Polar Bonded Phases

Alternatives to Silica for Normal Phase Separations

Normal phase separations were the first HPLC separations. Silica gel was the stationary phase and nonpolar solvents such as hexane and methylene chloride were common. Discrimination between isomers and related molecules was excellent.

Reversed phase media were later developed to overcome certain limitations of normal phase silica columns: reproducibility was a problem and the silica was frequently deactivated with polar solvents and trace water. By overcoming these limitations, and by providing the ability to separate molecules dissolved in polar solvents, reversed phase media came to dominate HPLC.

Despite the advantages of reversed phase media, chromatographers still sought "the best of both worlds," namely, a stationary phase that combines the water compatibility and superior reproducibility of reversed phase media with the ability of normal phase media to discriminate among positional isomers and to retain polar compounds in polar solvents. YMC offers its Polar Bonded Phase family to meet that need.

The YMC Polar Bonded Phases provide a wide variety of selectivity options for normal phase applications. This Technical Note illustrates the selectivity differences of nitroaromatics, phenols, steroids and tocopherols on the various stationary phases. For comparison, chromatography on bare silica is also shown.

You can expect better reproducibility and improved peak shape compared with bare silica. These phases also equilibrate much faster and provide more uniform surface activity than silica. Mobile phases containing water will not alter surface activity, so these packings clean easily and can be used over and over without fear of contamination or carryover. For preparative applications, these phases often support higher production rates than on the large irregular silica that is typically used.

The YMC Polar Bonded Phases include:

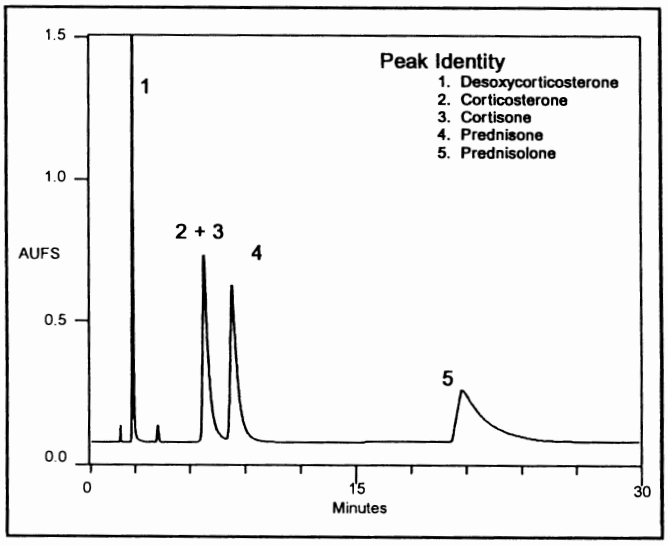
- YMC-Pack PVA-Sil™
- YMC-Pack Diol
- YMC-Pack Cyano
- YMC-Pack Amino.

These stationary phases are described in detail in the YMC catalog.

YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 (0)2064 427-0 · Fax +49 (0) 2064 427-222 · Email: info@ymc.de · www.ymc.de

Comparison of Normal Phase Media for Steroids

Silica

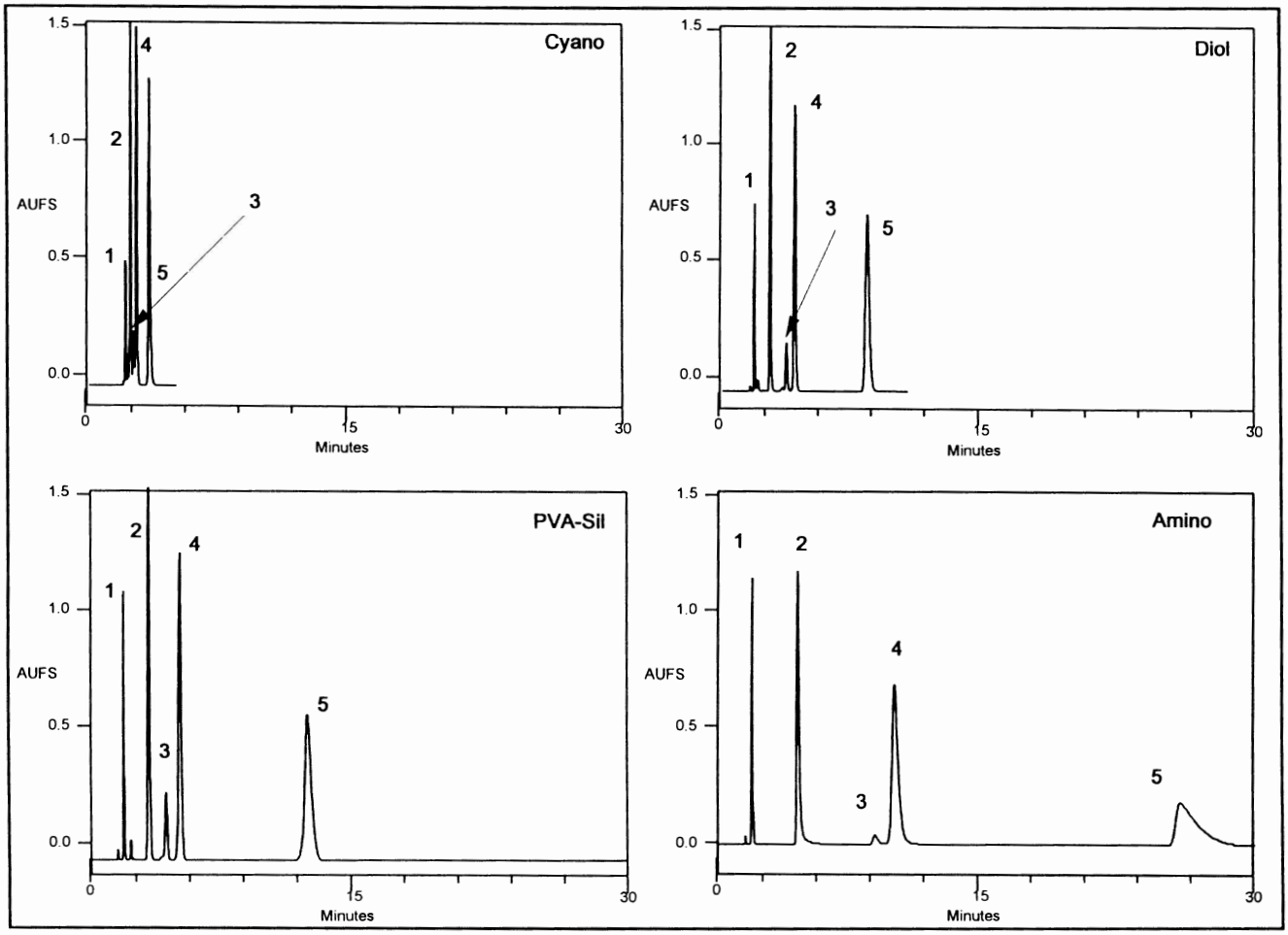


Observations:

- PVA-Sil and Diol provide the best resolution and peak shape
- Cyano requires a weaker (less polar) mobile phase for these compounds
- Amino phase shows strongest retention for steroids

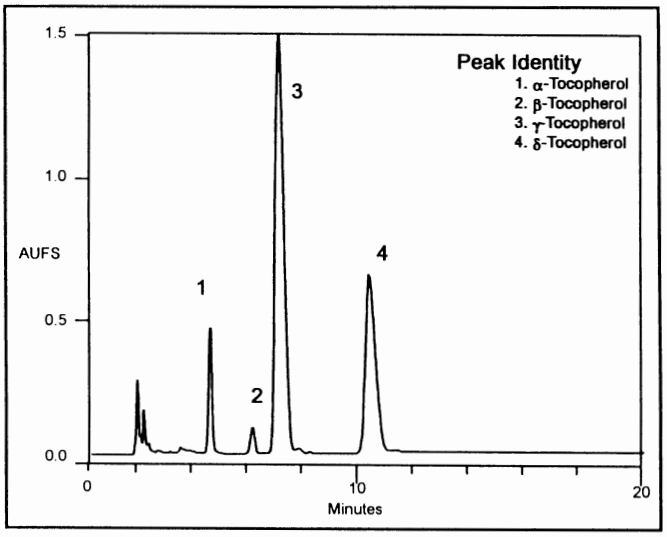
Polar Bonded Phases

HPLC Conditions: 4.6x250mm, 2mL/min, ambient temp., UV@254nm
Mobile Phase: 25% Isooctane, 70% 1,2-Dichloroethane, 5% isopropanol



Comparison of Normal Phase Media for Tocopherols

Silica

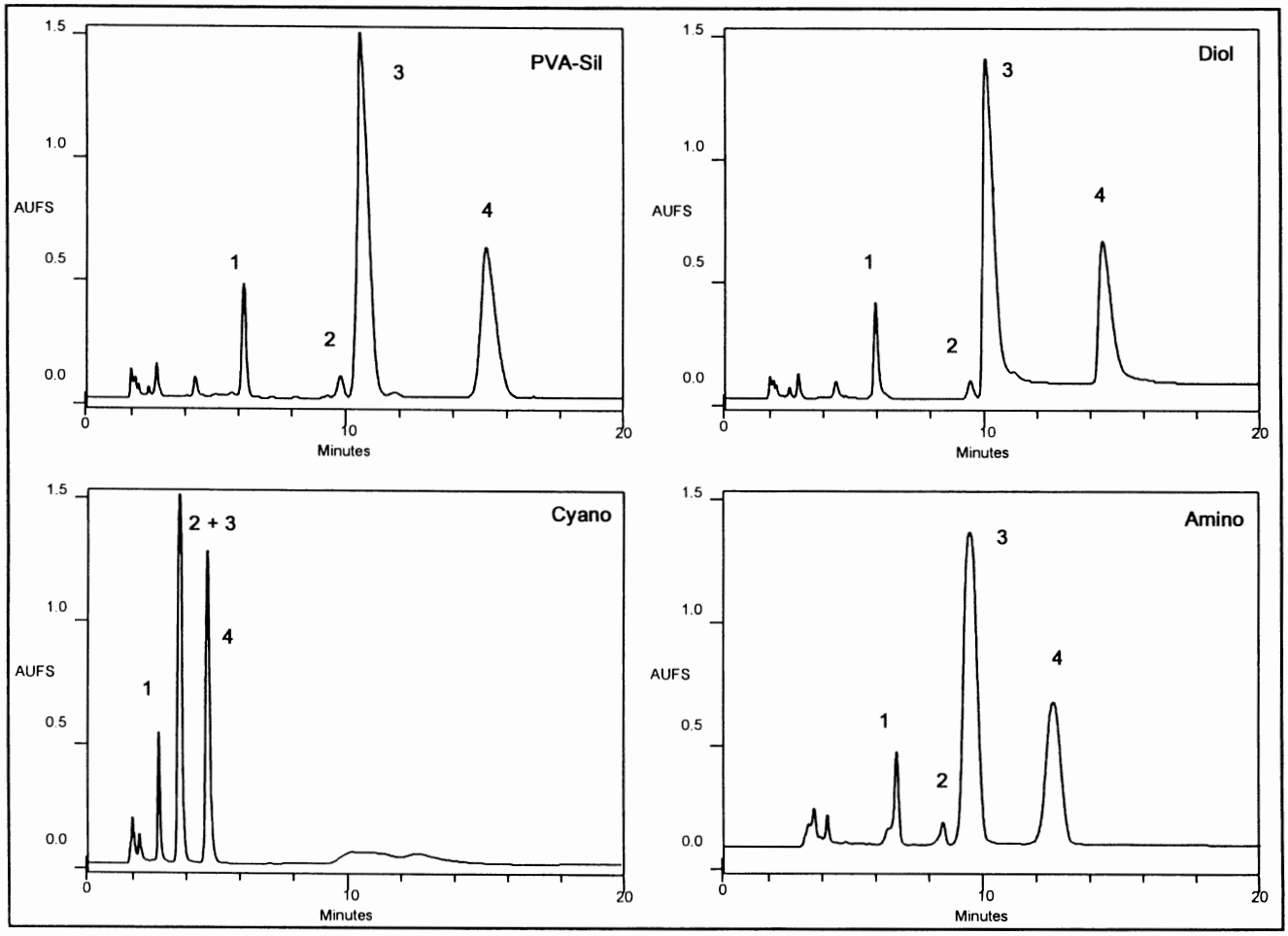


Observations:

- PVA-Sil offers good selectivity and resolves the late impurity
- Silica provides good peak shape, fast analysis time and the best separation between beta- and gamma-Tocopherol
- Amino phase requires a more polar solvent system than the other phases
- Diol provides good selectivity and retention for Tocopherols, but resolution of a late impurity is lost

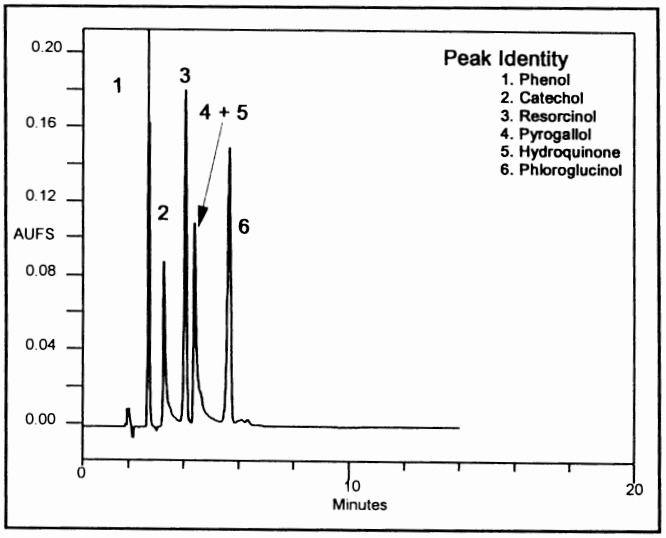
HPLC Conditions: 4.6x250mm, 2mL/min, ambient temp., UV@295nm
Mobile Phase: (All except Amino) 97% Isooctane, 3% Tetrahydrofuran
On Amino: 50% Isooctane, 45% Methyl t-butyl ether, 5% Ethyl acetate

Polar Bonded Phases



Comparison of Normal Phase Media for Phenols

Silica

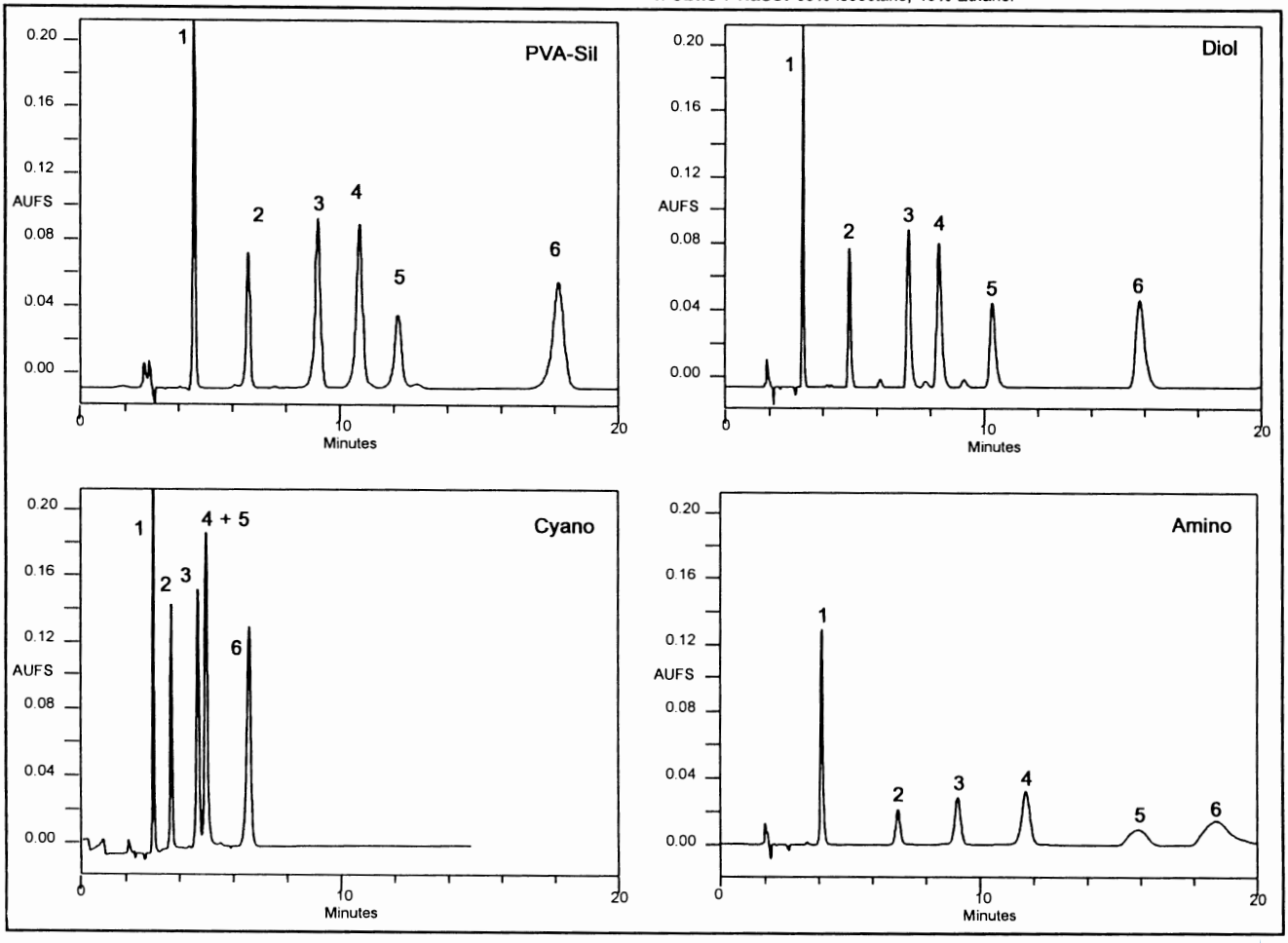


Observations:

- Diol offers highest efficiency, highest resolution, symmetrical peaks and the best selectivity between the di- (peaks 2, 3, 4) and tri-phenols (peaks 5, 6)
- Cyano phase provides good resolution between the mono- and di-phenols, but doesn't differentiate di- and tri-phenols
- PVA-Sil offers better resolution of all isomers compared with silica or cyano
- Silica demonstrates low resolution and tailing peaks

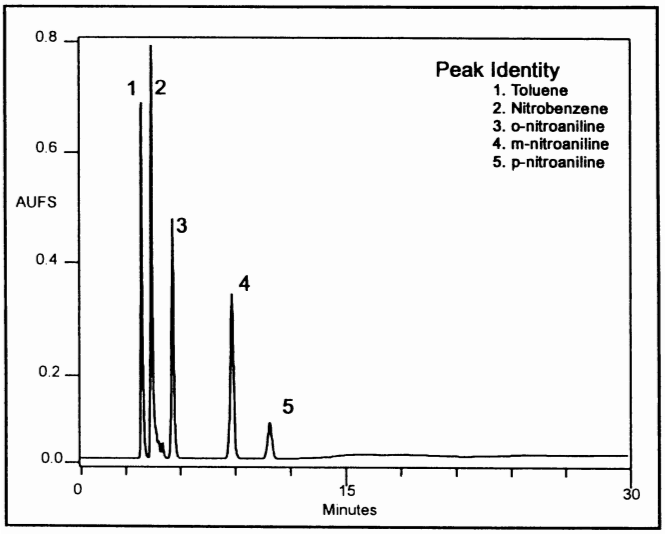
Polar Bonded Phases

HPLC Conditions: 4.6x250mm, 2mL/min, ambient temp., UV@254nm
Mobile Phase: 85% Isooctane, 15% Ethanol



Comparison of Normal Phase Media for Nitroaromatics

Silica

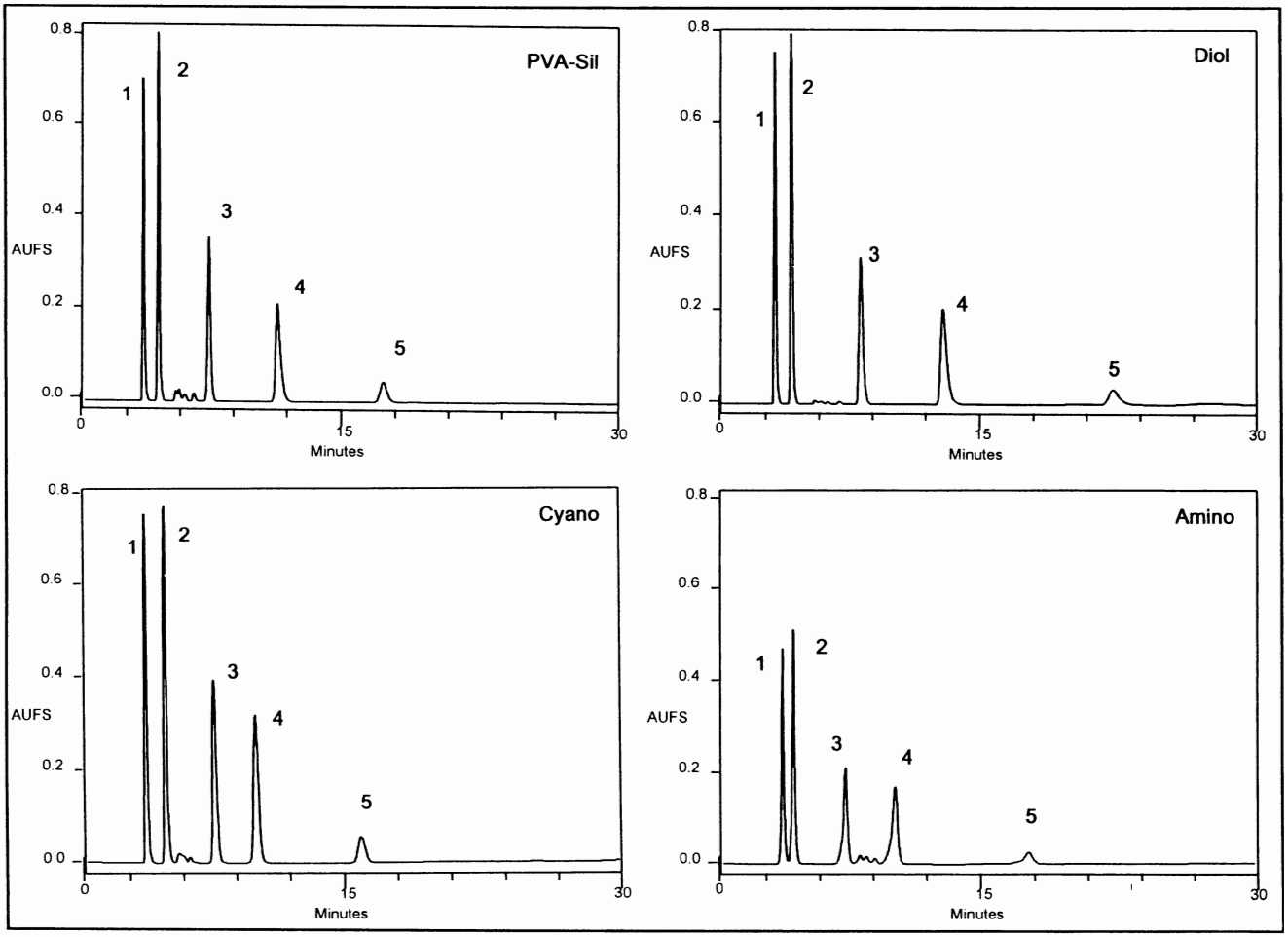


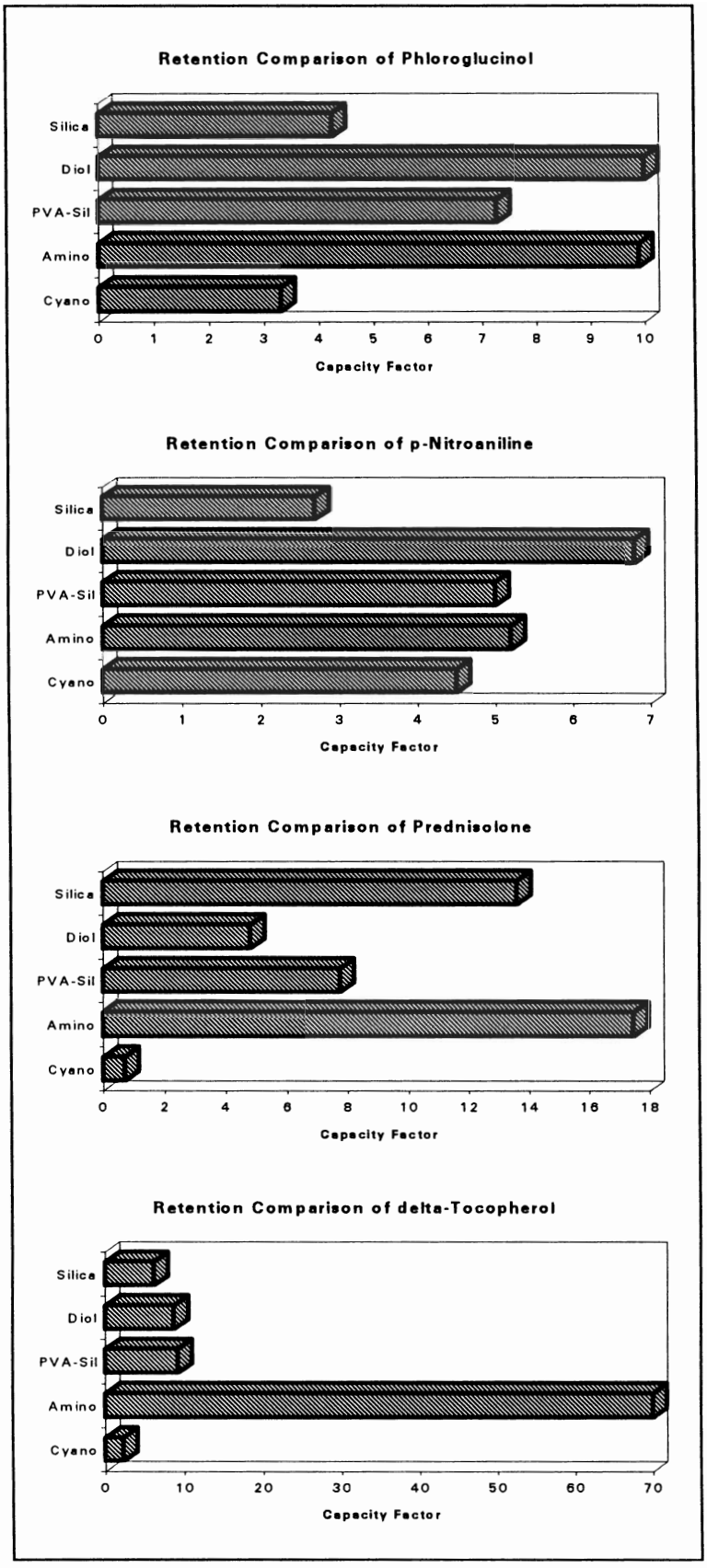
Observations:

- PVA-Sil provides excellent selectivity and peak symmetry on nitroaniline isomers (peaks 3,4,5)
- All Polar Bonded Phases provide better resolution than silica of nitrobenzene and later impurities
- Selectivity for minor impurities differs greatly among phases
- Diol is most retentive for nitroaromatics

Polar Bonded Phases

HPLC Conditions: 4.6x250mm, 2mL/min, ambient temp., UV@254nm
Mobile Phase: 85% Isooctane, 15% Isopropanol





Relative Polarity Index for the Polar Bonded Phases

In reversed phase HPLC, the relative polarity index is related directly to the chain length. For example, for nonpolar compounds, the retention time on C4 is always less than on C18. For the Polar Bonded Phase family, the relative polarity index varies with the polarity of the bonded phase and the hydrophilic interactions (hydrogen bonding) of the solute and bonded phase.

The bar charts to the left illustrate graphically the differences in retention character among the Polar Bonded Phases with the different samples. Notice also the changes in scale among the bar charts.

The hydrophilic interaction for phenolic and nitroaromatic type molecules is greatest on the Diol phase. For tocopherol and steroid type molecules, the Amino phase exhibits the highest retention, and often requires a stronger solvent system than the other bonded phases. Cyano is typically the least retentive of the Polar Bonded Phases.

The difference in hydrophilic interaction among the members of the Polar Bonded Phase family offers the chromatographer another dimension of selectivity. Separations are enhanced by both the stationary phase and the solvent system. This enhanced selectivity is useful both for analytical and preparative separations.

YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 (0)2064 427-0 · Fax +49 (0) 2064 427-222 · Email: info@ymc.de · www.ymc.de

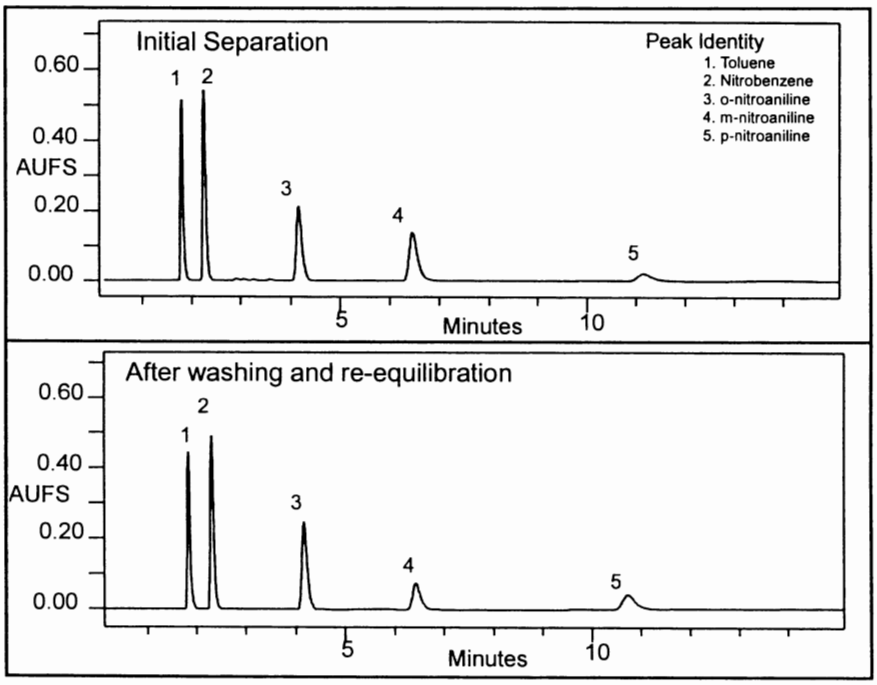
Effect of Polar Solvent Washes on Retention Times of Polar and Lipophilic Compounds

Polar Compounds

HPLC Conditions

Phase:	YMC-Pack Diol
Column size:	4.6 x 250mm
Eluent:	85% Isooctane 15% Isopropanol
Flow rate:	2 mL/min
Temperature:	Ambient
Detection:	UV @ 254 nm

Conditioning sequence:
- 30 mL Isopropanol
- 30 mL water
- 30 mL isopropanol
- 30 mL 85:15 isooctane:isopropanol

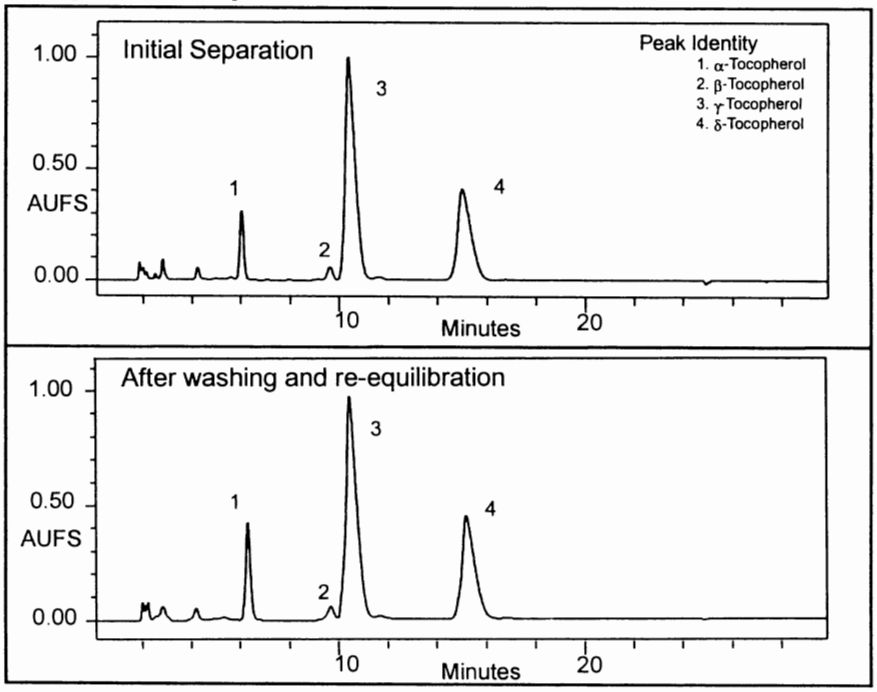


Lipophilic Compounds

HPLC Conditions

Phase:	YMC-Pack PVA-Sil
Column size:	4.6 x 250mm
Eluent:	97% Isooctane 3% THF
Flow rate:	2 mL/min
Temperature:	Ambient
Detection:	UV @ 295 nm

Conditioning sequence:
- 30 mL Isopropanol
- 30 mL water
- 30 mL isopropanol
- 30 mL 85:15 isooctane:isopropanol
- 30 mL 97:3 isooctane:THF



Retention times are identical before and after washing with polar, surface-deactivating solvents. This washing process is not possible with bare silica columns.

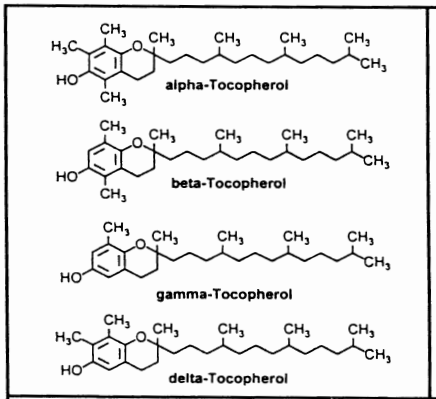
YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 (0)2064 427-0 · Fax +49 (0) 2064 427-222 · Email: info@ymc.de · www.ymc.de

Summary

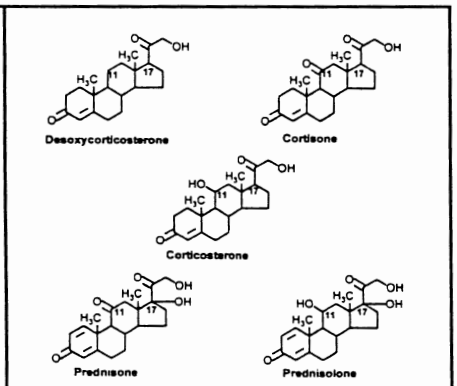
1. YMC Polar Bonded Phases offer the chemist an alternative to unbonded silica gel for normal hase separations with excellent discrimination of isomers and polar molecules.
2. The Polar Bonded Phases are easily cleaned with water and other polar solvents without deactivating the bonded phase surface.
3. The bonded phases equilibrate rapidly with new mobile phases.
4. The Relative Polarity Index for the Polar Bonded Phases changes based on a solute's functional groups, providing several selectivity options for difficult separations.
5. The Polar Bonded Phases provide unique selectivity opportunities compared with reversed phase columns, while offering the advantages of normal phase solvents for preparative separations.

Molecular Structures

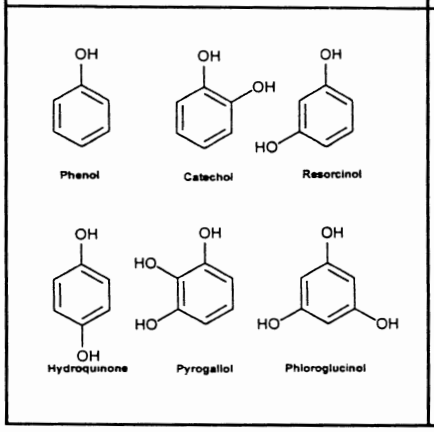
Tocopherols



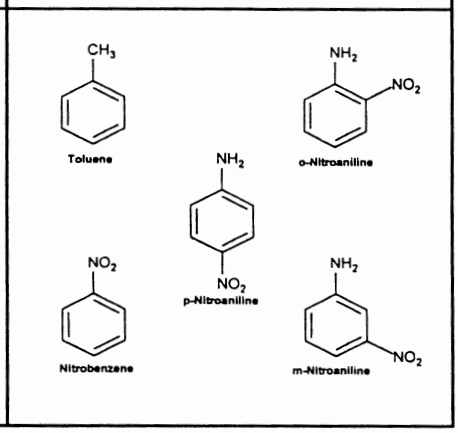
Steroids



Phenols



Nitroaromatics



Ordering information:

The Polar Bonded Phases and high purity YMC-Pack Silica are available in spherical particles ranging from 3 micron to 50 micron, in analytical and preparative columns. Bulk media for large scale separations is also available.