

Fluophase® and Fluofix® Columns

Introduction

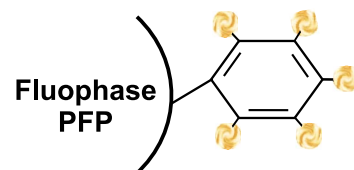
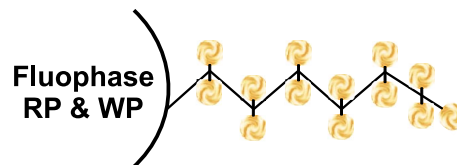
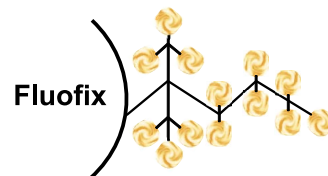
Fluorinated packings exhibit extra retention and selectivity for compounds that have fluorine and chlorine substituents. They also exhibit shape selectivity for isomers involving position of substituents on aromatic rings and other rigid systems. Fluophase packings are also useful for the separation of analytes possessing polar character and often show quite different retention characteristics when compared to alkyl chain bonded phases.

In this Technical Guide, we review the chromatographic behavior of the Fluophase packings developed at Thermo Hypersil-Keystone as well as Fluofix packings.

Fluophase RP and Fluophase WP are straight chain perfluorohexyl packings that are complementary to Fluofix, a branched perfluorohexyl packing. Fluophase PFP is a perfluorophenyl phase that often shows unique selectivity. In studying the chromatographic behavior of fluorinated phases, retention and selectivity are compared to the BetaBasic® 18 phase, a non-fluorinated highly base-deactivated C18 suitable for the analysis of neutral hydrophobic, polar basic, and polar acidic compounds.

Fluophase RP, WP and PFP phases show several useful chromatographic characteristics:

- Strong retention of polar analytes
- High selectivity toward closely related compounds
- Strong selectivity for halogenated compounds
- Excellent stability



Specifications:

Phase	Particle size	Chemistry	Pore Size	End-capping	Silica type
Fluophase RP	5µm	Perfluorohexyl straight chain	100Å	Yes	High purity, base deactivated
Fluophase WP	5µm	Perfluorohexyl straight chain	300Å	Yes	High purity, base deactivated
Fluophase PFP	5µm	Perfluorophenyl	100Å	Yes	High purity, base deactivated
Fluofix 120E	5µm	Perfluorohexyl branched chain	120Å	Yes	High purity, base deactivated
Fluofix 300E	5µm	Perfluorohexyl	300Å	Yes	High purity, base deactivated

Retention of Polar Compounds

The requirement to retain and analyze polar molecules by HPLC is one that has grown steadily over the last few years, and has been the driving force behind the generation of a range of new stationary phases dedicated to this purpose. The coupling of mass spectrometry to HPLC systems has become commonplace in many laboratories, increasing the demand for packings that can retain polar molecules. For example, electrospray ionization is more efficient when analytes are introduced in their ionic (and therefore most polar) form.

Chromatographic Characterization

Fluorinated packings offer additional modes of interaction compared to those of traditional alkyl bonded phases, and often provide different retention behavior and selectivity. We have compared the retention behavior of both Fluophase and Fluofix columns to the BetaBasic 18 phase, a highly base-deactivated and densely bonded C18 column appropriate for a range of analyte polarities.

Retention Based on Hydrophobic Interactions

Figure 1 demonstrates a comparison of retention using a homologous series of alkylbenzenes on fluorinated and C18 phases. The comparison indicates that where retention is based purely on hydrophobic interaction, it is considerably shorter on any of the fluorinated phases than on the C18 packing. The Fluofix® column shows the least retention in this comparison.

The results suggest that the mechanism of retention for nonpolar compounds such as alkyl benzenes is based on hydrophobic interactions that take place between the analyte and the stationary phase. Consequently, considerably shorter retention is observed for the three fluorinated packings when compared to the BetaBasic® 18 column. Carbon content for the fluorinated packings ranges from six to nine carbon units per ligand, and follows typical reversed phase behavior.

Retention of Polar Basic Compounds

In this study, we have chosen to use procainamides as test analytes. The chromatography of these basic compounds using the perfluorinated packings shows quite different retention behavior compared to the BetaBasic 18 column. Increased retention is observed on the Fluophase® PFP column, with very different selectivity compared to the BetaBasic 18 column.

The clear differences observed between the retention behavior of the procainamides on Fluophase PFP and Fluophase RP suggest a different mechanism of retention. There are several possibilities that may account for the difference in this behavior:

- Increased retention from silanol interactions on the Fluophase PFP phase
- Increased retention associated with the highly polarized electronic properties of the perfluorinated benzene ring on the Fluophase PFP packing
- Contributions from both of the above

This example illustrates the usefulness of the Fluophase PFP phase when different selectivity and retention are required for a separation.

Figure 1

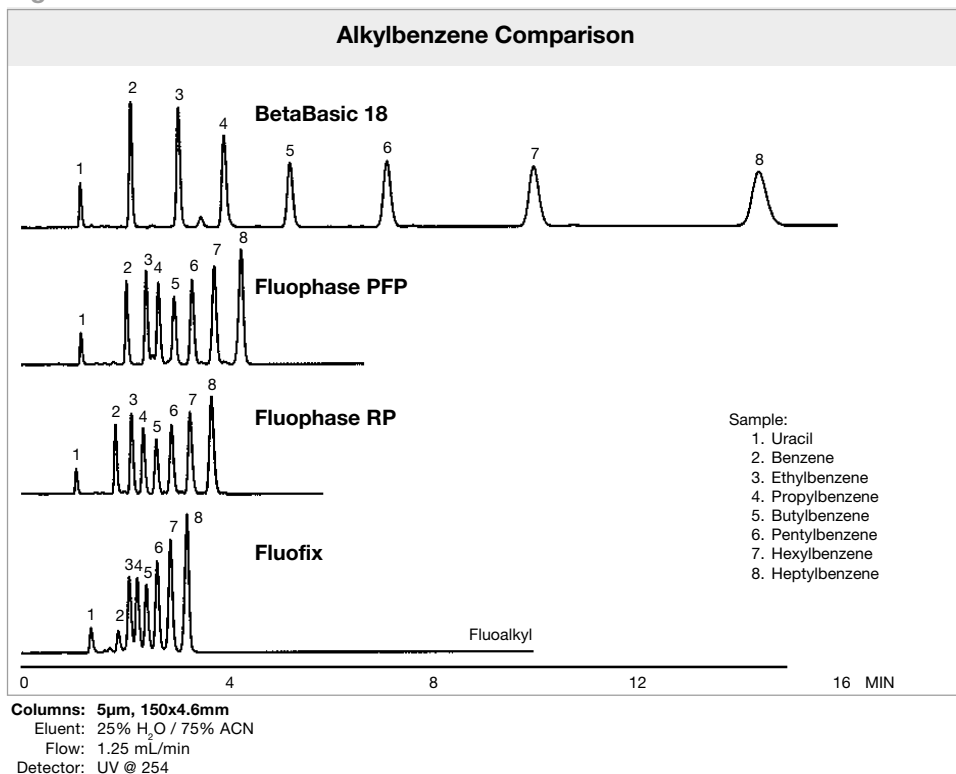
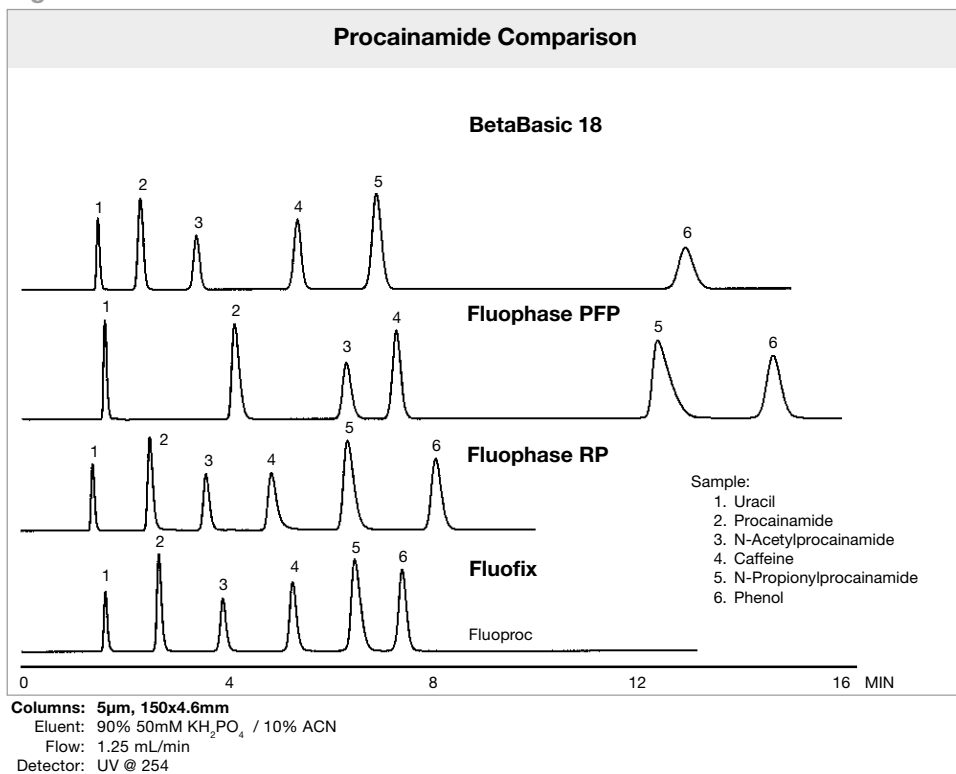


Figure 2



Retention of Polar Acidic Compounds

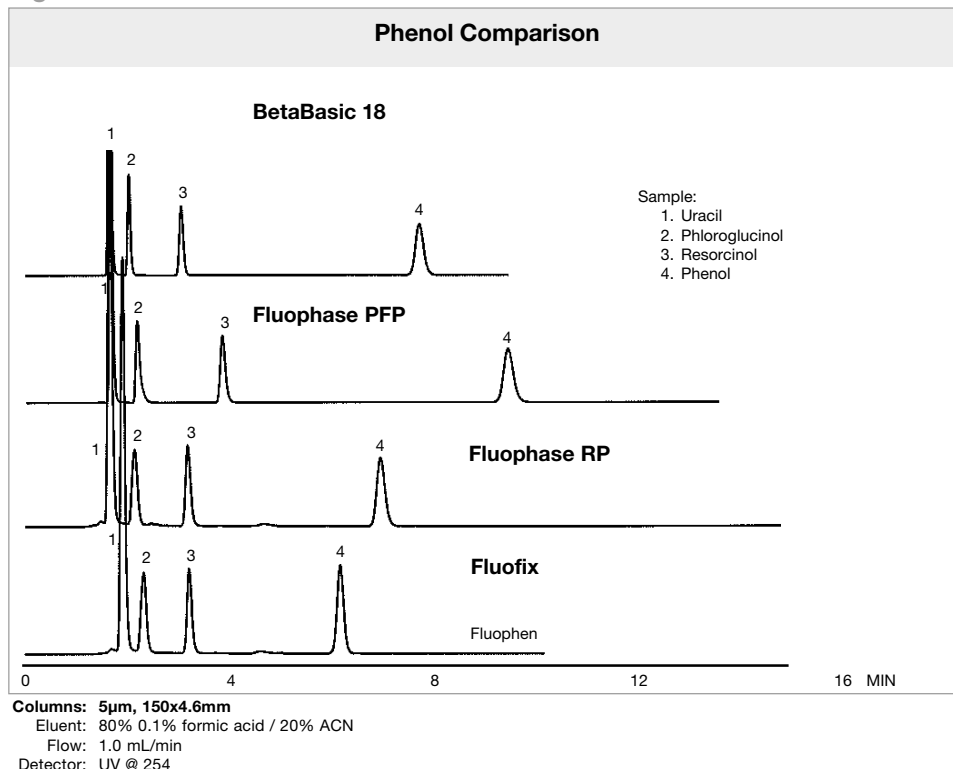
In order to study retention behavior of polar acidic compounds on fluorinated phases, a phenolic test mix was chosen to be representative of polar compounds with decreasing log P values:

- (1) Phenol log P 1.47
- (2) Resorcinol log P 0.81
- (3) Phloroglucinol log P 0.14

The log P value is the partition coefficient in octanol/water. The lower the log P value, the more polar the compound and less it will be retained on C18 or hydrophobic surfaces. The results from this study are shown in Figure 3.

The Fluophase® PFP column shows the strongest retention compared to the other columns used in the study, including the BetaBasic® 18 column. The Fluophase RP column shows similar retention character to the Fluofix® packing, but with slightly increased retention.

Figure 3

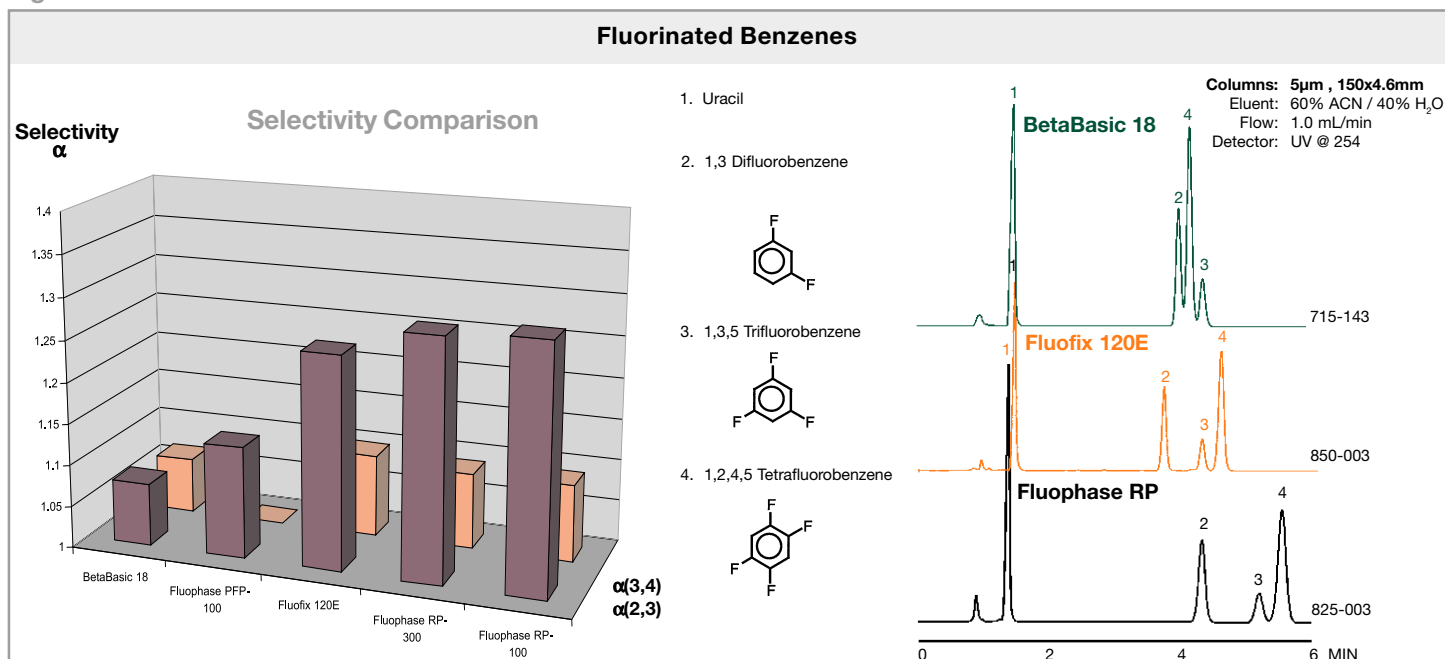


Special Selectivity toward Fluorinated Compounds

Fluophase packings show quite different selectivity and retention behavior for the analysis of halogenated, and in particular, fluorinated compounds by reversed phase HPLC. Fluorinated packings have been shown to offer increased retention and enhanced selectivity when compared to their non-fluorinated counterparts for halogenated analytes. The carbon-fluorine bond is more polar than the carbon-hydrogen bond, which may explain why extra selectivity is observed for compounds containing halogens and other polar groups.

Retention of fluorine-containing compounds increases with increasing number of fluorine substituents. This increased retention can be attributed to specific fluorine-fluorine interactions. Figure 4 shows the analysis of polyfluoro-substituted benzenes on both the branched-chain Fluofix 120E phase and the straight-chain Fluophase RP packing. The retention is slightly longer on the Fluophase RP column, but selectivity is similar.

Figure 4



Application to Taxanes

Locke et al reported an HPLC separation of 15 taxanes, including Taxol®, using a perfluorinated phenyl (PFP) stationary phase¹. The remarkable selectivity of fluorinated phases for these natural products is confirmed in Figure 5. The Fluofix® and all three Fluophase columns were able to separate this complex mixture of approximately 15 naturally-occurring compounds in a short time on 150x4.6mm columns. Note that each fluorinated phase exhibits a slightly different elution order. The fact that Fluophase® RP and WP, which have the same phase on a different pore size silica, exhibit different elution order suggests that pore size is also an important variable for these rather large molecules.

1. Shao, L. K. and Locke, D.C. Anal. Chem. 1997, 69, 2008-2016.

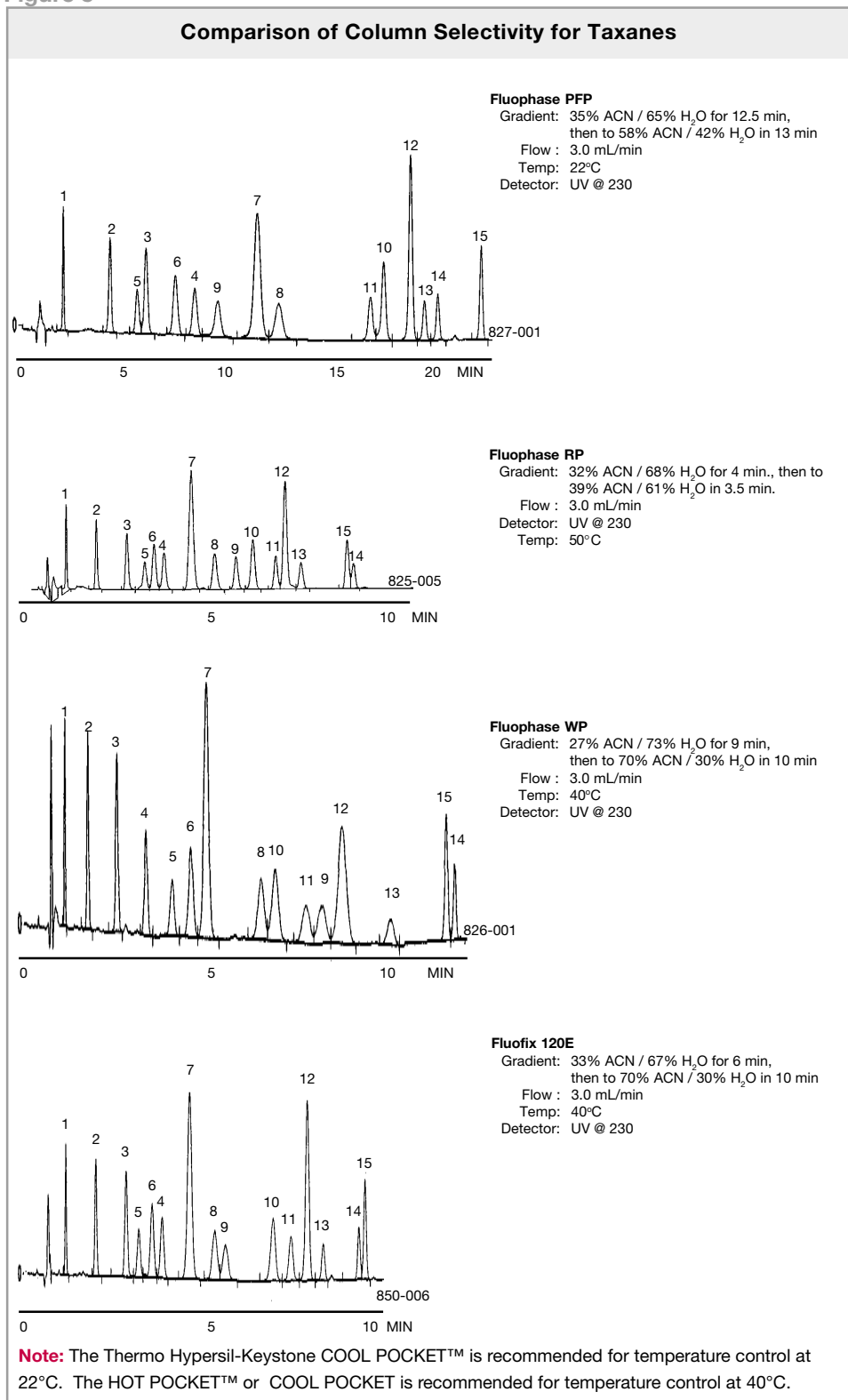
Sample: Taxane Compounds

1. 10-deacetyl baccatin III
2. baccatin III
3. 13-acetyl-9-dihydro baccatin III
4. taxinine M
5. 10-deacetyl-7-xylosyl taxol B
6. 10-deacetyl-7-xylosyl taxol
7. 10-deacetyl taxol
8. 7-xylosyl taxol
9. 10-deacetyl-7-xylosyl taxol C
10. 10-deacetyl-7-epitaxol
11. cephalomanine
12. paclitaxel
13. benzyl analog
14. taxol C
15. 7-epitaxol

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Fluofix is a registered Trademark of Neos Corp.
Taxol is a registered Trademark of Bristol-Myers Squibb.

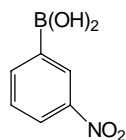
Figure 5



Pharmaceuticals

Sample:

1. 3-Nitrophenyl boric acid
2. 4-Nitrophenyl boric acid
3. Impurity



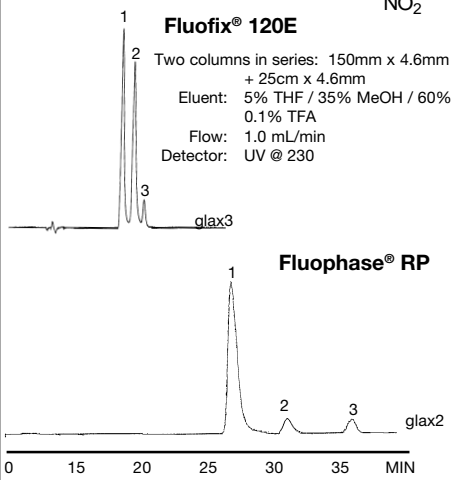
Fluofix® 120E

Two columns in series: 150mm x 4.6mm + 25cm x 4.6mm

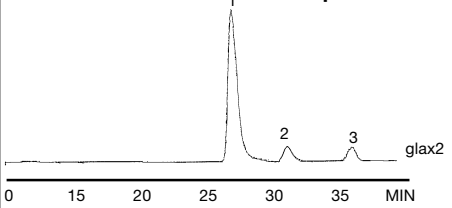
Eluent: 5% THF / 35% MeOH / 60% 0.1% TFA

Flow: 1.0 mL/min

Detector: UV @ 230



Fluophase® RP



Fluophase RP, 5µm, 250x4.6mm

Eluent: 20% THF / 80% 0.1% TFA

Flow: 1.0 mL/min

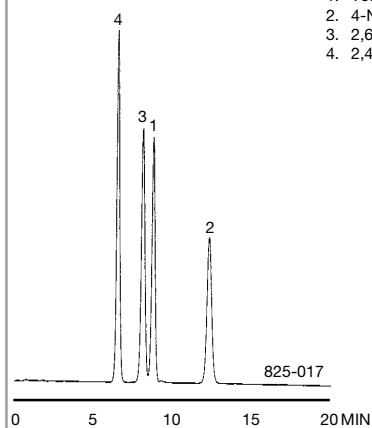
Detector: UV @ 230

Nitroaromatics

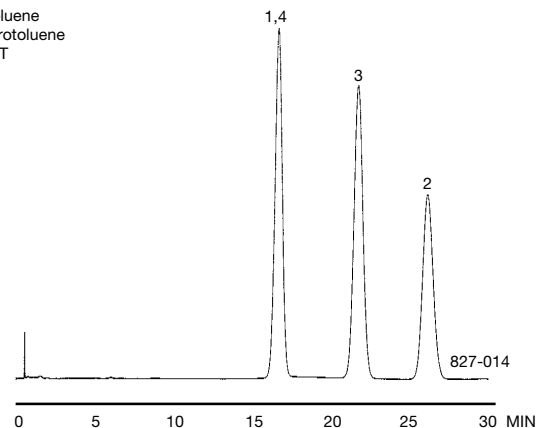
Fluophase RP

Sample:

1. Toluene
2. 4-Nitrotoluene
3. 2,6-Dinitrotoluene
4. 2,4,6-TNT



Fluophase PFP



Columns: 5µm, 150x4.6mm

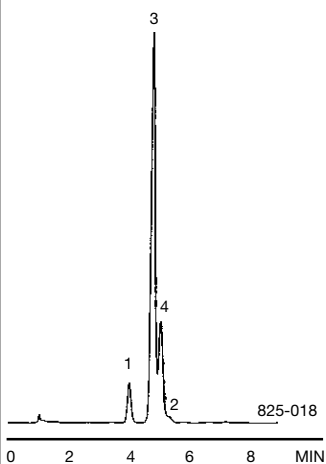
Eluent: 50% H₂O / 50% MeOH

Flow: 1.0 mL/min

Detector: UV @ 254

Explosives

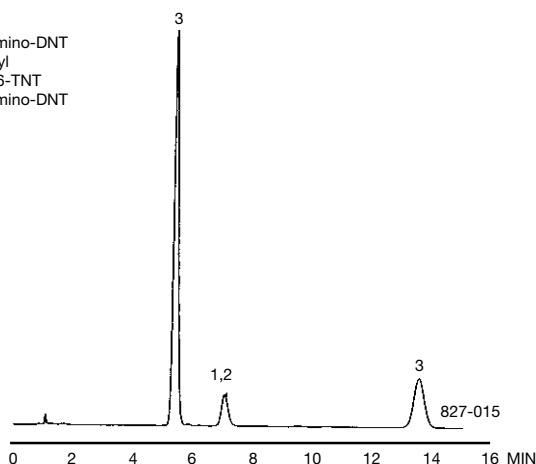
Fluophase RP 50% H₂O / 50% MeOH



Fluophase PFP 30% H₂O / 70% MeOH

Sample:

1. 4-Amino-DNT
2. Tetryl
3. 2,4,6-TNT
4. 2-Amino-DNT



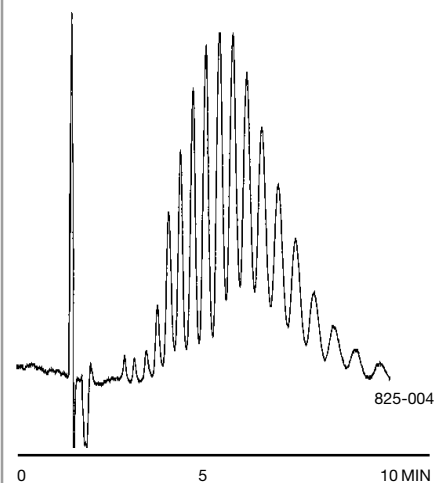
Columns: 5µm, 150x4.6mm

Flow: 1.0 mL/min

Detector: UV @ 254

Surfactant

Sample: Triton X-100 (nonionic surfactant)
Isocratic assay



Fluophase RP, 5µm, 150x4.6mm

Eluent: 45% MeOH / 55% H₂O

Flow: 1.0 mL/min

Detector: UV @ 280