Analysis of Positional Isomers with Agilent Poroshell 120 PFP Columns

Application Note

Environmental

Authors

William Long and Jonathan Horton

Agilent Technologies, Inc.

Introduction

The Agilent Poroshell 120 PFP (pentafluorophenyl) stationary phase can give extra retention and selectivity for positional isomers of halogenated compounds. PFP columns can also be used for selective analysis of non-halogenated compounds, such as polar compounds containing hydroxyl, carboxyl, nitro, or other polar groups. This selectivity is enhanced when the functional groups are located on an aromatic or other rigid ring system [1].

In this application note, the separation of three positional isomers of trimethoxybenzene and dinitrobenzene is accomplished on an Agilent Poroshell 120 PFP column. In addition, a separation of the 14 explosive compounds listed in EPA 8330 is shown [2]. This mixture contains several pairs of positional isomers. In many cases, the full separation of these positional isomers occurs only on the Poroshell 120 PFP column. In other cases, the full separation can be achieved on some other columns when the organic content is reduced.

Materials and Methods

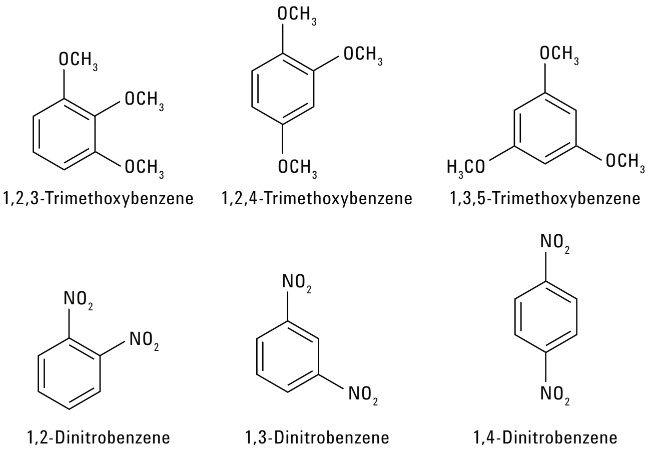
An Agilent 1260 Infinity LC was used, consisting of:

- Agilent Binary Pump SL, capable of delivering up to 600 bar (G1312B)
- Agilent Thermostatted Column Compartment (G1316C)
- Agilent High Performance Autosampler SL Plus (G1376D)
- Agilent Diode Array Detector (G4212A) equipped with a G4212-60008 10-mm path length, 1-µL flow cell

The columns used were:

- Agilent Poroshell 120 PFP, 4.6 × 50 mm, 2.7 µm (p/n 699975-408)
- Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)
- Agilent Poroshell 120 Bonus-RP, 4.6 × 50 mm, 2.7 µm (p/n 699968-901)
- Agilent Poroshell 120 Phenyl-Hexyl, 4.6 × 50 mm, 2.7 µm (p/n 699975-912)

Agilent OpenLAB version C.1.05. was used to control the instrument and process the data. Compounds used in this study include 1,2,3-trimethoxybenzene, 1,2,4-trimethoxybenzene, 1,2,5-trimethoxybenzene, 1,2-dinitrobenzene, 1,3-dinitrobenzene, and 1,4-dinitrobenzene, purchased from Sigma-Aldrich Corp. These compounds are shown in Figure 1. In addition, a 14-explosives sample from Cerilliant Corp. (ERE-021) was used, containing 200 µg/mL of each of the 1,3,5-trinitrobenzene, 1,3-dinitrobenzene, 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene, 2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene, 2-nitrotoluene, 3-nitrotoluene, 4-amino-2,6-dinitrotoluene, 4-nitrotoluene, HMX, nitrobenzene, RDX, and tetryl (2,4,6-trinitrophenyl-methylnitramine). These compounds are shown in Figure 4. Methanol was purchased from Honeywell (Burdick and Jackson) and water was 0.2-µm filtered 18 MΩ from a Milli Q system (Millipore).



1,2,3-Trimethoxybenzene 1,2,4-Trimethoxybenzene 1,3,5-Trimethoxybenzene

1,2-Dinitrobenzene 1,3-Dinitrobenzene 1,4-Dinitrobenzene

Figure 1. Structures of trimethoxybenzene and dinitrobenzene positional isomers.

Results and Discussion

Selectivity is the most powerful tool to optimize separations in HPLC. This parameter is changed by using different bonded phases, including C18, polar embedded, phenyl bonded, and perfluorophenyl, or by changing the mobile phase. In this application note, Poroshell 120, 4.6 × 50 mm columns were used to quickly evaluate method development choices. The short column length and high efficiency provided short analysis times and rapid equilibration, leading to fast investigation of selectivity.

The ring system of the pentafluorophenyl group is electron deficient, making it a Lewis acid. This Lewis acidity allows for electronic interactions with electron-donating Lewis bases. The electron donor/acceptor relationship can increase the interaction of the PFP phase with electron-rich compounds. Methoxy- and nitro-substituted benzenes are two examples of Lewis bases that allow for electronic interactions with the PFP phase. Furthermore, the aromatic nature of the methoxy- and nitro-substituted benzenes enhances interactions with the stationary phase through π-π stacking, taking full advantage of the molecular architecture of the PFP phase. PFP columns are electron acceptors, and compounds that are Lewis bases interact strongly with this phase. A Lewis base is any substance, such as the OH^- ion, that can donate a pair of nonbonding electrons. A Lewis base is, therefore, an electron-pair donor. These bases are sometimes referred to as nucleophiles, or seekers of a positive nucleus. Lewis bases include compounds such as methoxybenzenes and nitrobenzenes [3].

As can be seen in Figure 2, only Poroshell 120 PFP separates all three trimethoxybenzene isomers (shown in Figure 1). The C18, Phenyl-Hexyl, and Bonus-RP phases only separate the 123/124 and 125 compounds. Poroshell 120 Bonus-RP and Phenyl-Hexyl columns retain the trimethoxybenzenes nearly identically under these conditions. In all cases, the 1,25-trimethoxybenzene is eluted last.

In Figure 3, dinitrobenzene compounds are separated on the four columns, and, as with the example in Figure 1, Poroshell 120 PFP has the best separation. In this case, Poroshell 120 Bonus-RP and Poroshell 120 PFP elute the three compounds in the same order of 1,4 < 1,3 < 1,2, although the PFP column retains the three compounds more as the strength of the interaction is much greater. The C18 column elutes the material in the order 1,2 < 1,4 < 1,3, and the Phenyl-Hexyl column in the order 1,4 < 1,2 < 1,3.

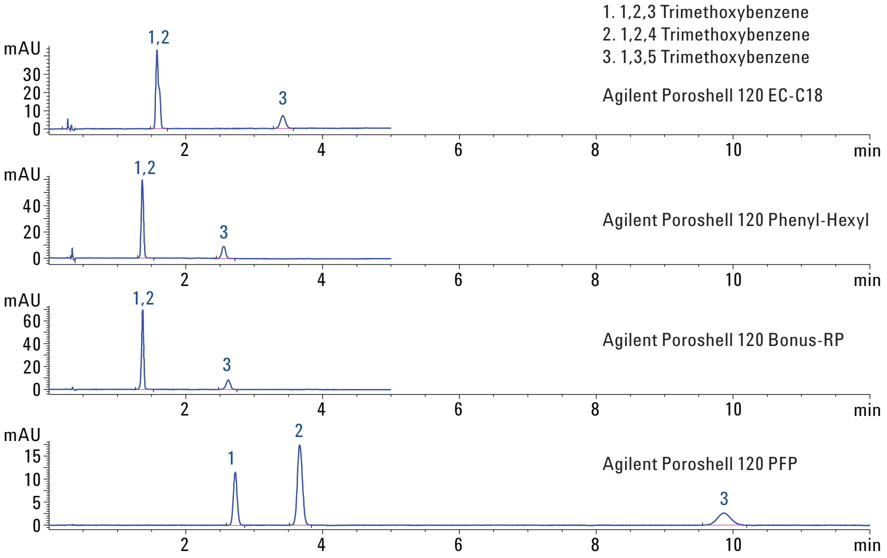


Figure 2. Separation of positional isomers of trimethoxybenzene on different Agilent Poroshell 120 phases (trimethoxybenzene mix on Agilent Poroshell 120, 4.6 × 50 mm columns, 70:30 water:methanol, 1.5 mL/min, 40 °C, 254 nm).

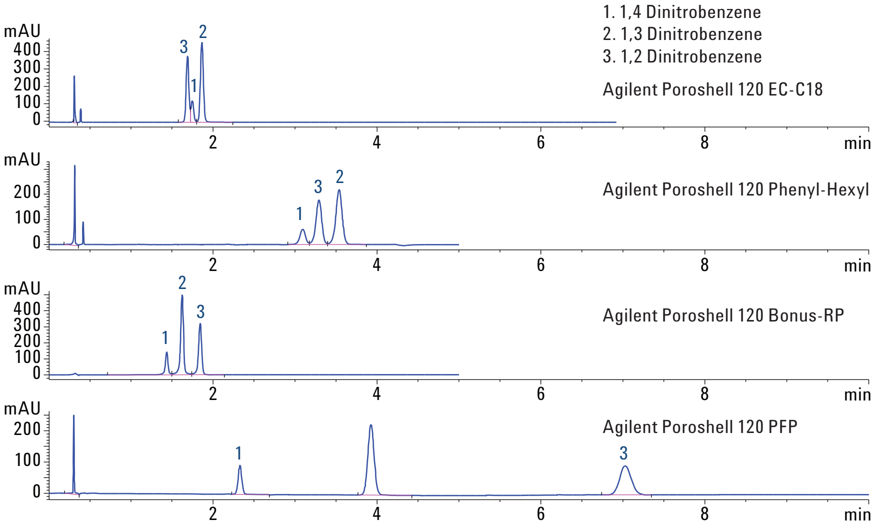
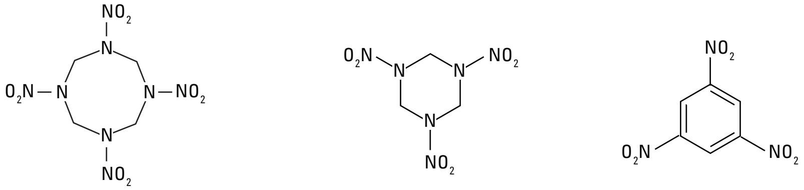


Figure 3. Separation of positional isomers of dinitrobenzene on different Agilent Poroshell 120 phases (dinitrobenzene mix on Agilent Poroshell 120, 4.6 × 50 mm columns, 35:65 methanol:water, 1.5 mL/min, 40 °C, 215 nm).



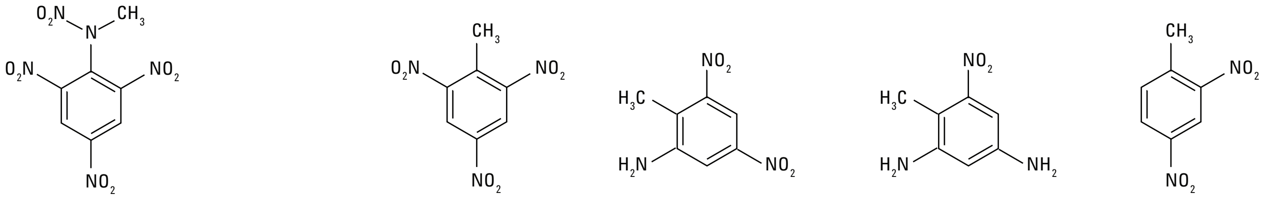
1. HMX
1,3,5,7-Tetranitro-1,3,5,7-tetrazocane

2. RDX
1,3,5-Trinitro-1,3,5-triazinane

3. 1,3,5-TNB
1,3,5-Trinitrobenzene

4. 1,3-DNB
1,3-Dinitrobenzene

5. NB
Nitrobenzene



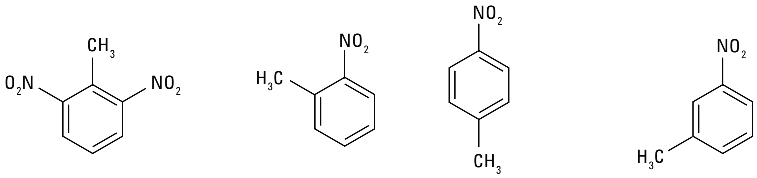
6. Tetryl
N-methyl-N-(2,4,6-trinitrophenyl) nitramide

7. TNT
2,4,6-Trinitrotoluene

8. 2A-2,6-DNT
2-Amino-4,6-dinitrotoluene

9. 4A-2,6-DNT
4-Amino-2,6-dinitrotoluene

10. 2,4-DNT
2,4-Dinitrotoluene



11. 2,6-DNT
2,6-Dinitrotoluene

12. 2-NT
2-Nitrotoluene

13. 4-NT
4-Nitrotoluene

14. 3-NT
3-Nitrotoluene

Figure 4. Structures of compounds in an explosive mixture (containing several positional isomer pairs).

In Figure 5, the separation of the 14 compounds listed in EPA 8330A is run on all four columns. The Poroshell 120 PFP column and the Phenyl-Hexyl column separate the mixtures best. While this separation is possible on C18 and Phenyl-Hexyl columns, the solvent strength on the Poroshell 120 PFP column is greater.

The Poroshell 120 PFP possesses a novel selectivity – alternative to the predominantly hydrophobic mechanism of C18 as well as π-π hydrophobic mechanism of phenyl hexyl. The selectivity of PFP is based on π-π, charge transfer, dipole, hydrogen bonding, and electrostatic interactions.

The separations of these compounds have been accomplished using C18 and phenyl-hexyl phases, and could most likely be accomplished using a Bonus-RP Phase as well. However, it can be seen that the Poroshell 120 PFP column has greater retention than other columns using this mobile phase. In this case, since the organic content is stronger than the other possible choices (C18 and Phenyl Hexyl), this column is a clear choice for methods involving sample preparation such as Solid Phase Extraction. A larger sample could be injected, or the sample could be injected in a stronger mobile phase (more organic) than C18 or Phenyl Hexyl.

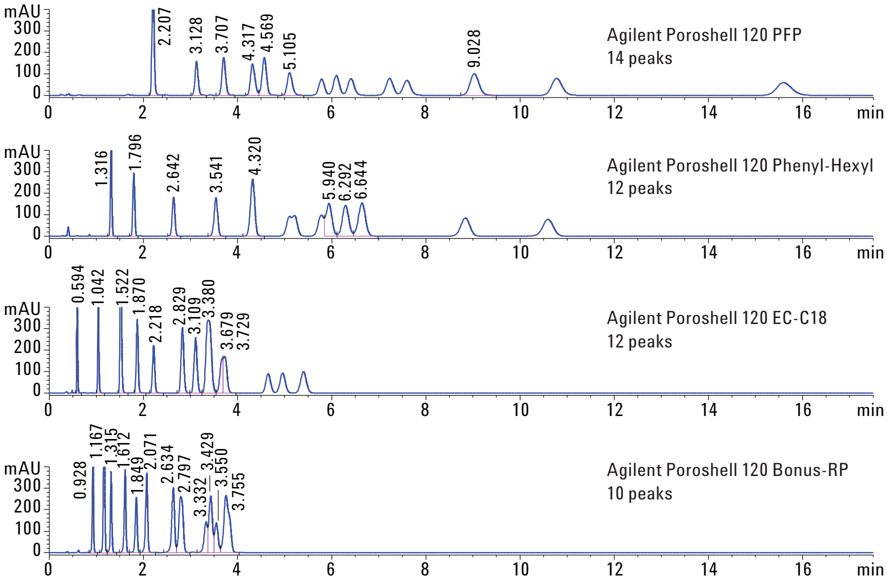


Figure 5. Separation of an explosive mixture on different Agilent Poroshell 120 phases (Agilent Poroshell 120, 4.6 × 50 mm columns, 35:65 methanol:water, 1.5 mL/min, 40 °C).

Conclusions

In this application note, several positional isomers groups are separated using a short Agilent Poroshell 120 PFP column and attempted on several other columns. For the separation of trimethoxybenzenes, only the Poroshell 120 PFP column successfully separates all three compounds. In the case of the dinitro positional isomers, all columns yield some separation, but the Poroshell 120 PFP column clearly yields the best separation. Finally, a complex separation of the EPA 8330A sample containing several pairs of positional isomers is separated on a Poroshell 120 PFP column. For samples containing positional isomers, Poroshell 120 PFP is a clear choice for method development.

References

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