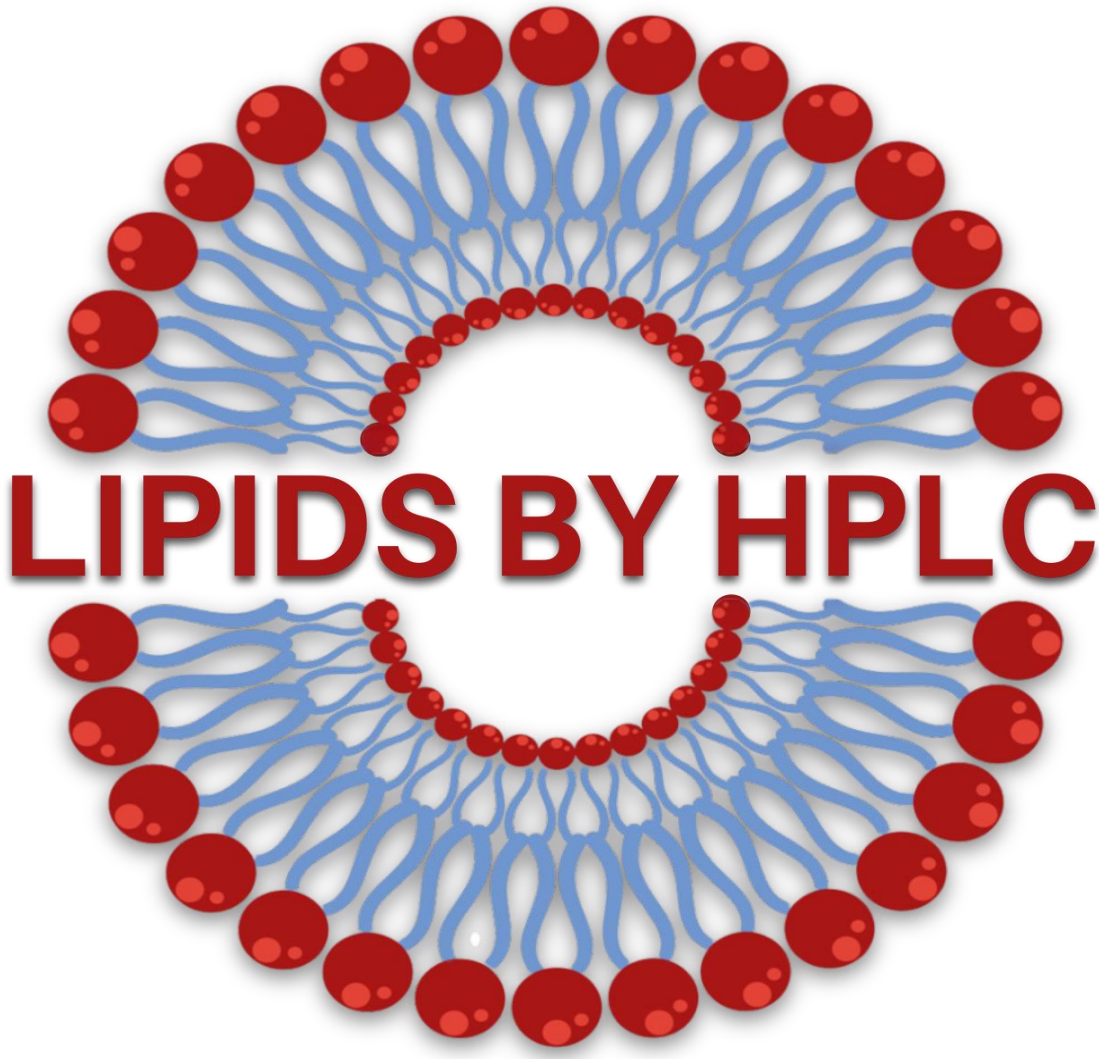
SIELC Technologies, Inc.
Wheeling, IL 60090 USA
P. 847-229-2629 **F.** 847-655-6079
mail@sielc.com www.sielc.com

SIELC



Lipids Analysis Background

Lipids are essential biological compounds that play crucial roles in cellular structure, energy storage, and signaling. They are found in various forms in living organisms and are characterized by their hydrophobic nature, with a small hydrophilic region that enables the formation of structures like membranes and micelles.

Different types of lipids are present depending on the source and function within the organism, and they can vary widely in structure and properties.

Chromatographic separation of lipids, particularly in reverse-phase chromatography, poses unique challenges due to their strong hydrophobicity. This hydrophobic nature results in strong interactions with the hydrophobic stationary phase, which can make separation difficult. Furthermore, the low solubility of lipids in aqueous mobile phases complicates the process, requiring the use of organic solvents.

Lipids are generally classified into three main categories based on their structural and functional characteristics.

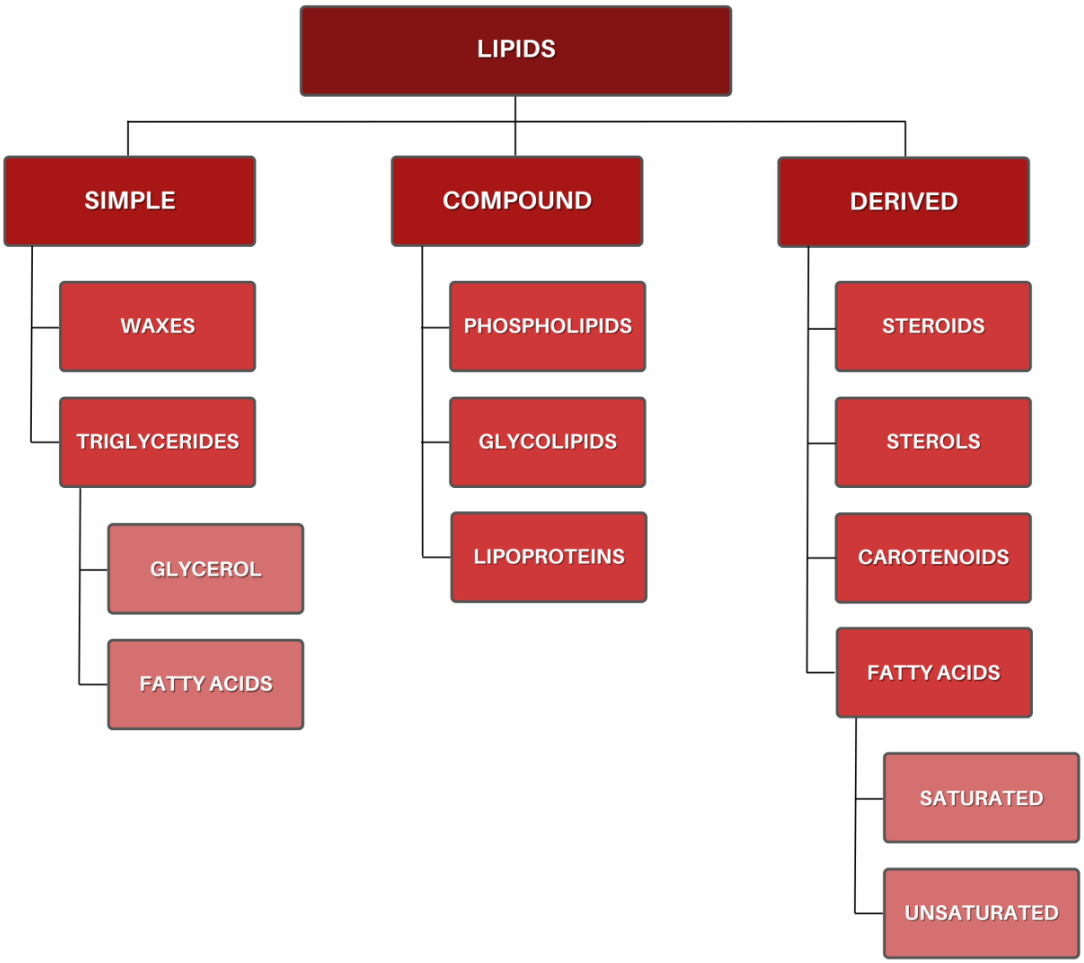


Fig. 1. Lipid Classification Overview

Lipids Analysis Background

Lipids encompass a vast array of classes with a wide range of polarities, from nonpolar triglycerides to amphipathic molecules such as phospholipids and glycolipids. These diverse chemical structures and properties make lipid analysis particularly challenging. Traditional chromatographic techniques, such as reverse-phase (RP) chromatography, often rely on a single interaction mode, typically involving hydrophobic interactions. However, these single-mode approaches are insufficient to fully resolve the complex composition of lipid samples, as they fail to account for the unique intermolecular forces present among various lipid classes.

A single RP column and mobile phase often cannot accommodate both polar and nonpolar lipid species, leading to incomplete separation or poor resolution. This limitation is particularly problematic in comprehensive lipidomics studies, where precise identification and quantification of individual lipid species are crucial.

To address these limitations, our new Lipak™ column utilizes mixed-mode chromatography, integrating reverse-phase functionality with a negatively charged group in its stationary phase. This innovative approach enables simultaneous retention and separation of both polar and nonpolar lipid species, accommodating the diverse chemical properties inherent to lipid classes. By leveraging dual interaction modes, the Lipak™ column offers superior resolution and reproducibility, even for complex lipid mixtures. This makes it an ideal solution for comprehensive lipidomics studies, ensuring precise identification and quantification across a wide range of lipid species while simplifying the analytical workflow.

In addition to lipids, this column is highly effective for separating other compound classes, such as fat-soluble vitamins, chlorophylls, surfactants, and more. The column is compatible with MS, ELSD, and CAD detection methods and is optimized for high-organic mobile phases. This eliminates solubility issues with fatty molecules, ensuring good peak shape and efficient separation.

The Lipak™ column is available in both 5 µm and 3 µm particle sizes and can be customized in any standard length and diameter to meet your specific analytical needs.

To order a column or ask a question send your message to sales@sielc.com or call us at +1 (847) 229-2629

Create a column part number by using chart below

LP - 46.150.0510

Type of Packing

Lipak	LP

Column ID

22 mm	220
1.0 mm	100
4.6 mm	46
3.2 mm	32
2.1 mm	21

Column Length

250 mm	250
150 mm	150
100 mm	100
50 mm	050

Particle Size

3 µm	03
5 µm	05

Pore Size

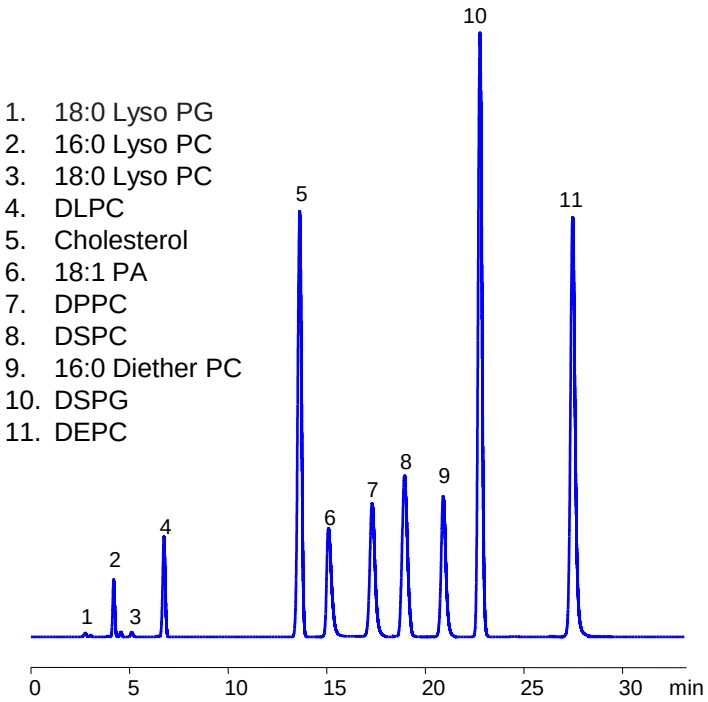
100 A	10

SIELC Technologies, Inc.

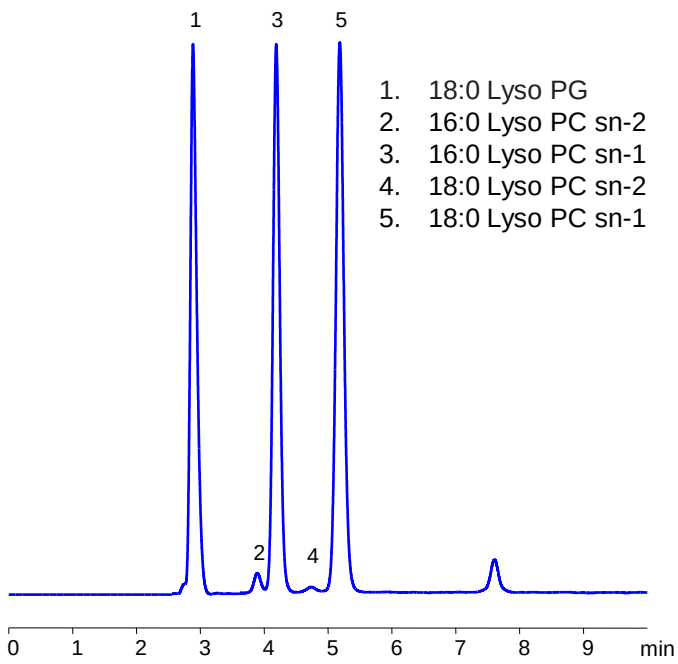
www.sielc.com email: mail@sielc.com ph. 847-229-2629 fax 847-655-6079

Phospholipids

Phospholipids are essential biomolecules that play critical roles in cellular structure and signaling, making their accurate analysis a cornerstone of lipidomics research. However, their amphiphilic nature and structural diversity—ranging from varying headgroups to differing fatty acid chain lengths and degrees of saturation—pose significant challenges for chromatographic separation. To address these complexities, our **Lipak™** column offers a groundbreaking solution for phospholipid analysis. By combining reverse-phase interactions with a strong polar interaction, the Lipak™ column provides exceptional retention and resolution of phospholipids across a wide polarity spectrum. This unique mixed-mode approach ensures precise separation of phospholipid classes, enabling comprehensive profiling and quantification critical for understanding their biological functions and implications in health and disease.



Column:	Lipak™
Column size:	4.6 × 150 mm, 5 µm, 100A
Column part number:	LP-46.150.0510
Mobile phase:	Gradient MeOH – 90 - 100%, 20 min, 10 min hold
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	1.0 mL/min
Detection:	ELSD



Column:	Lipak™
Column size:	4.6 × 150 mm, 5 µm, 100A
Column part number:	LP-46.150.0510
Mobile phase:	MeOH – 90%
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	1.0 mL/min
Detection:	ELSD

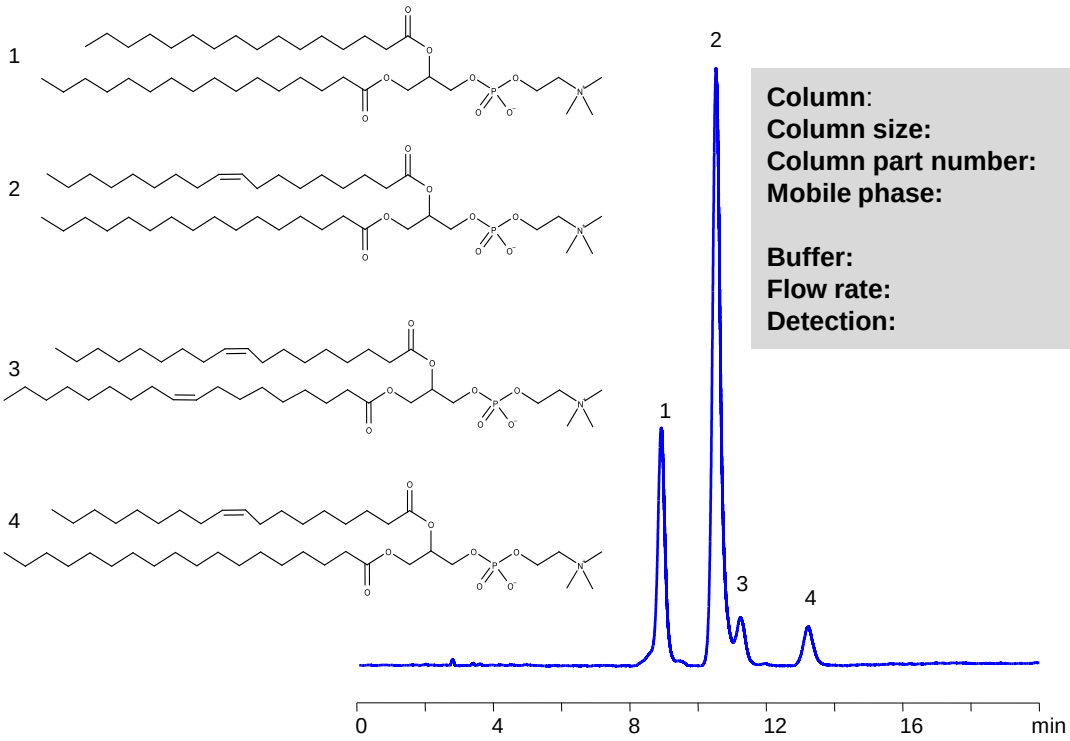
Fig. 2. Separation of Phospholipids

These chromatograms showcase the Lipak™ column's effectiveness in separating phospholipids with different chromatographic methods. The left chromatogram, using a gradient approach, separates a broad range of phospholipids, including Lyso PG, Lyso PC, and cholesterol. The right chromatogram, employing an isocratic method, targets the separation of Lyso PC species. Together, these examples highlight the column's versatility in phospholipid analysis, offering flexibility for both comprehensive and targeted separations.

Phospholipids

L-α-Phosphatidylcholine, commonly known as lecithin, is a crucial phospholipid found in cell membranes, playing a key role in maintaining their structural integrity. Composed of a glycerol backbone, two fatty acids, a phosphate group, and choline, L-α-Phosphatidylcholine exhibits amphipathic properties, with both hydrophilic and hydrophobic regions. This unique structure is essential for the formation of lipid bilayers in cellular membranes, promoting membrane fluidity and stability. As a major component of egg yolk, L-α-Phosphatidylcholine is involved in the structural organization of lipid membranes and has applications in various industries, including pharmaceuticals and biotechnology, due to its emulsifying and stabilizing properties.

1. Dipalmitoylphosphatidylcholine (DPPC) ~ 10 - 15% 16:0/16:0
2. Palmitoyl-oleoylphosphatidylcholine (POPC) ~ 32 - 35% 16:0/18:1
3. Dioleoylphosphatidylcholine (DOPC) ~ 10 - 15% 18:1/18:1
4. Stearoyl-oleoylphosphatidylcholine (SOPC) – 15 - 20% 18:0/18:1



Column:	Lipak™
Column size:	4.6 × 150 mm, 5 µm, 100A
Column part number:	LP-46.150.0510
Mobile phase:	Gradient MeOH – 95 - 100%, 20 min
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	1.0 mL/min
Detection:	ELSD

Fig. 3. Analyzing Phospholipids in Egg Yolk

The phospholipids, including L-α-Phosphatidylcholine and its derivatives such as DPPC, POPC, DOPC, and SOPC, are commonly extracted from egg yolk, a rich natural source. Using the Lipak™ column, these lipids can be successfully separated and quantified. This method enables the effective detection of these phospholipids, which are vital for cellular membrane function and have numerous applications in the pharmaceutical, biotechnology, and food industries. The separation achieved by using the Lipak™ column allows for precise analysis of complex lipid mixtures, ensuring accurate quantification and characterization of individual lipid species, a capability not attainable with traditional chromatographic methods.

Acylcarnitines

Lipak™ column is suitable for separation of many other hydrophobic compounds. Acylcarnitines are a group of molecules formed by the esterification of carnitine with fatty acids. Acylcarnitines are involved in fatty acid metabolism and are essential for maintaining cellular energy homeostasis. These compounds circulate in the blood and can serve as biomarkers for various metabolic conditions, including disorders related to fatty acid oxidation.

Octanoyl-L-carnitine and Stearoyl-L-carnitine, as medium- and long-chain acylcarnitines, respectively, play essential roles in transporting fatty acids across mitochondrial membranes for energy production—a process crucial to fatty acid β-oxidation in the mitochondria.

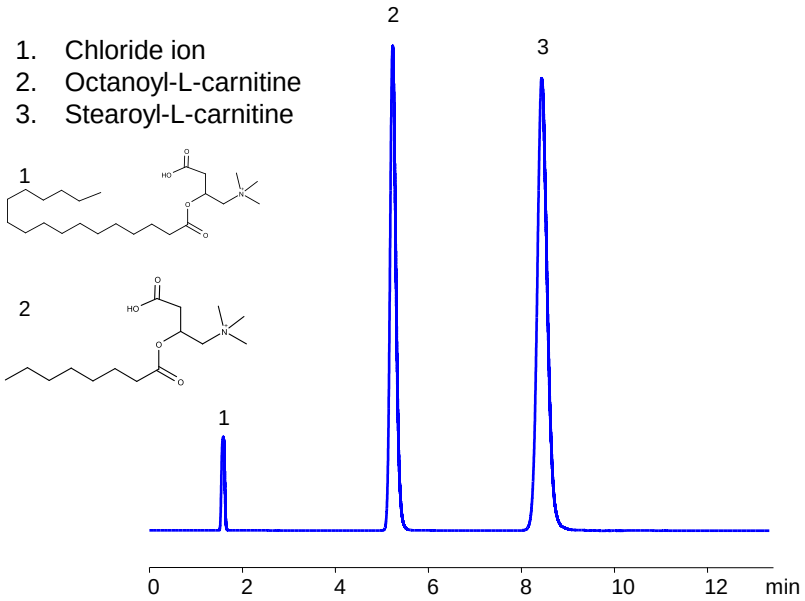


Fig. 4. Separation of Acylcarnitines

Column:	Lipak™
Column size:	4.6 × 150 mm, 5 µm, 100A
Column part number:	LP-46.150.0510
Mobile phase:	MeOH – 85%
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	1.0 mL/min
Detection:	ELSD

Lutein and Chlorophylls A & B

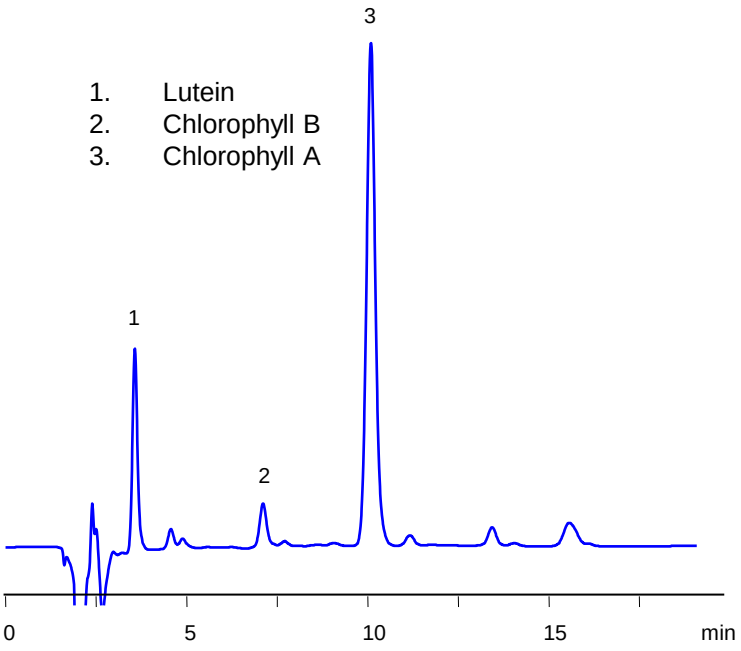


Fig. 5. Separation of Lutein and Chlorophylls A & B

Lutein, chlorophyll A, and chlorophyll B are essential compounds often analyzed using HPLC for their crucial roles in biological systems. Lutein, with its strong absorbance at 445–450 nm, is notable for protecting cells from UV damage, while chlorophyll A and B, absorbing at 665–680 nm and 645–660 nm respectively, are vital for photosynthesis and light energy absorption. These compounds act as powerful antioxidants, safeguarding cells from oxidative stress and maintaining cellular integrity in plants and other systems.

Column:	Lipak™
Column size:	4.6 × 150 mm, 5 µm, 100A
Column part number:	LP-46.150.0510
Mobile phase:	Gradient MeOH/EtOH/H2O – 95/0/5 – 60/40/0% in 10 min, 8 min hold
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	1.0 mL/min
Detection:	VIS 430nm ESI MS SIM 268+, 907+, 893+

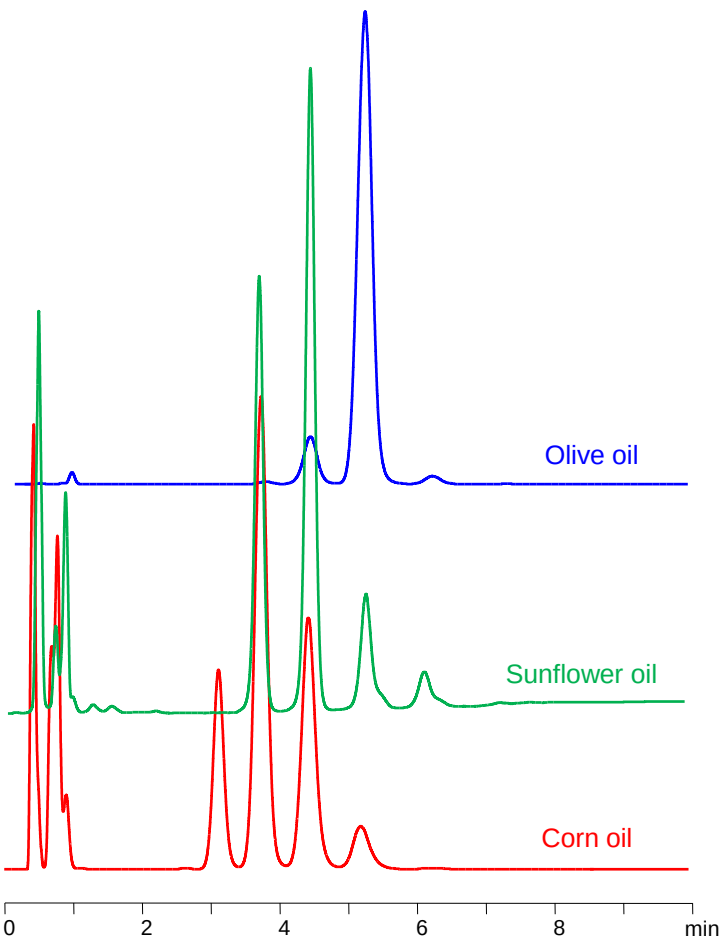
Triglycerides

Triglycerides are the primary form of fat storage in the body, consisting of three fatty acids attached to a glycerol backbone. Their structure influences their physical and chemical properties, making them crucial in fields like nutrition and clinical diagnostics.

High-Quality Triglyceride Chromatograms for Accurate Analysis

Our advanced HPLC solutions offer high-resolution chromatograms for precise triglyceride (TG) analysis, providing:

- **Clear Separation**: Achieve reliable identification and quantification of triglycerides, from short to long chains.
- **Enhanced Reliability**: Minimize interference for consistent, repeatable results in complex mixtures or purified samples.
- **Versatile Applications**: Ideal for clinical diagnostics, food quality control, and more, ensuring fast, accurate analysis in any lab setting.



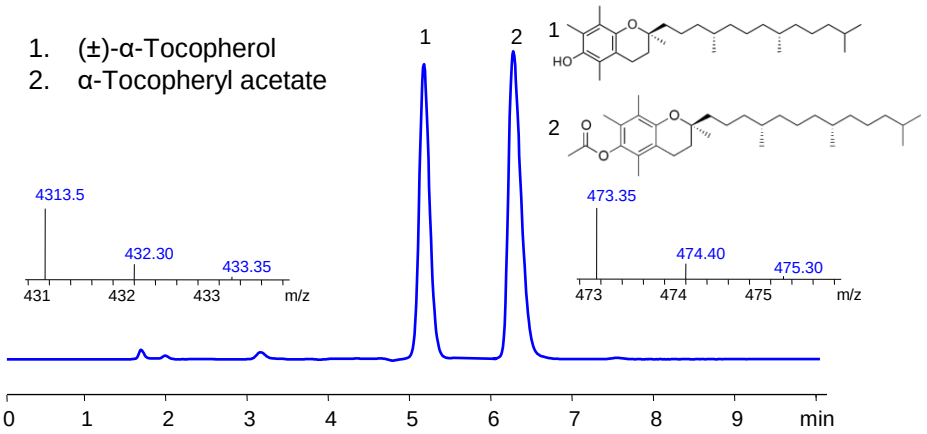
Olive Oil Triglycerides	
Triolein (OOO): ~ 40 - 60%	18:1/18:1/18:1
Oleic-Oleic-Palmitic (OOP): ~ 10 - 20%	18:1/18:1/16:0
Oleic-Oleic-Linoleic (OOL): ~ 15 - 20%	18:1/18:1/18:2
Oleic-Linoleic-Palmitic (OLP): ~ 5 - 10%	18:1/18:2/16:0
Sunflower Oil Triglycerides	
Trilinolein (LLL): 50 - 65%	18:2/18:2/18:2
Linoleic-Oleic-Linoleic (LOL): 10 - 20%	18:1/18:2/18:2
Oleic-Linoleic-Linoleic (OLL): 5 - 15%	18:1/18:2/18:2
Corn Oil Triglycerides	
Trilinolein (LLL): 25-50%	18:2/18:2/18:2
Linoleic-Oleic-Linoleic (LOL): 15-30%	18:1/18:2/18:2
Oleic-Linoleic-Linoleic (OLL): 15-25%	18:1/18:2/18:2

Column:	Lipak™
Column size:	4.6 × 50 mm, 5 µm, 100A
Column part number:	LP-46.50.0510
Mobile phase:	Gradient MeOH/EtOH/H2O – 90/0/10 – 50/50/0% 10 min
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	2.0 mL/min
Detection:	ELSD

Fig. 6. Analyzing Triglycerides in Vegetable Oils

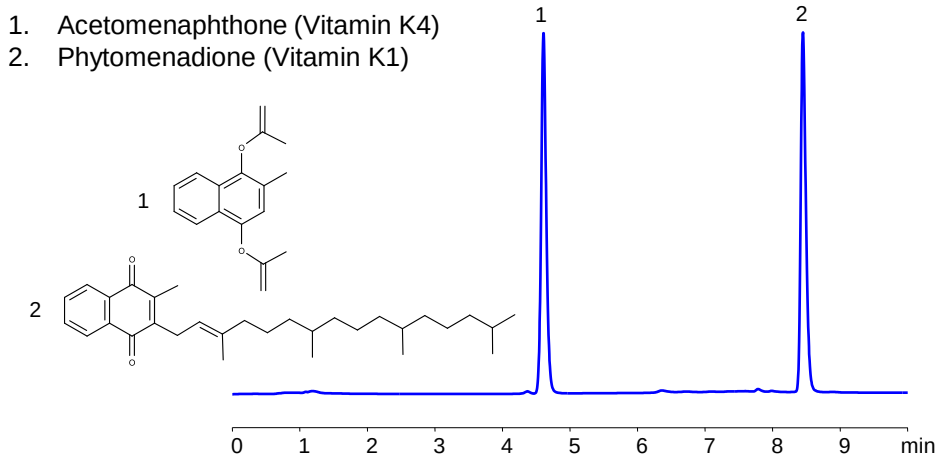
Fat Soluble Vitamins

(±)-α-Tocopherol is a form of vitamin E, a fat-soluble antioxidant that protects cell membranes from oxidative damage by neutralizing free radicals. Widely found in dietary sources like nuts and vegetable oils, it plays a critical role in maintaining skin health, immune function, and overall cellular protection. α-Tocopheryl acetate, on the other hand, is a stable esterified form of α-tocopherol. Commonly used in dietary supplements and skincare products, it is converted to active α-tocopherol in the body, offering a prolonged shelf life and enhanced stability in formulations.



Column:	Lipak™
Column size:	3.2 × 150 mm, 3 µm, 100A
Column part number:	LP-32.150.0310
Mobile phase:	Gradient MeOH/H2O – 90/10 - 100/0% in 10 min, 5 min hold
Buffer:	AmFm – 10mM, FA – 0.05%
Flow rate:	0.5 mL/min
Detection:	UV 292 nm ESI MS SIM 431+, 473+

Fig. 7. Separation of (±)-α-Tocopherol and α-Tocopheryl acetate



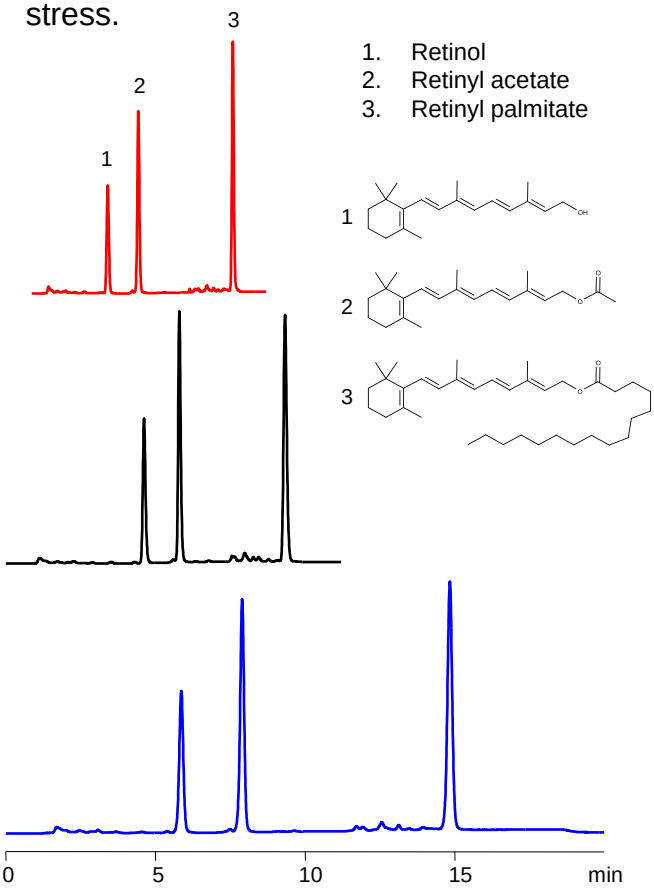
Column:	Lipak™
Column size:	3.2 × 100 mm, 3 µm, 100A
Column part number:	LP-32.100.0310
Mobile phase:	Gradient MeOH/H2O/EtOH – 40/60/0 - 0/0/100% in 5 min, 5 min hold
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	0.5 mL/min
Detection:	UV 270 nm

Vitamin K is a fat-soluble vitamin that plays a crucial role in blood clotting, bone health, and cardiovascular function. It activates proteins needed for coagulation, helping prevent excessive bleeding. It also supports bone health by binding calcium to strengthen bones and reduce fracture risk. In addition, Vitamin K prevents calcium buildup in blood vessels, lowering the risk of arterial calcification and heart disease. Vitamin K4, a synthetic form, is mainly used in research and some pharmaceutical applications, offering similar benefits to other forms of vitamin K. A deficiency can lead to bleeding disorders, weakened bones, and cardiovascular complications.

Fig. 8. Separation of Acetomenaphthone (Vitamin K4) and Phytomenadione (Vitamin K1)

Vitamins

Retinol, a form of vitamin A, is a potent antioxidant known for promoting skin renewal, reducing wrinkles, and supporting vision and immune health. It is commonly used in skincare for its ability to boost collagen production and enhance skin texture. Retinyl acetate and Retinyl palmitate are esterified derivatives of retinol. Retinyl acetate offers improved stability and is often used in supplements and cosmetics for its gradual conversion to active retinol. Retinyl palmitate, a milder and more stable form, is widely used in skincare products for sensitive skin, providing gentle vitamin A benefits while protecting against oxidative stress.



Column:	Lipak
Column size:	3.2 ×100 mm, 3 µm, 100A
Column part number:	LP-32.100.0310
Mobile phase:	Gradient MeOH/H2O/EtOH – 80/20/0 – 80/0/20% 5 min, 5 min hold
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	1.0 mL/min
Detection:	325 nm

Column:	Lipak
Column size:	3.2 ×100 mm, 3 µm, 100A
Column part number:	LP-32.100.0310
Mobile phase:	Gradient MeOH/H2O/EtOH – 80/20/0 – 80/0/20% 5 min, 5 min hold
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	0.5 mL/min
Detection:	325 nm

Column:	Lipak
Column size:	3.2 ×150 mm, 5 µm, 100A
Column part number:	LP-32.150.0510
Mobile phase:	Gradient MeOH/H2O/EtOH – 80/20/0 – 80/0/20% 10 min, 8 min hold
Buffer:	AmFm – 10 mM, FA – 0.05%
Flow rate:	0.5 mL/min
Detection:	325 nm

Fig. 9. Separation of Retinol, Retinyl Acetate, and Retinyl Palmitate

Lipak™ columns, available in 3 µm and 5 µm particle sizes, offer flexibility and performance. The 3 µm particles deliver higher plate numbers and better resolution on the same column length. So the column can be shorter if the resolution is sufficient on 5 µm column.

For instance, a 150 mm column with 5 µm particles can be replaced with a 100 mm column with 3 µm particles, maintaining excellent resolution for complex mixtures (see Figure 9). Smaller particles allows to use higher flow rate without loosing efficiency of the column. It enables faster run times increasing productivity while preserving quality.

Lipak™ columns provide an excellent solution for the efficient and precise analysis of lipid mixtures.

Contact Us

Learn more on our website or contact us for consultations and demonstrations.

For Product Information
Email: sales@sielc.com

For Accounts Payable
Email: finance@sielc.com

For Method Development
Email: research@sielc.com

Call: 847 229-2629
Fax: 847 655-6079

SIELC Technologies
804 Seton Ct.
Wheeling, IL USA 60090

We offer free HPLC method development tailored to your specific compounds, ensuring optimized separations with reliable, reproducible results. Free custom solutions — designed for seamless analytical performance.