PRODUCT MANUAL

for

Acclaim® C30 Columns

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

Product Manual

for

Acclaim® C30 Columns

075718 Acclaim C30, 5µm, Analytical, (4.6 x 250mm)
075719 Acclaim C30, 5µm, Analytical, (4.6 x 150mm)
075723 Acclaim C30, 3µm, Analytical, (4.6 x 150mm)
075726 Acclaim C30, 3µm, Analytical, (3.0 x 250mm
075724 Acclaim C30, 3µm, Analytical, (3.0 x 150mm)
075725 Acclaim C30, 3µm, Analytical, (2.1 x 150mm)

075720 Acclaim C30, 5µm, Guard, (4.6x10mm)
075721 Acclaim C30, 5µm, Guard, (3.0x10mm)
075722 Acclaim C30, 5µm Guard, (2.1x10mm)

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SECTION 1 – INTRODUCTION

The 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 C30 stationary phase is based on a covalent modification of C30 alkyl silane onto high-purity, spherical, porous silica gel. This column is complementary to other reversed-phase columns (e.g. C18) in a broad range of applications in pharmaceutical, food & beverage, chemical, environmental, and academia research.

1.1. Main features

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

1.2. Physical data

Bonding Chemistry: C30 alkyl
Silica Substrate: Spherical, high-purity
Particle size – 3, 5 µm
Surface area – 200 m^2/g
Pore size – 200 Å

1.3. Specifications and Operational Parameters

pH range: pH 2 – 8
Temperature (max): 60 °C
Solvent compatibility: 0 to 100%
Aqueous compatibility: 0 to 100%
Pressure rating: See Table 1
Flow rate: See Table 1

Table 1
Operating pressure and flow rate specifications

Column Dimension	Particle Size	P/N	Maximum Pressure (psi)	Typical Flow Rate (Recommended) (mL/min)	Maximum Flow Rate (mL/min)
4.6 x 250mm	5µm	075718	9,000	0.8 – 1.5	2.0
4.6 x 150mm	5µm	075719	8,000	0.8 – 1.5	2.0
4.6 x 150mm	3µm	075723	8,000	0.8 – 1.5	1.0
3.0 x 250 mm	3µm	075726	12,000	0.4 – 0.6	1.0
3.0 x 150mm	3µm	075724	8,500	0.4 – 0.6	1.0
2.1 x 150mm	3µm	075725	8,500	0.2 – 0.3	0.5

1.4. Operational Guidelines

- The direction of flow is marked on the column.
- While it is not harmful to the column, reverse flow should be avoided except to attempt removal of inlet blockage (see "Column Care").
- A new column is shipped in a solution of 70% acetonitrile and 30% D.I. water. Initially, care should be taken to avoid any mobile phase that might cause a precipitate.
- Acclaim C30 columns are compatible with up to 100% aqueous and all common organic solvents.
- The use of a guard column is recommended to protect the analytical column and extend its useful lifetime.
- Avoid use of this column below pH 2 or above pH 8.
- Recommended operating pressures are column format dependent (see Table 1 for details).
- Maximum operation temperature is 60 °C.
- Columns should be stored in 100% acetonitrile for long-term storage. For short-term storage, avoid exposing the column to harsh conditions, such as pH < 3, pH > 6 or elevated temperature (> 40 °C).

1.5. Ordering Information

	Particle Size	Column Dimensions	P/N	Required Holder
Analytical	5µm	4.6 x 250 mm	075718	
		4.6 x 150 mm	075719	
	3µm	4.6 x 150 mm	075723	
		3.0 x 250 mm	075726	
		3.0 x 150 mm	075724	
		2.1 x 150 mm	075725	
Guard	5µm	4.6 x 10 mm	075720	P/N 069580
		3.0 x 10 mm	075721	P/N 069580
		2.1 x 10 mm	075722	P/N 069580

SECTION 2 – STEP-BY-STEP USER GUIDE

Dionex recommends that you test on your Acclaim C30 column upon receiving it, to ensure no damage has occurred during shipping. Steps 1 – 5 below outline the necessary steps. Test the column using the conditions described on the Quality Assurance (QA) Report enclosed in the column box. Repeat the test periodically to track the column performance over time. Slight variations may be obtained on two different HPLC systems due to system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Step 1 – Visually inspect the column

Report any visual damage to Dionex Corporation.

Step 2 – Mobile phase preparation

Obtaining reliable, consistent and accurate results require mobile phases that are free of ionic and spectrophotometric impurities. Chemicals, solvents and de-ionized water used to prepare mobile phase should be of the highest purity available. Maintaining low trace impurities and low particle levels in mobile phases helps to protect your columns and system components. Dionex cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare the mobile phase has been compromised.

Depending on specific application, the mobile phase system consists of an organic solvent (e.g. usually acetonitrile) and an aqueous portion (e.g. D.I. water, ammonium acetate or phosphate buffer). Both pre-mixed and proportioning valve generated mobile phases give satisfactory results. The use of proportioning valve provides better flexibility in method optimization, while the pre-mixed mobile phase provides more reproducible results.

Solvents

The solvents used must be free from ionic and UV-absorbing impurities. Use of ultrahigh purity solvents, HPLC grade, will usually ensure that your chromatography is not affected by impurities in the solvent.

De-ionized Water

The de-ionized water used to prepare the mobile phase should be Type 1 Reagent Grade water or HPLC Grade water. The de-ionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Many commercial water purifiers are designed for HPLC applications and are suitable for these applications.

NOTE ***Degas the aqueous component of the mobile phase and then add the solvent component. Avoid excessive purging or degassing of mobile phases containing solvents, if possible, since the volatile solvent can be 'boiled' off from the solution.***

Mobile Phase for Column Performance Test (QA test):

Mobile phase can be generated either by pre-mixing or by using a proportioning valve; both give satisfactory results. The use of a proportioning valve provides better flexibility in method optimization, while the pre-mixed mobile phase provides less baseline noise.

Step 3 – Set up the LC system

Use a standard LC system equipped with a LC pump, a column oven, a UV detector (or an ELS detector depending on the application), and an injector (or an autosampler). The system should be thoroughly primed before use.

Step 4 – Condition the column

Each new column is shipped in the mobile phase used for column performance test (containing 70% acetonitrile). Before use, the column should be washed thoroughly with the mobile phase (~20 column volumes depending on the aqueous content in the mobile phase) before any injection is made.

When switching to a different mobile phase, make sure that the new mobile phase is compatible with the existing mobile phase to avoid column clogging due to precipitation. A good practice is to purge the column with 50% acetonitrile in D.I. water (v/v) for approximately 10 column volumes before switching to a new mobile phase.

Step 5 – Reproduce the chromatogram in the Quality Assurance Report

Perform the column QA test using the conditions described in the Quality Assurance Report (QAR), and compare the result with the reported values. The column should be fully equilibrated before any injection. At least three injections should be made until reproducible results are obtained.

NOTE ***Due to various reasons, such as: difference of LC systems, mobile phases, oven temperature control, etc, you may observe somewhat different separation from that in the report.***

Actual QC test and performance of your column is described in the Column Performance Report enclosed with your column.

Step 6 – Real sample analysis

Once you are satisfied with the column performance report result, the column is ready for real applications.

SECTION 3 – COLUMN CARE

The inlet and outlet frits on these columns have a nominal porosity of 0.5µm. Particulates in the sample or the mobile phase larger than 0.5µm will plug the column inlet frit.

If solvent flow appears to be restricted (high column back-pressure), check first to see that solvent flow is unobstructed up to the column inlet. If the column has the restriction, there may be particulate matter on the inlet frit. An attempt should be made to remove any inlet debris by back-flushing 25 to 30 mL of mobile phase through the column. If this fails to return the column to its original operating pressure, consider replacing the column.

To remove strongly retained materials from the column, flush the column with stronger (less polar) solvents. Solvents such as methanol, acetonitrile, or a 95% / 5% mixture of dichloromethane and methanol should remove most highly retained compounds. When switching between solvents with vastly different polarities, it may be necessary to first purge the column with mutually miscible solvent, such as isopropanol.

Long-term storage of a silica-based, bonded-phase column should be in a pure organic solvent; preferably an aprotic one such as 100% acetonitrile. If the column has been previously used with a buffered mobile, the buffer should first be removed by purging the column with 20 – 30 column volumes of a 50/50 mixture of methanol or acetonitrile and water, followed by 20-30 column volumes of the pure solvent. Before storing the column, the end-fittings should be tightly capped with end-plugs to prevent the packing from drying out.

The column may be safely stored for short periods in most mobile phases. However, to protect equipment, it is desirable to remove salts from the instrument and column by purging the column with the same organic solvent/water ratio without the buffer. For example, using 60/40 acetonitrile/water to remove 60/40 acetonitrile/0.02 M phosphate buffered mobile phase. Re-equilibration is rapid with the original mobile phase when using this approach, and any danger of corrosion from the salts is eliminated.

SECTION 4 – APPLICATIONS

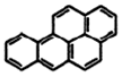
The Acclaim C30 is a reversed-phase HPLC column. Like other reversed-phase columns, such as C18, this column is useful in a wide range of separations including food & beverage, chemical, environmental, pharmaceutical, academia, etc. Unlike other general-purpose reversed-phase columns, the Acclaim C30 has several unique features. First, it exhibits higher shape selectivity suited to separation of structural isomers. Second, it is fully compatible with various aqueous buffers, resulting in a broader application range and more flexibility in method development. Therefore, the Acclaim C30 can serve as an ideal candidate to complement general-purpose C18 columns in a variety of applications. The following figures are example applications demonstrating the capability of this column.

4.1. Shape Selectivity

SRM 869a is useful for characterizing liquid chromatographic (LC) column selectivity for separation of PAHs. This Standard Reference Material (SRM) is a mixture of three polycyclic aromatic hydrocarbons (PAHs) in acetonitrile: benzo[a]pyrene (BaP), 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN, alternate name, dibenzo[g,p]chrysene), and phenanthro[3,4-c]phenanthrene (PhPh). Depending on the elution order of the three components, column selectivity can be predicted for complex PAH mixtures (particularly isomeric PAHs). Note that a smaller α (TBN/BaP) (= k'_{TBN}/k'_{BaP}) value indicates higher shape selectivity. As show in Figure 1, the Acclaim C30 column exhibits the highest shape selectivity among other Acclaim reversed-phase columns *(Note: lower α(TBN/BaP) suggests higher shape selectivity)*

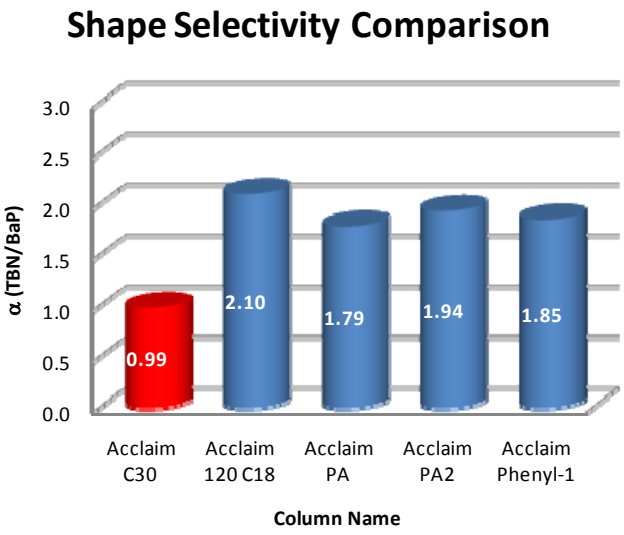
Column: 4.6 x 150 mm
Mobile Phase: CH_3CN/H_2O v/v 85/15
Temperature: 25 °C
Flow Rate: 2 mL/min
Inj. Volume: 5 µL
Detection: UV (254 nm)
Sample: NIST SRM 869a standard mix

1. Benzo[a]pyrene (BaP)



2. Phenanthro[3,4-c]phenanthrene (PhPh)

3. Dibenzo[g,p]chrysene (TBN)



$$\alpha_{(TBN/BaP)} = k'_{TBN} / k'_{BaP}$$

Figure 1
Shape Selectivity

4.2. Aqueous Compatibility

Most reversed-phase columns have high ligand densities, designed to improve the peak shape of basic analytes and stability at extreme pHs. However, dense surface coverage of silica particles with hydrocarbon chains often results in inconsistent retention times in 100% aqueous conditions, also called de-wetting or phase collapse. Although the low ligand density bonding is used to achieve better compatibility with 100% aqueous mobile phases, these phases provide poor peak shapes for bases and lower hydrolytic stability. Because of the intelligent phase design and unique bonding process, Acclaim C30 column overcomes aforementioned difficulties and provides high surface coverage as well as 100% aqueous compatibility (shown Figure 2)—no loss of retention was observed after 50 stop-flow cycles.

Column:	Acclaim C30, 5 µm
Dimensions:	4.6 x 150 mm
Mobile Phase:	25 mM Ammonium acetate, pH 5.0
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV (254 nm)
Sample:	0.1 mg/mL
Probe:	Thymine

Stop-Flow Protocol:

1. Test the column under above condition
2. Stop the pump for 5 min (pump pressure drops to 0)
3. Resume the flow and condition the column under above condition for 10 min
4. Inject the sample and run the test under the same conditions.
5. Repeat 2 through 4

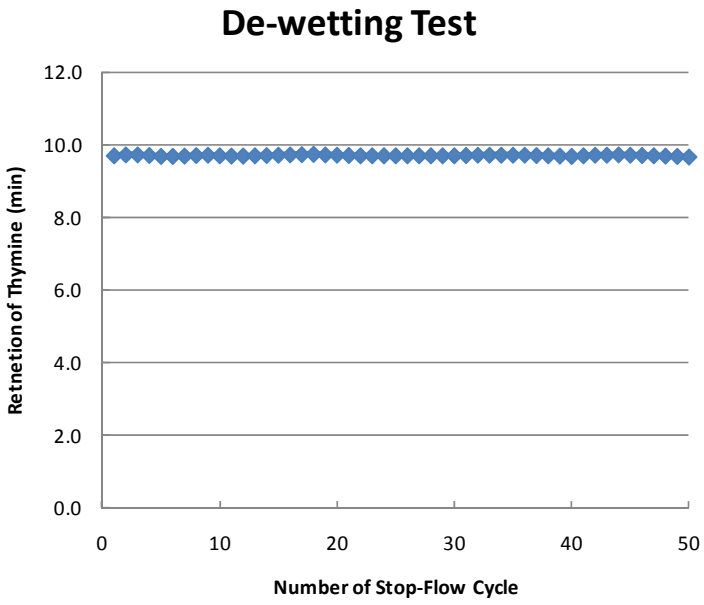


Figure 2
Aqueous Compatibility

4.3. Analysis of Carotenoids

Carotenoids are naturally occurring in the chloroplasts and chromoplasts of plants, and some fungi and bacteria. They serve two key roles in plants and algae – absorb light energy for use in photosynthesis, and 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. In the eye, certain other carotenoids (lutein and zeaxanthin) apparently act directly to absorb damaging blue and near-ultraviolet light, in order to protect the macula lutea. As shown in Figure 3, six common carotenoids and chlorophyll are separated on the Acclaim C30 column with excellent selectivity and resolution.

Column:	Acclaim C30, 5 µm
Dimensions:	4.6 x 150 mm
LC System:	UltiMate 3000 RS
Mobile Phases:	A) Acetonitrile
	B) Methanol:Ethyl acetate 1:1 (v/v)
	C) 200 mM Acetic acid in water

Gradient:

Min	-5	0	2	15	25
%A	85.0	85.0	85.0	65.0	65.0
%B	14.5	14.5	14.5	34.5	34.5
%C	0.5	0.5	0.5	0.5	0.5

Flow:	1.50 mL/min
Temperature:	30 °C
Injection:	8 µL
Detector:	Diode Array; VIS 450 nm, spectra 260 – 800 nm
Samples:	A. Carrot extract in acetone
	B. Spinach extract in acetone
	C. Maize extract in acetone

Peaks:
1. Lutein
2. Zeaxanthin
3. Chlorophyll-b
4. α-Cryptoxanthin
5. β-Cryptoxanthin
6. Chlorophyll-a
7. α-Carotene
8. β-Carotene

Reference: "HarvetsPlus Handbook for Carotenoid Analysis", D.B. Rodriguez-Amaya and M. Kimura, International Food Policy Research Institute, 2004.

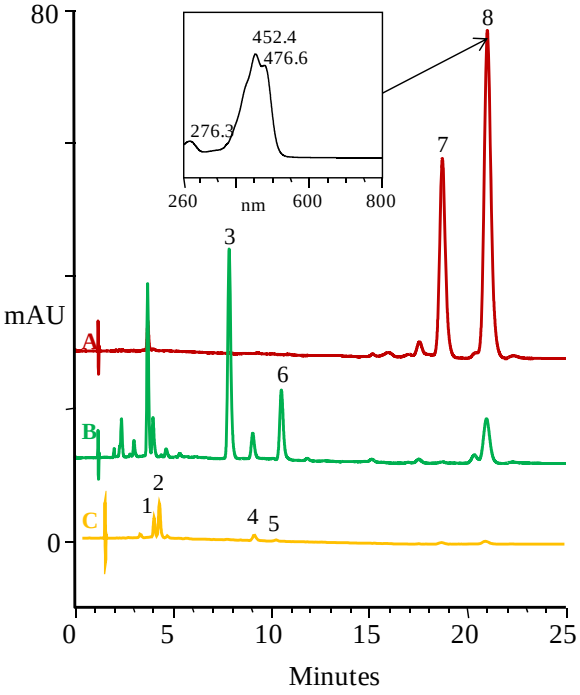


Figure 3
Carotenoids in Vegetables

4.4. Analysis of Glucocorticosteriods

Glucocorticosteroids are a group of naturally occurring and synthetic hormones that moderate inflammation and other stress responses. Glucocorticosteroids are on the World Anti-Doping Agency's list of substances prohibited in-competition. Separation of these substances is challenging due to their structural similarity. The method of McWhinney is commonly used in clinical laboratories for monitoring of these substances. While the original method uses a C18 column, the Acclaim C30 offers improved resolution and throughput with same elution order used the literature method. Figure 4 shows a baseline separation of eight glucocorticosteroids using an Acclaim C30 column.

Column:	Acclaim C30, 3 µm
Dimensions:	3.0 x 150 mm
LC System:	UltiMate 3000 RS
Mobile Phase:	Methanol:tetrahydrofuran:water 3:25:72(v/v)
Flow:	0.50 mL/min
Temperature:	30 °C
Injection:	2 µL
Detector:	Diode Array; UV 240 nm

Peaks:	**µg/mL**
1. Prednisone	50
2. Cortisone	
3. Prednisolone	
4. Hydrocortisone	
5. Dexamethasone	
6. 6-Methylprednisolone	
7. Corticosterone	
8. 11-Deoxyhydrocortisone	

Reference: McWhinney B C, Ward G, Hickman P E; Clin. Chem, 1996, 42:979-981.

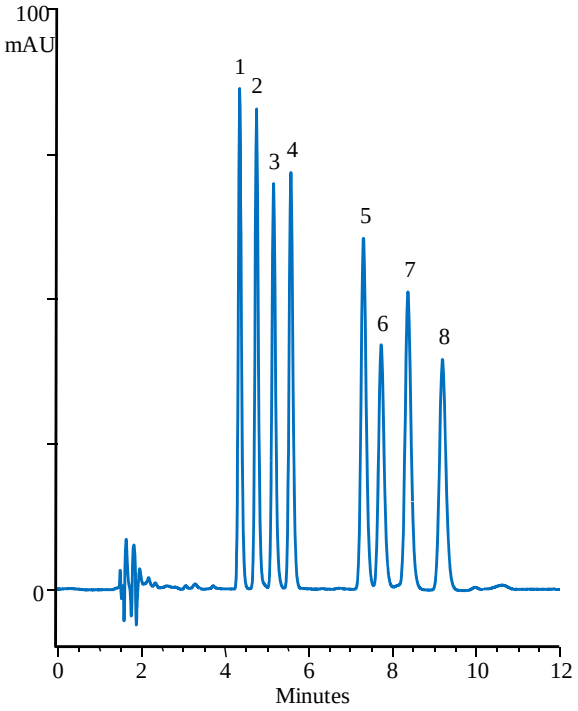


Figure 4
Glucocorticosteroids

4.5. Analysis of Water-soluble Vitamins

Water-soluble vitamins are essential nutrients. They are found naturally in food, added to food, or formulated in supplements. Chemically they are a heterogeneous group of anions, cations, zwitterions and neutrals. Due to the variety and complexity of matrices where vitamins are found, the analysis is often challenging. The Acclaim C30 column has desirable combination of high hydrophobic retention and aqueous compatibility, which enables a simple reversed-phase separation of these difficult analytes (Figure 5).

Column:	Acclaim C30, 5 µm				
Dimensions:	4.6 x 150 mm				
LC System:	UltiMate 3000 RS				
Mobile Phases:	A) Acetonitrile				
	B) 7 mM Phosphoric acid + 40 mM sodium sulfate (pH 2.65)				
Gradient times (min):	-3.0	0.0	2.0	10.0	12.0
%A	0	0	0	25	25
%B	100	100	100	75	75
Flow Rate:	1.50 mL/min				
Temperature:	30 °C				
Injection:	10 µL				
Detector:	Diode Array; UV 210 nm, spectra 260 – 400 nm				
Samples:	Vitamin standards in mobile phase				

Peaks:	**µg/mL**
1. Thiamine	25
2. Ascorbic acid	62
3. Niacinamide	25
4. Pyridoxine	25
5. Pantothenic acid	25
6. Folic acid	6.2
7. Riboflavin	5.0

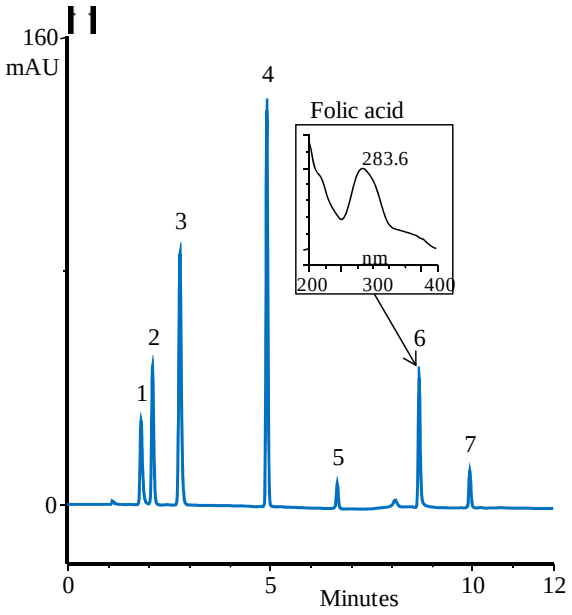


Figure 5
Water-Soluble Vitamins

4.6. Analysis of Triglycerides in Cooking Oils

Cooking oils are purified lipids from plants, and are usually liquid at room temperature. They contain triglycerides as major components, and small quantity of free fatty acids, mono- and di-glycerides. 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 minor components, major components, and obtaining more detailed "fingerprint." Because of its high shape selectivity, the Acclaim C30 provides higher resolution than the C18 column for oil analysis (Figure 1). Separations of several cooking oils are illustrated in Figure 6 and 7.

Column:	Acclaim C30 or Acclaim 120 C18, 5 µm
Dimension:	4.6 x 150 mm
Mobile Phase:	Acetonitrile (MeCN)/Iso-propanol (IPA)/ Ammonium Acetate (0.1 M, pH 5.0) (Buffer)
Temperature:	40 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	2 µL
Detection:	Corona ultra (Gain = 100 pA; Filter = medium; Nebulizer. Temp. = 25 °C)
Sample:	Peanut oil (5 mg/mL in iso-propanol)

Time (min)	MeCN	IPA	Buffer
-15	90	5	5
0	90	5	5
0.1	90	5	5
60	0	95	5
70	0	95	5

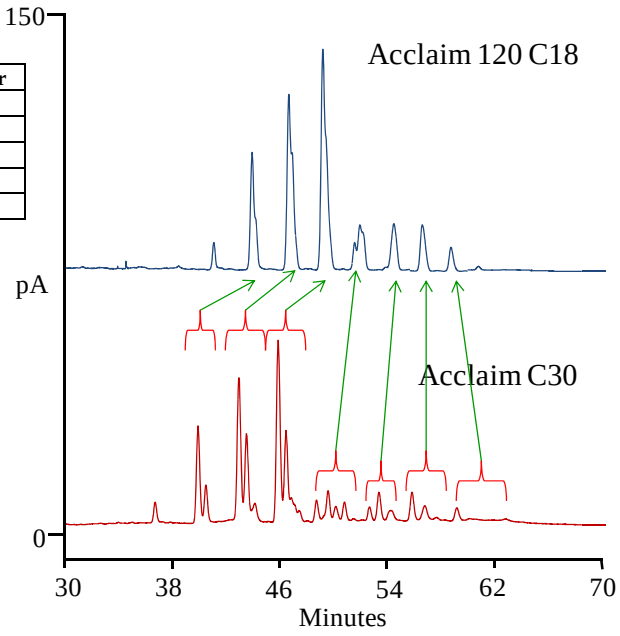


Figure 6
Analysis of Cooking Oil: C30 vs. C18

4.7. Analysis of Cooking Oils

Column: Acclaim C30, 5 µm
Dimension: 4.6 x 150 mm
Mobile Phase: Acetonitrile (MeCN)/Iso-propanol (IPA)/ Ammonium Acetate (0.1 M, pH 5.0) (Buffer)
Temperature: 40 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 2 µL
Detection: Corona ultra (Gain = 100 pA; Filter = medium; Nebulizer Temp. = 25 °C)
Sample: Peanut, Olive, or Wheat Germ Oil (5 mg/mL in iso-propanol)

Time (min)	MeCN	IPA	Buffer
-15	90	5	5
0	90	5	5
0.1	90	5	5
60	0	95	5
80	0	95	5

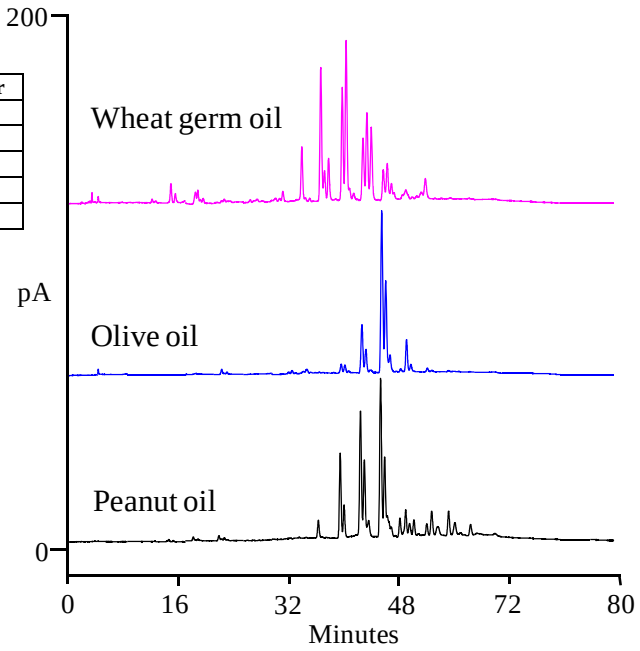


Figure 7
Analysis of Cooking Oils

4.8. Phospholipids in Egg Lecithin

Phospholipids are a class of lipids and are a major component of all cell membranes as they can form lipid bilayers. Most phospholipids contain a diglyceride, a phosphate group connected a hydrophilic organic group such as choline. The first phospholipid identified in biological tissues was lecithin in egg yolk. Analysis of phospholipids is challenging due to its complexity (hydrocarbon length, degree of saturation of diglyceride moiety, and the type of the hydrophilic organic group connecting to the phosphate). Figure 8 shows the profile of commercial grades of lecithin from egg yolk and soybean obtained on an Acclaim C30 column using a Corona ultra CAD detector. It indicates that lecithin from egg yolk contains both phospholipids and triglycerides, with minor impurities. To obtain detailed information on phospholipids composition, a higher resolution method can be used for is used (Figure 9).

Column:	Acclaim C30, 5 µm
Dimension:	4.6 x 150 mm
Mobile Phase:	Acetonitrile (MeCN)/Iso-propanol (IPA)/ Ammonium Acetate (0.1 M, pH 5.0) (Buffer)
Temperature:	40 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	2 µL
Detection:	Corona ultra (Gain = 100 pA; Filter = medium; Nebulizer Temp = 25 °C)
Sample:	Egg Lecithin – PL30S (5 mg/mL in iso-propanol)

Time (min)	MeCN	IPA	Buffer
-15	70	0	30
0	70	0	30
0.1	70	0	30
10	90	0	10
35	10	80	10
50	0	95	5
60	0	95	5

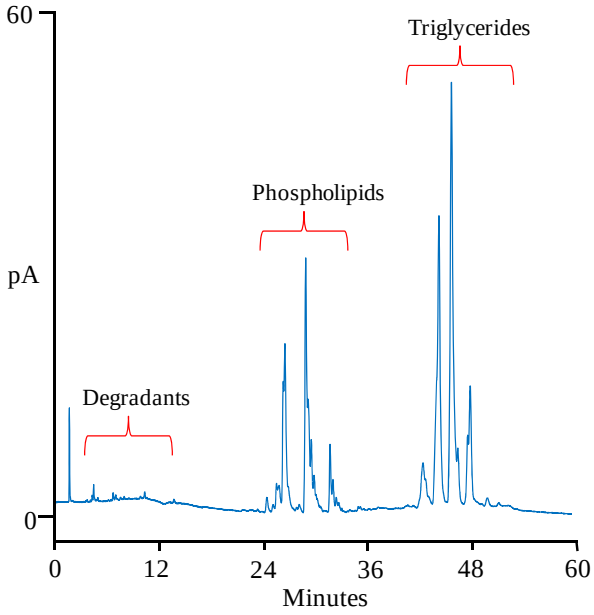


Figure 8
Profile of Egg Lecithin

4.9. Phospholipids in Egg Lecithin

Column: Acclaim C30, 5 µm
Dimension: 4.6 x 150 mm
Mobile Phase: Acetonitrile (MeCN)/Iso-propanol (IPA)/ Ammonium Acetate (0.1 M, pH 5.0) (Buffer)
Temperature: 40 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 5 µL
Detection: Corona ultra (Gain = 100 pA; Filter = medium; Nebulizer. Temp. = 25 °C)
Sample: Egg Lecithin – PL100M
(5 mg/mL in iso-propanol)

Time (min)	MeCN	IPA	Buffer
-15	60	20	20
0	60	20	20
0.1	60	20	20
150	38	50	12
151	5	90	5
160	5	90	5

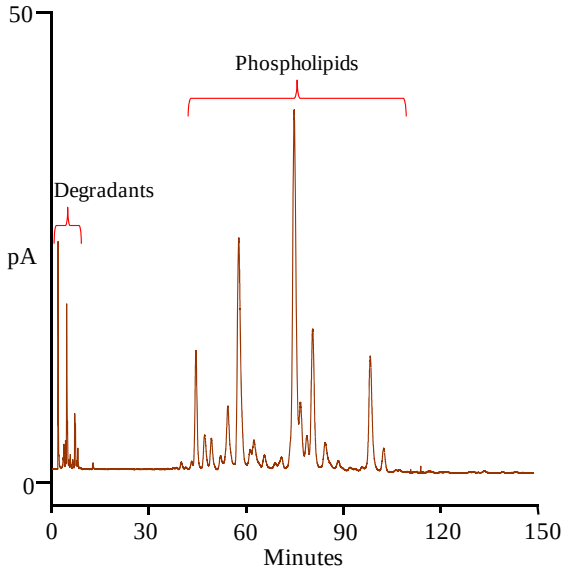


Figure 9
Phospholipids in Egg Lecithin

