

Use of C30 as a General-Purpose Stationary Phase for a Broad Range of Applications

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Introduction

The C30 column is a reversed-phase HPLC column. Like C18 phase, the C30 phase can be used in a broad range of applications in food and beverage, chemical, environmental, pharmaceutical, academia, and other industries. The C30 column offers several unique features that set it apart from its C18 counterparts. First, it exhibits higher shape selectivity suited to separation of hydrophobic, long-chain, structural isomers (e.g., carotenoids, steroids, lipids, etc.). Second, it is fully compatible with various aqueous buffers, allowing for a broader application range (e.g., water-soluble vitamins, organic acids, etc.) and more flexibility in method development. Third, it is a viable alternative to normal-phase columns for lipid analysis. This work demonstrates use of the C30 column, not only as a general-purpose column that complements C18 columns, but also as a specialty column when a C18 column fails to provide satisfactory results.

Acclaim C30 and Its Features

The Thermo Scientific Acclaim C30 column is designed to provide high shape selectivity for separating hydrophobic structurally related isomers and unique selectivity complementary to other reversed-phase columns (e.g., C18). The column is based on covalent attachment to C30 alkyl silane on high-purity, spherical, porous silica gel particles. A combination of advanced surface modification technology and careful matching of C30 alkyl chain with the pore size of the silica substrate provides the following benefits:

- High shape selectivity
- Unique selectivity complementary to other reversed-phase columns
- Compatibility with highly aqueous mobile phases
- High-quality rugged columns with low-column bleed and high efficiency.

Specifications

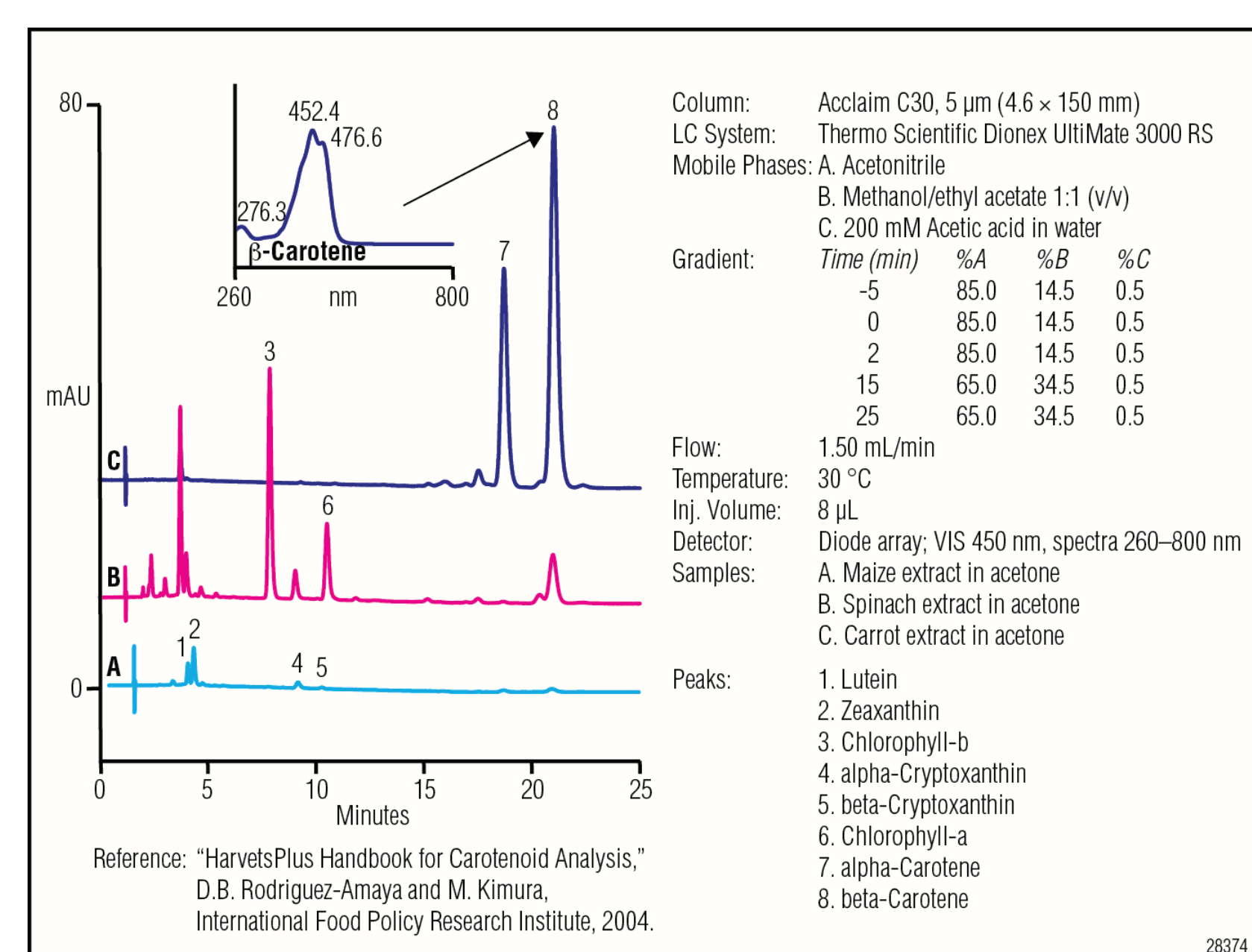
Acclaim C30 Column	
Column Chemistry	C30 alkyl chain
Silica Substrate	Spherical, high-purity
Particle Sizes	3 and 5 µm
Surface Area	200 m ² /g
Pore Size	200 Å
Operating pH Range	pH 2–8
Operating Temperature	up to 60 °C
Aqueous Compatibility	0 to 100%
Solvent Compatibility	0 to 100%

Applications

Carotenoids

Carotenoids occur naturally in the chloroplasts and chromoplasts of plants, and some fungi and bacteria. They serve two key roles in plants and algae: 1) absorb light energy for use in photosynthesis, and 2) protect chlorophyll from photo damage. In humans, four carotenoids (β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin) have vitamin A activity, and can also act as antioxidants. As shown in Figure 1, six common carotenoids and chlorophyll in vegetables are separated on the Acclaim C30 column with excellent selectivity and resolution.

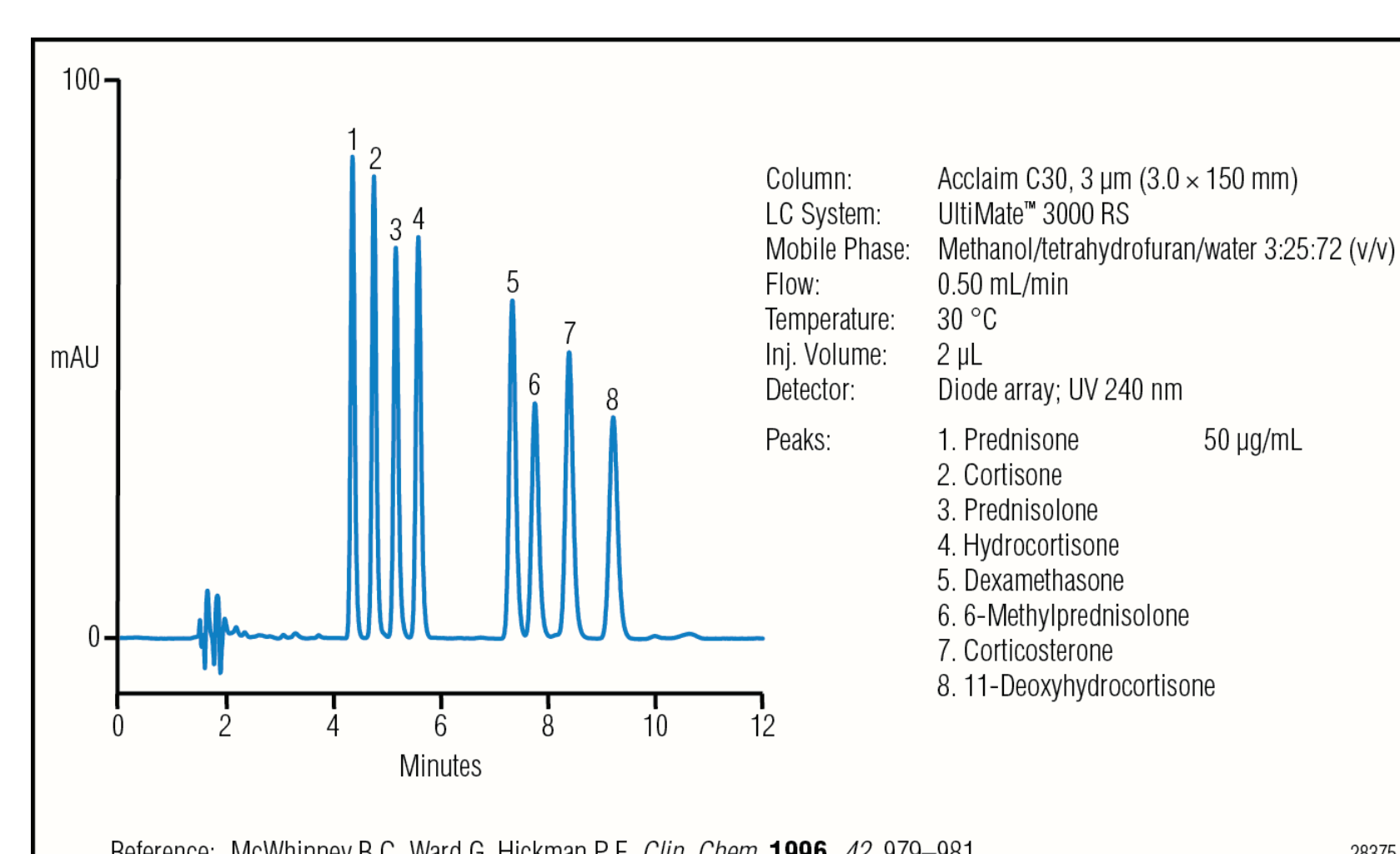
FIGURE 1. Carotenoids in vegetables.



Glucocorticosteroids

Glucocorticosteroids are a group of naturally occurring and synthetic hormones that moderate inflammation and other stress responses. Separation of these substances is challenging due to their structural similarity. While the method referenced uses a C18 column, the Acclaim C30 offers improved resolution and throughput with the same elution order under the same conditions (Figure 2).

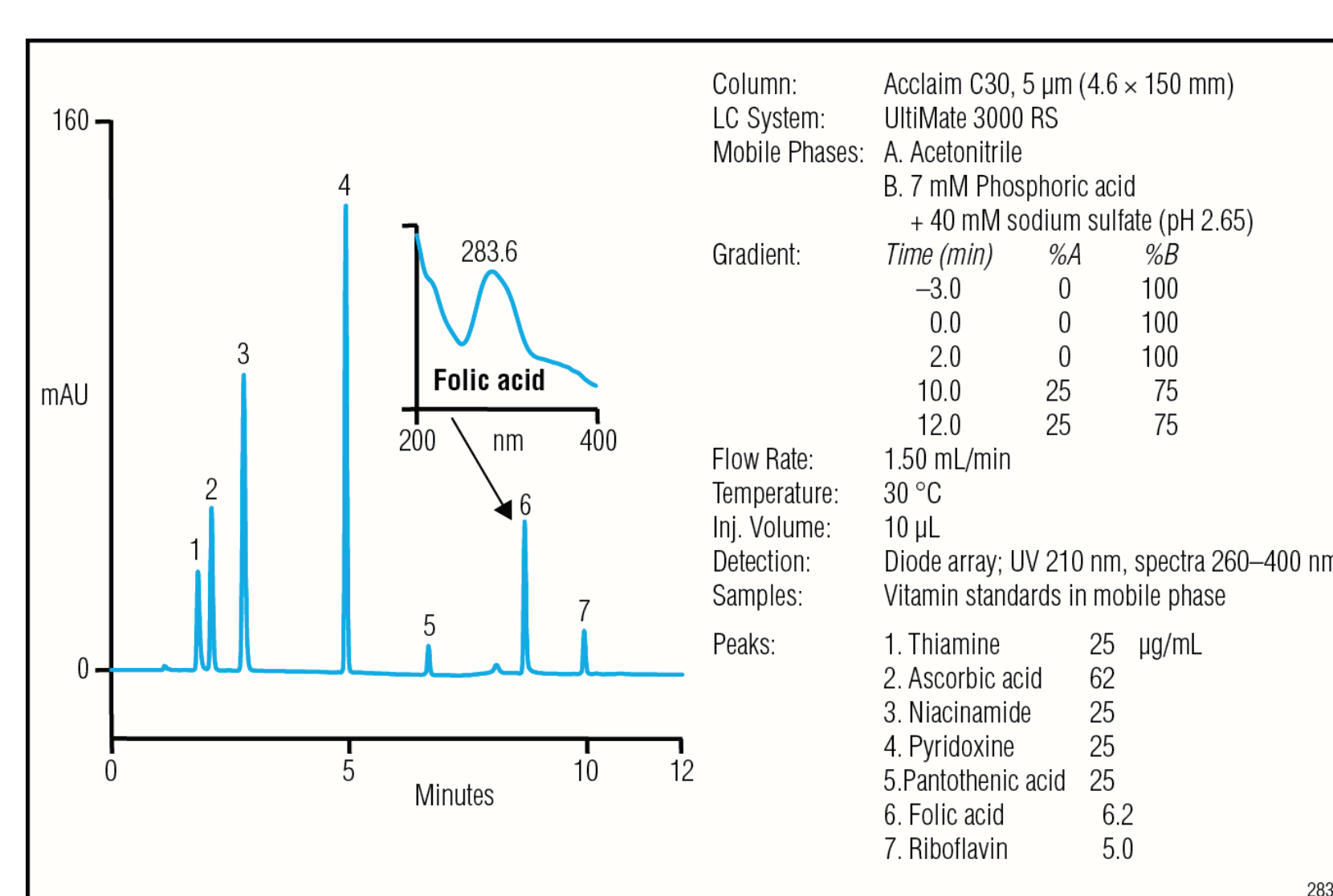
FIGURE 2. Glucocorticosteroids.



Water-Soluble Vitamins

Water-soluble vitamins are essential nutrients present in foods and formulated supplements. Chemically, they are a heterogeneous group of anions, cations, zwitterions, and neutrals. Because vitamins are present in a variety of matrices of varied complexity, their analysis is often challenging. The Acclaim™ C30 column demonstrates both high hydrophobic retention and aqueous compatibility providing a desirable combination of features enabling simple reversed-phase separation of these complex analytes (Figure 3).

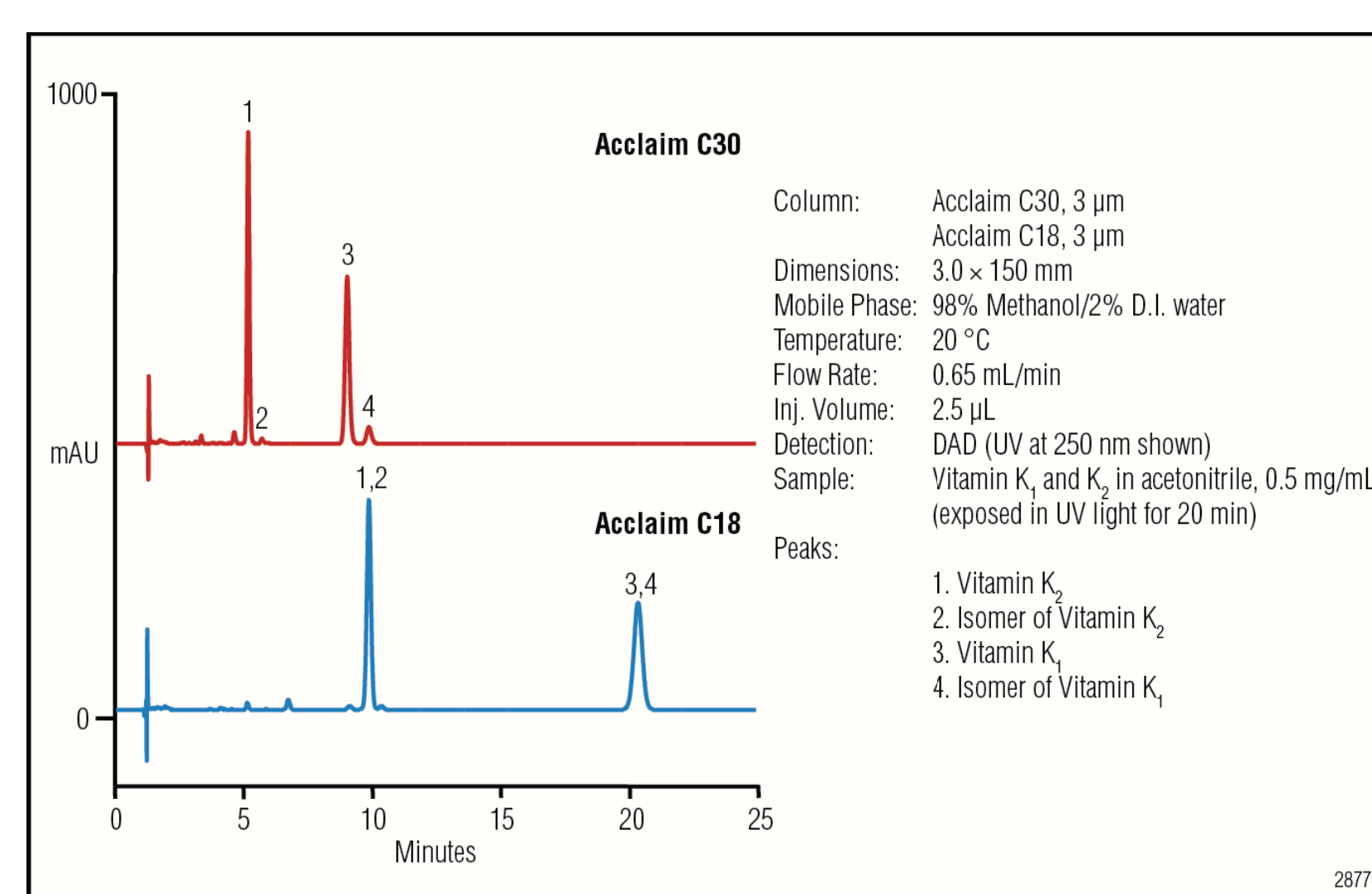
FIGURE 3. Water-soluble vitamins.



Fat-Soluble Vitamins

Vitamin K is a group of structurally similar, fat soluble vitamins that are needed for the posttranslational modification of certain proteins, mostly required for blood coagulation but also involved in metabolic pathways in bone and other tissue. They are 2-methyl-1,4-naphthoquinone derivatives. This group of vitamins includes vitamin K₁ and vitamin K₂. Plants synthesize vitamin K₁, while bacteria can produce a range of vitamin K₂ forms, including the conversion of K₁ to K₂ by bacteria in the small intestines. As shown in Figure 4, the Acclaim C30 column provides good resolution between both vitamin K₁ and vitamin K₂ and corresponding structural isomers, which the C18 column fails to separate these isomers.

FIGURE 4. Vitamin K.



Triglycerides in Cooking Oils

Cooking oils are purified lipids from plants, and they are typically liquid at room temperature. These compounds contain triglycerides as major components, and small quantities of free fatty acids and mono- and diglycerides. The composition of cooking oils is highly complex due to the wide variety of alkyl chain length, degree of unsaturation, origin, etc. While normal-phase chromatography is often used to characterize oils by their hydrophilicity, reversed-phase chromatography provides high resolution for analyzing major and minor components, and a detailed fingerprint. High shape selectivity of the Acclaim C30 provides higher resolution than the C18 column for oil analysis (Figure 5). Separations of several different cooking oils are illustrated in Figure 6.

FIGURE 5. Analysis of cooking oil: Acclaim C30 vs C18.

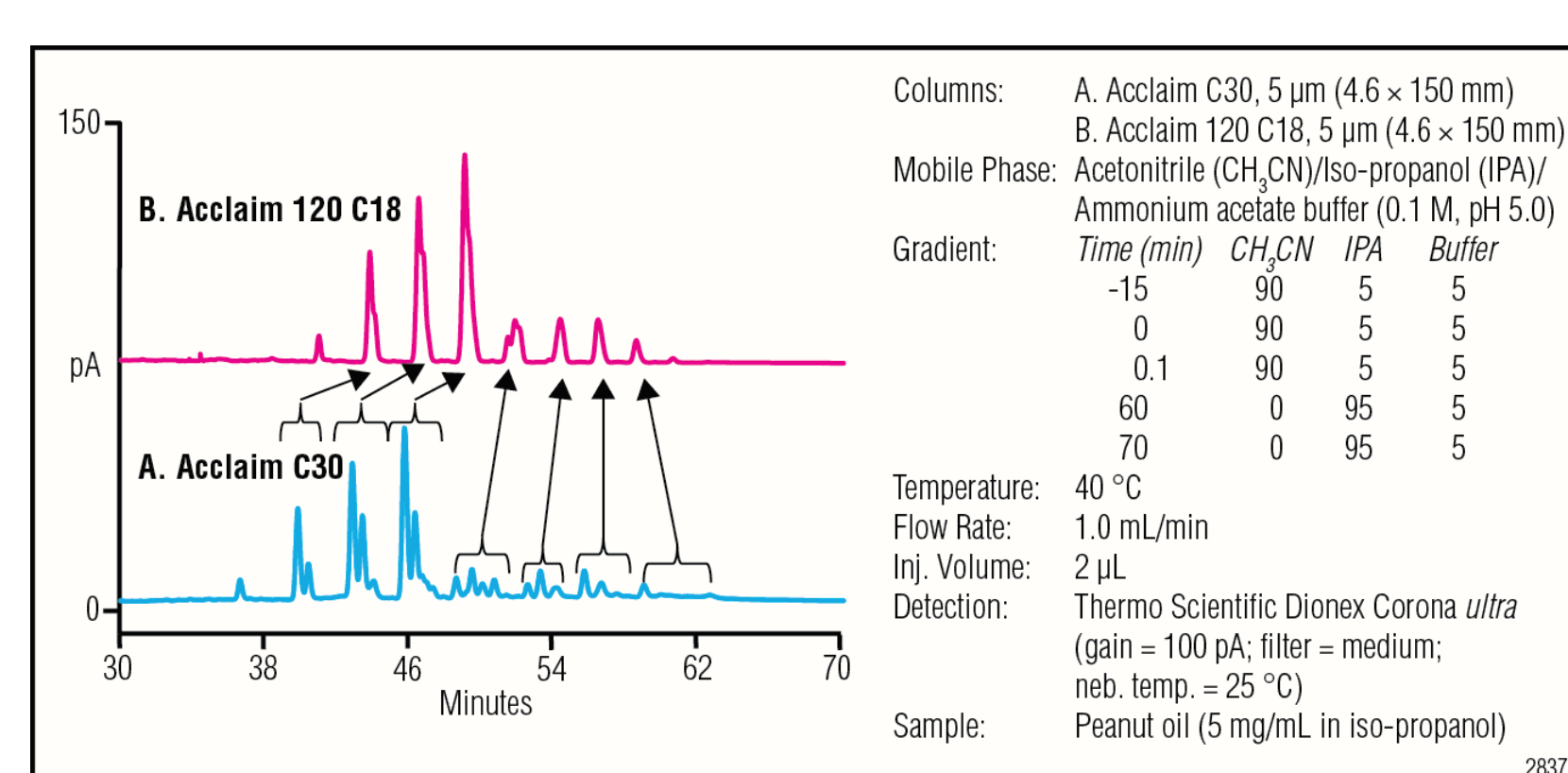
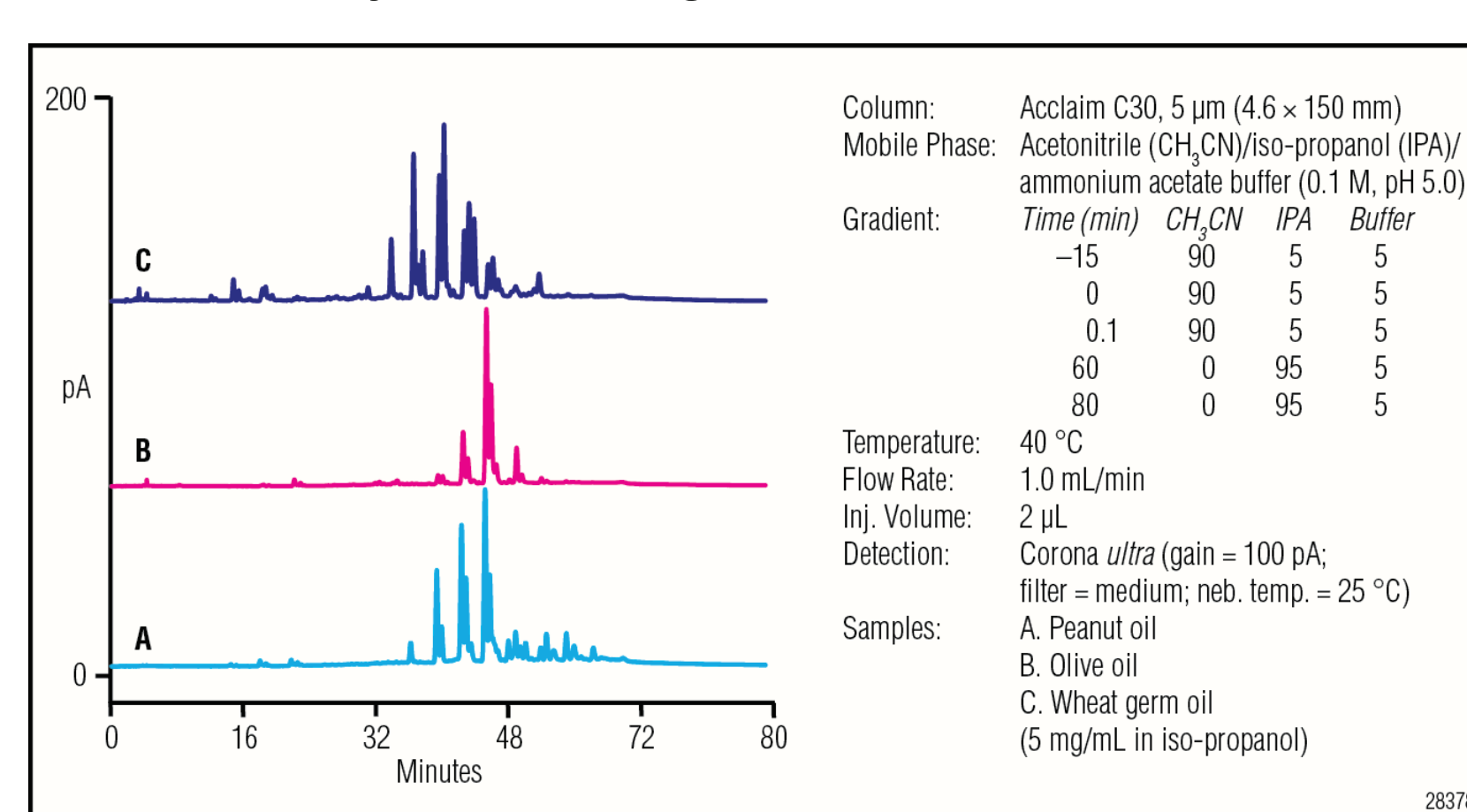


FIGURE 6. Analysis of cooking oils.



Phospholipids in Egg Lecithin

Phospholipids are major components of all cell membranes as they can form lipid bilayers. Most phospholipids contain a diglyceride, a phosphate group and a hydrophilic organic group such as choline. The first phospholipid identified in biological tissues was lecithin in the egg yolk. Analysis of phospholipids is challenging due to its complexity (hydrocarbon length, degree of saturation of diglyceride moiety, and type of the hydrophilic organic group). Figure 7 shows the profile of lecithin from egg yolk and soybean obtained using an Acclaim C30 column and a Thermo Scientific Dionex Corona ultra Charged Aerosol Detector. It indicates that lecithin from egg yolk contains both phospholipids and triglycerides, with other minor impurities. To obtain detailed information on phospholipid composition, a higher-resolution method can be used (Figure 8).

FIGURE 7. Profile of egg lecithin.

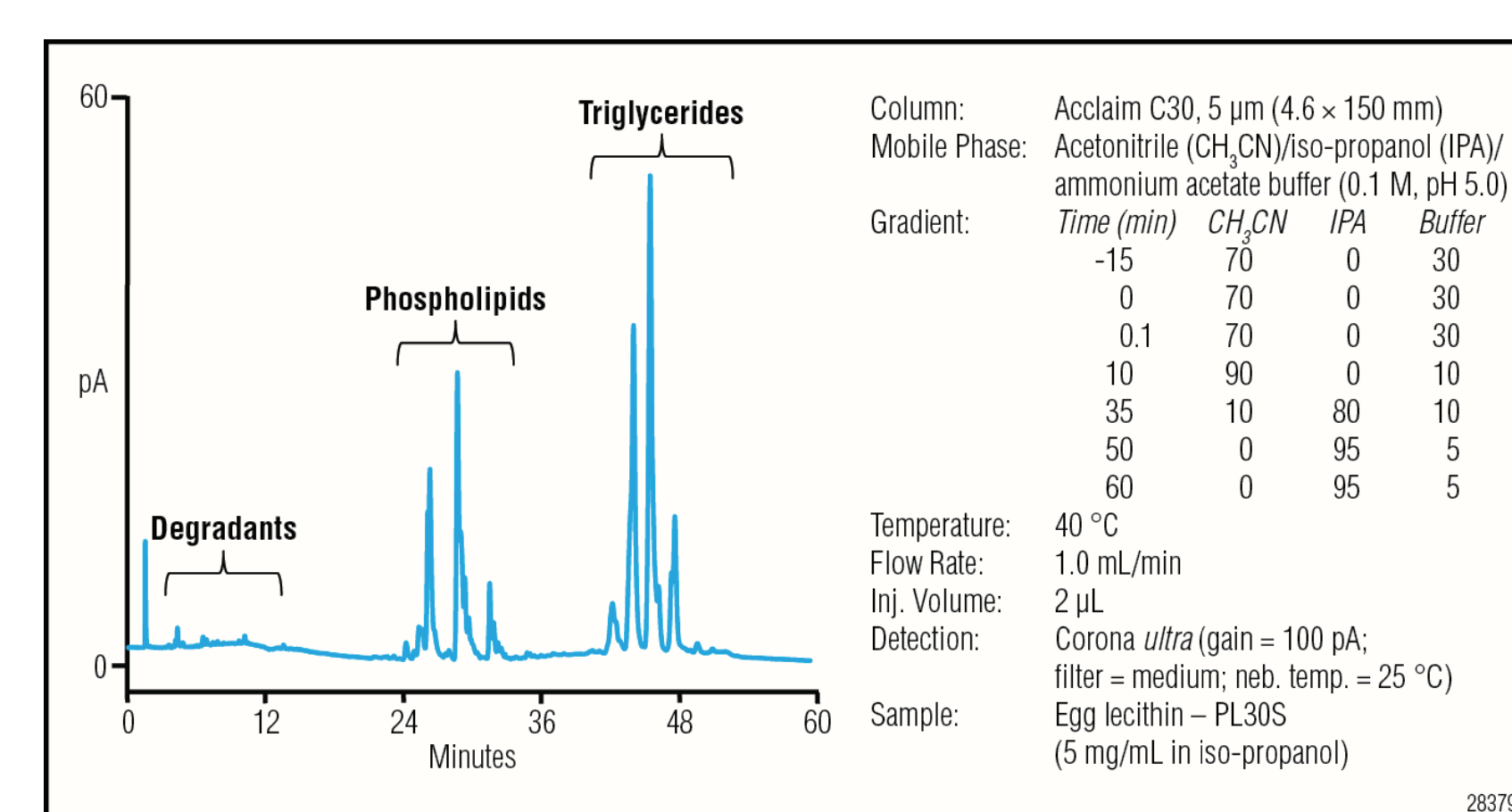
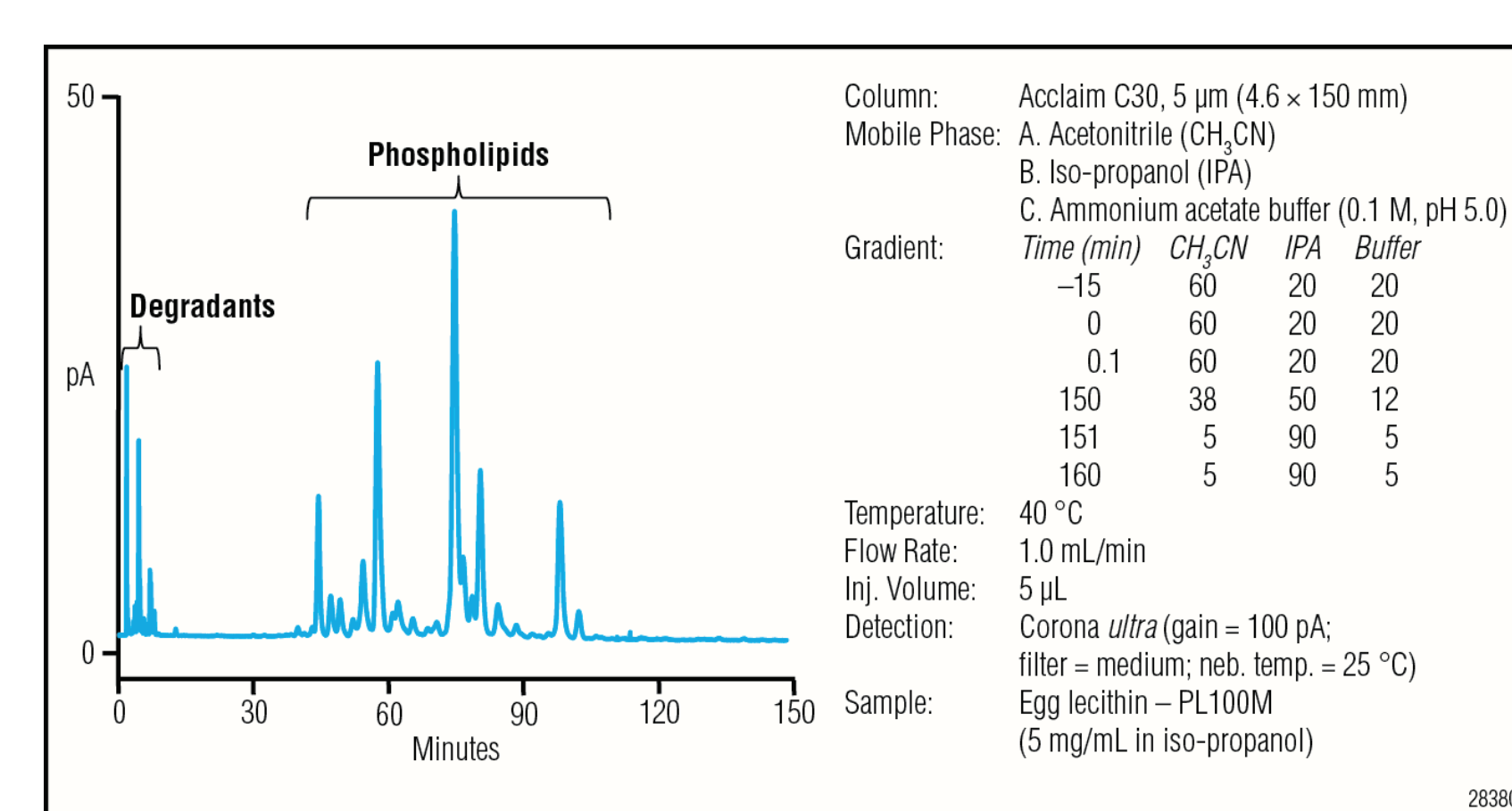


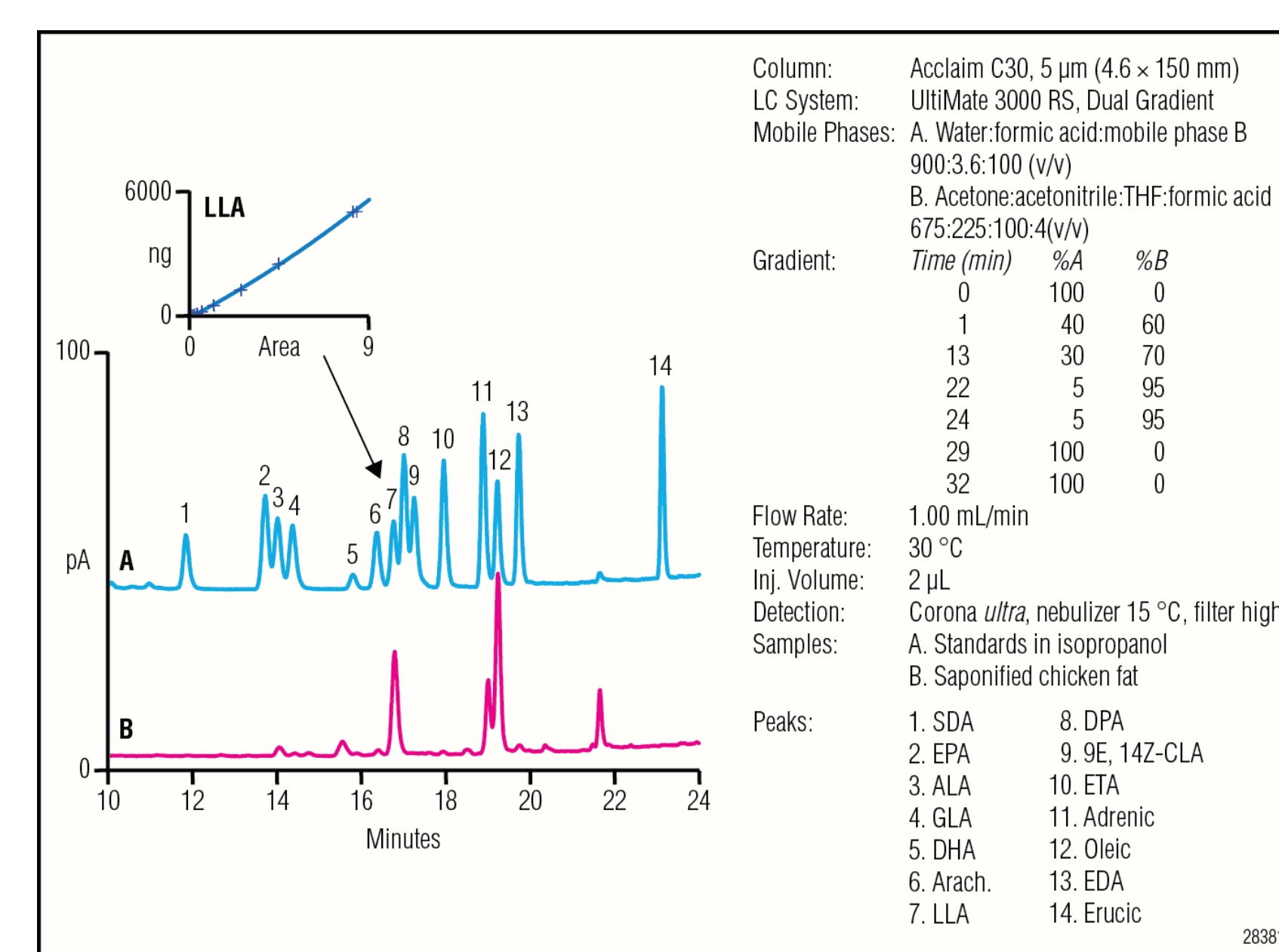
FIGURE 8. Phospholipids in egg lecithin.



Omega Fatty Acids

Omega-3 fatty acids are a family of unsaturated fatty acids that share a final carbon-carbon double bond in the n-3 position. Omega-3 fatty acids, such as α-linolenic acid (18:3, n-3; ALA), eicosapentaenoic acid (20:5, n-3; EPA), and docosahexaenoic acid (22:6, n-3; DHA), are important in human nutrition. The biological effects of omega-3 are largely mediated by their interactions with the omega-6 fatty acids. Some medical research suggests that excessive levels of omega-6 relative to omega-3 fatty acids may increase the probability of several diseases and depression. As shown in Figure 9, the Acclaim C30 column exhibits excellent resolution power for a suite of closely related omega fatty acids.

FIGURE 9. Omega fatty acids.



Conclusion

Point 4. Compared to C18 columns, the Acclaim C30 column provides higher shape selectivity and improved aqueous compatibility. Therefore, it can be used as a general-purpose reversed-phase column for a broad range of applications including food and beverage, chemical, environmental, pharmaceutical, academia, and others. Because of its unique chromatographic properties, the Acclaim C30 column can also be used as a complementary or a specialty column for lipid analysis when C18 columns fail to provide satisfactory results.

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