

HPLC Stationary Phase Selection for Environmental Analysis

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Abstract

This study compares the selectivity of various stationary phases for environmental analysis, including pesticides, explosives, carbamates, and PAH's. Key stationary phase variables are identified and strategies for phase selection are provided.

Introduction

There are several variables to consider in HPLC column selection, but the most critical one is the stationary phase. Stationary phases can be broadly classified by the primary ligand such as octadecyl (C18), octyl (C8), cyano, and phenyl. However there are many other variables that can play a critical role in selectivity such as ligand density and degree of polymeric character.

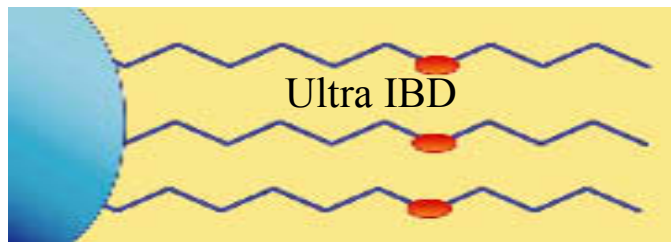
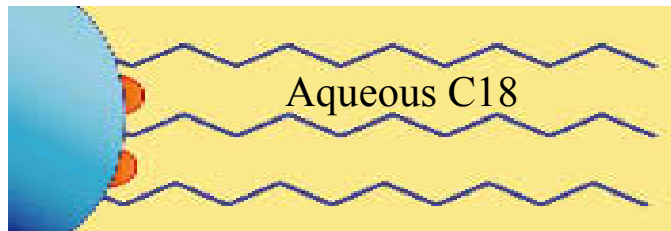
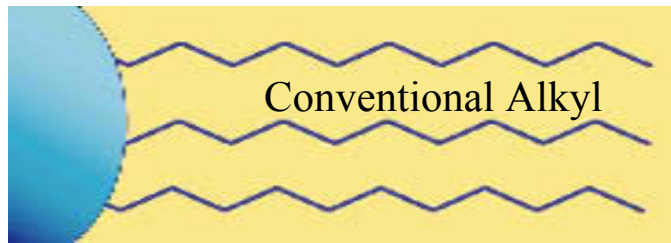
Introduction

In addition to the common alkyl, phenyl, and cyano stationary phases, there are many newer stationary phases with more complex chemistries.

Many of these are based on alkyl chains with some secondary functionality added to aid selectivity.

Another approach for adjustment of selectivity is to use more than one stationary phase (i.e multiple columns) with complimentary selectivity in order to achieve better resolution with difficult separations.

Stationary Phases

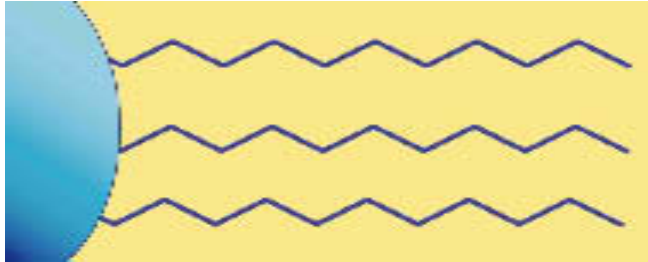


 Color denotes Polar Functionality

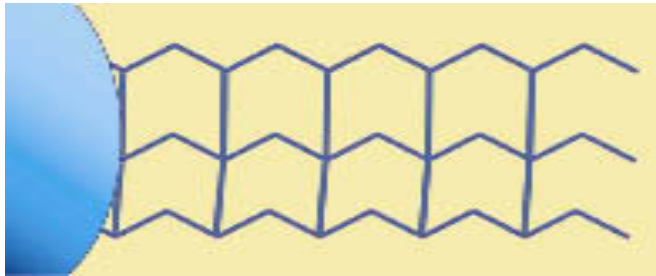
The Aqueous C18 and Ultra IBD stationary phases have secondary polar functionalities that enhance the retention of polar compounds, eliminate retention loss caused by chain folding (even in 100% aqueous mobile phase), and, in some cases, provide unique selectivity.

Stationary Phases

Conventional Alkyl



Cross-linked Alkyl



Alkyl phases can be cross-linked to enhance structural integrity against folding. Also, the correct degree of polymeric character of a cross-linked alkyl phase is essential to enhancing selectivity for PAH separations.

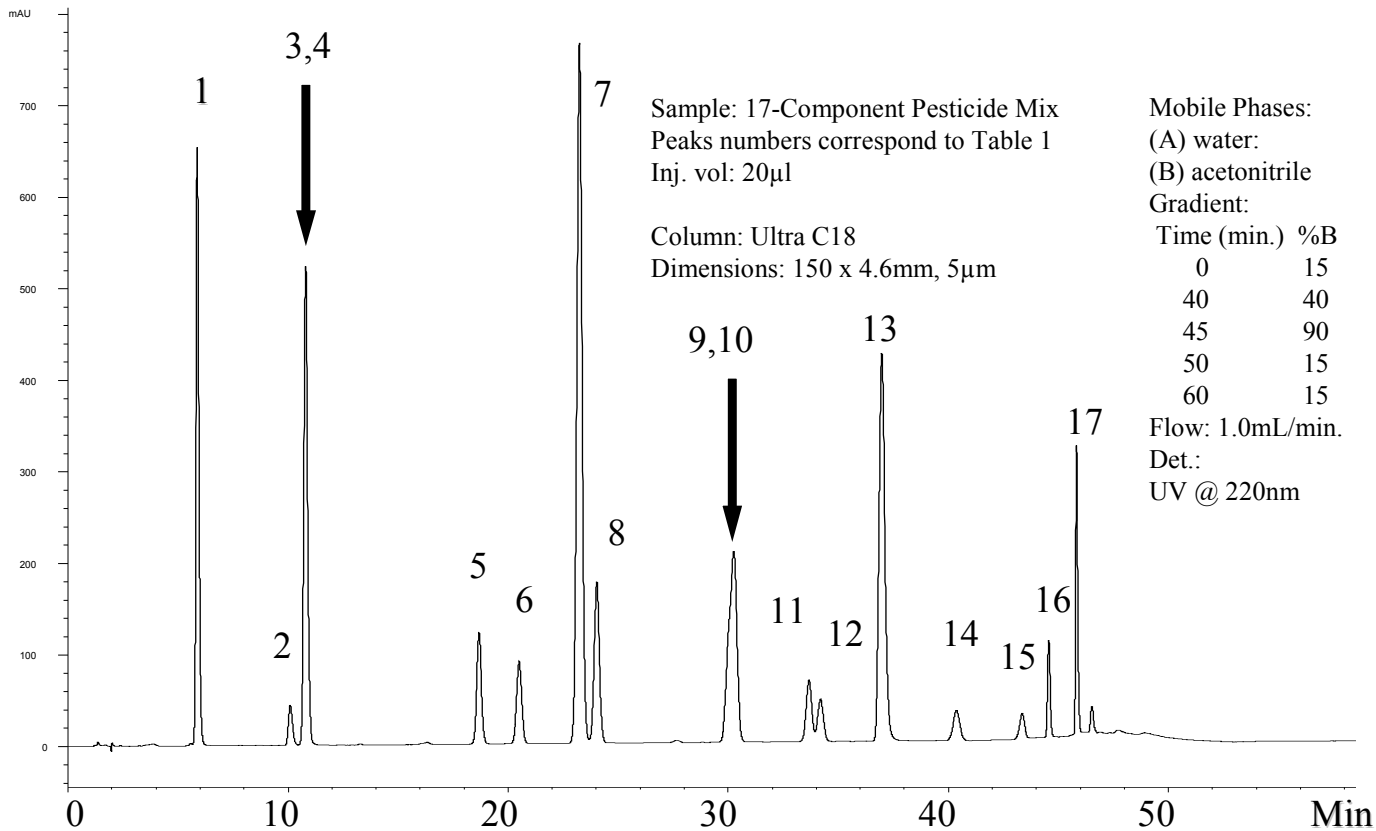
Pesticides

The unique selectivity of the Ultra IBD column was used to develop a two-column method for analyzing a mixture of the 17 triazine, phenyl urea, and carbamate pesticides and metabolites listed on Table 1. On the Ultra C18 column, 15 of the 17 compounds are resolved. An Ultra IBD column with the same conditions also resolves 15 of the 17 compounds, but the pesticides that co-elute on the C18 are well resolved, as indicated by the arrows.

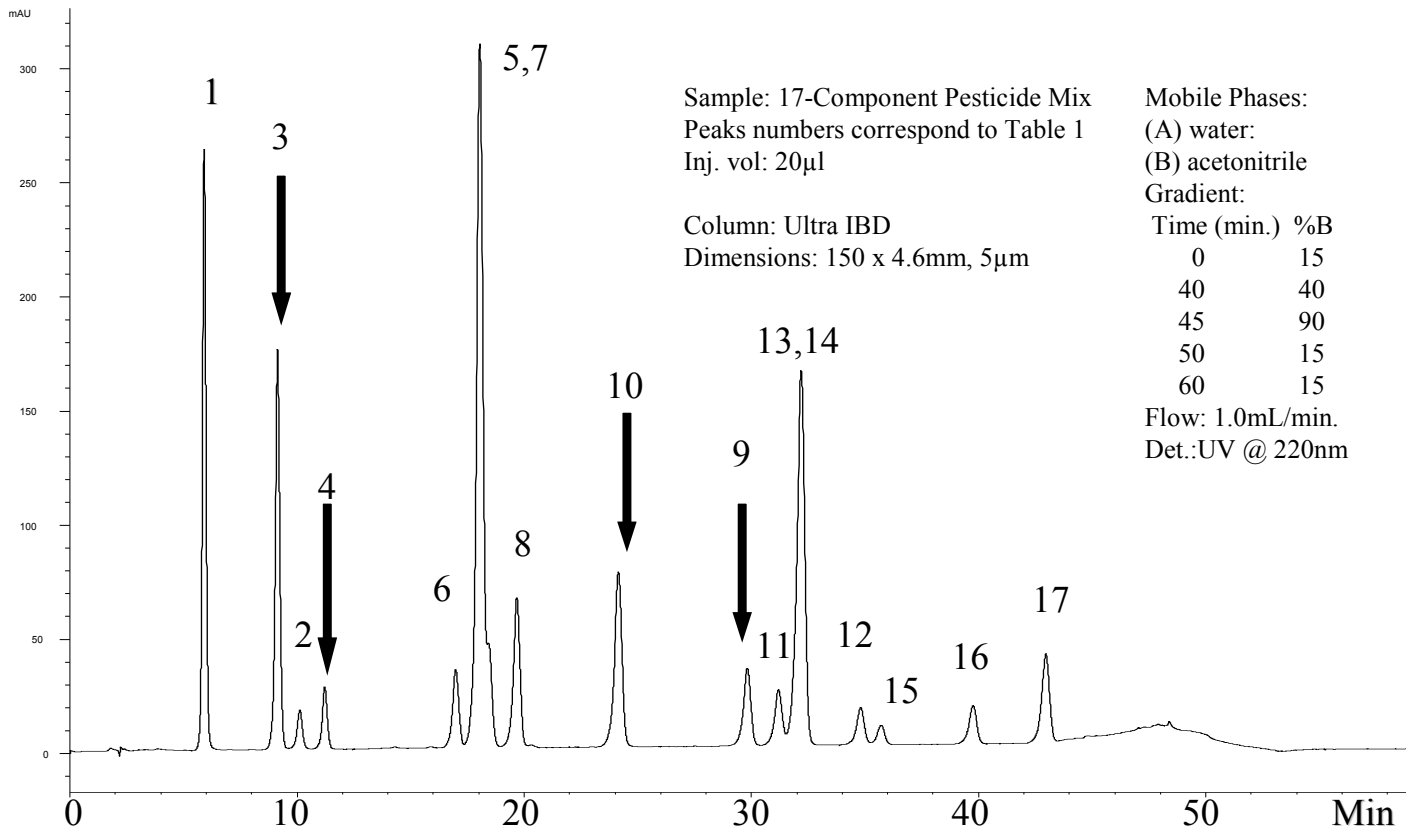
Table 1. Pesticides: 17-Component Mixture

- | | |
|--------------------------|-------------------|
| 1. Desisopropylatrazine | 9. Chlortoluron |
| 2. Hydroxyatrazine | 10. Atrazine |
| 3. Desethylatrazine | 11. Isoproturon |
| 4. Carbendazim | 12. Diuron |
| 5. Metoxuron | 13. Terbumeton |
| 6. Simazine | 14. Propazine |
| 7. Cyanazine | 15. Terbutylazine |
| 8. Desethylterbutylazine | 16. Linuron |
| | 17. Terbutryn |

Pesticides on Ultra C18 Column



Pesticides on Ultra IBD Column



Explosives

Typically EPA 8330 explosives are run using complimentary columns of CN and C18 phases.

By placing a 5 μ m, 20mm x 4.0 mm ID Pinnacle II Cyano guard column in front of the 5 μ m, 25cm x 4.6 mm Pinnacle II C18, the co-eluting peaks tetryl and nitrobenzene can be separated.

This enhanced separation takes advantage of the high selectivity for tetryl and nitrobenzene on the CN column.

Due to the high selectivity, only a 20mm length CN column is needed to increase resolution.

EPA 8330 Explosives Method Conditions

Mobile Phase: 50: 50 Water:Methanol

Flow Rate: 1.5 mL/min

Temperature: 27.0 °C

Detection: UV @ 254 nm

Injection volume: 3 µL

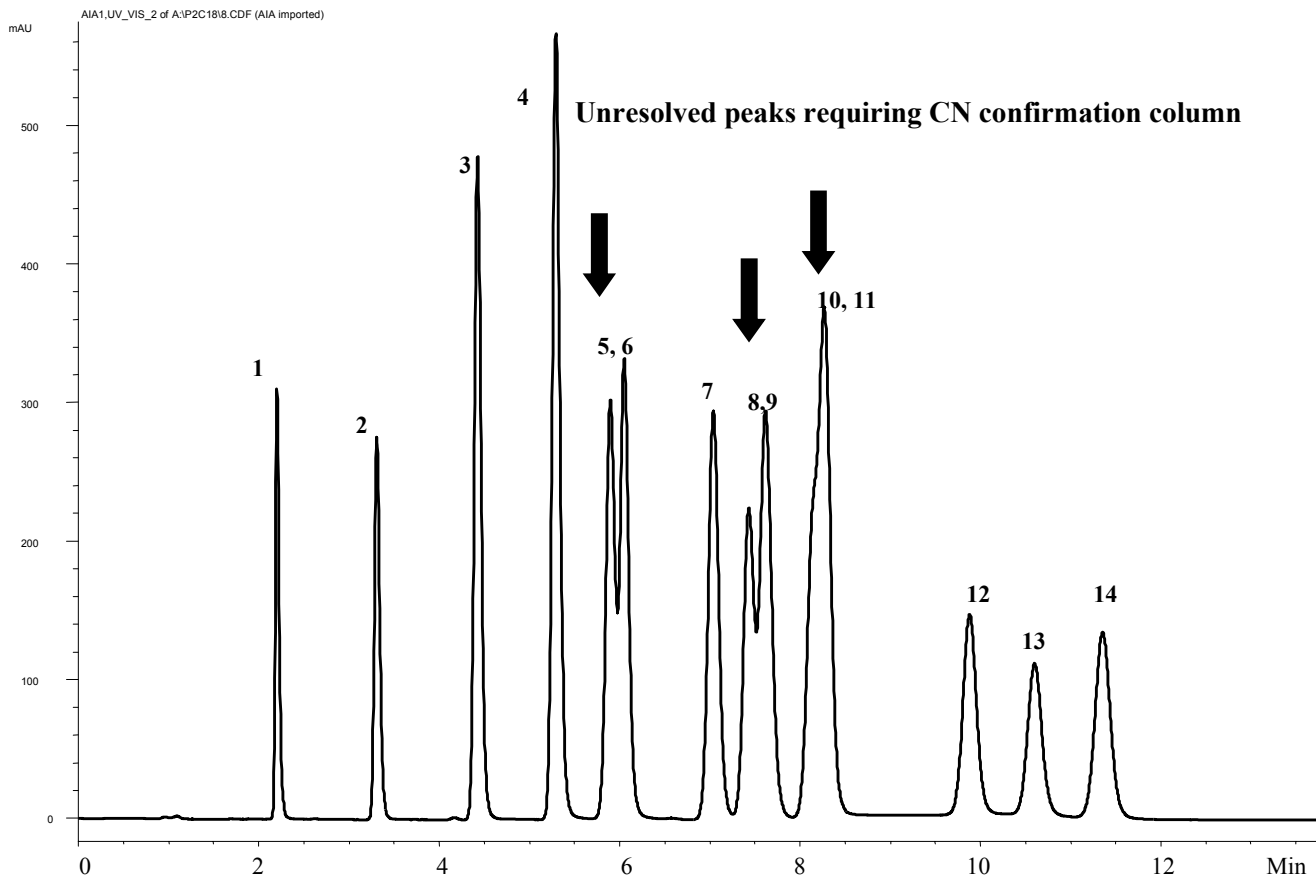
Concentration: 500 µg/mL each component

Sample Diluent: Acetonitrile

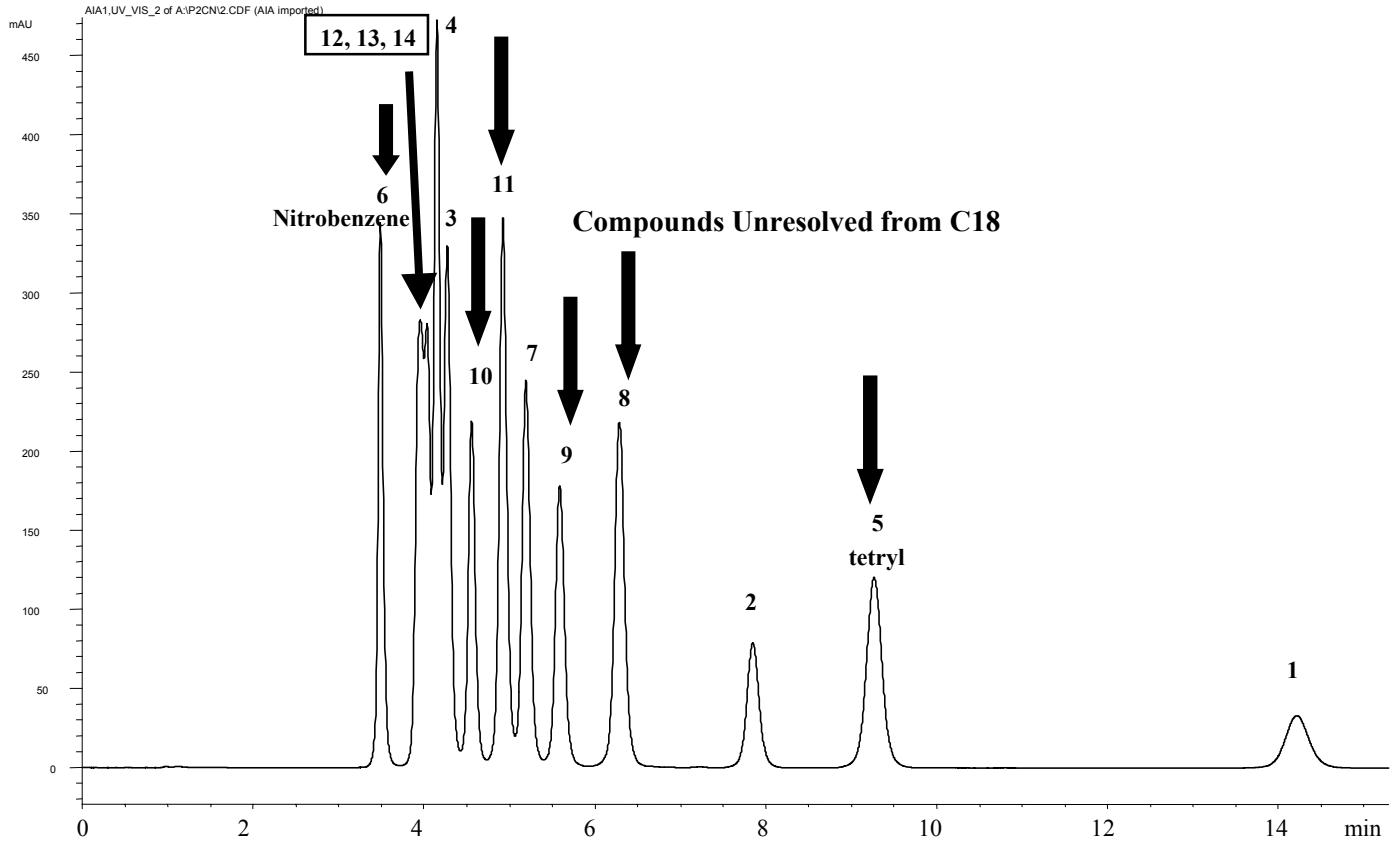
Peak List:

- | | |
|--------------------------|-------------------------------|
| 1. HMX | 8. 2-Amino-4,6-dinitrotoluene |
| 2. RDX | 9. 4-Amino-2,6-dinitrotoluene |
| 3. 1,3,5-Trinitrobenzene | 10. 2,6-Dinitrotoluene |
| 4. 1,3-Dinitrobenzene | 11. 2,4-Dinitrotoluene |
| 5. Tetryl | 12. 2-Nitrotoluene |
| 6. Nitrobenzene | 13. 4-Nitrotoluene |
| 7. 2,4,6-Trinitrotoluene | 14. 3-Nitrotoluene |

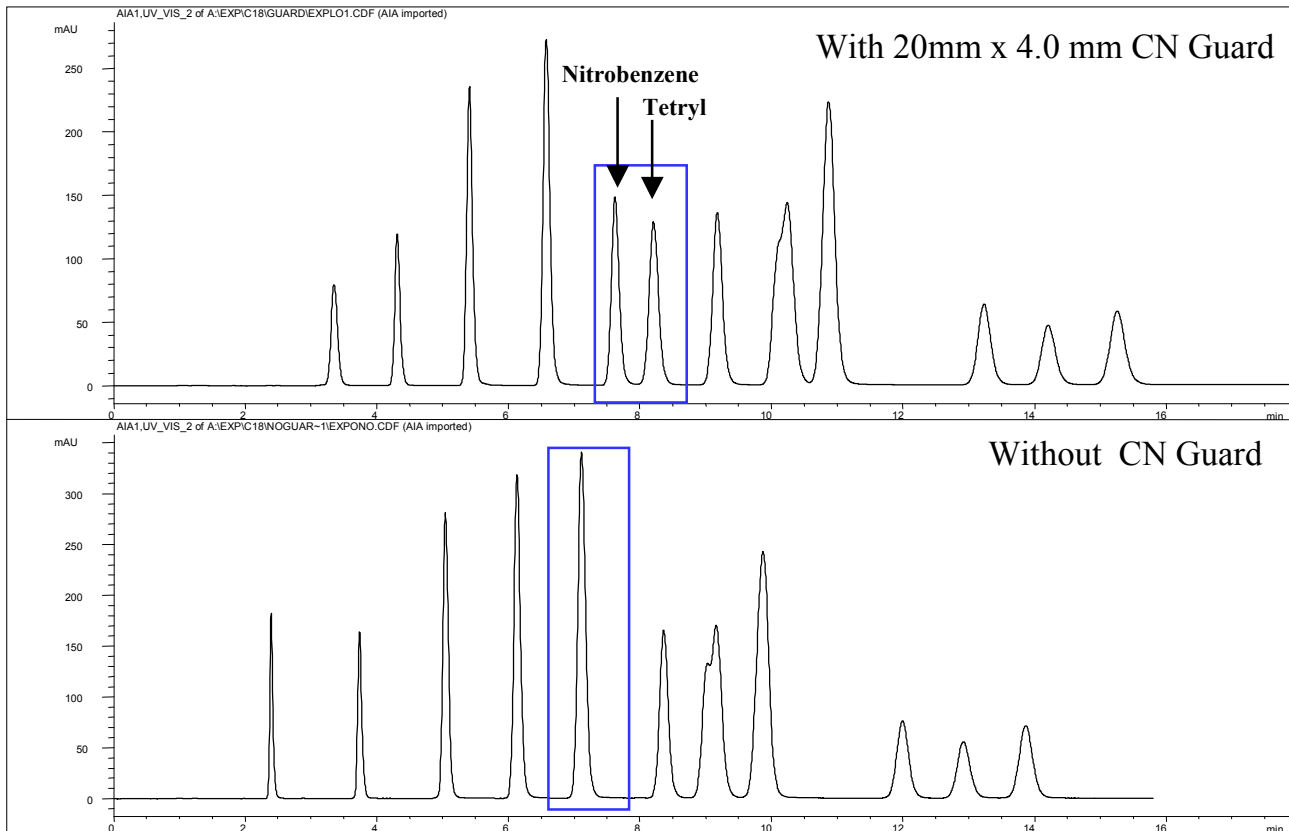
Explosives Separation on Pinnacle II C18, 25cm x 4.6mm, 5um



Explosives Separation Pinnacle II CN, 25cm x 4.6mm, 5um



Explosives Co-elution Resolved Using Pinnacle II CN Guard with Pinnacle II C18 Column



Carbamates

For EPA Method 8318 (Carbamates), the Pinnacle Carbamate column provides an excellent separation in under 40 minutes. This column separates analytes based upon their hydrophobicity.

Retention times can be shortened and greater selectivity obtained by taking direct advantage of polar differences as well as hydrophobicity with the Ultra IBD column.

Other columns as the experimental carbamate obtain higher selectivity toward related analytes through alterations of the ligand to promote shape selectivity.

Pinnacle Carbamate Column – Reference Separation

Carbamate Pesticides on Pinnacle Carbamate

Applications Note: LC_0192

Peak List:

1. aldicarb sulfoxide
2. aldicarb sulfone
3. oxamyl
4. methomyl
5. 3-hydroxycarbofuran
6. aldicarb
7. propoxur
8. carbofuran
9. carbaryl
10. 1-naphthol
11. methiocarb

Column: Pinnacle Carbamate

Catalog#: 9173575

Dimensions: 250 x 4.6mm

Particle size: 5µm

Pore size: 120Å

Solvent A: Water

Solvent B: Methanol

Gradient Program:

20-68% B; 0-20 min.

68-79% B; 0-34 min.

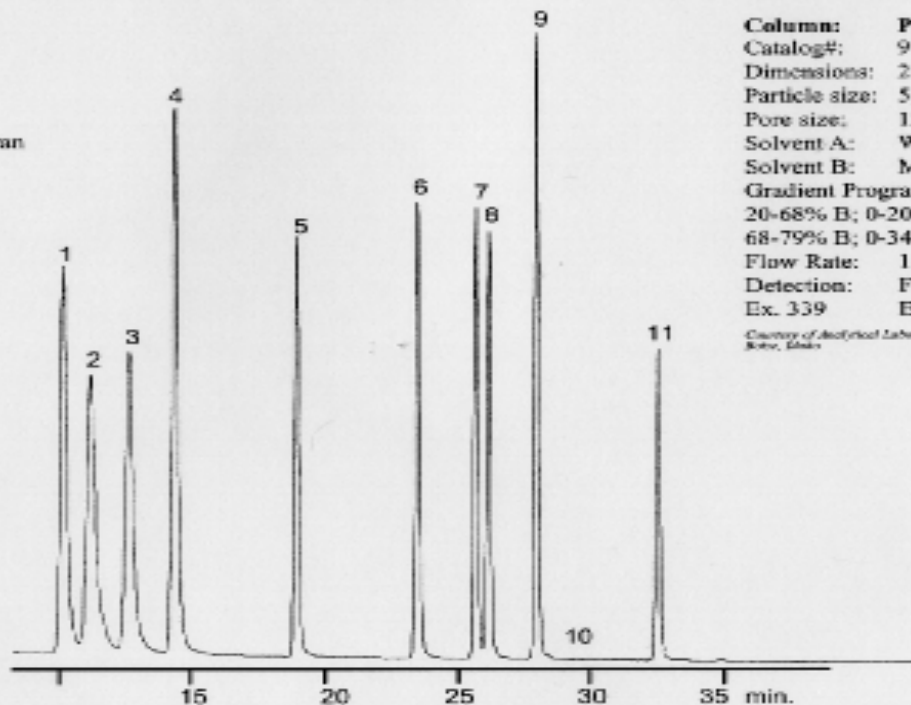
Flow Rate: 1.0mL/min

Detection: Fluorescence

Ex. 339

Em. 445nm

Country of Analytical Laboratories, Inc.
Billerica, MA



Ultra IBD Allows Faster Separation of Carbamates

Carbamates on Ultra IBD

Application Note: LC_0134

Peak List:

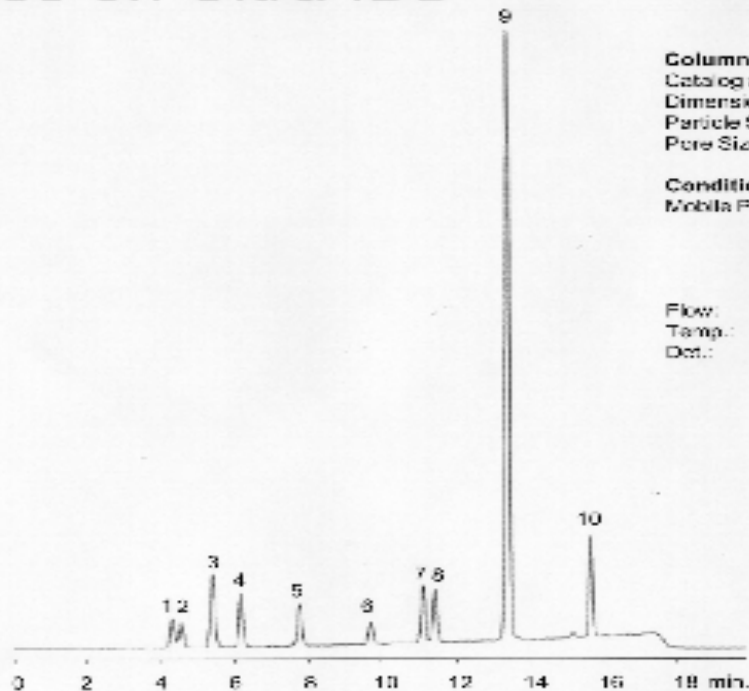
1. Aldicarb Sulfoxide
2. Aldicarb Sulfone
3. Oxamyl
4. Methomyl
5. 3-hydroxycarbafuran
6. Aldicarb
7. Propoxur
8. Carbafuran
9. Carbaryl
10. Methidathion

Sample:

Inj: 5µL
Conc: 1 mg/mL
each component
Solvent: Methanol

Column: Ultra IBD
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: Gradient
A: Water
B: Methanol
Initial: 80% A; 20% B
15 min.: 20% B; 80% B
Flow: 1.0mL/min
Temp.: ambient
Det.: UV @ 220nm



EPA Method 8318 (Carbamates) on Experimental Column

Degradation products are more easily observed.

Calibration Mix Peak List:

1. aldicarb sulfoxide?
2. aldicarb sulfone
3. oxamyl
4. methidathion
5. 3-hydroxycarbofuran
6. aldicarb
7. propoxur (baygon)
8. carbofuran
9. carbaryl
10. methidathion

19 unknowns present in degraded Standard.

Degradation Products of:

- A. aldicarb sulfone
- B. oxamyl
- C. 3-hydroxycarbofuran
- D. propoxur and carbofuran
- E. diluent impurity
- F. carbaryl
- G. methidathion
- H. 3-hydroxycarbofuran
- I. 3-hydroxycarbofuran
- J. methidathion
- K. methidathion

Sample:

Restek EPA 531.1 Carbamate calibration standard catalog# 52273.
Standard components diluted to 40 µg/mL in water before injection.

Inj.: 15 µL
Conc.: 40 µg/mL
Solvent: water:methanol (50:50)

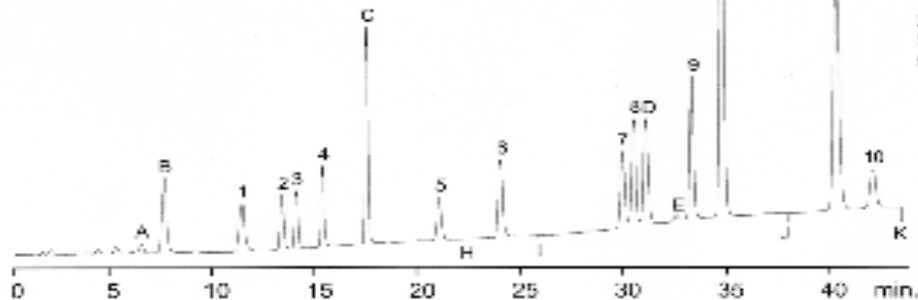
Note: Standard degrades in Methanol/Water Solutions. Most of the impurities are actually hydrolysis products (degradents). The retention time of the Aldicarb Sulfoxide analyte needs to be determined. Initial tests gave a retention time of 22.6 minutes. The standard has been called into question.

Column: Exp
Catalog#: Custom
Dimensions: 150mm x 4.8mm
Particle Size: 5µm
Pore Size: 200Å

Conditions:
Flow: 1.2ml/min
Temp.: 30°C
Det.: UV @ 210nm

Mobile Phase: Gradient
A: water
B: methanol

Time (minutes)	%B
0.00	5.0
10.00	10.0
15.00	25.0
25.00	35.0
32.00	40.0
50.00	60.0



Polyaromatic Hydrocarbons

EPA Methods require the analysis of 16 Polyaromatic hydrocarbons (PAH's). Ordinary monomerically bonded C18 stationary phases do not resolve all 16 PAH's. C18 phases with the proper degree of polymeric structure (cross-linking) have the shape selectivity to resolve all 16 PAH's

PAH Selectivity Test Probe

A simple isocratic test using a three component PAH test mix has been shown to be an excellent test probe for comparing the relative degree polymeric character of C18 stationary phases¹. This test can accurately predict the ability of C18 phases to resolve all 16 PAH's.

1. Sander, L.C. and Wise, S.A., Synthesis and Characterization of Polymeric C18 Stationary Phases For Liquid Chromatography, *Anal. Chem.*, **56**, pp.504-510, (1984).

PAH Selectivity Test

Column Dimensions: 150 x 3.2mm, 5 μ m

Mobile Phase: 85/15 acetonitrile/water

Flow rate: 1.0 ml/min

Temp.: 25C

Detection: UV 254nm

Sample: 10 μ l NIST SRM 869a containing:

benzo(a)pyrene (BaP),

1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN), and

phenanthro[3,4-c]phenanthrene (PhPh)

Results of PAH Selectivity Test

Column	Alpha (TBN/BaP)
Exp.PAH#1	0.93
Exp.PAH#2	0.62
Exp.PAH#3	0.56
Exp.PAH#4	0.85

The lower the alpha value, the greater the polymeric character of the cross-linked C18 phase.

16 Component PAH Test

Column Dimensions: 150 x 3.2mm, 5 μ m

Mobile Phases: (A) water, (B) acetonitrile

Gradient: 40%B to 100% B in 30min,

Hold 100%B for 10min.

Flow rate: 1.0 ml/min

Temp.: 25C

Detection: UV 254nm

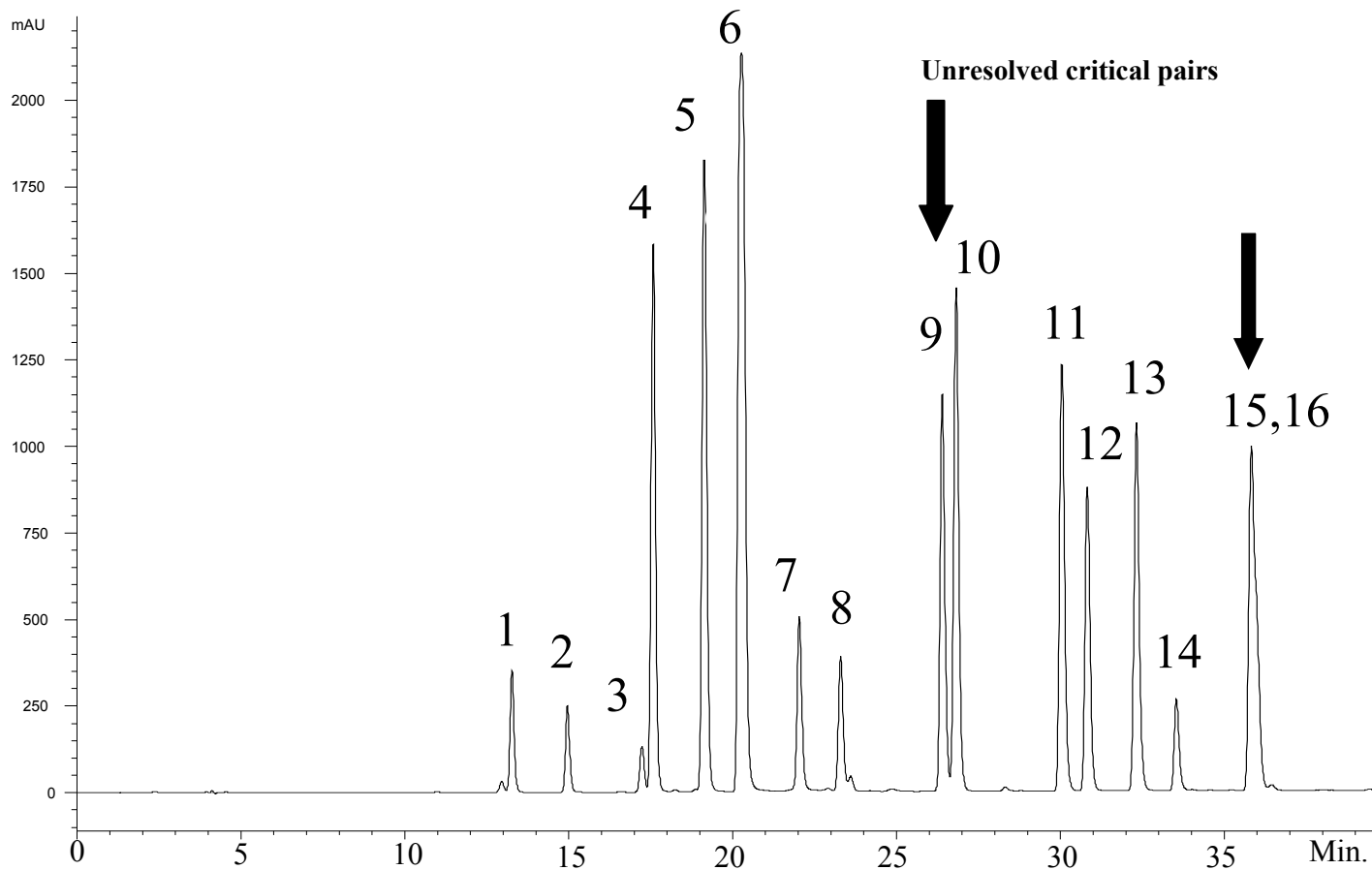
16 Component PAH Test (Cont.)

Sample: 5 μ L Restek 610 PAH Calibration Mix A

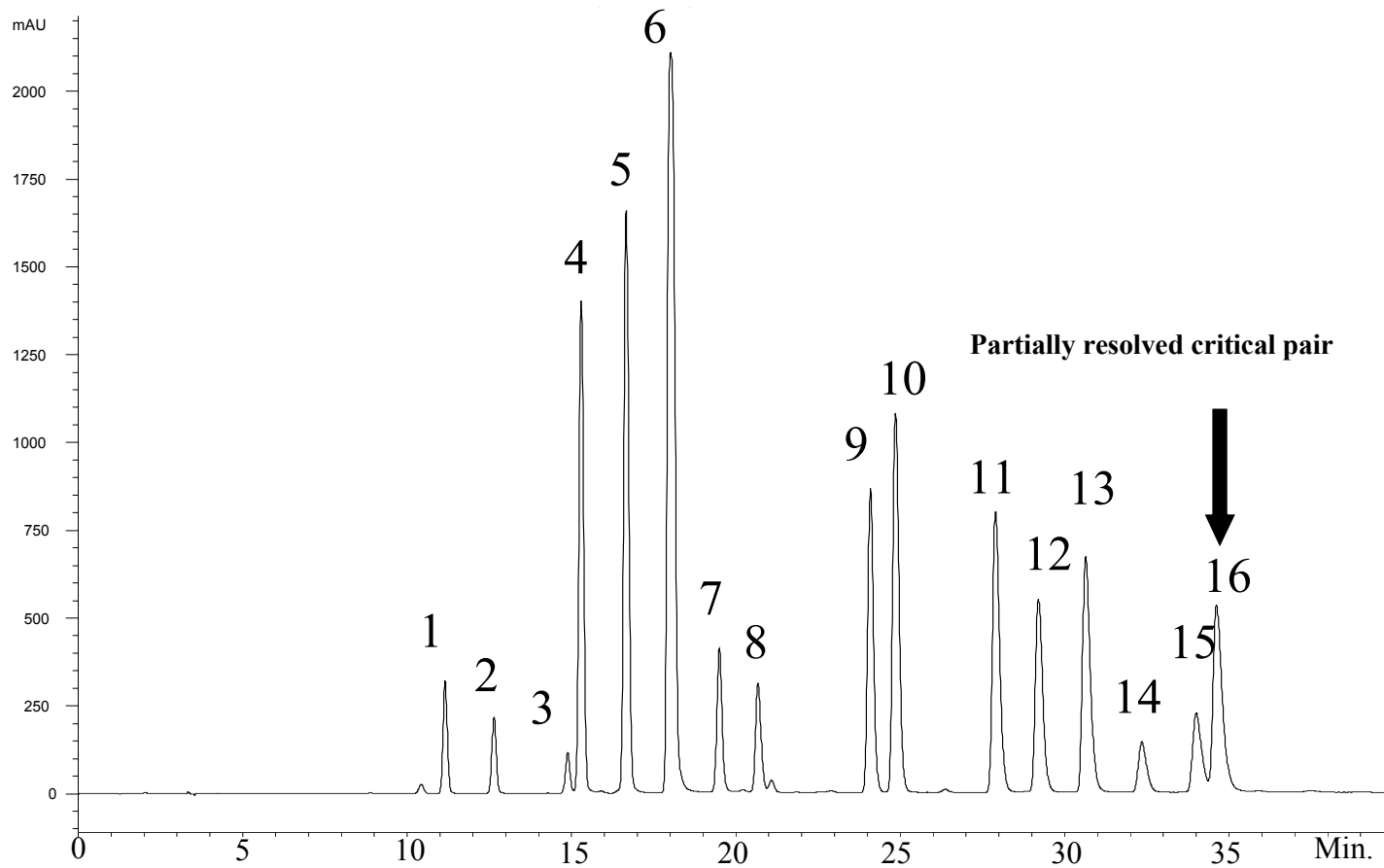
Peak List:

- | | |
|-------------------|-----------------------------|
| 1. Naphthalene | 9. Benzo(a)anthracene |
| 2. Acenaphthylene | 10. Chrysene |
| 3. Acenaphthene | 11. Benzo(b)fluoranthene |
| 4. Fluorene | 12. Benzo(k)fluoranthene |
| 5. Phenanthrene | 13. Benzo(a)pyrene |
| 6. Anthracene | 14. Dibenzo(a,h)anthracene |
| 7. Fluoranthene | 15. Benzo(g,h,i)perylene |
| 8. Pyrene | 16. Indeno(1,2,3-c,d)pyrene |

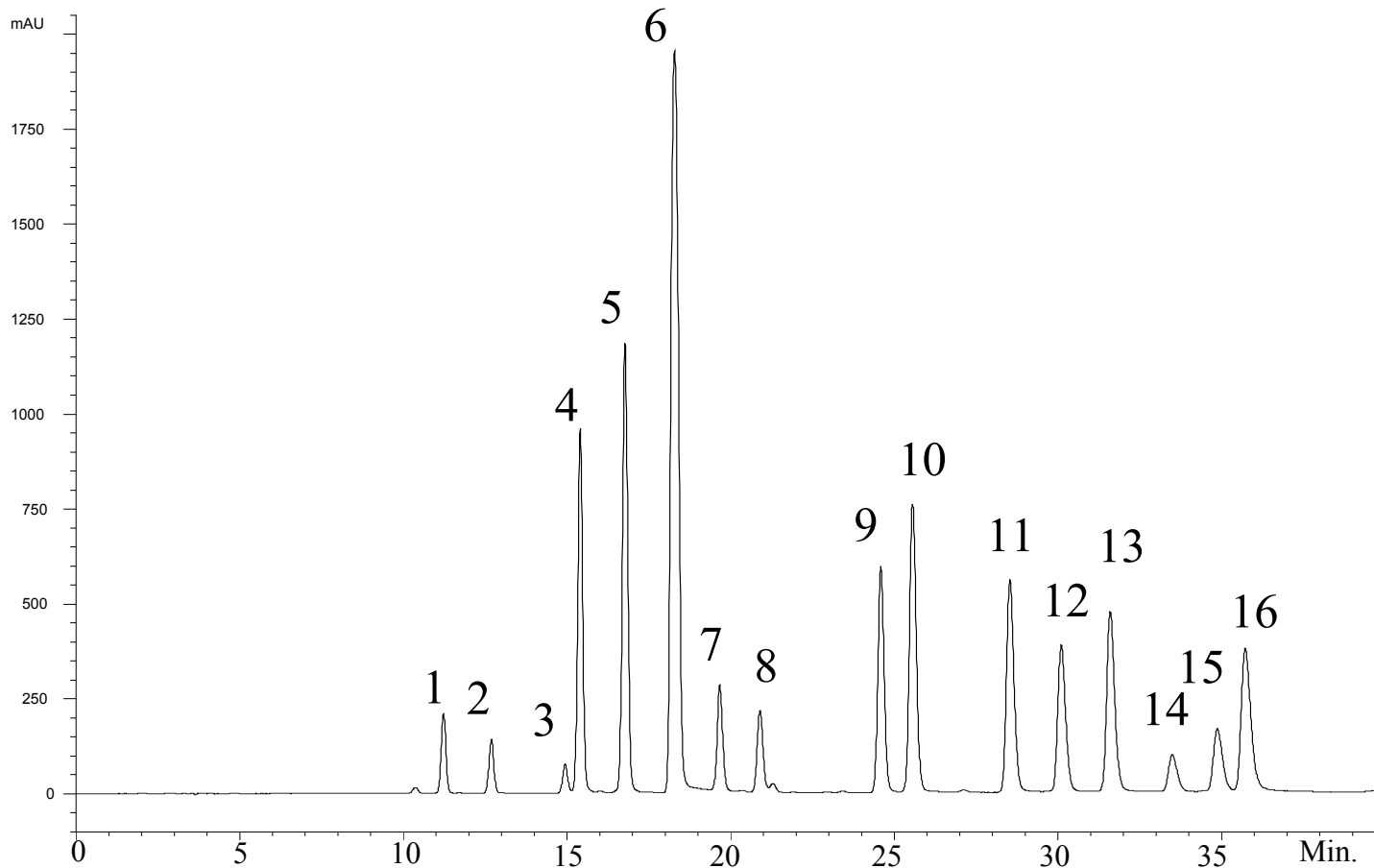
Exp. PAH #1 - One co-elution



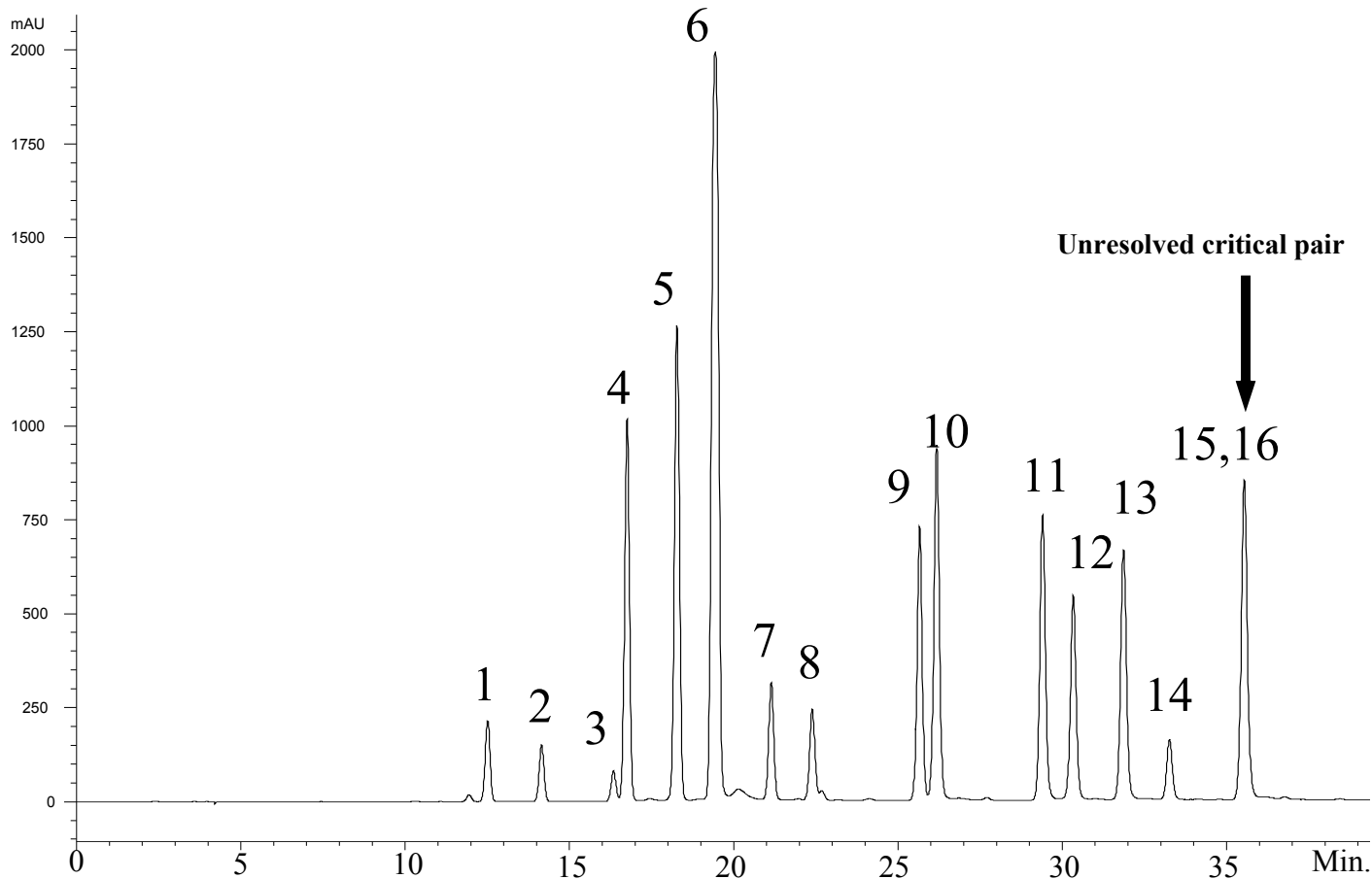
Exp. PAH#2 - All 16 PAH's resolved



Exp. PAH#3 - Best Separation - All 16 PAH's baseline resolved



Exp. PAH#4 - One Co-elution



Conclusions

- Using alternative stationary phases can give complimentary selectivity to confirm analytes unresolved by standard alkyl phases.
- Phases with high and complimentary selectivity can be used within a guard cartridge to enhance critical pair separations.
- Taking advantage of mixed mode interactions can substantially decrease analysis time.
- Controlling polymeric character of an alkyl stationary phase is critical to achieving shape selectivity of PAH analytes.