Thermo Scientific

Acclaim Phenyl-1

Column Product Manual

P/N: 065323-04 September 2013

Thermo SCIENTIFIC

Product Manual

for

Acclaim Phenyl-1 Columns

3µm, Analytical, 4.6 x 150mm (071969)
3µm, Analytical, 4.6 x 100mm (078017)
3µm, Analytical, 4.6 x 50mm (078018)
3µm, Analytical, 3.0 x 250mm (074694)
3µm, Analytical, 3.0 x 150mm (071970)
3µm, Analytical, 3.0 x 100mm (074693)
3µm, Analytical, 3.0 x 50mm (071972)
3µm, Analytical, 2.1 x 250mm (078014)
3µm, Analytical, 2.1 x 150mm (071971)
3µm, Analytical, 2.1 x 100mm (078015)
3µm, Analytical, 2.1 x 50mm (078016)

5µm, Analytical, 4.6 x 250mm (079697)
5µm, Analytical, 4.6 x 150mm (088016)
5µm, Analytical, 2.1 x 150mm (079698)

Guard, 4.6 x 10mm (071973)
Guard, 3.0 x 10mm (071974)
Guard, 2.1 x 10mm (079934)

For Research Use Only. Not for use in diagnostic procedures.

Revision History:

Revision 03, January, 2013, Rebranded for Thermo Scientific. Added 5 µm column formats.

Revision 04, September, 2013, Changed 5µm, Analytical, 4.6 x 150mm P/N from 079696 to 088016.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

 **SAFETY**	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
 **WARNING**	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
 **CAUTION**	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.***
 **NOTE**	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

Contents

1. Introduction

Thermo Scientific™ Acclaim™ Phenyl-1 is based on covalent modification of high-purity, spherical, porous silica particles, with a specially designed silane ligand bearing proprietary alkyl aromatic functionality. This novel column chemistry offers high aromatic selectivity, high hydrophobic retention, and resistance to dewetting (in 100% aqueous mobile phase).

1.1 Main features of the Acclaim Phenyl-1 Column include:

- High aromatic selectivity
- High hydrophobic retention
- Unique and complementary selectivity
- Compatibility with highly aqueous mobile phase
- High efficiency and rugged packing

1.2 Physical data

Bonding Chemistry: Proprietary covalently bonded alkyl aromatic moiety

Silica Substrate: Spherical, high-purity
Particle size – 3 and 5 µm
Surface area – 300 m^2/g
Pore size – 120 Å

Table 1. Ordering Information

	Particle Size	Column Dimensions	P/N	Required Holder
Analytical	3 µm	4.6 x 150 mm	071969	
		4.6 x 100 mm	078017	
		4.6 x 50 mm	078018	
		3.0 x 250 mm	074694	
		3.0 x 150 mm	071970	
		3.0 x 100 mm	074693	
		3.0 x 50 mm	071972	
		2.1 x 250 mm	078014	
		2.1 x 150 mm	071971	
		2.1 x 100 mm	078015	
		2.1 x 50 mm	078016	
	5 µm	4.6 x 250 mm	079697	
		4.6 x 150 mm	088016	
		2.1 x 150 mm	079698	
Guard pkg of 2	3 µm	4.6 x 10 mm	071973	P/N 069580
		3.0 x 10 mm	071974	P/N 069580
		2.1 x 10 mm	079934	P/N 069580

1.3 Specifications and Recommended Operational Parameters

Shipping solution: 70 / 30 acetonitrile / water
Storage solution: 100% acetonitrile
Buffer pH Range: pH 2 – 8
Temperature Range: < 60 °C

Table 2. Operating pressure and flow rate specifications

Particle Size	Column Dimensions	P/N	Maximum Recommended Pressure	Typical Flow Rate (Recommended)	Maximum Flow Rate
3 µm	4.6 x 150 mm	071969	8,800 psi	0.8 – 1.5 mL/min	2.0 mL/min
	4.6 x 100 mm	078017	6,000 psi	0.8 – 1.5 mL/min	2.0 mL/min
	4.6 x 50 mm	078018	4,500 psi	0.8 – 1.5 mL/min	2.0 mL/min
	3.0 x 250 mm	074694	12,000 psi	0.4 – 0.8 mL/min	1.0 mL/min
	3.0 x 150 mm	071970	8,800 psi	0.4 – 0.8 mL/min	1.0 mL/min
	3.0 x 100 mm	074693	8,800 psi	0.4 – 0.8 mL/min	1.0 mL/min
	3.0 x 50 mm	071972	4,500 psi	0.4 – 0.8 mL/min	1.0 mL/min
	2.1 x 250 mm	078014	12,000 psi	0.2 – 0.4 mL/min	0.5 mL/min
	2.1 x 150 mm	071971	8,800 psi	0.2 – 0.4 mL/min	0.5 mL/min
	2.1 x 100 mm	078015	6,000 psi	0.2 – 0.4 mL/min	0.5 mL/min
	2.1 x 50 mm	078016	4,500 psi	0.2 – 0.4 mL/min	0.5 mL/min
5 µm	4.6 x 250mm	079697	6,000 psi	0.8 - 2.0 mL/min	2.5 mL/min
	4.6 x 150mm	088016	6,000 psi	0.8 - 2.0 mL/min	2.5 mL/min
	2.1 x 150mm	079698	6,000 psi	0.2 - 0.4 mL/min	0.5 mL/min

1.4 Operational Guidelines

- Operate the column according to “Operational Parameters” described in Section 1.3.
- Follow the direction of flow that is marked on the column. Reverse flow should be avoided except for removal of inlet blockage.
- Avoid sudden pressure surge on to the column.
- Use guard columns to protect the analytical column to prolong column lifetime.
- Keep the column plugged when not in use to prevent column from drying out.

2. Step-By-Step User Guide

Thermo Scientific recommends that you perform an efficiency test on your Acclaim Phenyl-1 column before use. The purpose of column performance validation is to ensure no damage has occurred during shipping. Steps 1 – 5 below outline the necessary steps to perform this validation test. 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 Thermo Scientific.

Step 2 – Mobile phase preparation

Obtaining reliable, consistent and accurate results requires 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. Thermo Scientific 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 proportioning valve provides better flexibility in method optimization, while the pre-mixed mobile phase provides less baseline noise.

NOTE

These two mobile phases by weight (not by volume) for best reproducibility.

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 to 50 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 in the column 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.

A typical quality control (QC) test chromatogram for a 4.6 mm × 150 mm column is shown in Appendix 1. The 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 your application.

3. Column Care

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

If the 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 near 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 iso-propanol should remove most highly retained compounds. When switching between solvents with vastly different polarities, it may be necessary to first purge the column with a mutually miscible solvent such as isopropanol.

Long-term storage of silica-based, bonded-phase columns 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 phase, the buffer should first be removed by purging the column with 20 column volumes of a 50/50 mixture of methanol or acetonitrile and water, followed by 10 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.

Columns 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 a 60/40 acetonitrile /0.02 M phosphate buffered mobile phase. Because re-equilibration is rapid with the original mobile phase when using this approach, and any danger of corrosion from the salts is eliminated.

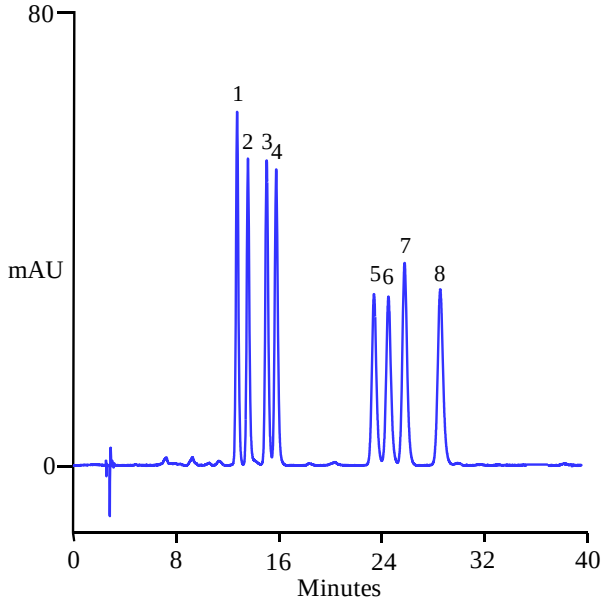
4. Example Applications

The Acclaim Phenyl-1 column is ideally suited for the analysis of aromatic analytes and has demonstrated its use in a wide variety of applications.

4.1 Separation of Glucocorticosteroids

Glucocorticosteroids are a group of naturally occurring and synthetic hormones that moderate inflammation and other stress responses. As shown in Figure 1, eight glucocorticosteroids are baseline resolved on a 3 x 250-mm Acclaim Phenyl-1 column using a methanol/water mobile phase system.

Figure 1. Separation of Glucocorticosteroids



Column:	Acclaim Phenyl-1, 3 µm
Dimension:	3.0x250 mm
Mobile Phase:	Methanol/Water v/v 46/54
Temperature:	40 C
Flow Rate:	0.5 mL/min
Inj. Volume:	5 µL
Detection:	UV, 254 nm
Peaks:	(50 ppm each)

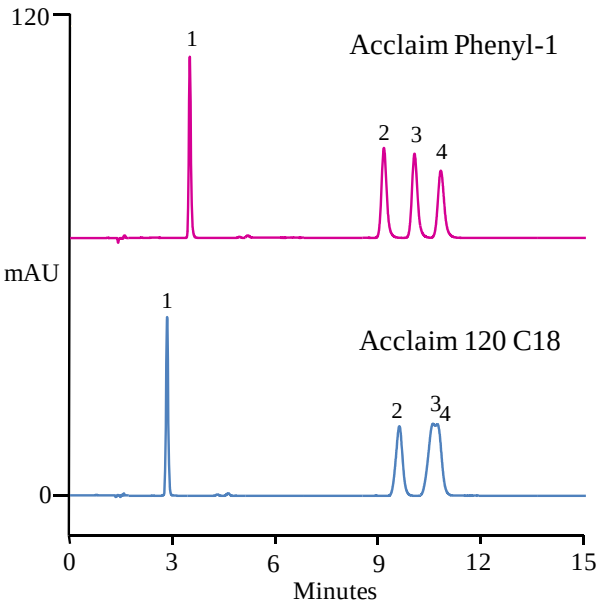
1. Prednisone
2. Cortisone
3. Prednisolone
4. Hydrocortisone
5. Dexamethasone
6. 6-Methylprednisolone
7. Corticosterone
8. Deoxyhydrocortisone

4.2 Separation of Estrogens

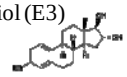
Estrogens are a group of steroid compounds, named for their importance in the estrous cycle, and functioning as the primary female sex hormone. Estrogens are used as part of some oral contraceptives and in estrogen replacement therapy for postmenopausal women.

Three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). Estradiol (E2) is the predominant form in nonpregnant females, estrone is produced during menopause, and estriol is the primary estrogen of pregnancy. Ethynylestradiol, a derivative of estradiol, is an orally bio-active estrogen used in almost all modern formulations of combined oral contraceptive pills. In Figure 2 the Acclaim Phenyl-1 shows the baseline resolve estrogens using isocratic conditions.

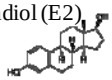
Figure 2. Separation of Estrogens



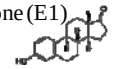
Column: 4.6x150 mm, 3 µm
Mobile Phase: Acetonotrile/Methanol/Water v/v/v 15/40/45
Temperature: 40 C
Flow Rate: 1.2 mL/min
Inj. Volume: 5 µL
Detection: UV, 220 nm
Peaks: (100 ppm each)
1. Estriol (E3)



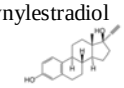
2. Estradiol (E2)



3. Estrone (E1)



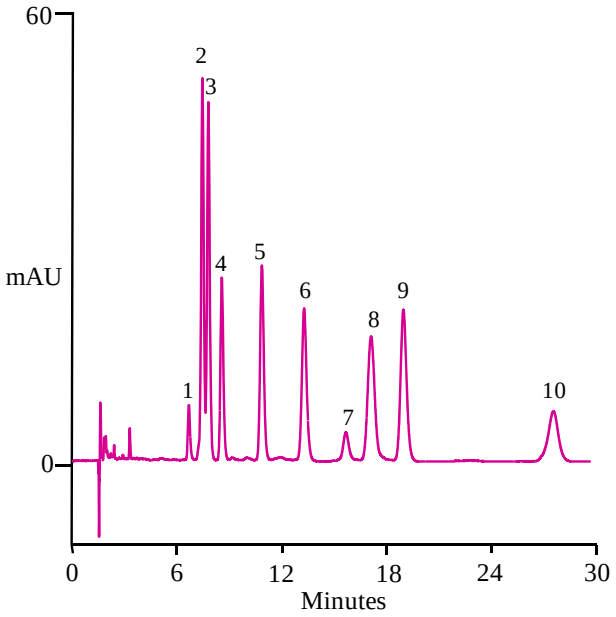
4. Ethynylestradiol



4.3 Fat-soluble vitamin separation

Fat-soluble vitamins analysis is an important and challenging essay for various products like pharmaceuticals, foods and nutritional supplements. As shown in Figure 3, the Acclaim Phenyl-1 column provides excellent selectivity for separating vitamins A, D2, D3, K1 , K2, as well as E and E acetate along with related substances - δ - and γ - tocopherols, under both gradient (not shown) and isocratic conditions.

Figure 3. Fat-soluble vitamin separation



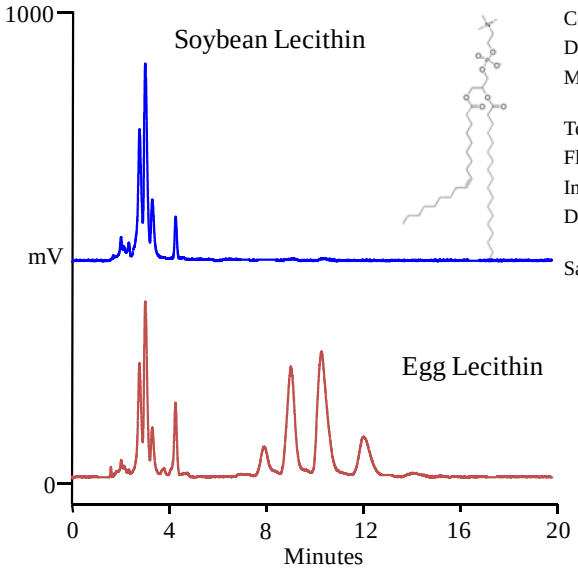
Column:	Acclaim Phenyl-1, 3 µm
Dimension:	3.0x150 mm
Mobile Phase:	Methanol/Water v/v 90/10
Temperature:	30 C
Flow Rate:	0.5 mL/min
Inj. Volume:	2 µL
Detection:	UV, 220 nm
Peaks:	(100 ppm each)

1. Retinol acetate (Vitamin A acetate)
2. Vitamin D2
3. Vitamin D3
4. δ-Tocopherol
5. γ-Tocopherol
6. α-Tocopherol (Vitamin E)
7. Impurity (unknown)
8. Vitamin E acetate
9. Vitamin K2
10. Vitamin K1

4.4 Separation of Phospholipids

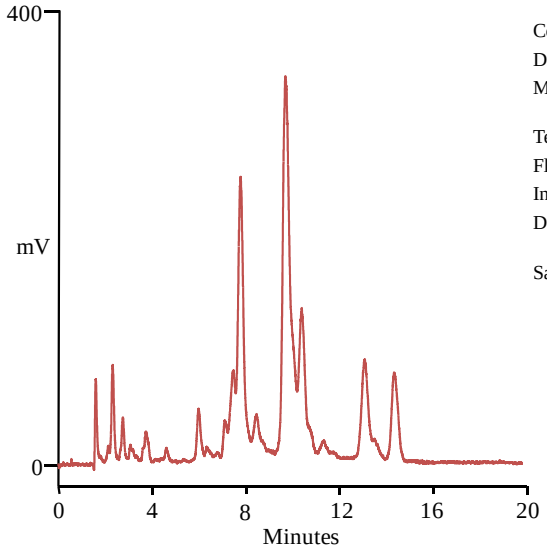
Lecithin is a generic term to designate the yellow-brownish fatty substances occurring in animal and plant tissues. Lecithin has emulsification and lubricant properties, and is a surfactant. Thus it is widely used for applications in human food, animal feed, pharmaceutical, paint, and other industrial applications. Phospholipids are a class of lipids and are a major component of all cell membranes as they can form lipid bilayers. Depending on the source, the composition of lecithin can vary. Figure 4 shows the profiles of lecithin from egg yolk and soybean obtained on an Acclaim Phenyl-1 column using a Corona *ultra* CAD detector. While both egg yolk and soybean contain phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol) as early-eluting peaks (in 2 to 4 min range), it is indicated that egg yolk significant quantity of triglycerides as later eluting peaks (from 7 to 13 min). To obtain detailed information on phospholipids composition, a mobile phase containing higher aqueous and less isopropanol is used to resolve major components of phospholipids in soybean lecithin (Figure 5).

Figure 4. Analysis of Soybean Lecithin and Egg Lecithin



Column:	Acclaim Phenyl-1, 3 µm
Dimension:	3.0x150 mm
Mobile Phase:	Acetinitrile/Iso-propanol/Ammonium Acetate (100 mM, pH5.0) v/v/v 45/45/10
Temperature:	25 C
Flow Rate:	0.45 mL/min
Inj. Volume:	2 µL
Detection:	Corona *ultra* (Gain = 100 pA; Filter = low; Neb; Temp = 25 C)
Sample:	Soybean Lecithin (1 mg/mL in iso-propanol)
	Egg Lecithin (2.5 mg/mL in iso-propanol)

Figure 5. Phospholipids in Soybean Lecithin

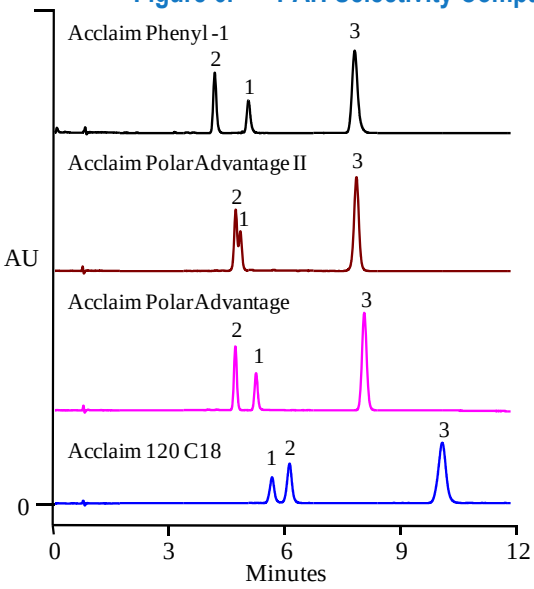


Column:	Acclaim Phenyl-1, 3 µm
Dimension:	3.0x150 mm
Mobile Phase:	Acetinitrile/Iso-propanol/Ammonium Acetate (100 mM, pH5.0) v/v/v 70/10/20
Temperature:	25 C
Flow Rate:	0.45 mL/min
Inj. Volume:	5 µL
Detection:	Corona *ultra* (Gain = 100 pA; Filter = low; Neb; Temp = 25 C)
Sample:	Soybean Lecithin (1 mg/mL in iso-propanol)

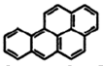
4.5 Polycyclic aromatic hydrocarbon (PAH): Unique and complementary selectivity

NIST *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. Figure 6 demonstrates the elution order of these three PAHs on the Acclaim Phenyl-1 column and three other Acclaim reversed-phase columns, namely Acclaim 120 C18, Acclaim PolarAdvantage (sulfonamide-embedded), and Acclaim PolarAdvantage II (amide-embedded). It is clear that the new phenyl column shows different and complementary selectivity. Compared to other commercial phenyl-type column, the Acclaim Phenyl also exhibits unique selectivity (Figure 7).

Figure 6. PAH Selectivity Comparison – NIST SRM 869a



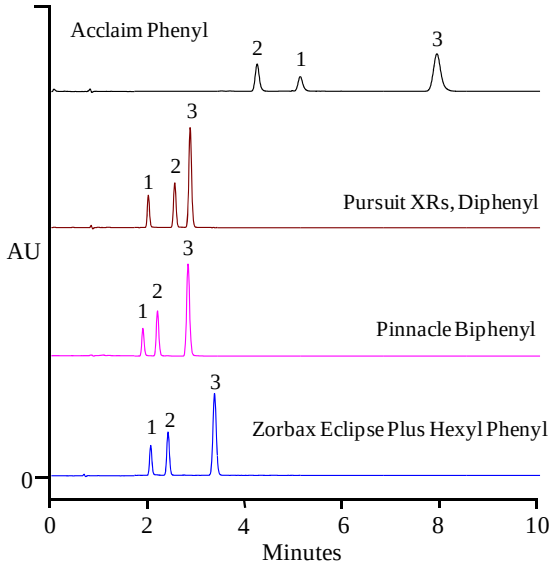
Column: 4.6x150 mm, 3 µm
Mobile Phase: CH_3CN/H_2O v/v 85/15
Temperature: 25 C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV (254 nm)
Sample: NIST SRM 869a standard mix
1. Benzo[a]pyrene (BaP)



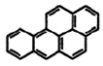
2. Phenanthro[3,4-c]phenanthrene (PhPh)

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

Figure 7. Shape Selectivity Comparison – NIST SRM 869a



Column: 4.6x150 mm, 3 µm
Mobile Phase: CH_3CN/H_2O v/v 85/15
Temperature: 25°C
Flow Rate: 1 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)

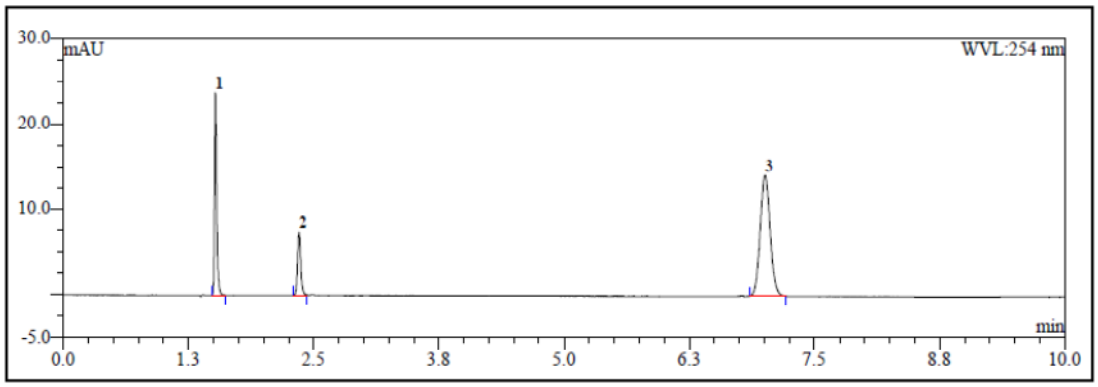
Appendix – Quality Performance Test Report

Acclaim® Phenyl-1
3µm 120Å (4.6 x 150 mm)
Product No. 071969

Date: 06-May-10 12:21
Serial No. :
Lot No. : 2009-22-74

Mobile Phase: 70:30:0.1 v/v/v Acetonitrile/Water/2M NH4OAc pH 5.4
Flow Rate: 1.00 mL/min **Temperature:** 30 °C
Detection: UV, 254 nm **Injection Volume:** 5.0 µL

Storage Solution: 70 %Acetonitrile



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Efficiency (EP)	Concentration
1	Uracil	1.52	1.35	26055	0.015 mg/mL
2	Dimethylphthalate	2.35	1.20	26545	0.075 µL/mL
3	Phenanthrene	7.01	1.08	24056	0.015 mg/mL

QA Results:

Analyte	Parameter	Specification	Results
Phenanthrene	Efficiency	>=16,200	Passed
Phenanthrene	Asymmetry	0.95-1.32	Passed
Phenanthrene	Retention Time	6.36-7.24	Passed
	Pressure	<=2200	1423

Production Reference:
Datasource: QAR
Directory: Acclaim\Phenyl
Sequence: 4P6X150MM
Sample No: 1

6.80 SR5 Build 2413 (137116) (Demo-Installation)

Chromeleon® Dionex Corp. 1994-2010

074647-01 (QAR)