Alternatives to Normal Phase Silica Separations for Rugged HPLC Separations

YMC AMERICA, INC.

Abstract

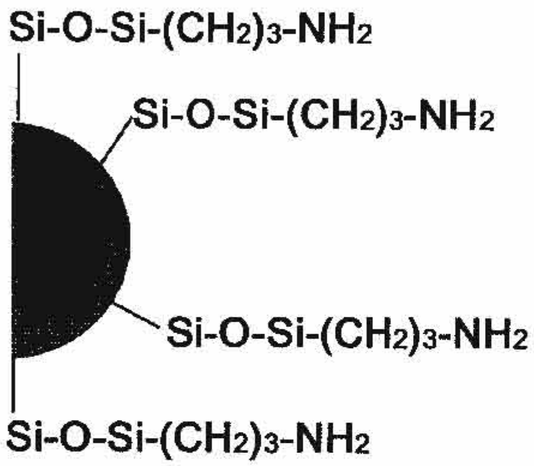
Initially, all HPLC separations were normal phase - silica gel was the stationary phase and non-polar solvents such as hexane and methylene chloride were common. Discrimination between isomers and related molecules was excellent. Due to reproducibility problems and deactivation of the stationary phase caused by polar solvents and trace water, reversed phase media were developed. The advantage of reversed phase is its ability to separate molecules dissolved in polar solvents or water. Its disadvantage is its inability to easily discriminate positional isomers and retain polar compounds. In addition, the selectivity differences between C4, C8 and C18 for many molecules is not significant.

To circumvent the historical problems of non-reproducible silica separations and poor discrimination of isomers with reversed phase, YMC has developed bonded polar phases. This family of media (BNP family) operates in the normal phase mode and provides reproducible separations. The bonded normal phase media easily discriminate between positional isomers and will separate polar molecules dissolved in polar solvents.

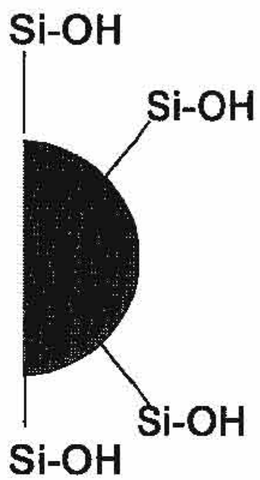
This work illustrates the separation capabilities of these bonded phase packings with a broad spectrum of samples including: steroids, phenols, nitroaromatics and tocopherols. The polarity and selectivity differences of these phases offer the chemist a new separation tool for the isolation, quantitation and preparative purification of isomers and polar samples.

In addition, these BNP's are unaffected by polar solvents, including water. The chemist is able to completely clean the column without losing selectivity or retention. The ability to clean the column greatly extends the column lifetime.

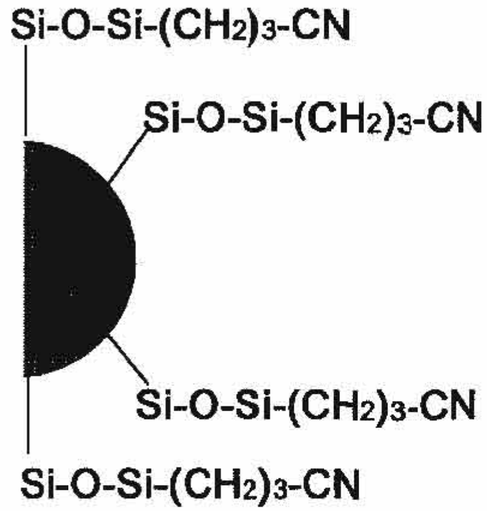
The Normal Phase Family Structures



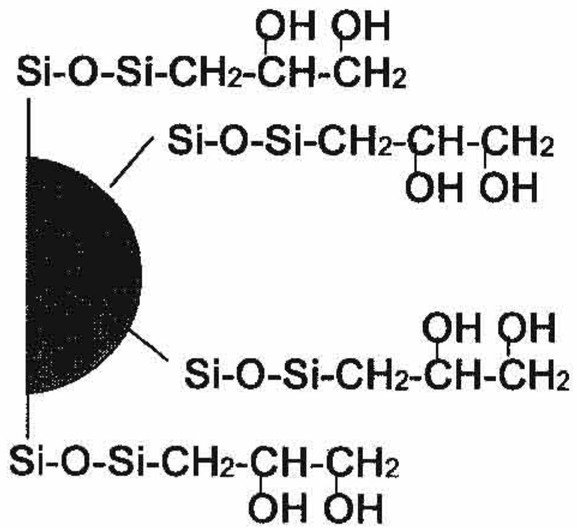
Amine



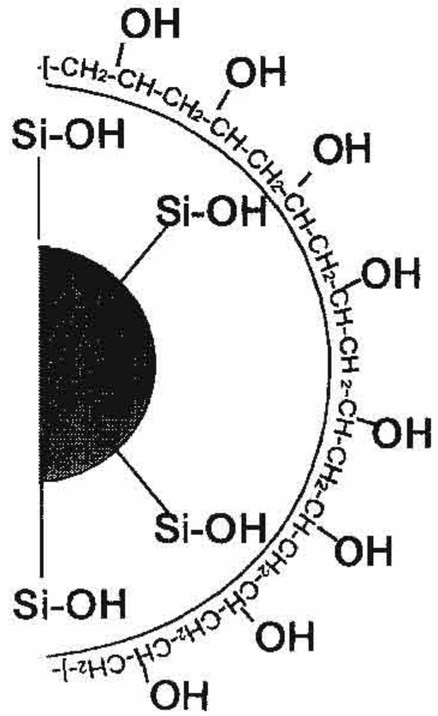
Silica



Cyano

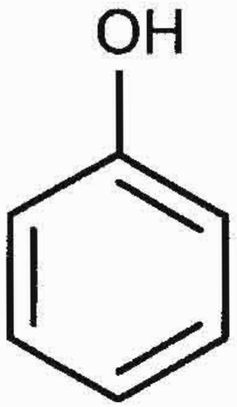


Diol

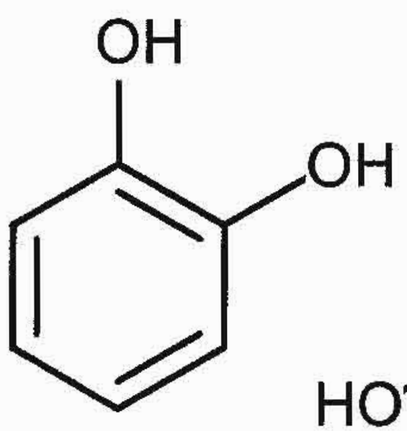


PVA-sil

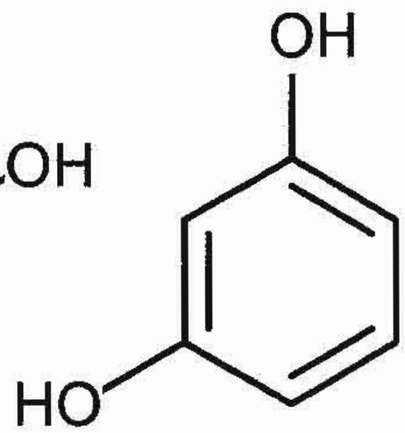
Phenolics



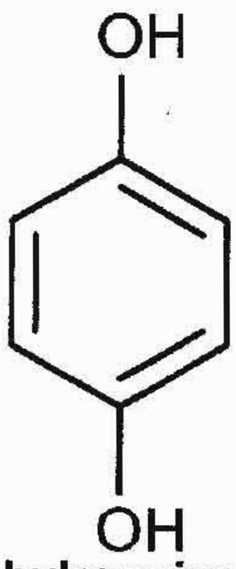
Phenol



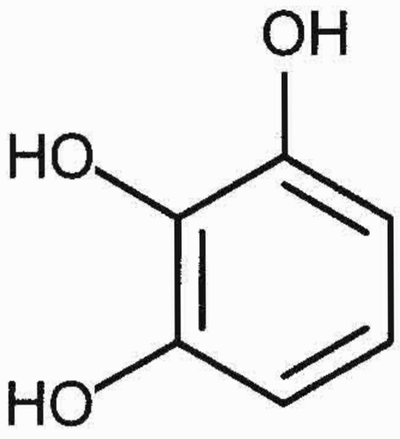
Catechol



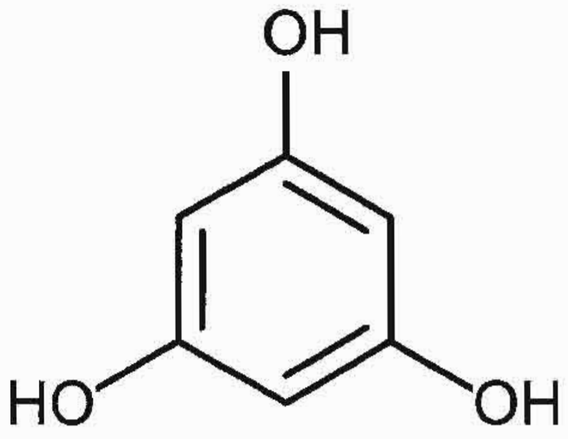
Resorcinol



Hydroquinone



Pyrogallol

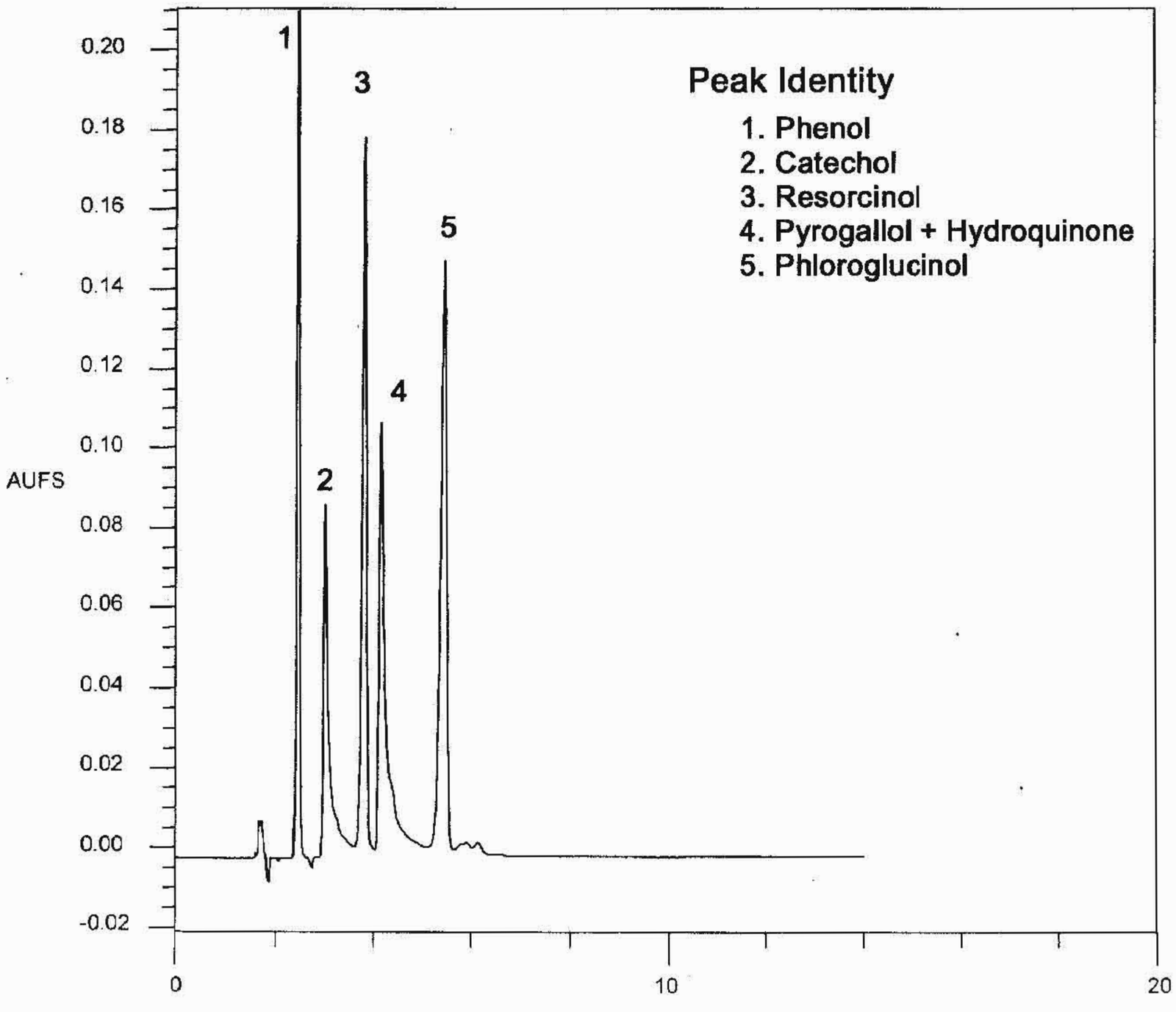


Phloroglucinol

HPLC Conditions

Column size: 4.6 x 250 mm
Mobile phase:
85 Isooctane
15 Ethanol
Flow rate: 2 mL/min
Temperature: Ambient
Detection: UV @ 254 nm

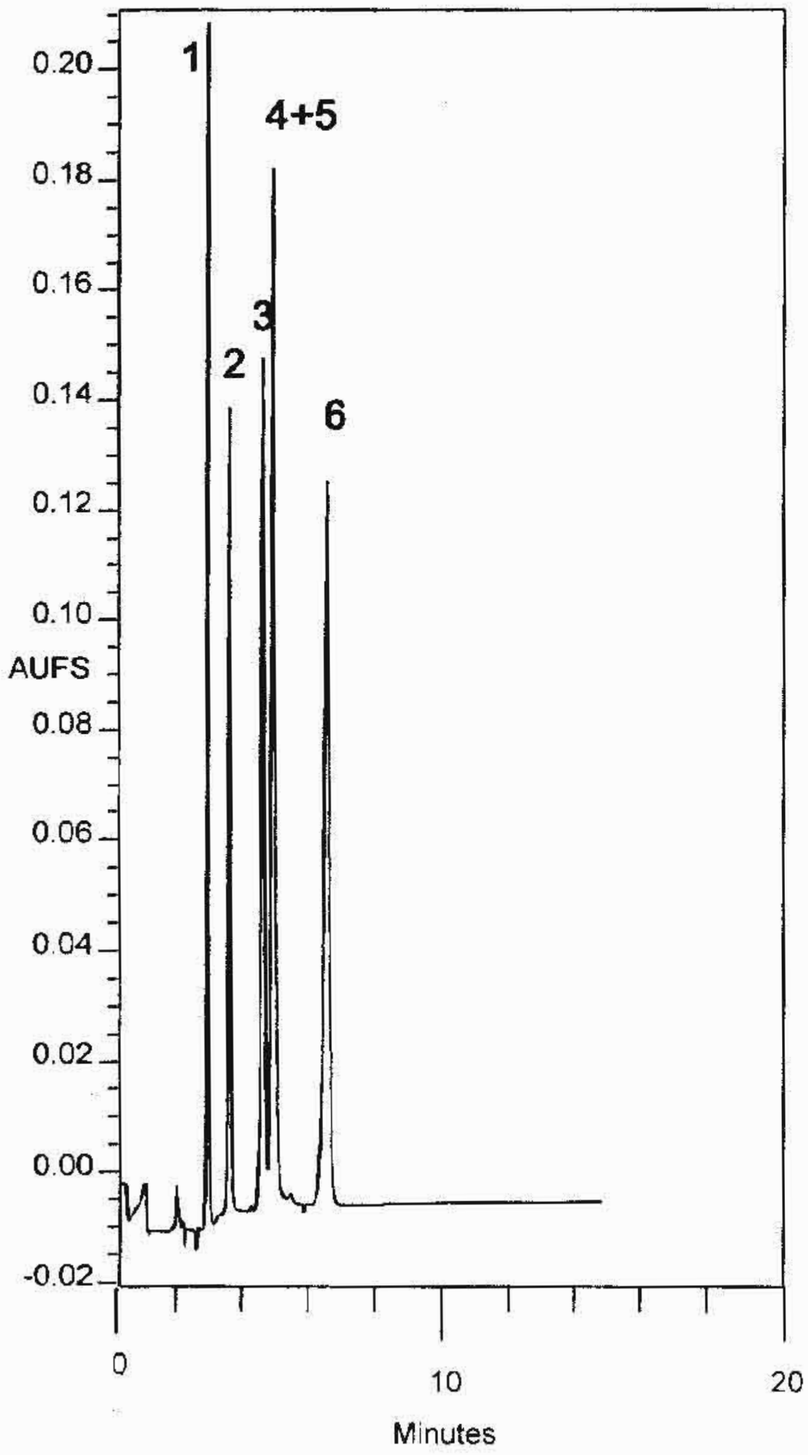
Phenolics on Silica



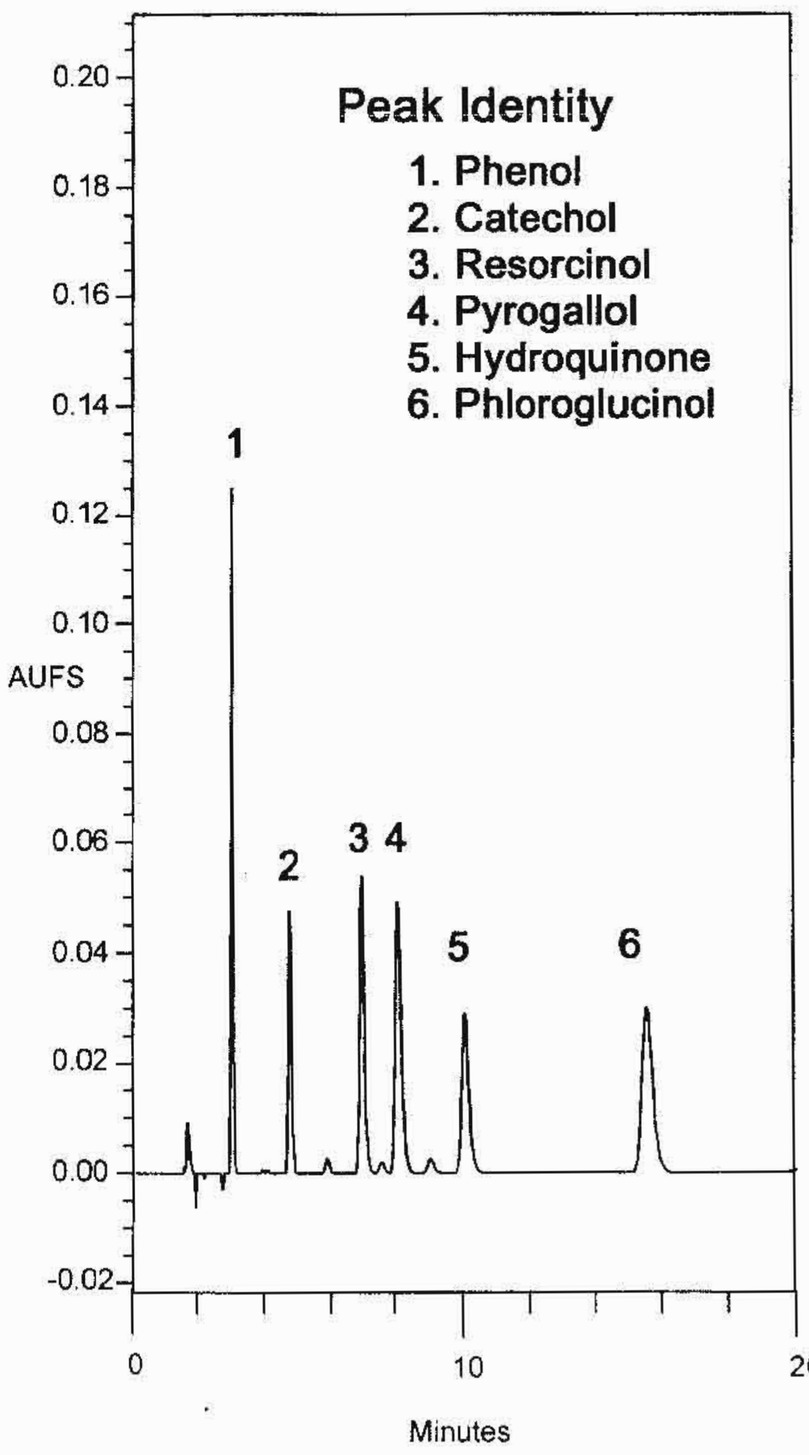
- No resolution between diphenolic hydroquinone and triphenolic pyrogallol
- Poorest resolution of other phenolics
- Shortest retention of all phases for phenolic compounds

Phenolics on Cyano and Diol

Cyano



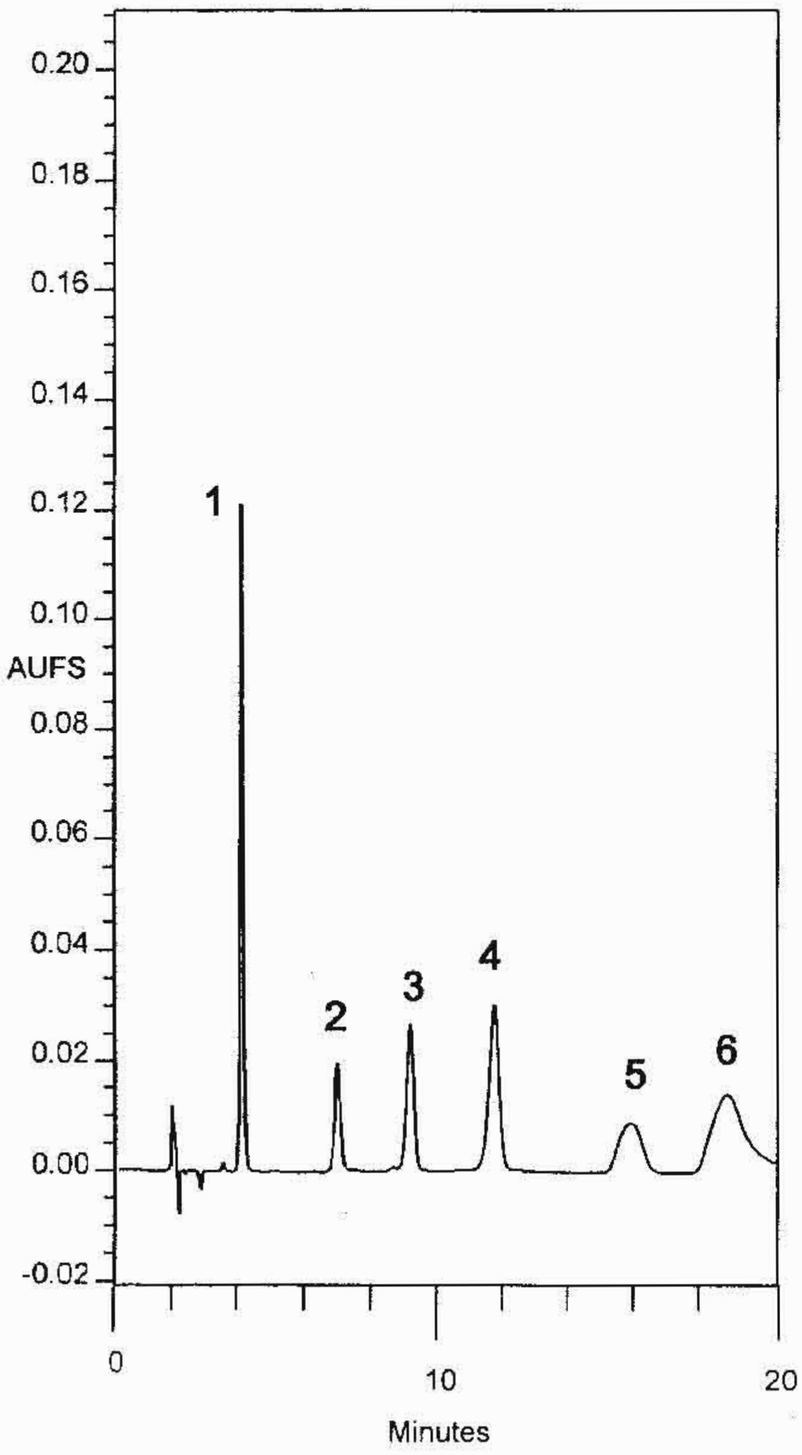
Diol



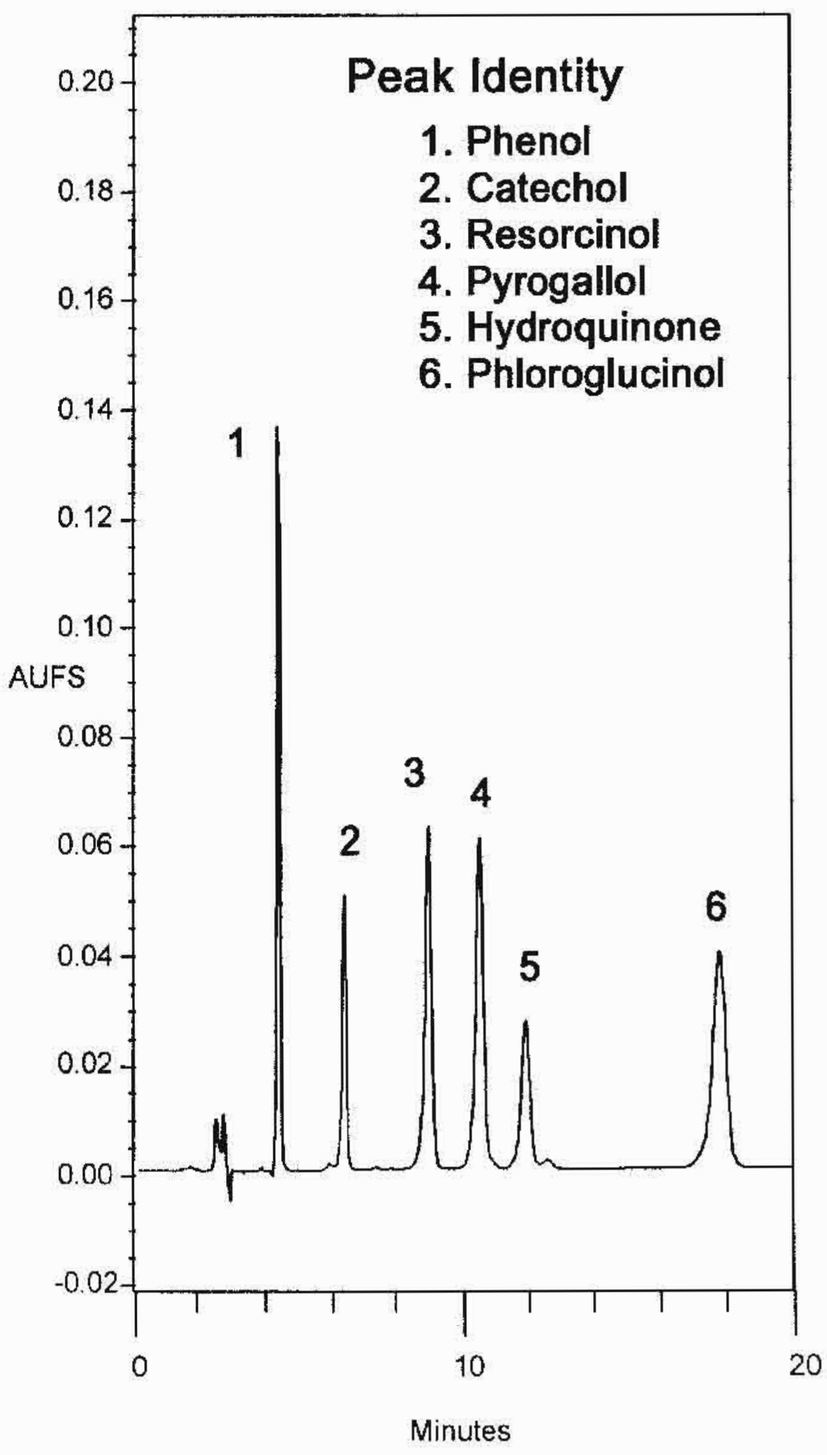
- Cyano is least retentive phase for phenolics
- Note coelution of pyrogallol and hydroquinone
- Diol shows superior resolution and selectivity for phenolics compared to both Cyano and Silica
- Retention increases on Diol with molecular symmetry

Phenolics on Amine and PVA-sil

Amine

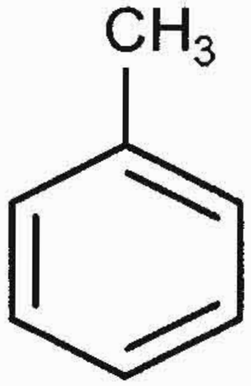


PVA-sil

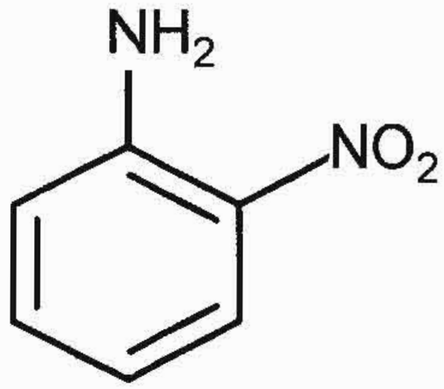


- Amine is most retentive phase for phenolics
- PVA-sil shows better resolution and selectivity for phenolics compared to both Cyano and Silica

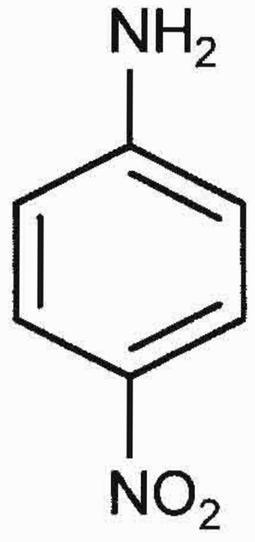
Nitroaromatics



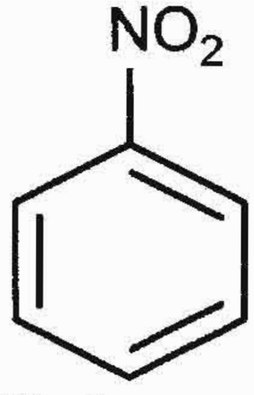
Toluene



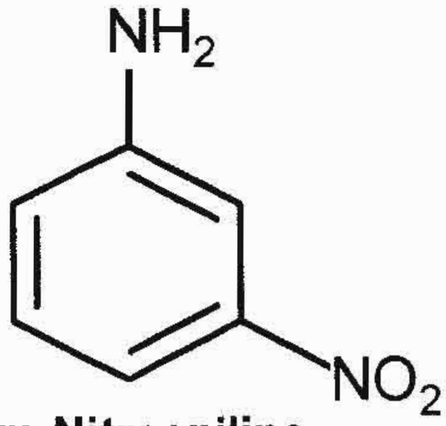
o-Nitroaniline



p-Nitroaniline



Nitrobenzene

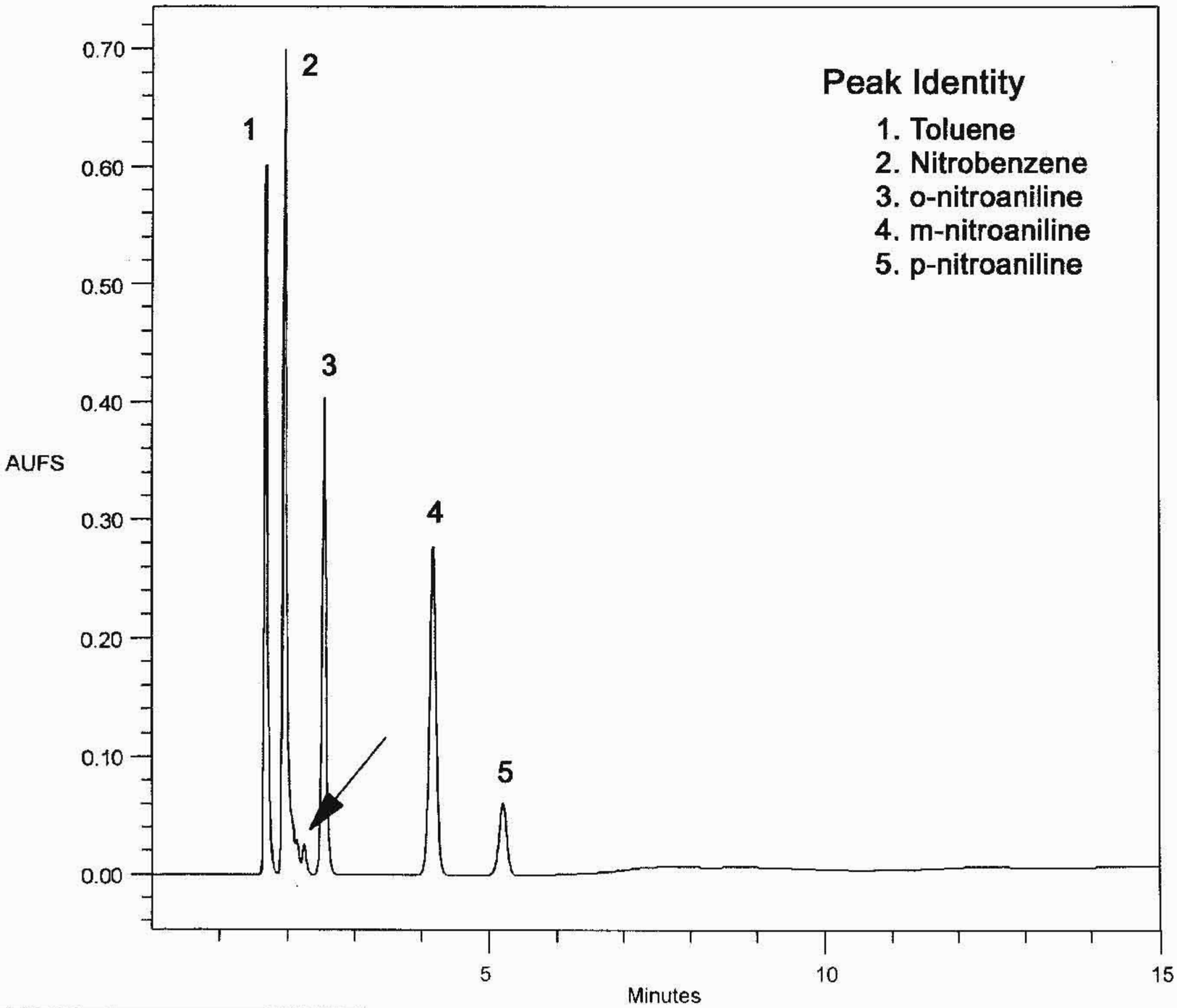


m-Nitroaniline

HPLC Conditions

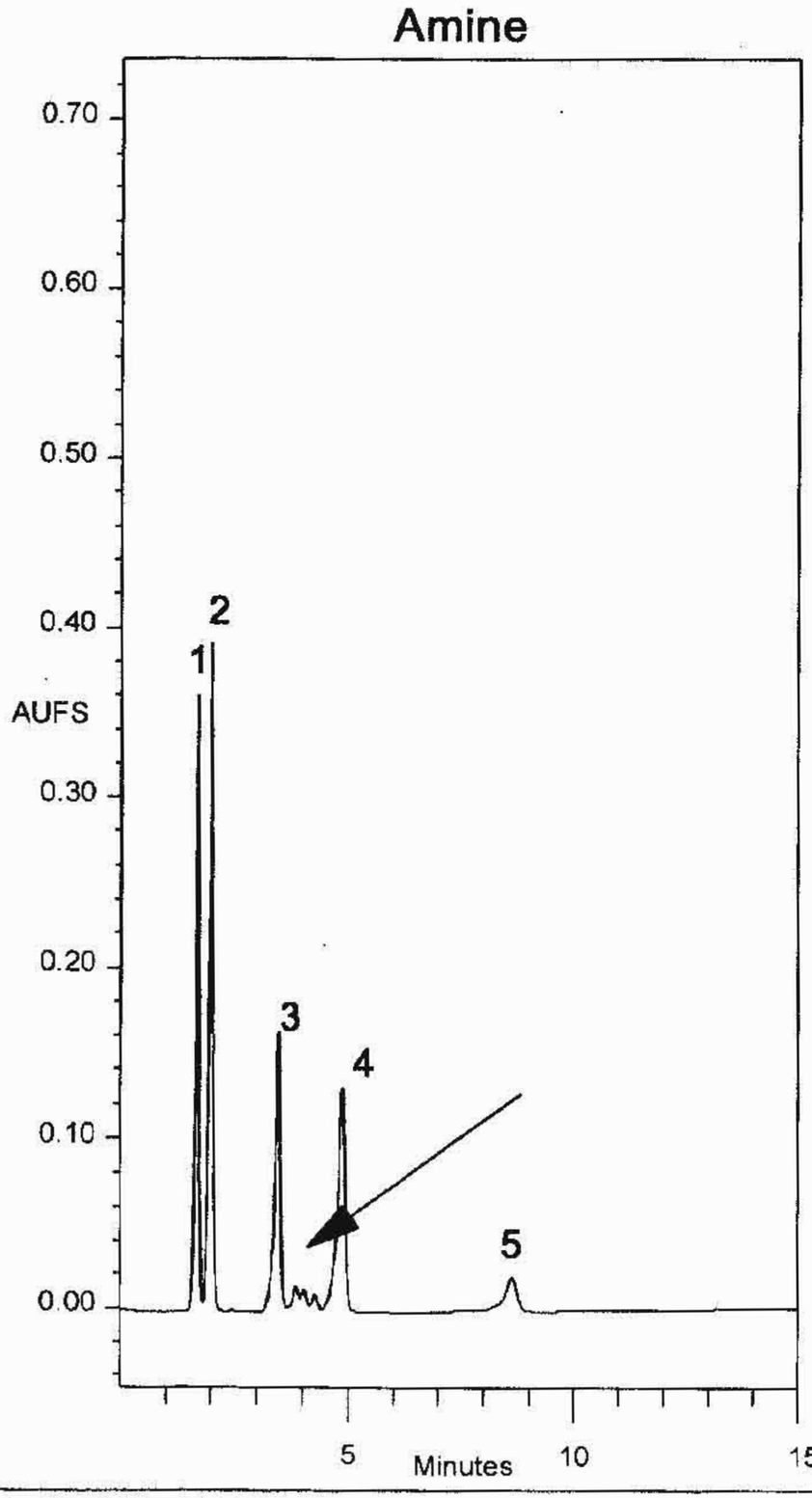
Column size: 4.6 x 250 mm
Mobile phase:
85 Isooctane
15 Isopropanol
Flow rate: 2 mL/min
Temperature: Ambient
Detection: UV @ 254 nm

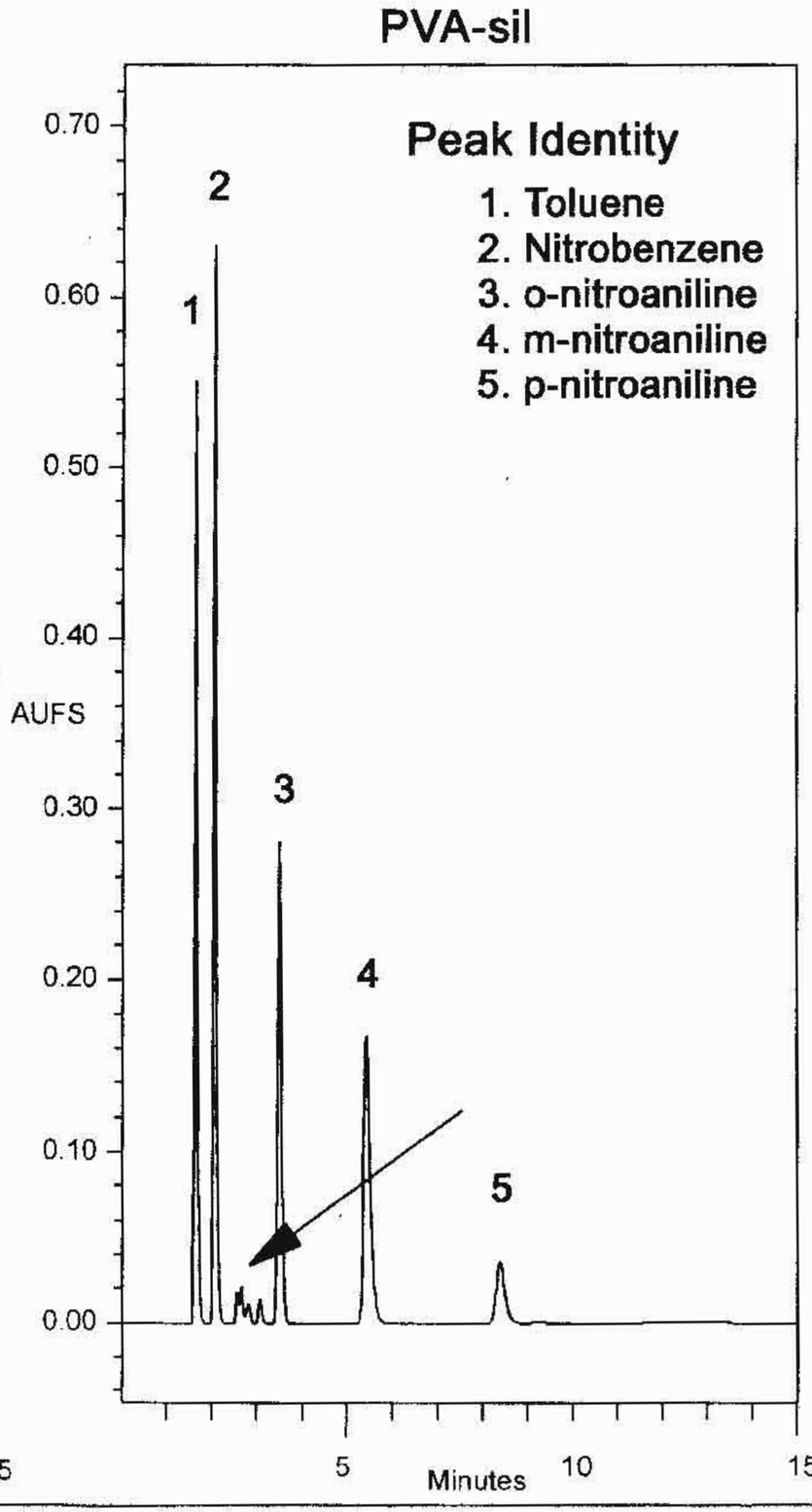
Nitroaromatics on Silica



- Selectivity between m-nitroaniline (peak 4) and p-nitroaniline (peak 5) changes compared to Diol and PVA-sil
- Good peak shape for most components
- Less resolution between Nitrobenzene (peak 2) and later impurities (⟶)

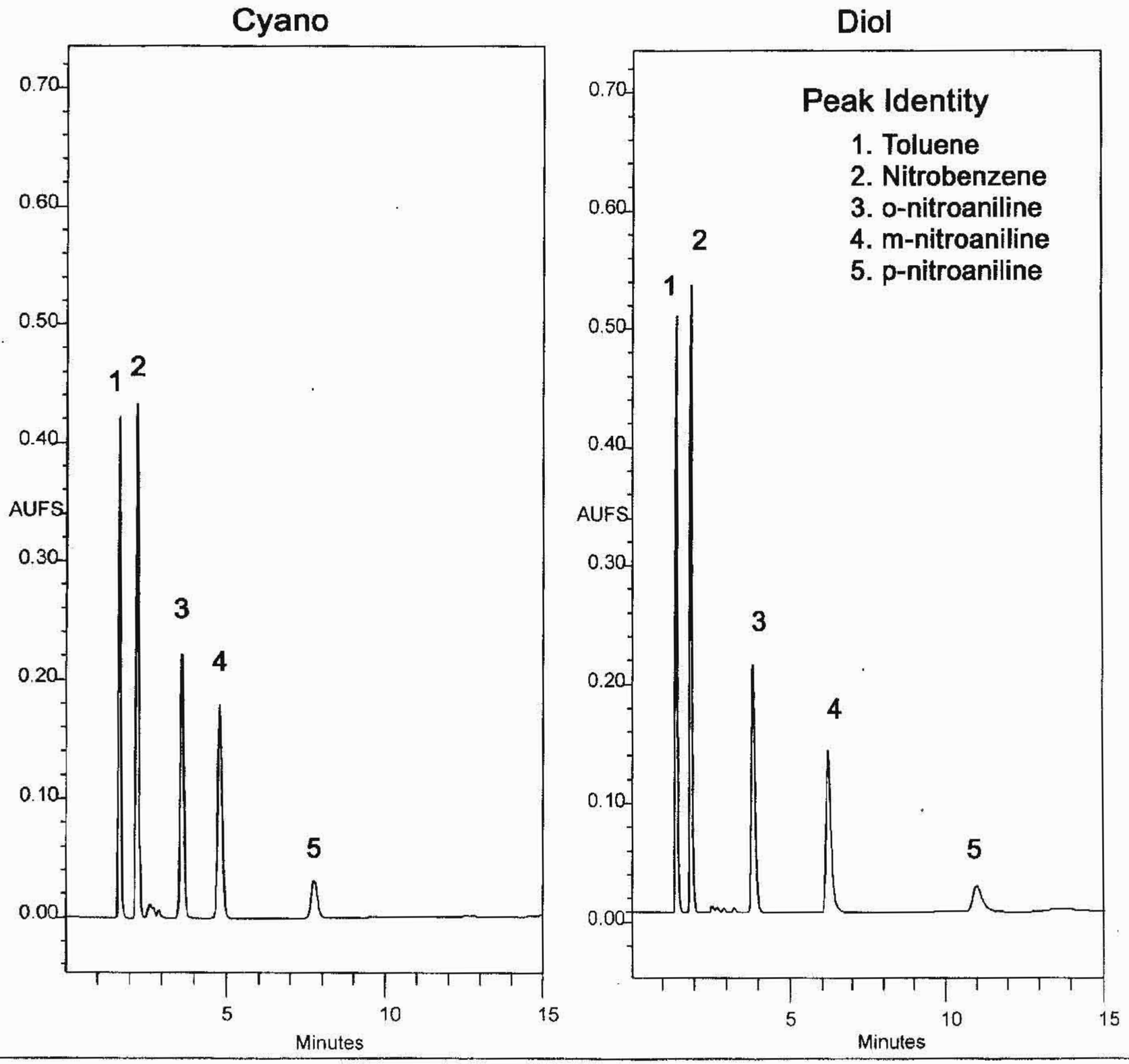
Nitroaromatics on Amine and PVA-sil Media





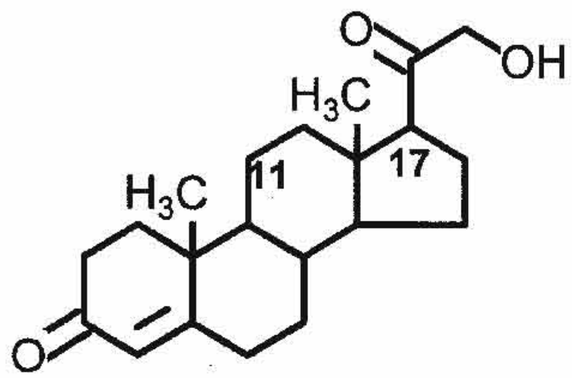
- Increased retention compared to Silica
- Low resolution between toluene (peak 1) and nitrobenzene (peak 2) on Amine
- Improved resolution and peak symmetry for nitroaniline isomers (peaks 3,4,5) on PVA-sil
- Note the selectivity difference for the minor impurities (⟶)

Nitroaromatics on Cyano and Diol Media

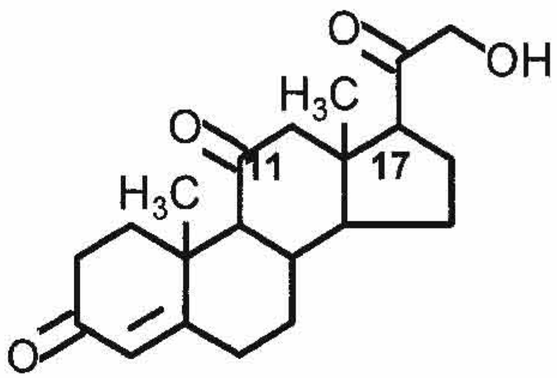


- Better resolution of toluene (peak 1) from nitrobenzene (peak 2) on Cyano and Diol compared to Silica
- Diol is most polar phase for nitroanilines (longest retention)

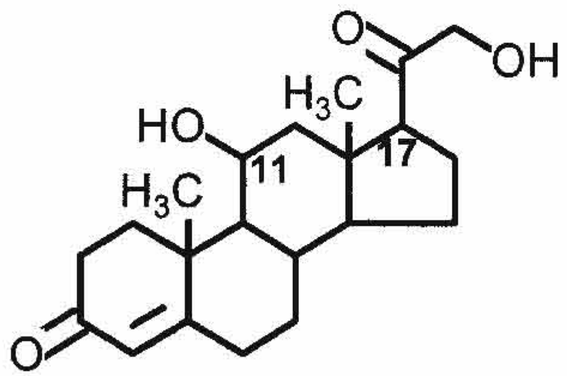
Steroids



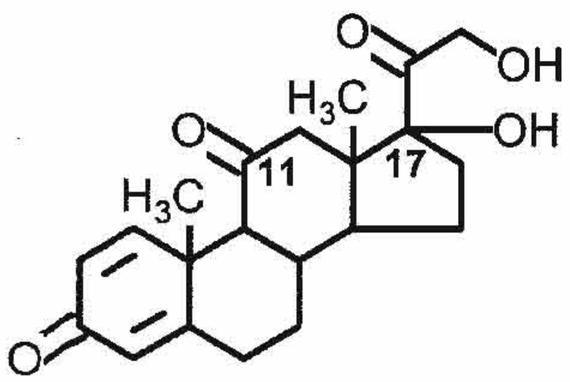
Desoxycorticosterone



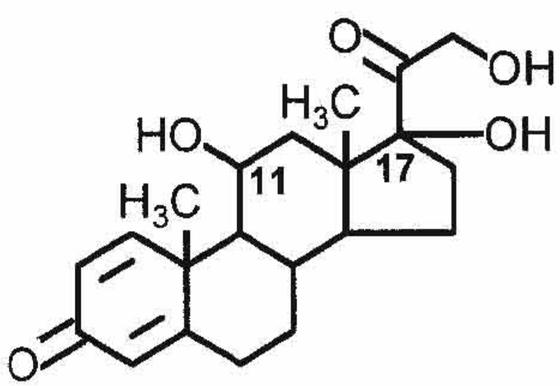
Cortisone



Corticosterone



Prednisone

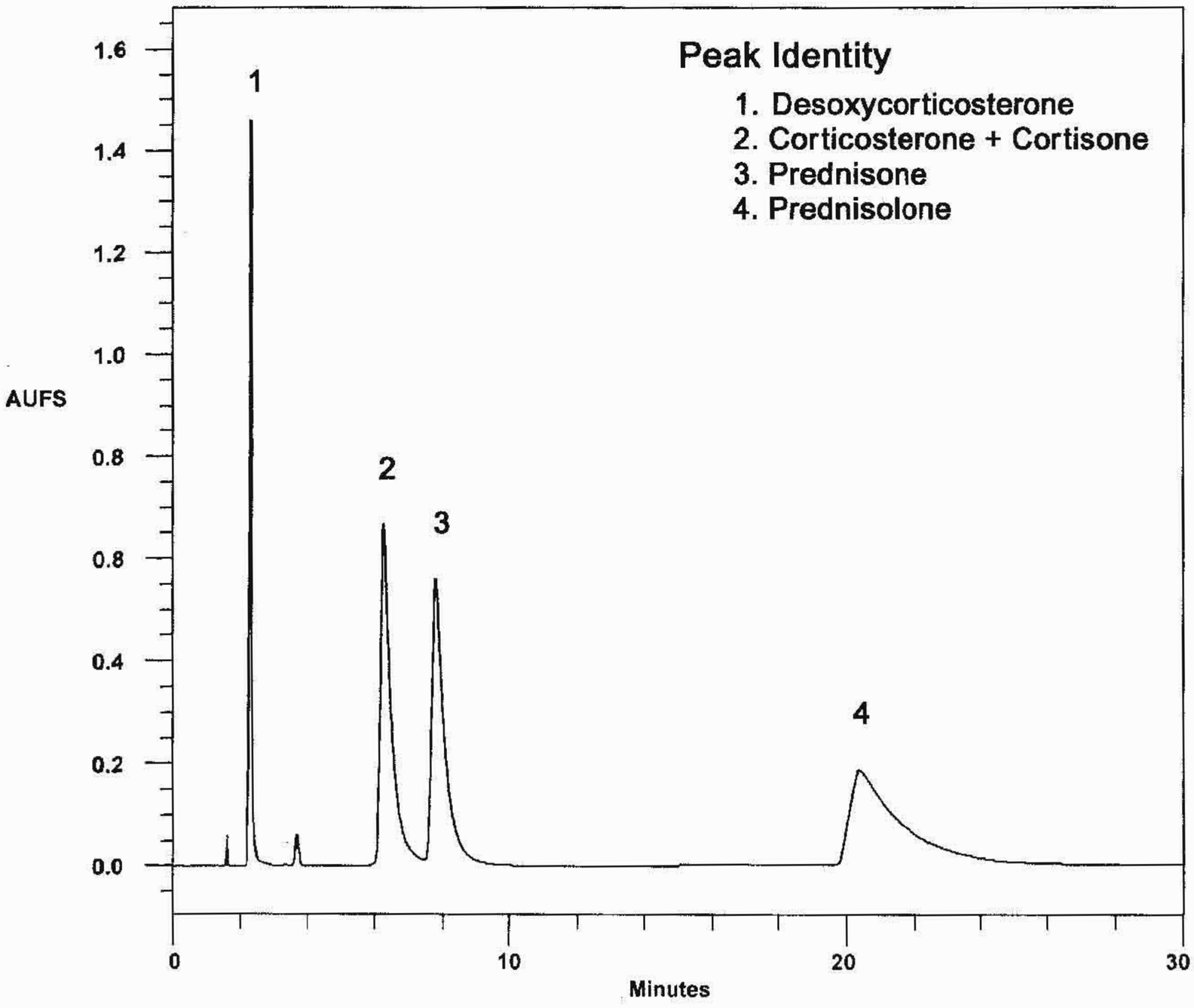


Prednisolone

HPLC Conditions

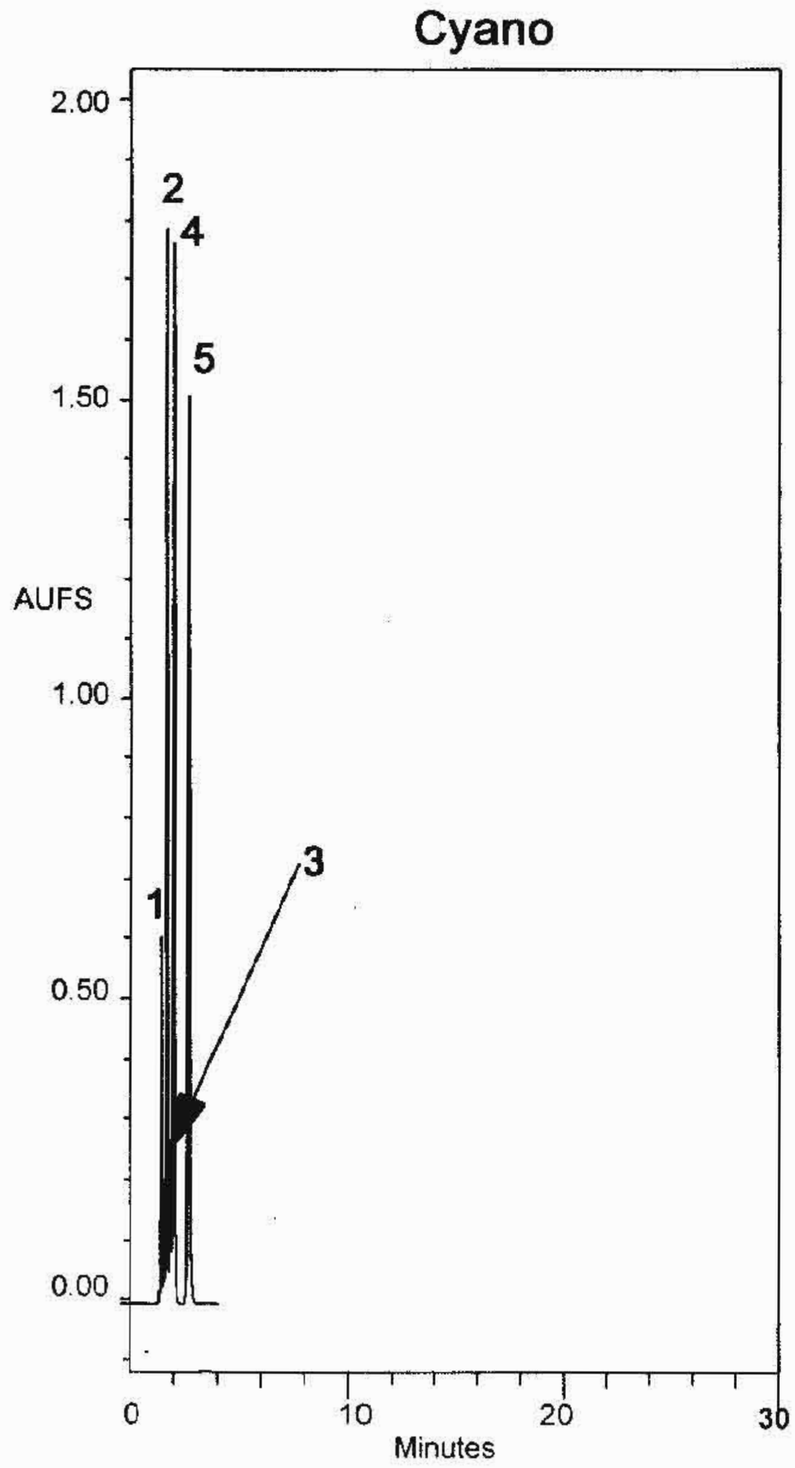
Column size: 4.6 x 250 mm
Mobile phase:
25 Isooctane
70 1,2-Dichloroethane
5 Isopropanol
Flow rate: 2 mL/min
Temperature: Ambient
Detection: UV @ 254 nm

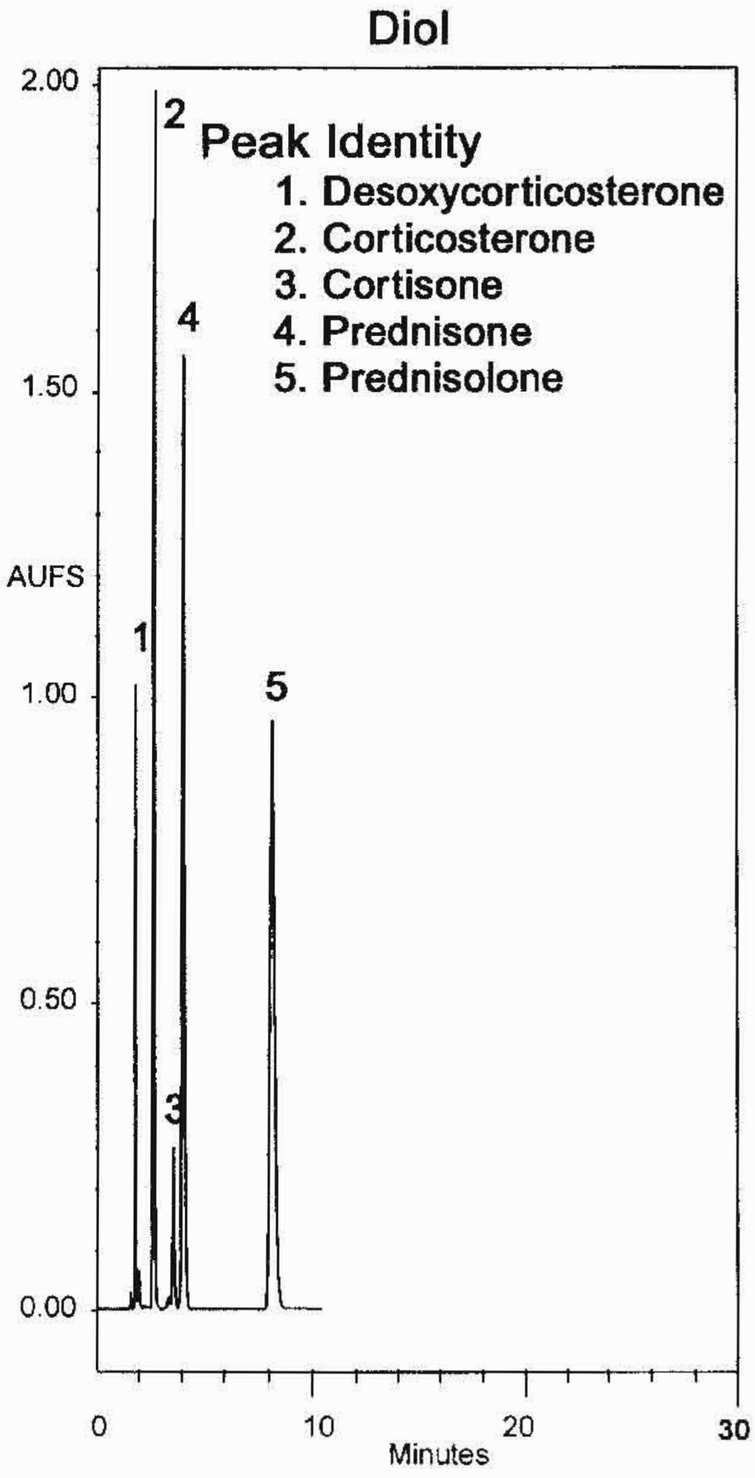
Steroids on Silica



- Poor resolution of steroids
- Coelution of cortisone and corticosterone (peak 2)
- Poor symmetry for prednisolone due to strong interaction between the -OH groups on positions 11 and 17 of the steroid structure and the silanols of the bare silica

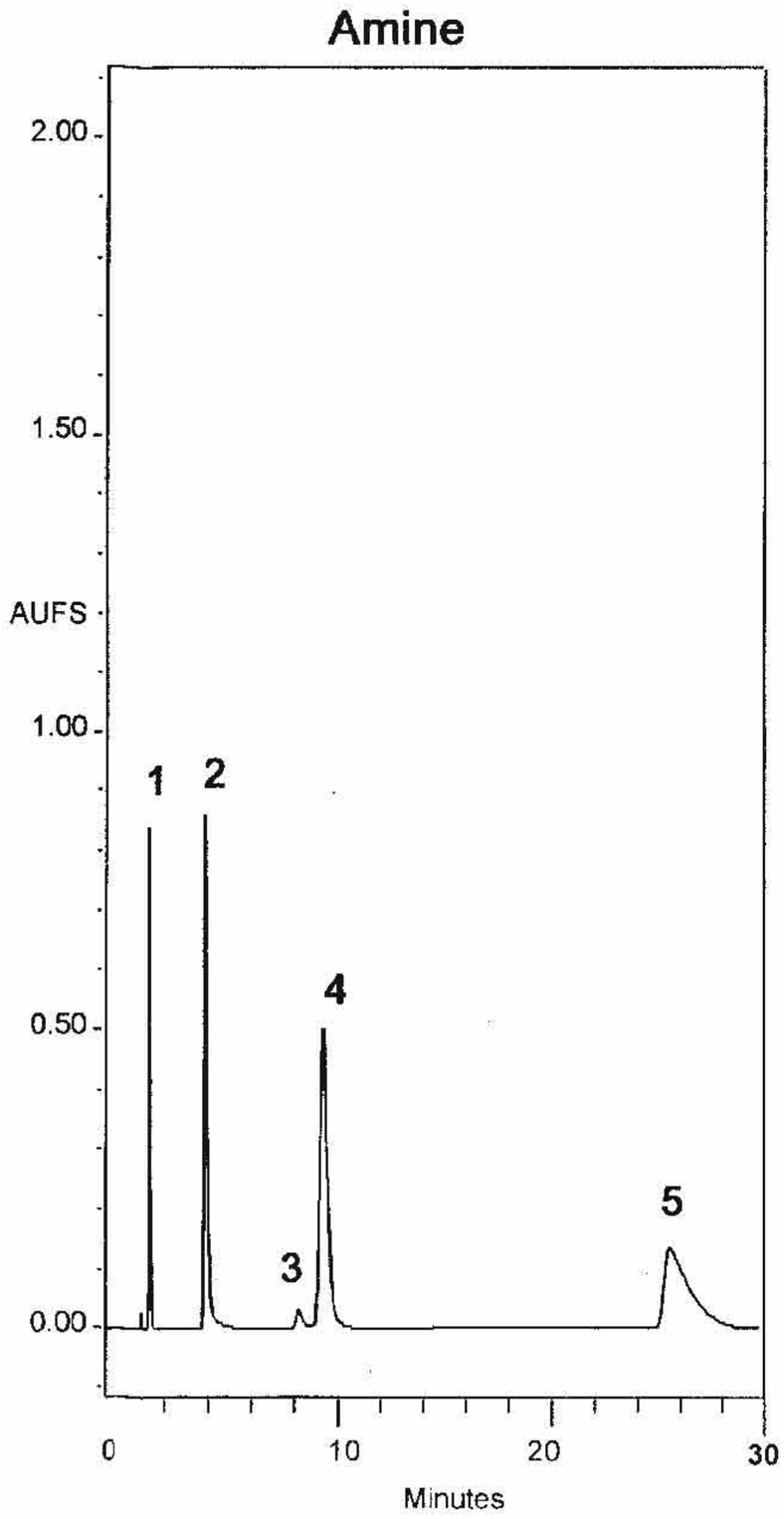
Steroids on Cyano and Diol

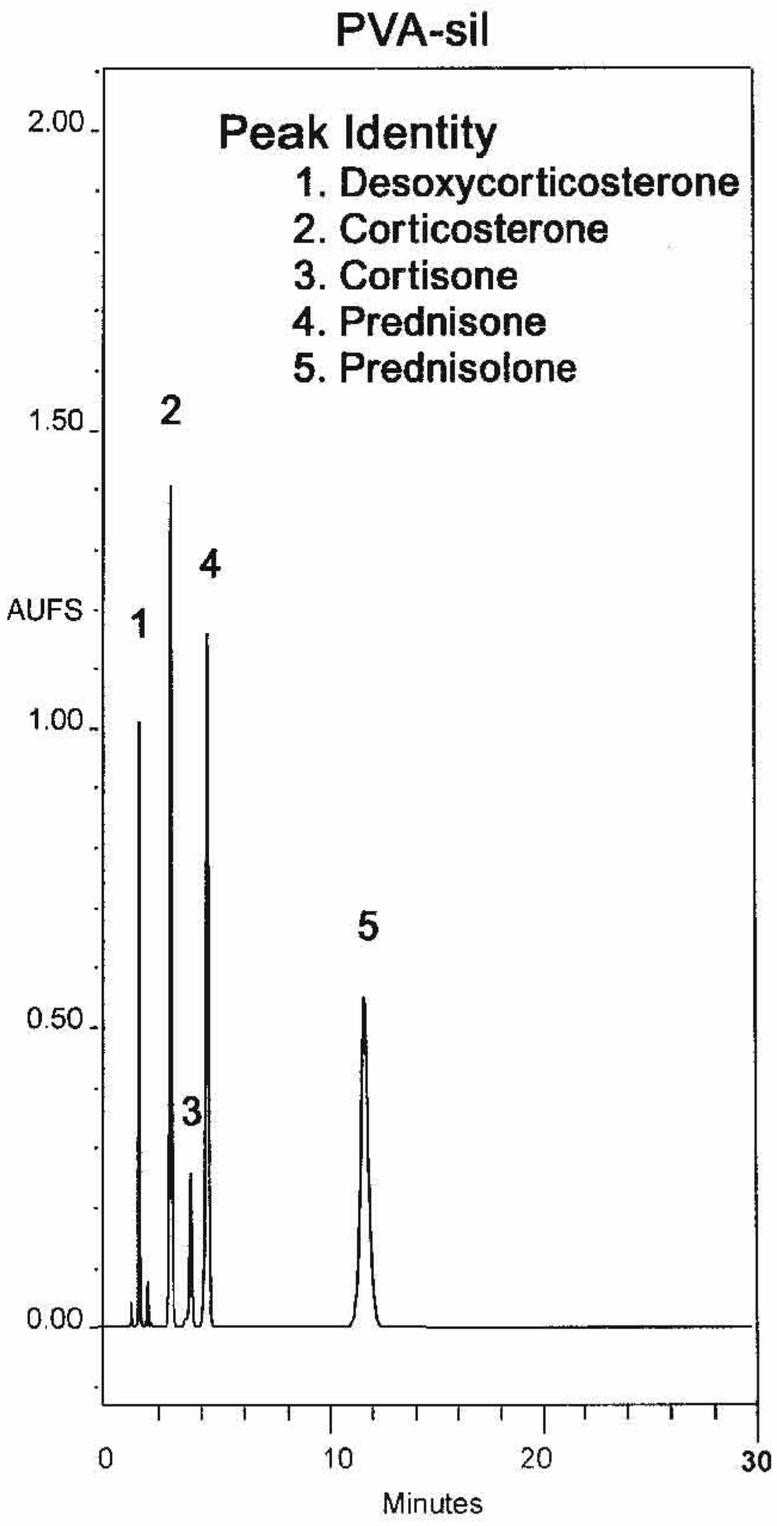




- PVA-sil and Diol provide the best resolution and peak shape
- Cyano requires a weaker mobile phase (least polar phase for these compounds)
- Amine phase shows strongest retention for steroids (most polar phase)

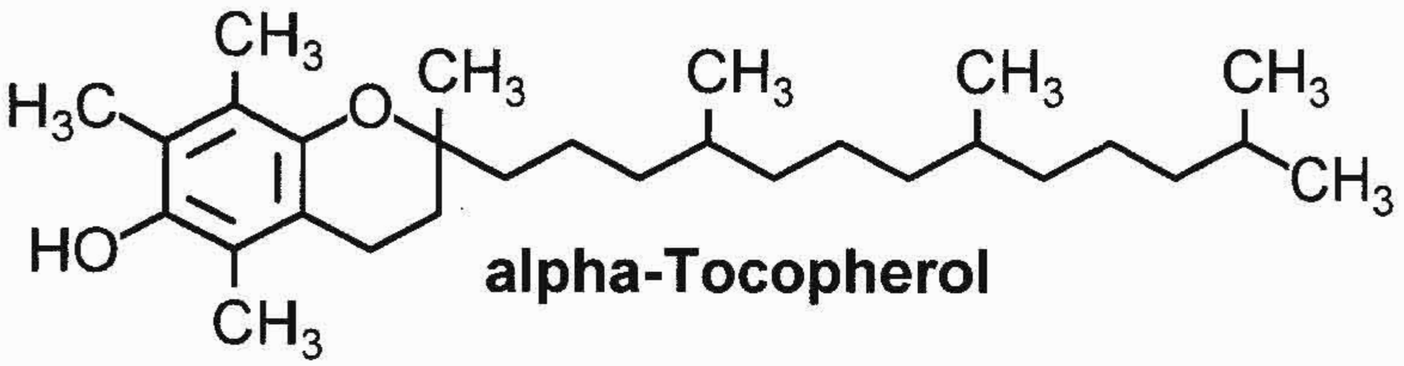
Steroids on Amine and PVA-sil



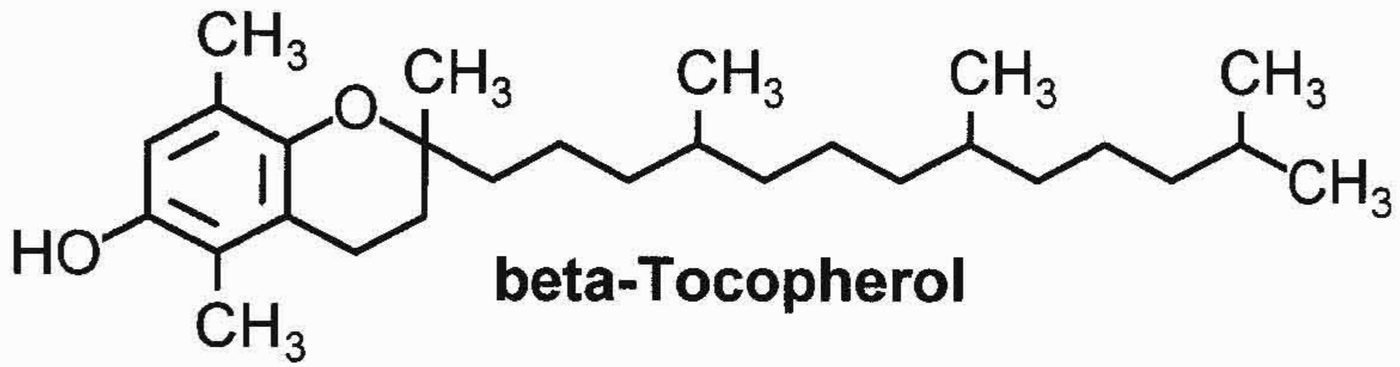


- PVA-sil and Diol provide the best resolution and peak shape
- Cyano requires a weaker mobile phase (least polar phase for these compounds)
- Amine phase shows strongest retention for steroids (most polar phase)

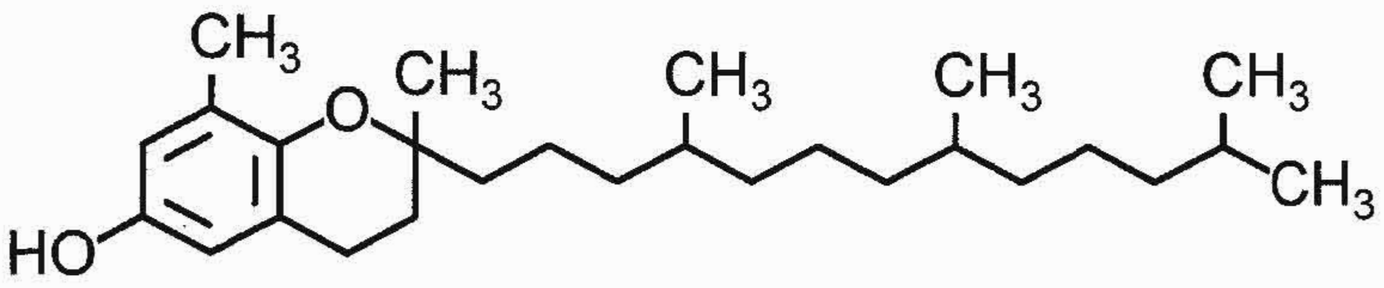
Tocopherols



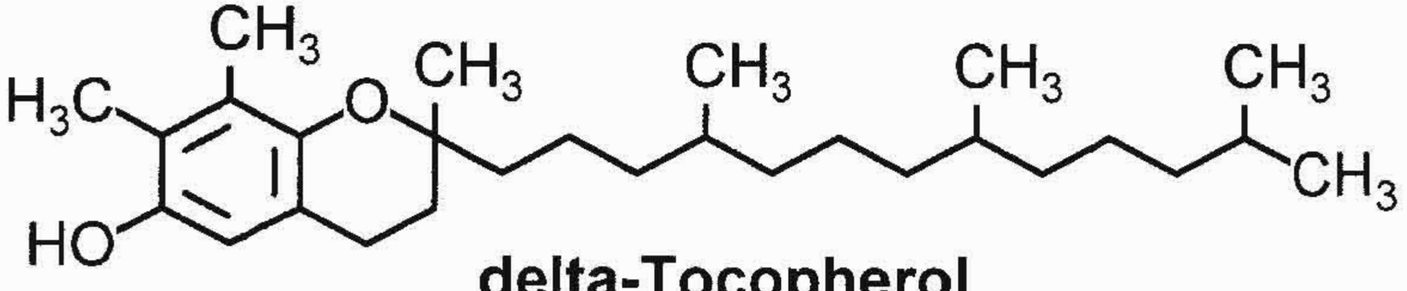
alpha-Tocopherol



beta-Tocopherol



gamma-Tocopherol

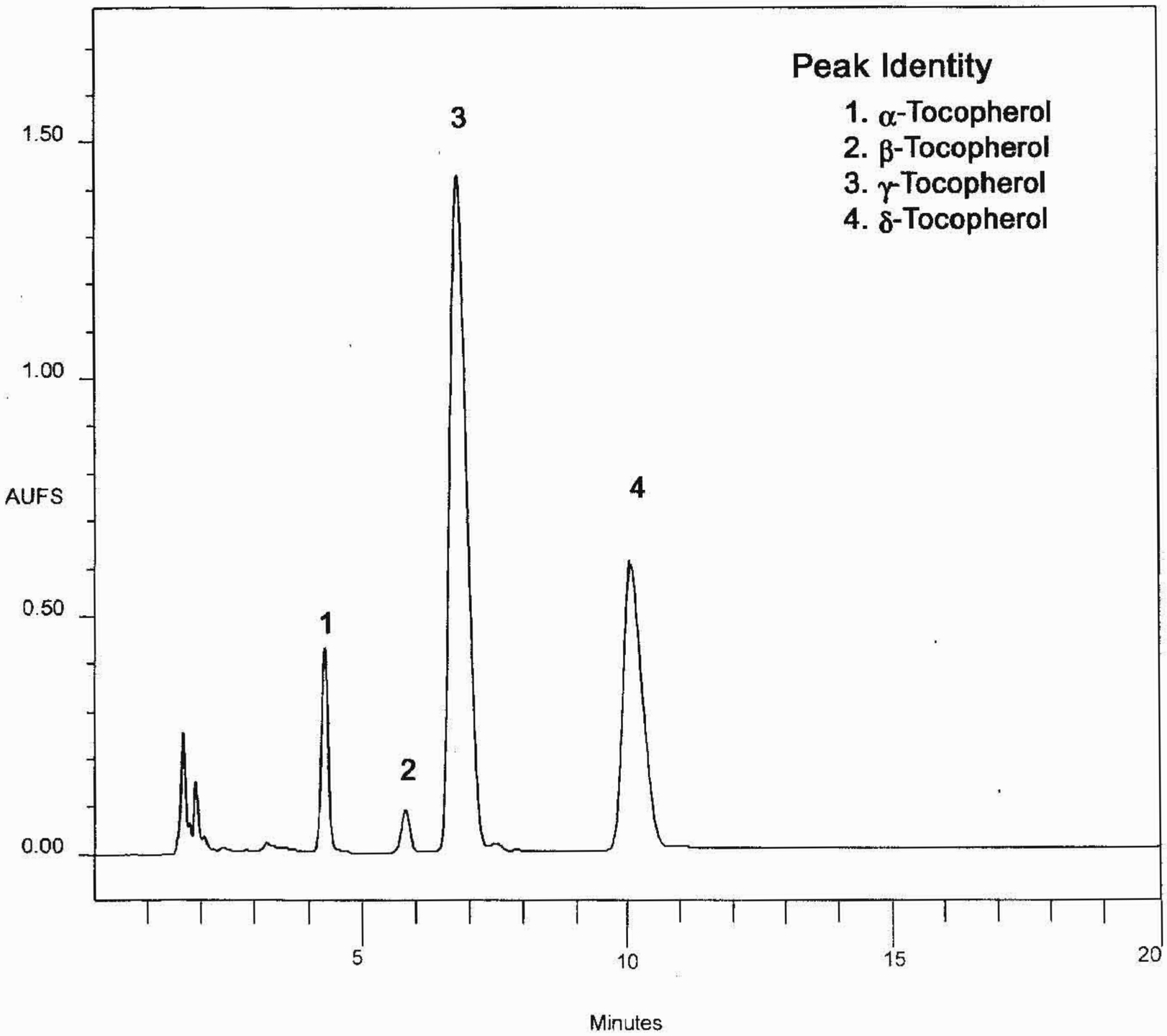


delta-Tocopherol

HPLC Conditions

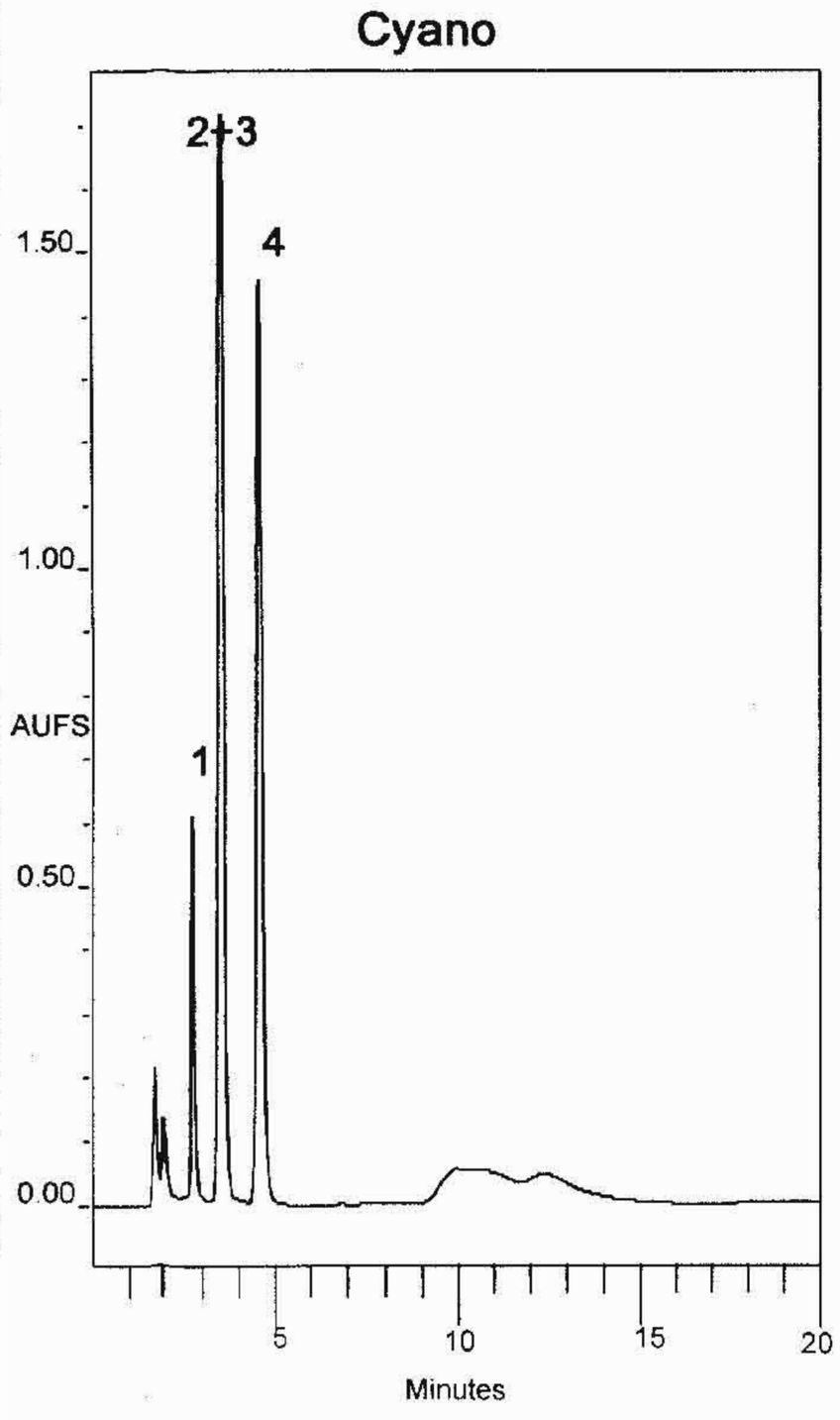
Column size: 4.6 x 250 mm
Mobile phase:
97 Isooctane
3 Tetrahydrofuran
Flow rate: 2 mL/min
Temperature: Ambient
Detection: UV @ 295 nm

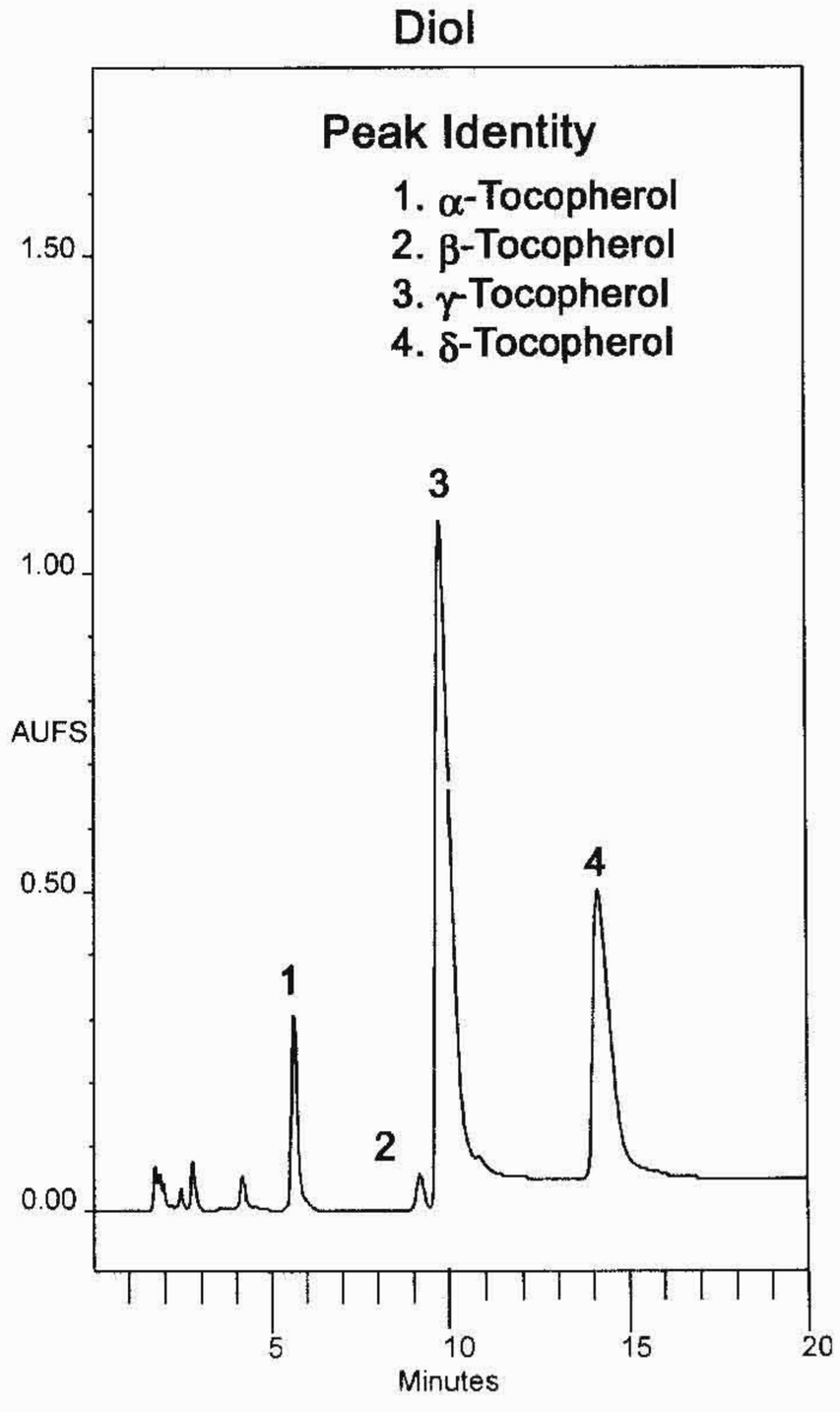
Tocopherols on Silica



- Best separation of tocopherols
- Excellent peak shape
- Fast analysis time
- Best resolution between β and γ-Tocopherol (peaks 2 and 3)

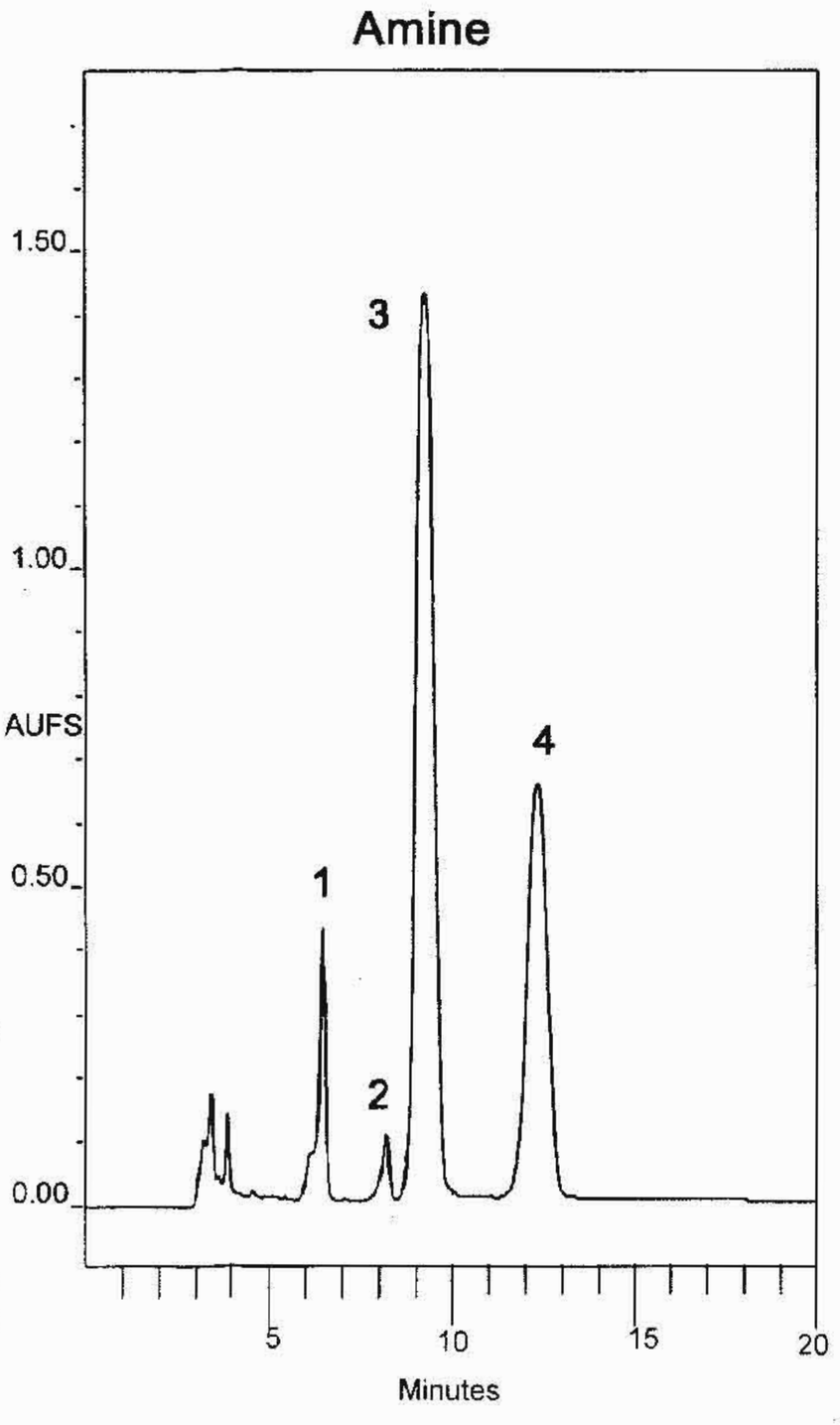
Tocopherols on Cyano and Diol

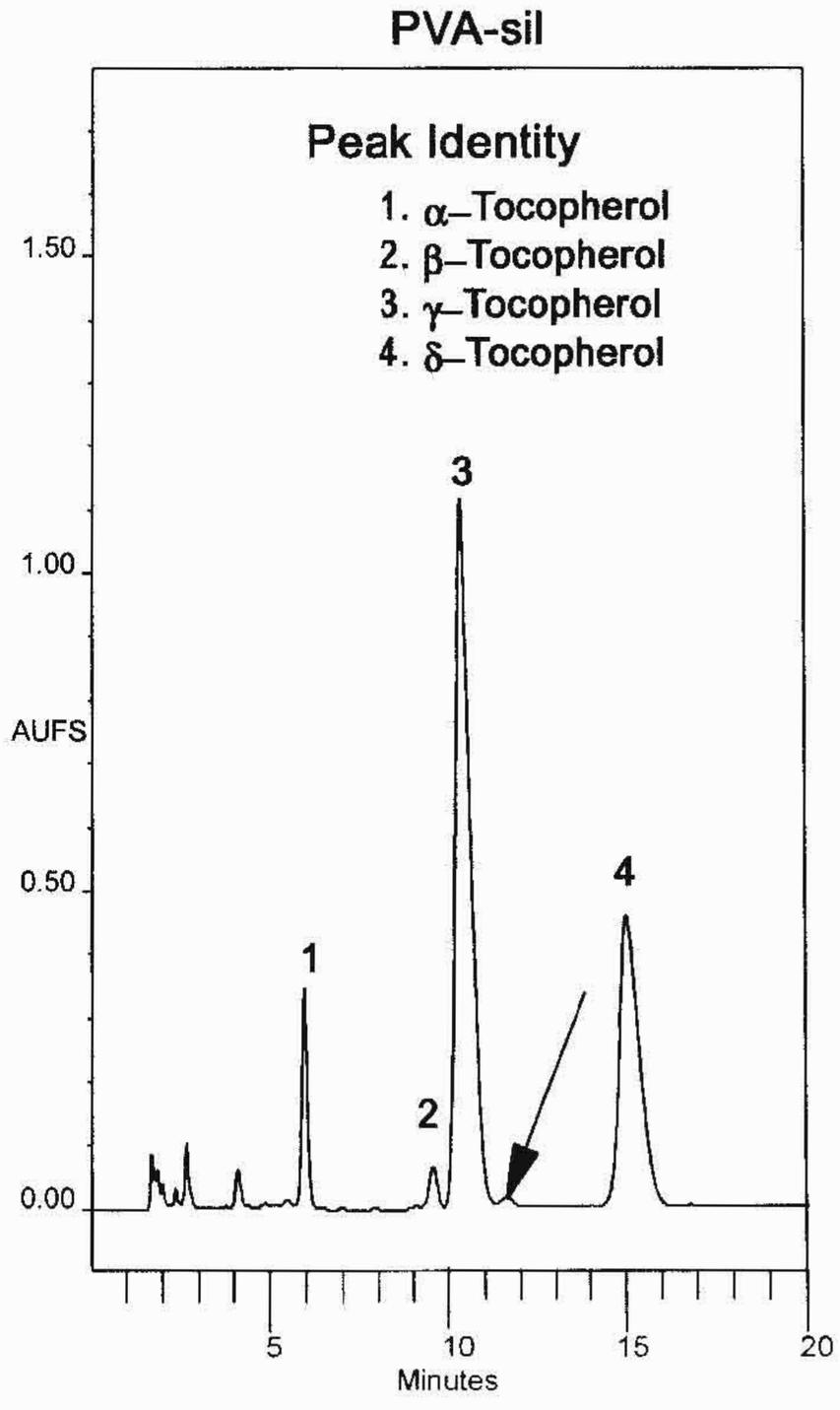




- Good selectivity for all tocopherols on Diol
- Coelution of β and γ– tocopherol on Cyano
- Better resolution between impurities and α-tocopherol on Diol than Silica

Tocopherols on Amine and PVA-sil





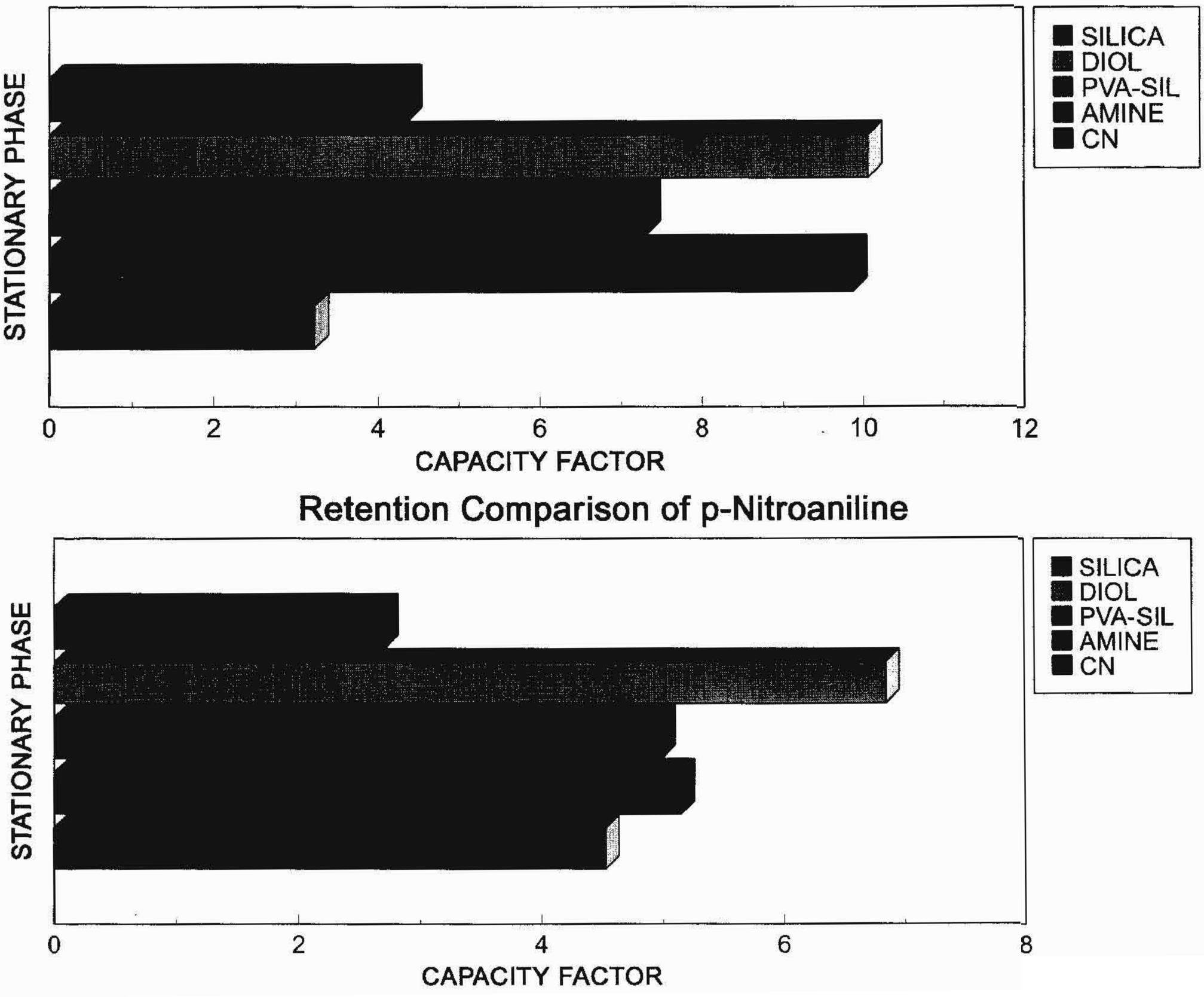
- Amine is most retentive for tocopherols (requires a more polar mobile phase) - IO/MTBE/EtOAc (50:45:5)
- PVA-sil is more retentive than Silica for tocopherols
- Better resolution between impurity (⟶) and γ-tocopherol on PVA-sil than Silica

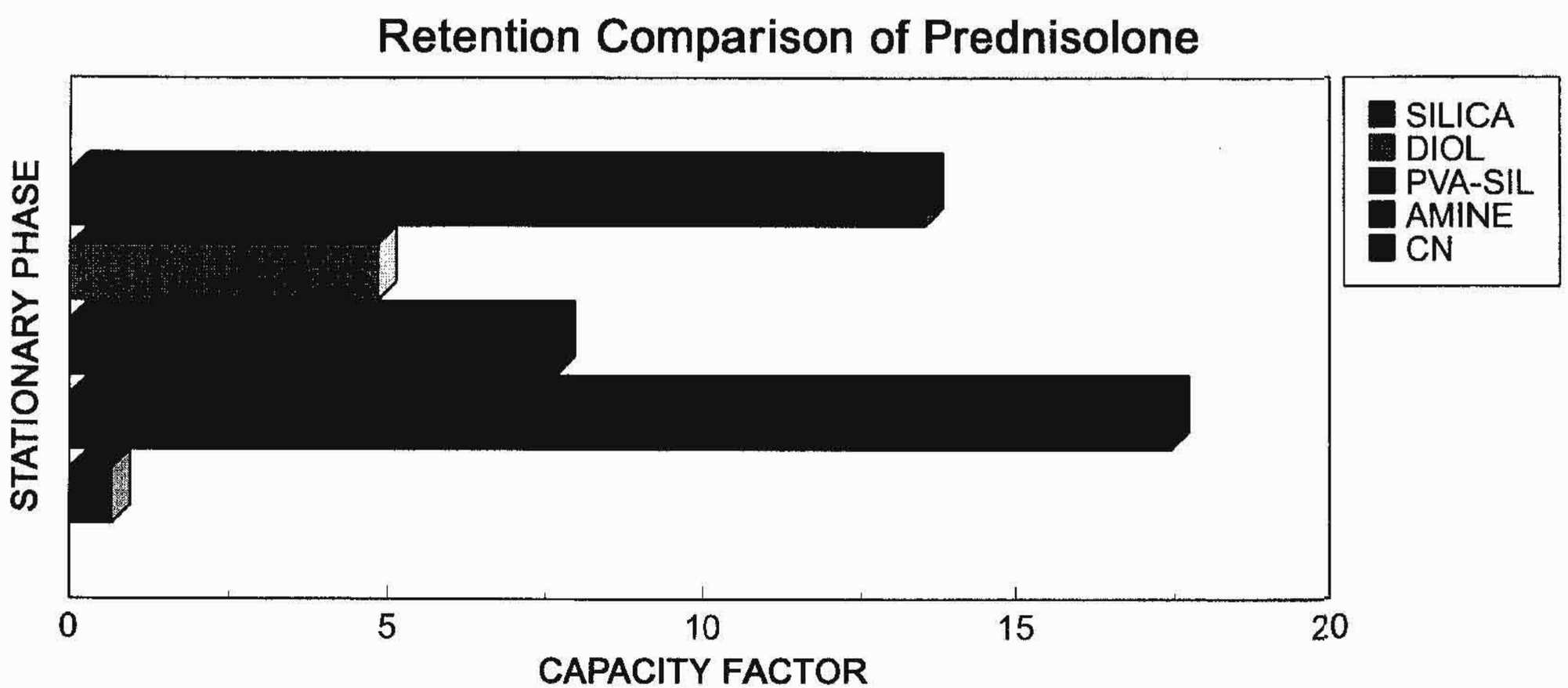
Relative Polarity Index or the BNP family

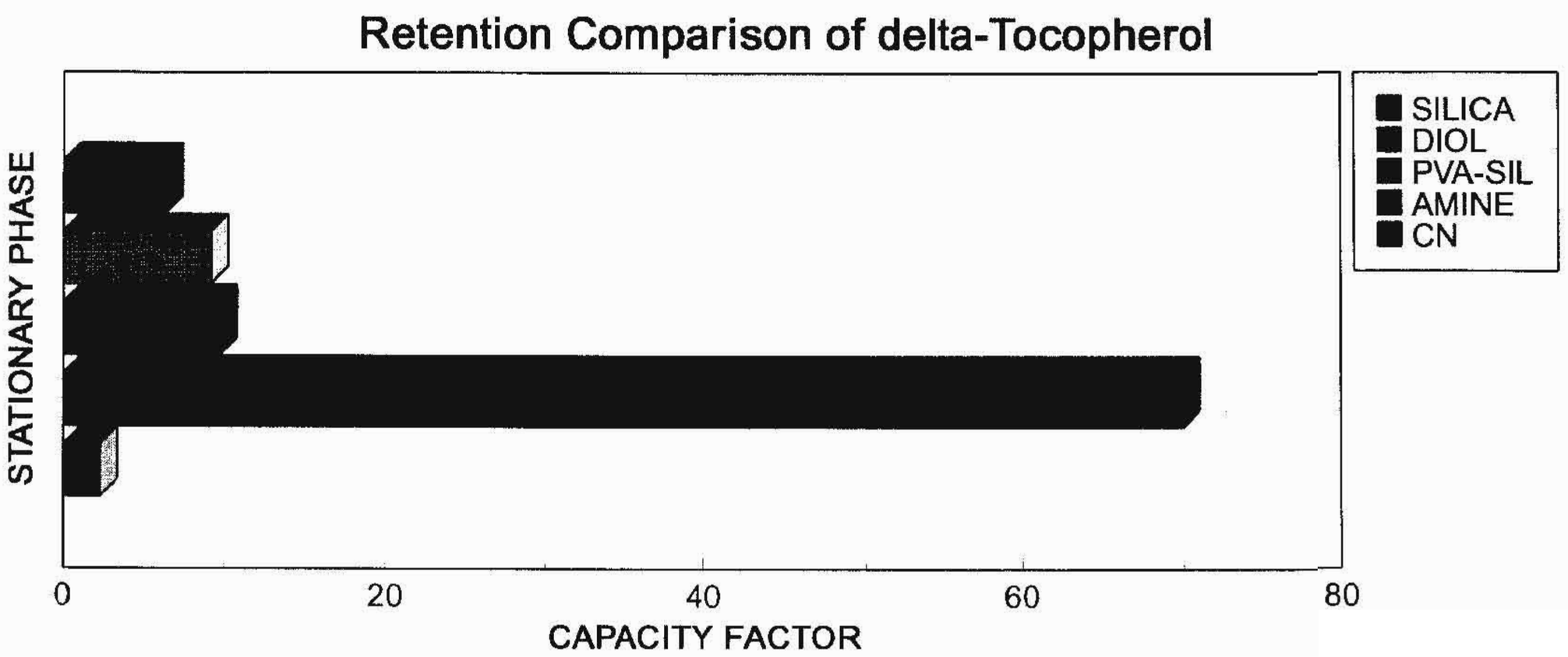
In reversed phase HPLC, the relative polarity index is directly related to chain length (i.e. for non-polar compounds the retention time on C4 is always less than C18). For the BNP family the relative polarity index varies with the polarity of the bonded phase and the hydrophilic interactions (e.g. hydrogen bonding) of the solute and bonded phase.

The hydrophilic interaction for nitroaromatic and phenolic type molecules is greatest on the Diol phase. For **tocopherol and steroid type molecules the Amine phase exhibits the highest retention.**

The hydrophilic interaction of the BNP family benefits the chromatographer by offering another dimension of selectivity. The separations are influenced by both the stationary phase and the solvent. This enhanced selectivity is very useful for analytical as well as preparative separations.


Retention Comparison of Phloroglucinol
STATIONARY PHASE
SILICA
DIOL
PVA-SIL
AMINE
CN
0
2
4
6
8
10
12
CAPACITY FACTOR
Retention Comparison of p-Nitroaniline
STATIONARY PHASE
SILICA
DIOL
PVA-SIL
AMINE
CN
0
2
4
6
8
CAPACITY FACTOR


Retention Comparison of Prednisolone
STATIONARY PHASE
SILICA
DIOL
PVA-SIL
AMINE
CN
0
5
10
15
20
CAPACITY FACTOR


Retention Comparison of delta-Tocopherol
STATIONARY PHASE
SILICA
DIOL
PVA-SIL
AMINE
CN
0
20
40
60
80
CAPACITY FACTOR

Cleaning Protocol for the BNP Family

To extend the lifetime of columns it is important to periodically clean the media surface. This is very difficult to do with bare silica. Alcohol or water will clean the silica surface but they also deactivate it. This results in the loss of retention and the employment of extreme re-activation measures.

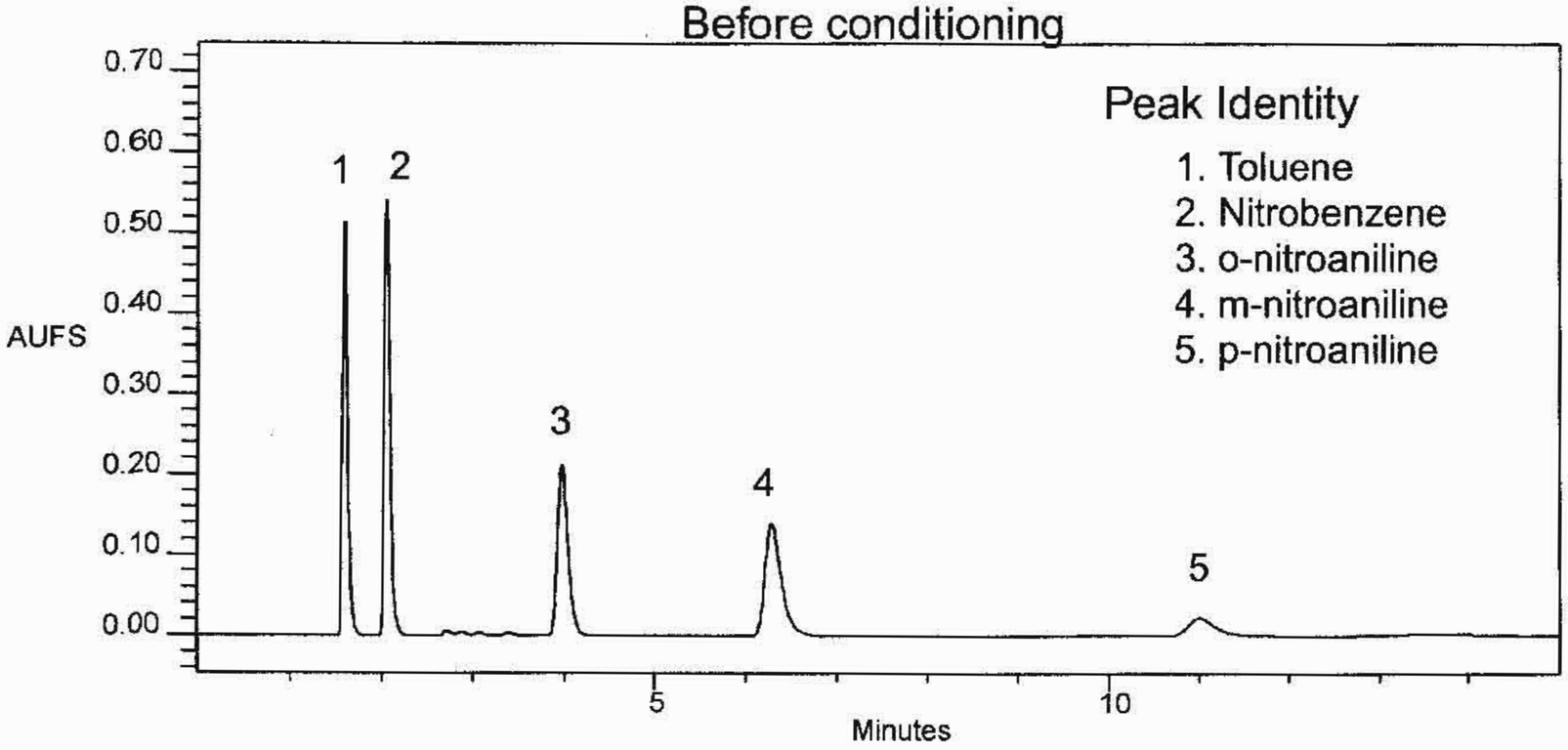
Bonded normal phase media are unaffected by the addition of alcohol or water. This washing protocol cleans the media surface and provides a column ready to begin the next separation.

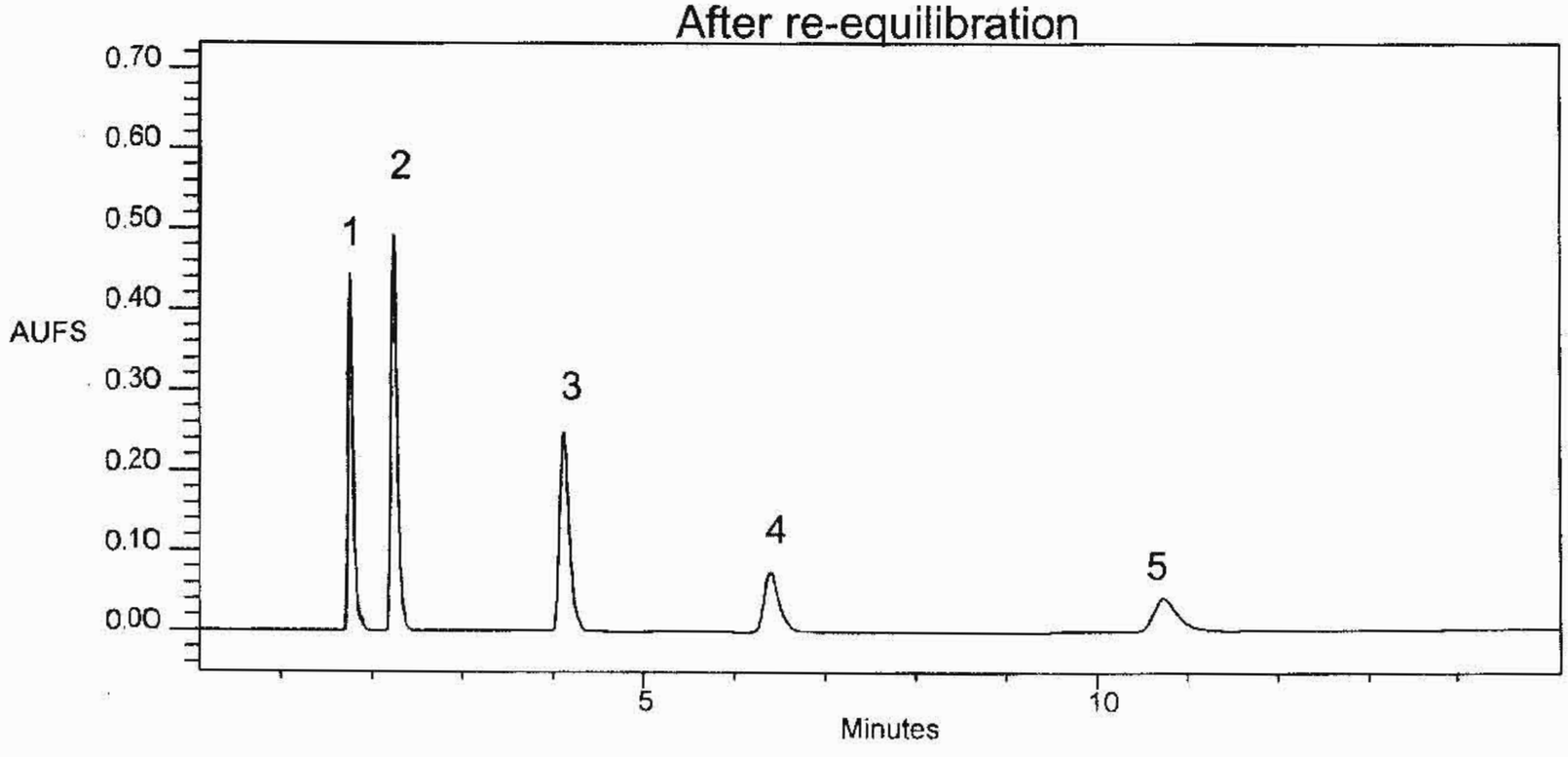
WASHING PROTOCOL

Solvent	Volume (mL)	Column Volumes
isopropanol	30	10
water	30	10
isopropanol	30	10
isooctane/isopropanol (85:15)	30	10

To re-equilibrate the column with non-polar solvent requires just 20 column volumes.

Effect of Polar Solvent Washes: Polar Compounds



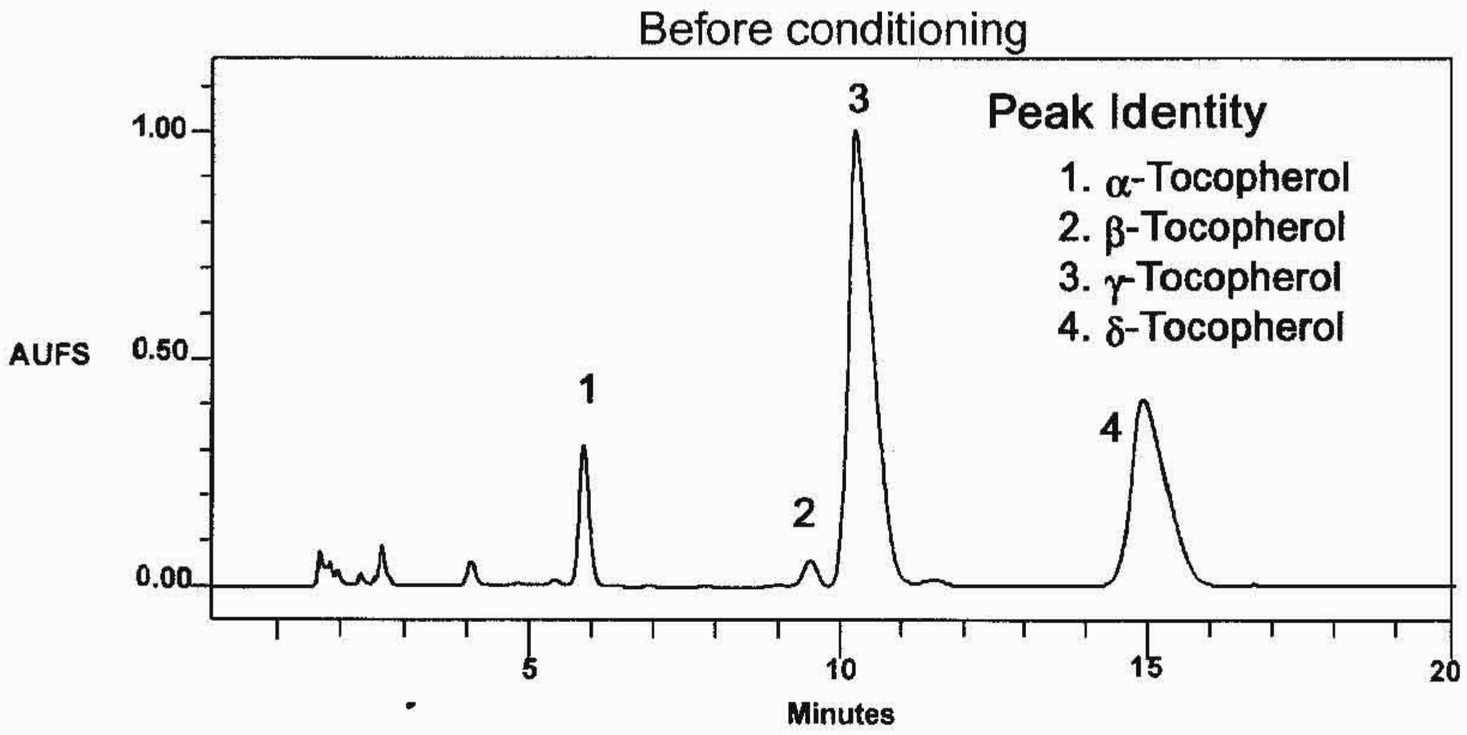


HPLC Conditions

Phase: Diol
Column size: 4.6 x 250 mm
Mobile phase:
85 Isooctane
15 Isopropanol
Flow rate: 2 mL/min
Temperature: Ambient
Detection: UV @ 254 nm

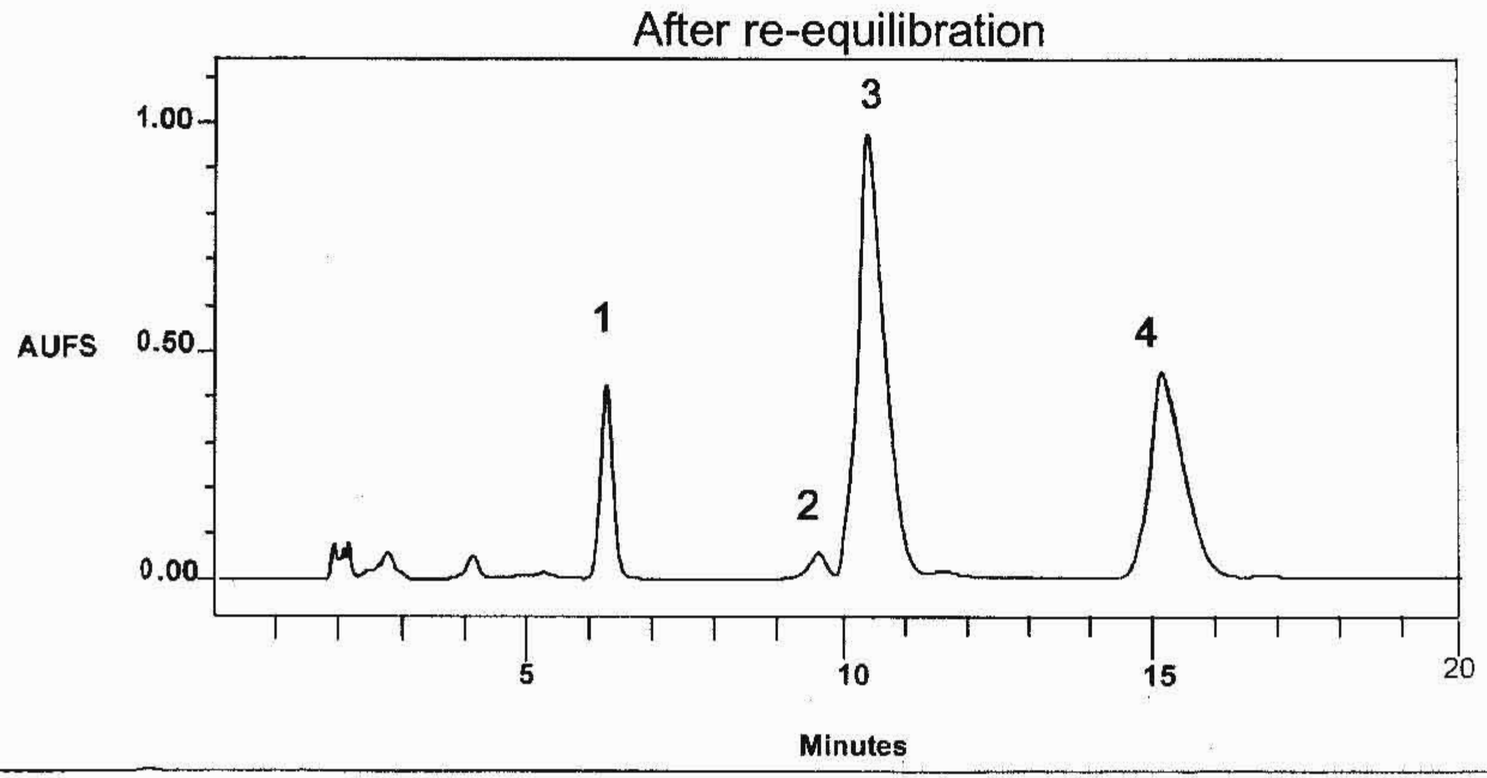
- The retention times before and after washing with polar solvents are identical. This is not possible with bare silica columns since the surface is deactivated by polar solvents.

Effect of Polar Solvent Washes: Lipophilic Compounds



HPLC Conditions

Phase: PVA-sil
Column size: 4.6 x 250 mm
Mobile phase:
97 Isooctane
3 THF
Flow rate: 2 mL/min
Temperature: Ambient
Detection: UV @ 295 nm



- The retention times are identical following washing with polar solvents. Silica columns would be deactivated with this wash protocol

Summary

- The Bonded Normal Phase (BNP) family offers a new alternative to unbonded silica gel for normal phase separations
  - Excellent discrimination of isomers and polar molecules
  - Unique selectivity for difficult separations
  - Available in spherical particles from 3 to 50 micron

- Easily cleaned with alcohols, water and other polar solvents
  - Rapidly equilibrate with new mobile phase (only 20 column volumes)
  - Not deactivated with water
  - Clean up extends lifetime reducing overall column and operating costs

- Relative Polarity Index for the BNP family changes based on a solute's functional groups
  - Diol is most retentive for nitroanilines while the Amine phase is most retentive for steroids.
  - Cyano is the least retentive phase in the BNP family
  - Amine phase is the most polar and requires a stronger solvent system than other bonded phases