>51</td>
<td>70</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>C: Triethylamine buffer pH3</td>
<td>26</td>
<td>70</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Mobile Phase:**

- A: Acetonitrile
- B: Dichloromethane
- C: Triethylamine buffer pH3

**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.

**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.

**Column:**

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>Time</th>
<th>%B</th>
<th>Flow Rate</th>
<th>Detector</th>
<th>Column Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Chloroform</td>
<td>0</td>
<td>12</td>
<td>0.5mL/min</td>
<td>ELSD</td>
<td>40°C</td>
</tr>
<tr>
<td>B: Methanol</td>
<td>16</td>
<td>60</td>
<td>0.5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>C: Triethylamine buffer pH3</td>
<td>17</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Mobile Phase:**

- A: Chloroform
- B: Methanol
- C: Triethylamine buffer pH3

**Gradient:**

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

**Flow Rate:**

- 0.5mL/min

**Detector:**

- ELSD

**Column Temp:**

- 40°C
Butter

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

1. CCLa
2. C11
3. CLaLa
4. CCM
5. LaLaLa
6. CLaM
7. LaLaM

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

1. C13
2. PLO
3. PLP
4. POO
5. PLL
6. POP
7. SOO/PPP
8. SLS
9. POS
10. PPS
11. SOS
12. PSS
13. SOA
14. C19

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

1. C11
2. LLLn
3. LLL
4. LLO
5. PLL
6. LOO
7. LPL
8. PLP
9. OOO
10. SLO
11. POO
12. POP
13. SOO/PPP
14. C17

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.
Triglycerides

Sunflower Oil

Column: Alltima™ HP C18 Hi-Load, 3µm, 150 x 3.0mm (Part No. 87610)
Mobile Phase: A: Acetonitrile
B: Dichloromethane
Gradient:
Flow Rate: 0.72mL/min
Detector: ELSD
Column Temp: 20°C

Mobile Phase: A: Acetonitrile
B: Dichloromethane
Gradient:
Flow Rate: 1.0mL/min
Detector: ELSD
Column Temp: 20°C

Sesame Seed Oil

Column: Alltima HP C18 Hi-Load, 5µm, 250 x 4.6mm (Part No. 87698)
Mobile Phase: A: Acetonitrile
B: Dichloromethane
Gradient:
Flow Rate: 0.72mL/min
Detector: ELSD
Column Temp: 20°C

Column: Alltima HP C18 Hi-Load, 3µm, 150 x 3.0mm (Part No. 87610)
Mobile Phase: A: Acetonitrile
B: Dichloromethane
Gradient:
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.
Chromatograms courtesy of Prof. Dr. Ir. K. Dewettinck and Ir. R. Rombaut - Ghent University (Belgium) Laboratory of Food Technology & Engineering.

**Palm Oil**

1. C13  9. POO
2. OLL  10. PLS
3. PLL  11. POP
4. LOO  12. SOO/PPP
5. PLO  13. POS
6. PLP  14. PPS
7. MOP  15. SOS
8. OOO  16. C19

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**

1. CCLa  8. LaMM
2. C11  9. LaMO
3. CLaLa  10. LaMP/MMM
4. CMM  11. MMO/LaPO
5. LaLaLa  12. MMP/LaPP/MMO
6. LaLaM  13. C17
7. LaLaO  14. LaLaO

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**

1. CCLa  10. LaMO
2. C11  11. LaMP/MMM
3. CLaLa  12. MMO/LaPO
4. CMM  13. MMP/LaPP/MMO
5. LaLaLa  14. PLP
6. LaLaM  15. POO
7. LaLaO  16. POP
8. LaLaO  17. C17

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
LACER  Lactosylceramide
SM1  Sphingomyelin 1
SM2  Sphingomyelin 2
U  Unknown
Polar Lipid Standards

1. GLUCER
2. LACER
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

1. LACER
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

1. LACER
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

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.
### Triglycerides – Alltima™ HP Columns

<table>
<thead>
<tr>
<th>Phase</th>
<th>Format</th>
<th>I.D. x Length</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18 Hi-Load, 3µm</td>
<td>Expedite MS</td>
<td>2.1 x 10mm</td>
<td>87691</td>
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<td></td>
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<td></td>
<td>Expedite MS</td>
<td>4.6 x 10mm</td>
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<td>3.0 x 150mm</td>
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### Polar Lipids – Prevail™ Columns

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<th>Format</th>
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</thead>
<tbody>
<tr>
<td>Silica, 3µm</td>
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### Alltima HP All-Guard™ Cartridges*

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<th>I.D. x Length</th>
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<th>Part No.</th>
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<td>C18 Hi-Load</td>
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All-Guard Cartridge Holder
(Includes Direct-Connect™ Column Coupler)

ea 80101

*All-Guard Holder required

### Prevail™ All-Guard Cartridges*

<table>
<thead>
<tr>
<th>Phase</th>
<th>I.D. x Length</th>
<th>QTY</th>
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</table>

All-Guard Cartridge Holder
(Includes Direct-Connect Column Coupler)

ea 80101

*All-Guard Holder required