Criteria for the selection of a suitable RP phase in HPLC, showed with NUCLEODUR® examples

Stationary phase selection

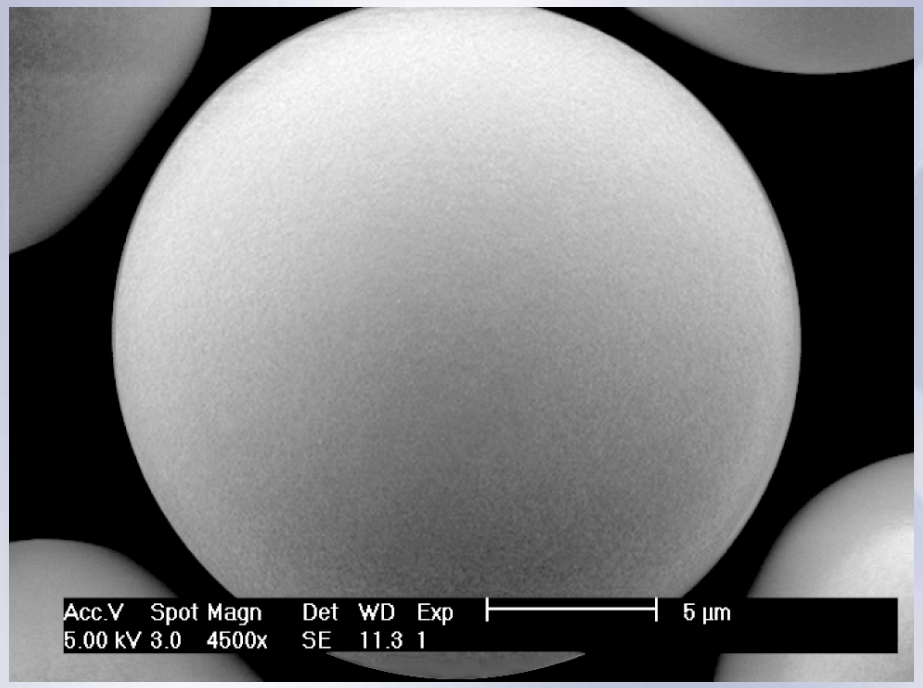
- Silica support

  ✓ **Mechanical properties/metal contaminants**

- Surface chemistry

  - Base deactivation
  - Retention and selectivity
  - pH-stability and selectivity
  - Stability in 100% aqueous eluents
  - Special selectivity features

Demands on high performance RP silicas



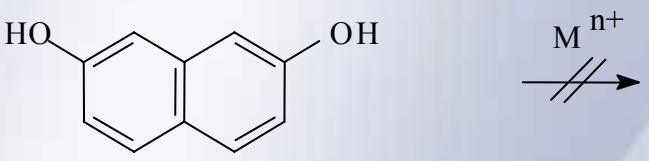
NUCLEODUR 100 Å

- **high level of purity**

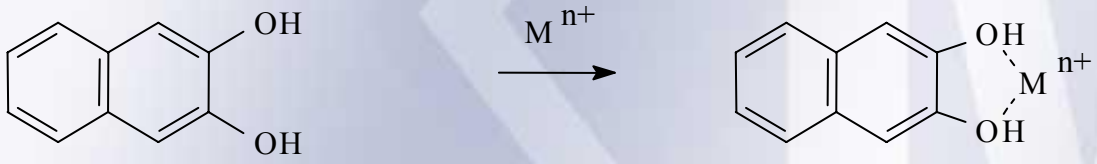
Al	< 5	ppm
Fe	< 5	ppm
Na	< 5	ppm
Ca	< 5	ppm
Ti	< 1	ppm
Zr	< 1	ppm
As	< 0.5	ppm
Hg	< 0.05	ppm

- **homogeneous surface for optimal coverage**

Determination of metal contaminants on the silica surface



2,7-Dihydroxynaphthalene (2,7-Dert)

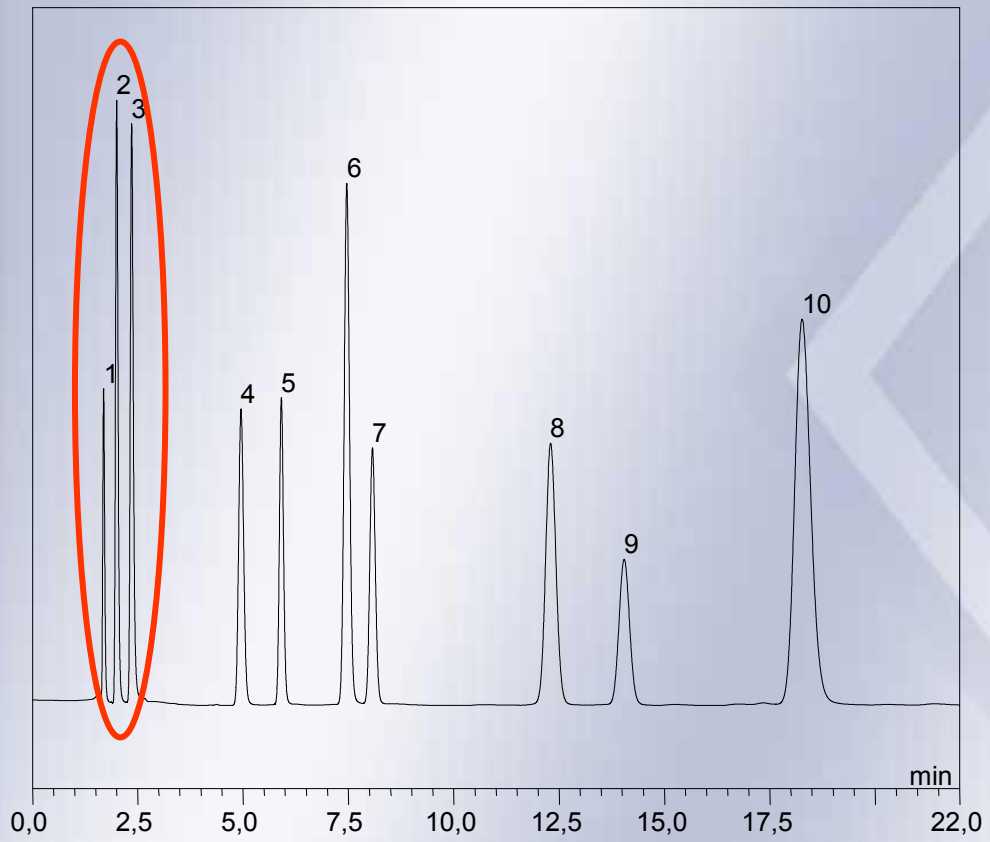


2,3-Dihydroxynaphthalene (2,3-Dert)

broad and
tailing peak

An indication of metal ions incorporated on the silica surface is the quotient As(2,3-Dert)/As(2,7-Dert)

Determination of metal contaminants on the silica surface



Column: EC 250/4 **Nucleodur** C18 Gravity,5 µm

Eluent: MeOH/ 20 mM KH_2PO_4, pH 7, 75:25 (v/v)

Temp.: 30°C

Flow: 1 ml/min

Detection: UV, 254 nm

1. Uracil
2. 2,7-Dihydroxynaphthalene
3. 2,3-Dihydroxynaphthalene
4. Lidocaine
5. Toluene
6. Naphthalene
7. Ethylbenzene
8. Dibuytphthalate
9. Acenaphthene
10. Amitriptyline

Stationary phase selection

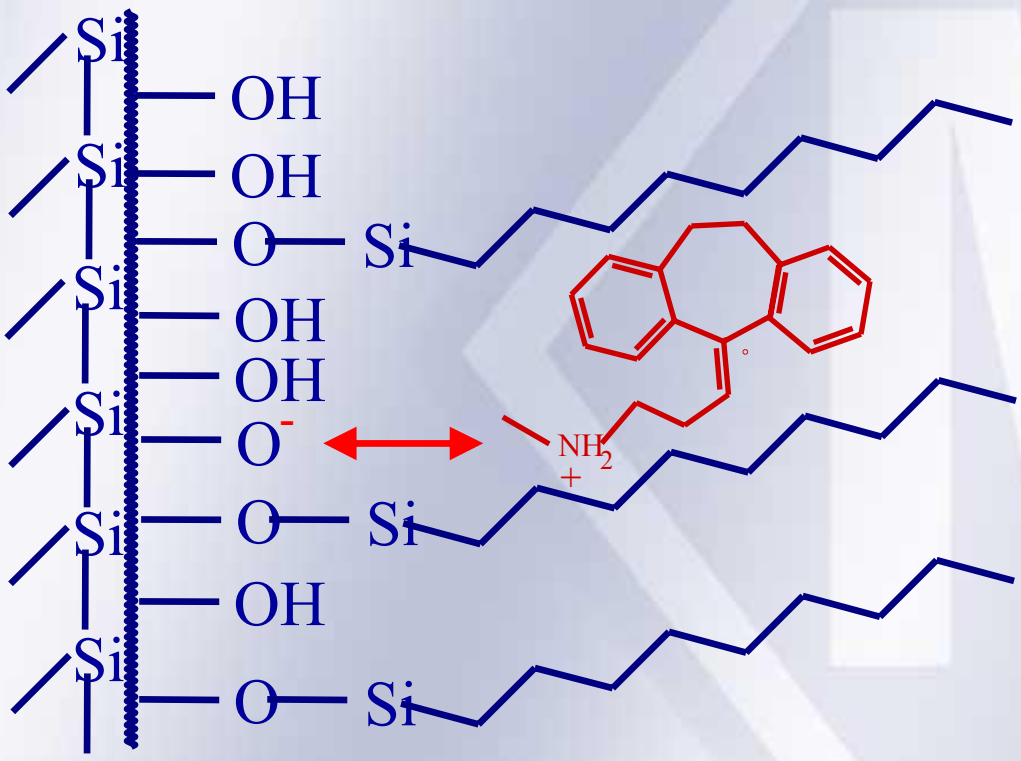
- Silica support

  ✓ Mechanical properties/metal contaminants

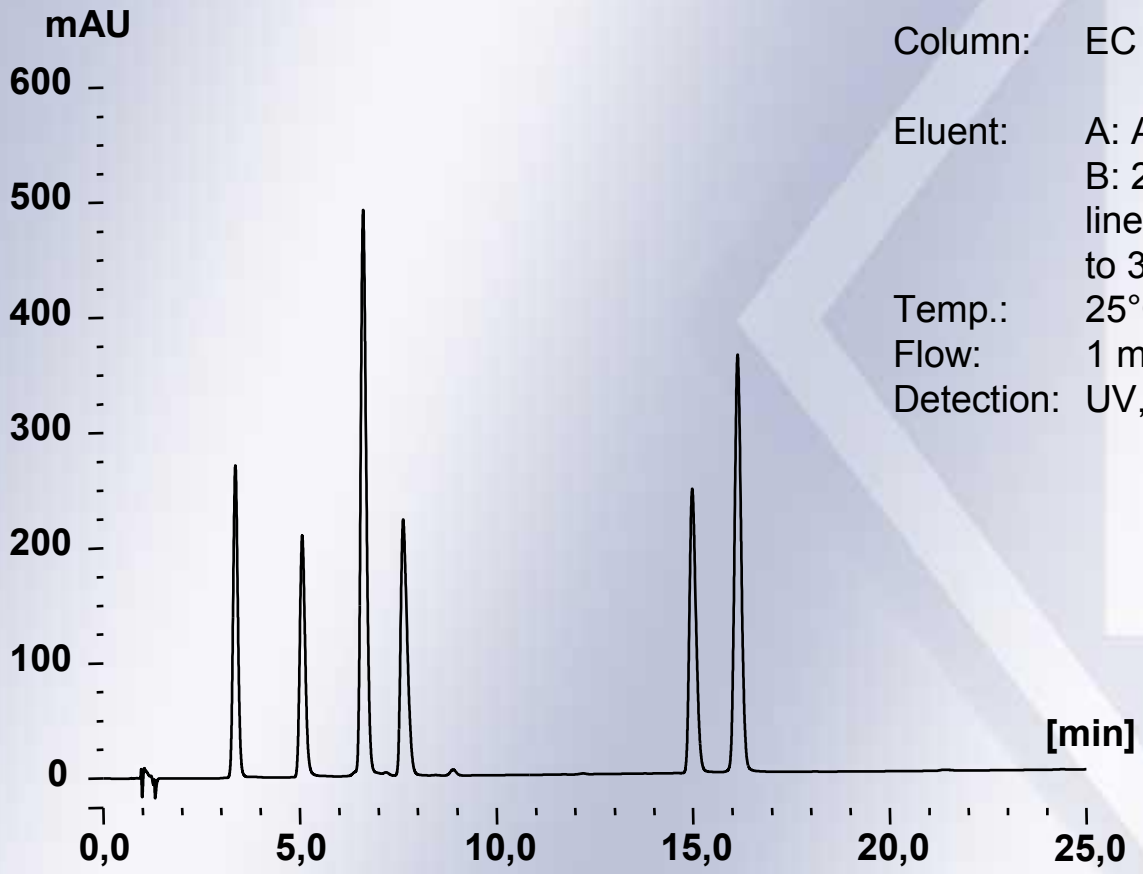
- Surface chemistry

  ✓ **Base deactivation**
  - Retention and selectivity
  - pH-stability and selectivity
  - Stability in 100% aqueous eluents
  - Special selectivity features

Retention of basic compounds



Alkaloids



Column: EC 125/4 Nucleodur **C18 Gravity**, 5 µm

Eluent: A: ACN
B: 20 mM KH_2PO_4, pH 2.5,
linear gradient from 10% A in 25 min
to 30% A

Temp.: 25°C

Flow: 1 ml/min

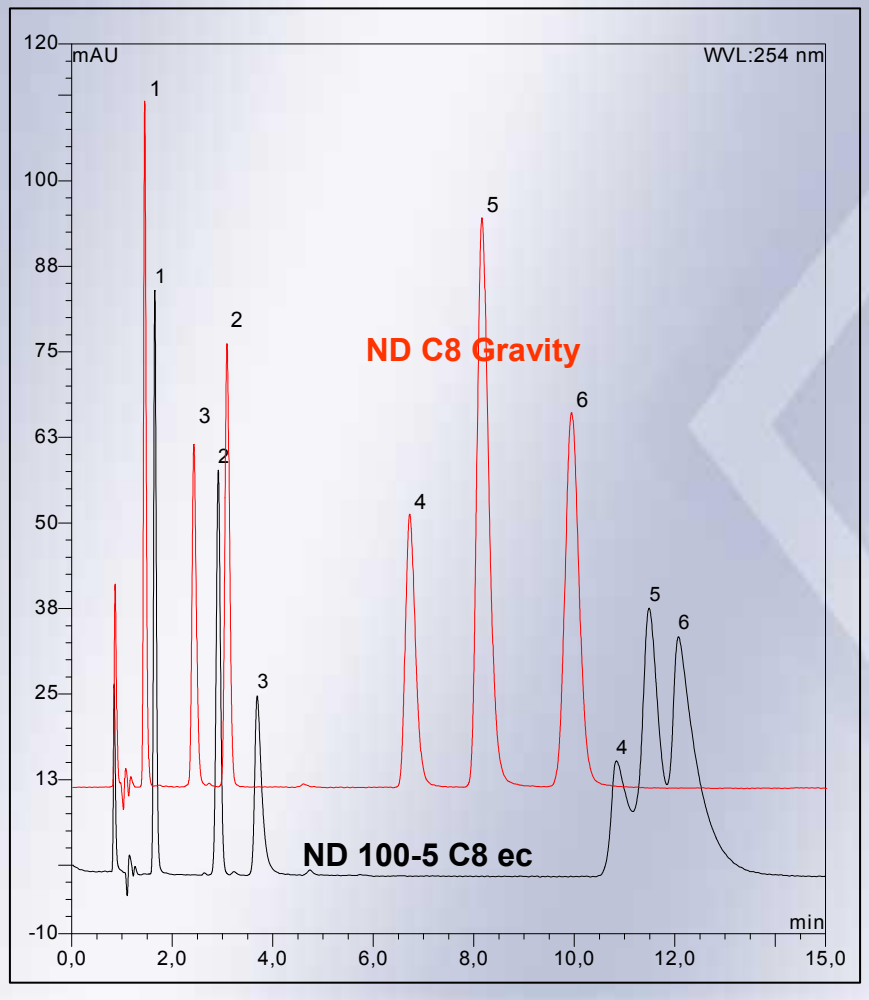
Detection: UV, 210 nm

1. Codeine
2. Quinine
3. Strychnine
4. Atropine
5. Papaverine
6. Noscapine

Stationary phase selection

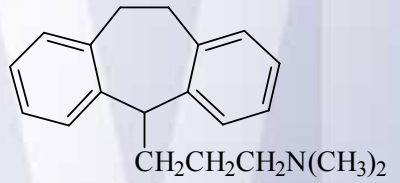
- Silica support
  - ✓ Mechanical properties/metal contaminants
- Surface chemistry
  - ✓ Base deactivation
  - ✓ **Retention and selectivity**
    - pH-stability and selectivity
    - Stability in 100% aqueous eluents
    - Special selectivity features

Selectivity of C8 phases with different silylation reagents



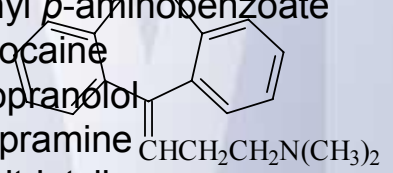
Column: EC 125/4 Nucleodur **C8 Gravity**, 5 µm
EC 125/4 Nucleodur 100-5 **C8 ec**
Eluent: MeOH/ 20 mM KH_2PO_4, pH 7, 70:30 (v/v)
Temp.: 30°C
Flow: 1 ml/min
Detection: UV, 254 nm

4



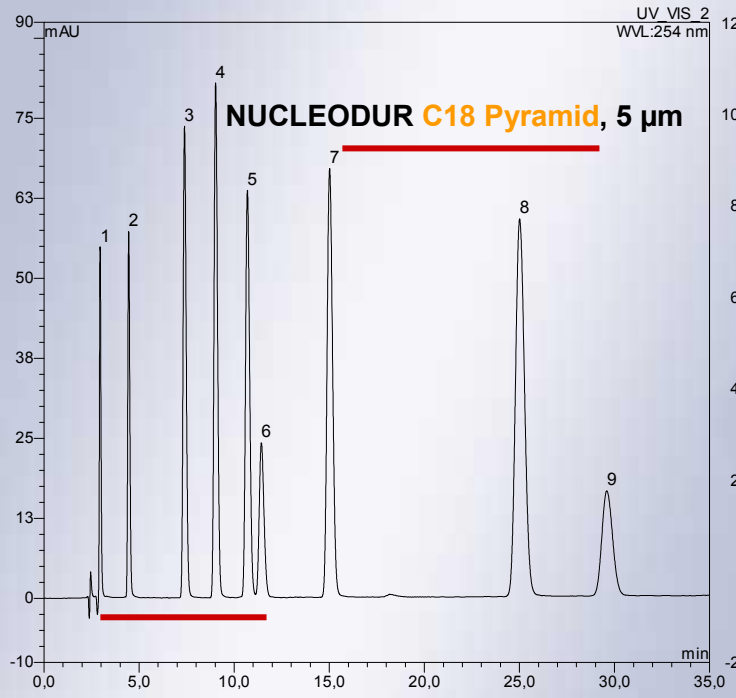
1. Ethyl *p*-aminobenzoate
2. Lidocaine
3. Propranolol
4. Imipramine
5. Amitriptyline
6. Trimipramine

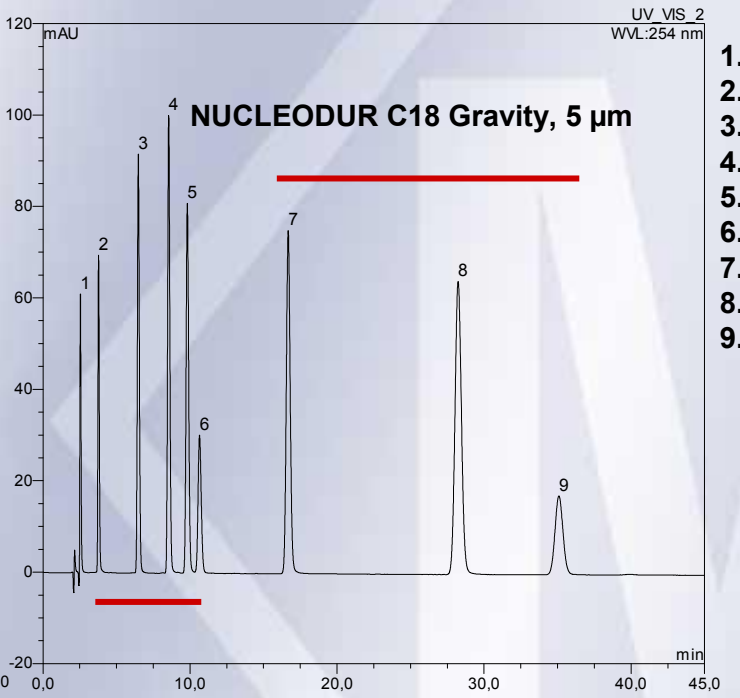
5



6

Polar and non-polar selectivity of C18 phases with different kind of endcapping





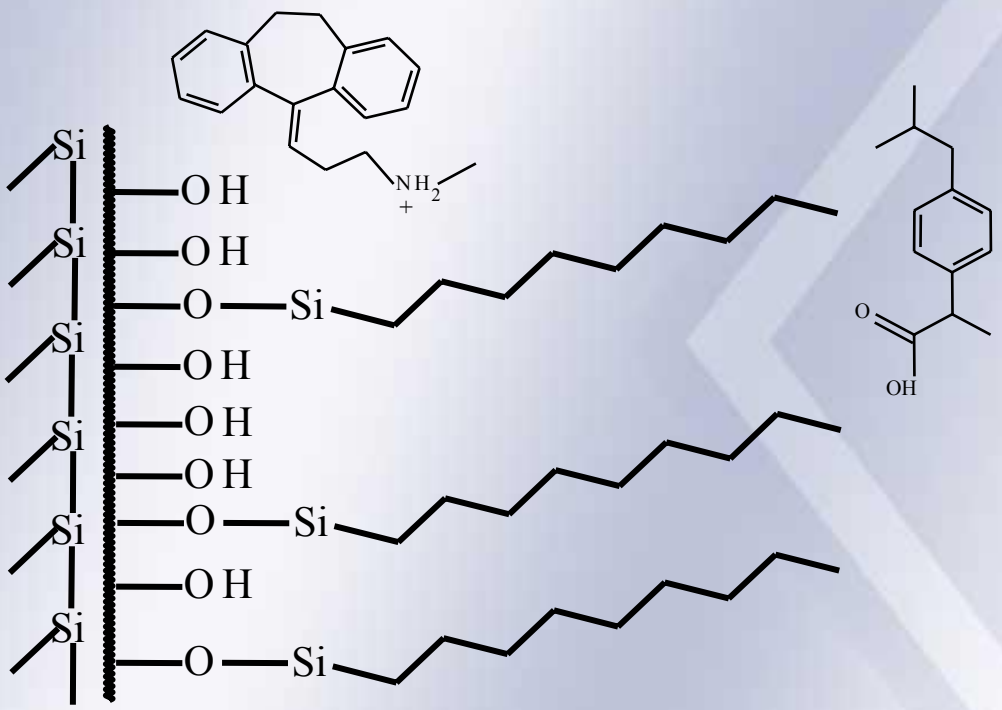
1. Chlorphenyramine
2. Dimethylphthalate
3. Benzamide
4. Ethylbenzoate
5. Benzophenone
6. Lidocaine
7. Naphthalene
8. Biphenyl
9. Acenaphthene

Column:	250 x4 mm
Eluent:	MeOH/25 mM $NH_4H_2PO_4$; pH 7 65:35 (v/v)
Flow rate:	0.8 ml/min
Temp.:	40 °C
Detection:	UV, 254 nm
Inj. Vol.:	5 µl

Stationary phase selection

- Silica support
  - ✓ Mechanical properties/metal contaminants
- Surface chemistry
  - ✓ Base deactivation
  - ✓ Retention and selectivity
  - **✓ pH-stability and selectivity**
    - Stability in 100% aqueous eluents
    - Special selectivity features

Retention of ionizable analytes in correlation with pH

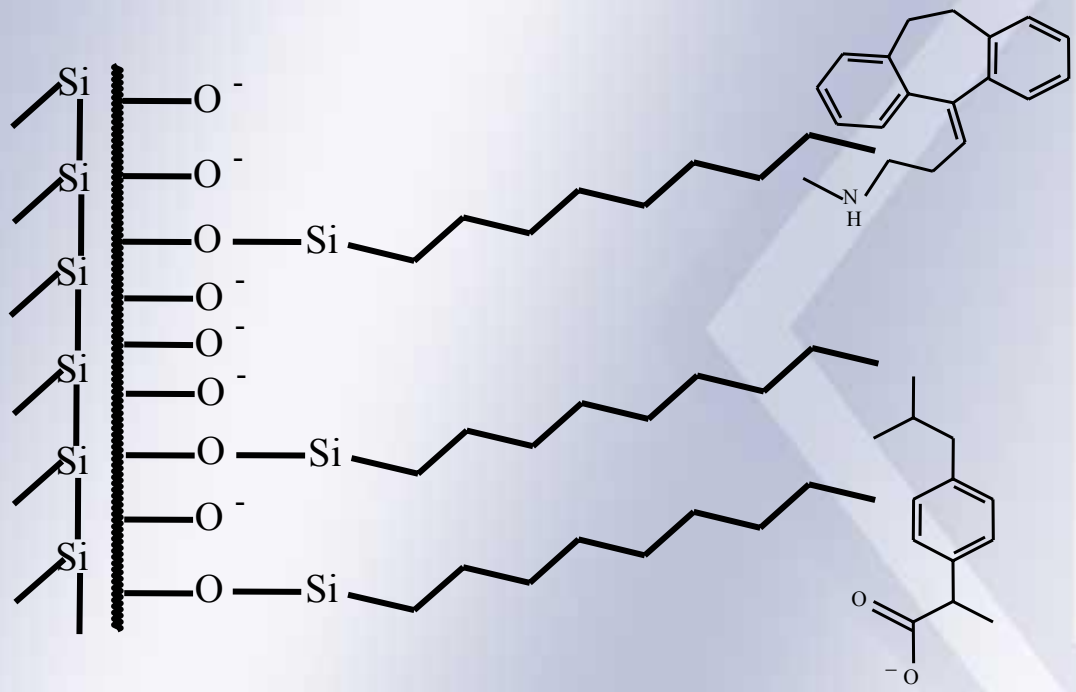


pH << 7

- surface silanols protonated
- basic analytes ionized
- poor retention for amino compounds
- acidic analytes neutrally charged
- good retention for acid compounds

-> Cleavage of the siloxane bond by hydrolysis!

Retention of ionizable analytes in correlation with pH

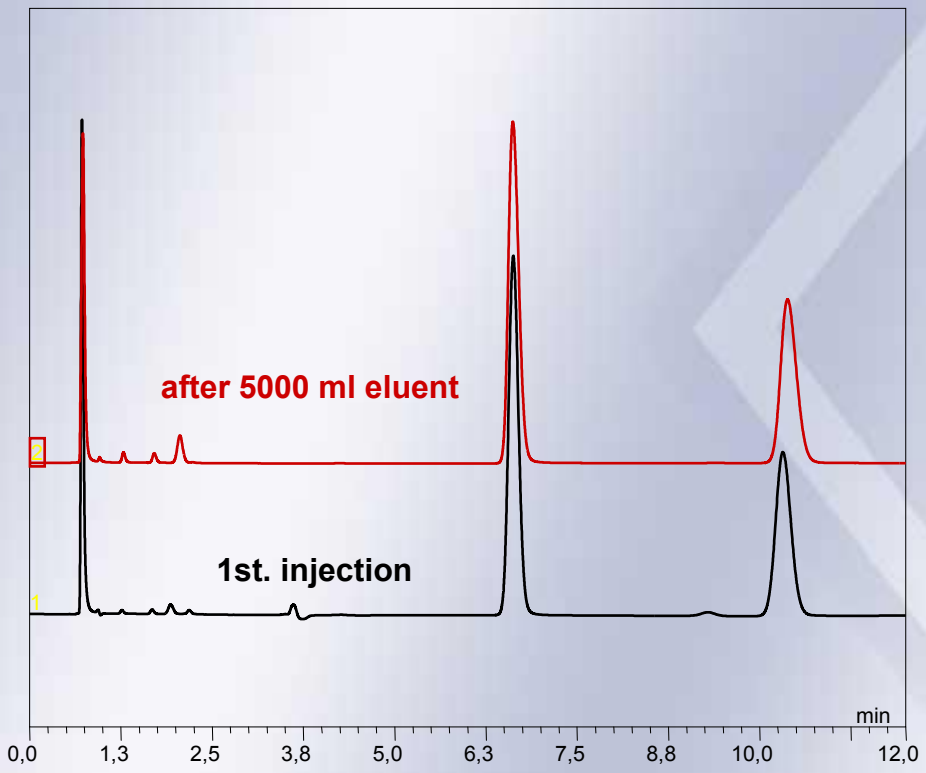


pH >> 7

- deprotonation of surface silanols
- basic compounds neutrally charged
- sufficient retention of amines
- acidic compounds ionized
- poor retention of acids

-> Attack on the silica support and gradual dissolution of the silica!

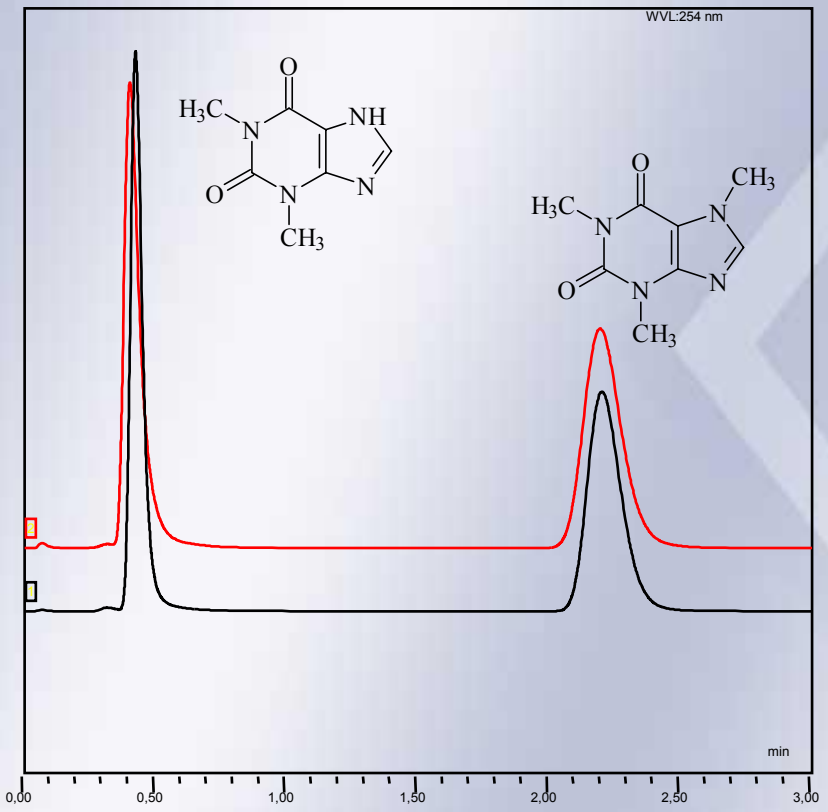
Stability at acidic pH



Column: EC 125/4 Nucleodur **C18 Gravity**, 5 µm
Eluent: Acetonitrile
1% TFA in water
50:50 (v/v), **pH 1.5**
Flow: 1 ml/min
Temp.: 30 °C
Detection: UV, 230 nm

Sample: Pyridine
Toluene
Ethylbenzene

RP 18 phases under basic pH



Column: EC 50/4.6 Nucleodur **C18 Gravity**, 5 µm

Eluent: $MeOH/H_2O/NH_3$
20:80:0,5 (v/v/v), **pH 11**

Temp.: 30°C

Flow: 1.3 ml/min

Detection: UV, 254 nm

Sample: Theophylline
Caffeine

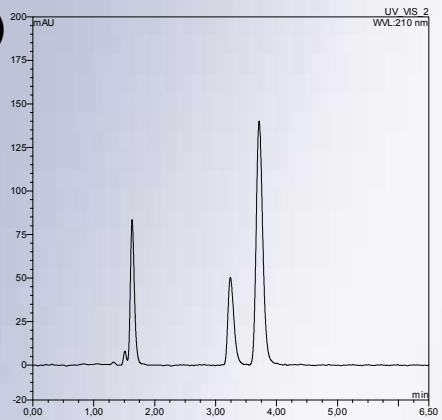
1. first injection
2. After 300 injections

Stationary phase selection

- Silica support
  - ✓ Mechanical properties/metal contaminants
- Surface chemistry
  - ✓ Base deactivation
  - ✓ Retention and selectivity
  - ✓ pH-stability and selectivity
  - ✓ **Stability in 100% aqueous eluents**
  - Special selectivity features

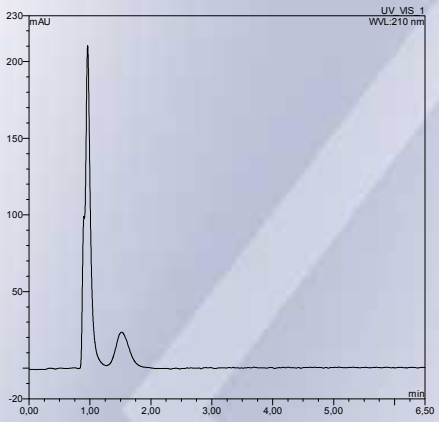
Stability test in 100% aqueous eluents

a)



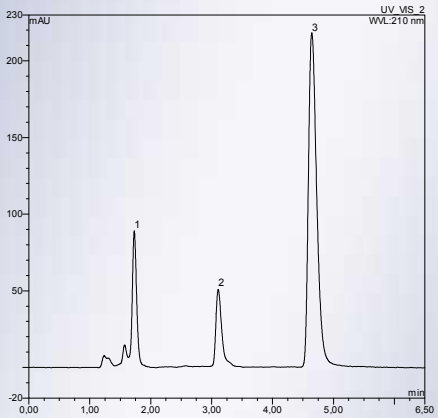
→ **initial injection**

pump stopped!



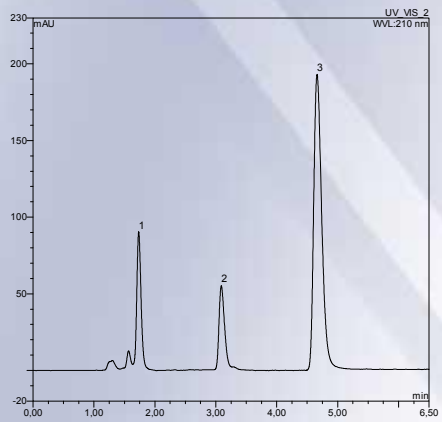
→ **restart after 5 min**

b)



→ **initial injection**

pump stopped!



→ **restart after 12 h**

Adsorbent: a) **Conventional RP 18 column**

b) NUCLEODUR **C18 Pyramid**

Column:	125 x 4 mm
Eluent:	50 mM KH_2PO_4, pH 2.5
Flow rate:	0.7 ml/min
Temp.:	25 °C
Detection:	UV, 210 nm
Inj. Vol.:	1 µl

1. Tartaric acid
2. Acetic acid
3. Maleic acid

- good stability in aqueous eluents
- similar selectivity features to RP18
- apparently lower bleeding characteristics (e.g. for MS detection)

Stationary phase selection

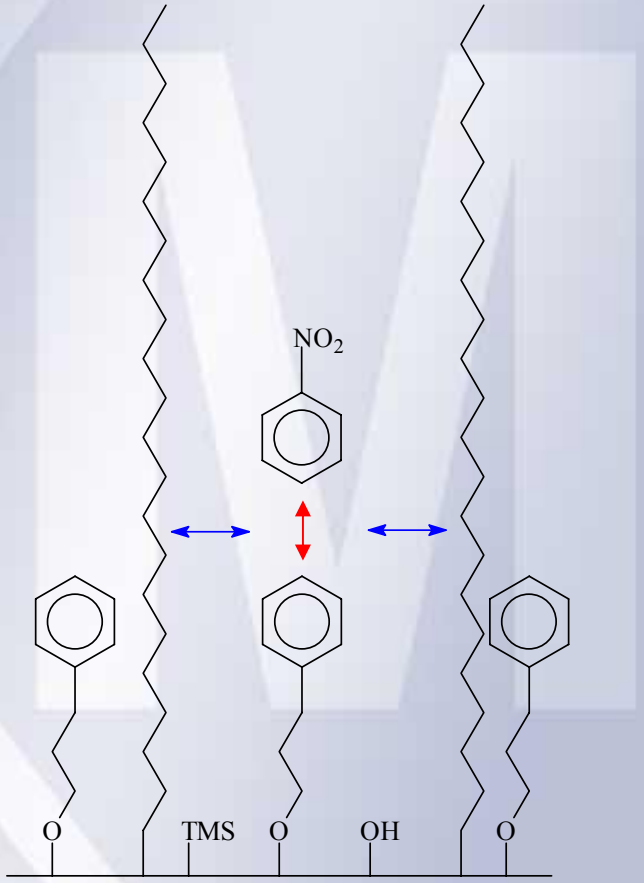
- Silica support
  - ✓ Mechanical properties/metal contaminants
- Surface chemistry
  - ✓ Base deactivation
  - ✓ Retention and selectivity
  - ✓ pH-stability and selectivity
  - ✓ Stability in 100% aqueous eluents
  - **✓Special selectivity features**

Phenyl Octadecyl phase

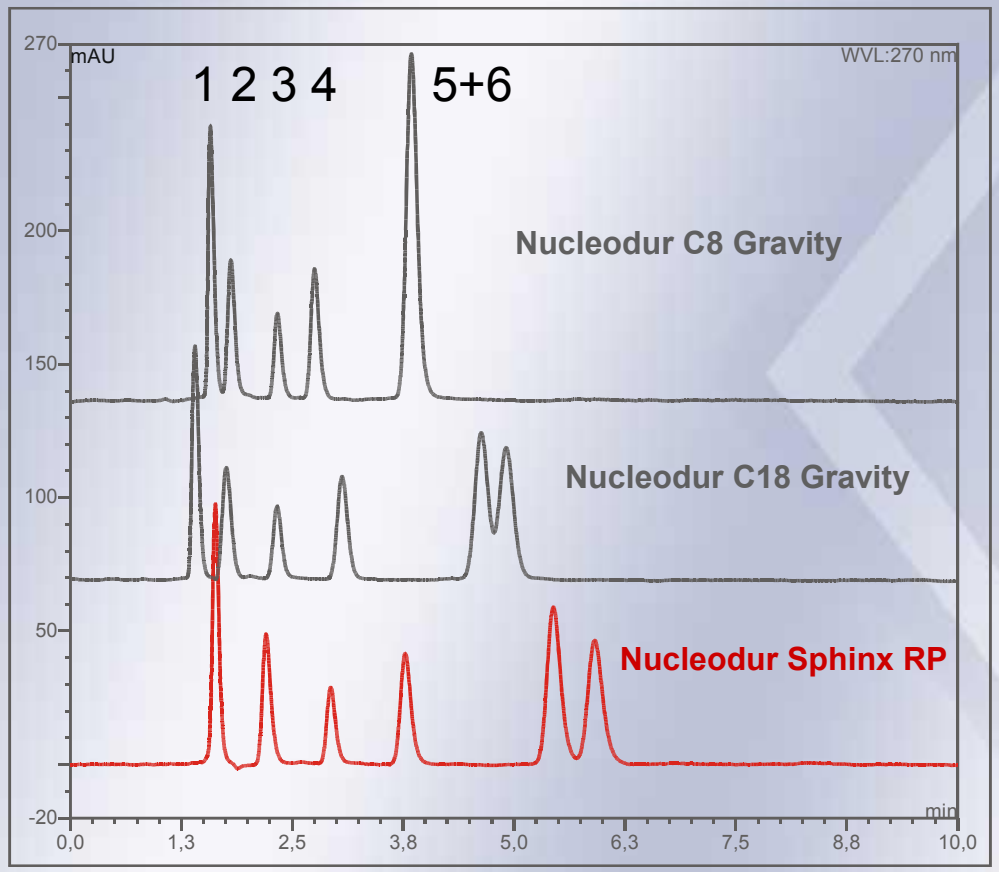
Unique Concept of
Nucleodur Sphinx RP !

Optimized proportion of octadecyl- and phenylpropylsilanes

Hydrophobic and π- π interactions combined



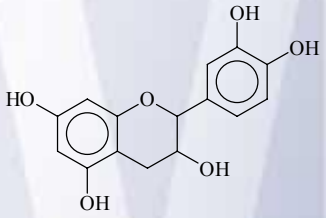
Separation of Flavonoids



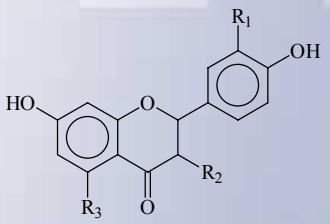
Chromatographic conditions:

150/4.6 mm Nucleodur **Sphinx RP**, 5µm
methanol/water 40:60 (v/v)
1 ml/min, UV, 270 nm, 30 °C

1. Catechin



2. Rutin $R_1 = R_3 = OH$, R_2 = O-Rutinose
3. Fisetin $R_1 = R_2 = OH$, $R_3 = H$
4. Quercetin $R_1 = R_2 = R_3 = OH$
5. Kaempferol $R_1 = H$, $R_2 = R_3 = OH$
6. Isorhamnetin R_1= OMe, $R_2 = R_3 = OH$



C18 phase with steric selectivity

- Steric selectivity is the ability to separate compounds in relation to its moleculare structure and geometry.
- Compounds with rigid structure show a steric behaviour:

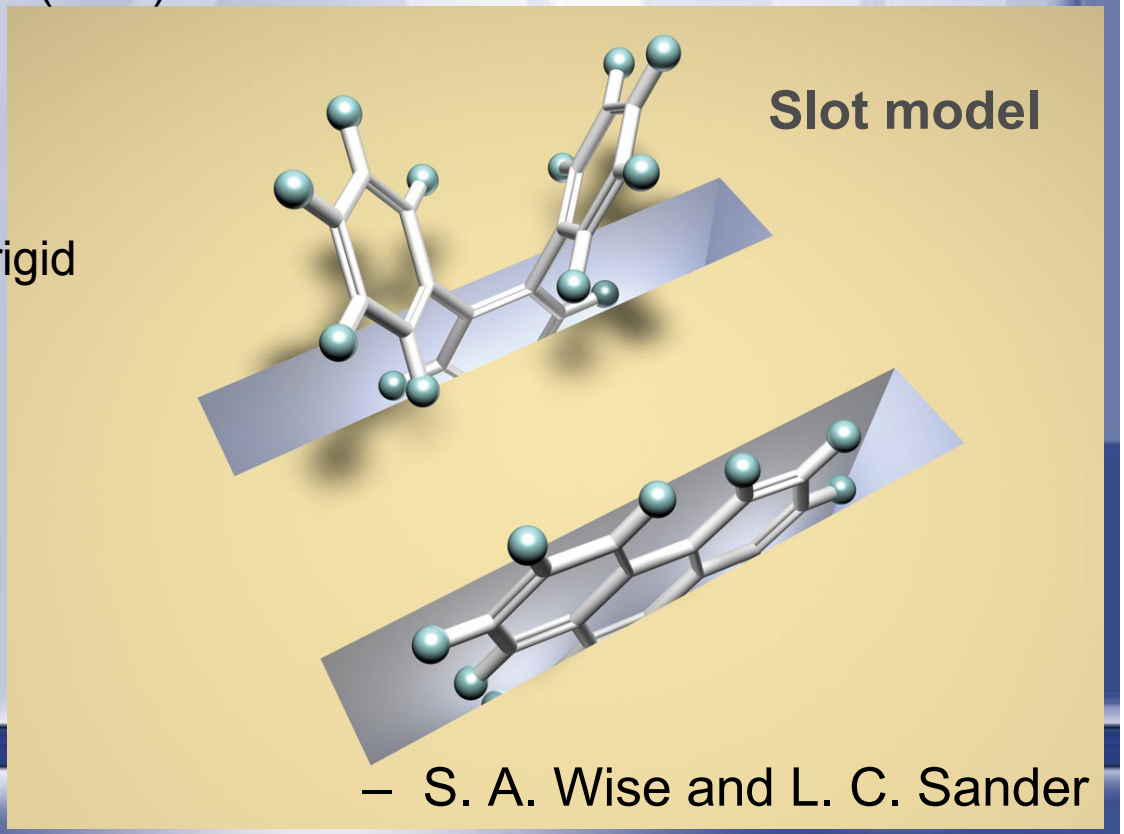
e.g.: polycyclic compounds (PCB)

steroids

carotinoides

vitamines (D2/D3)

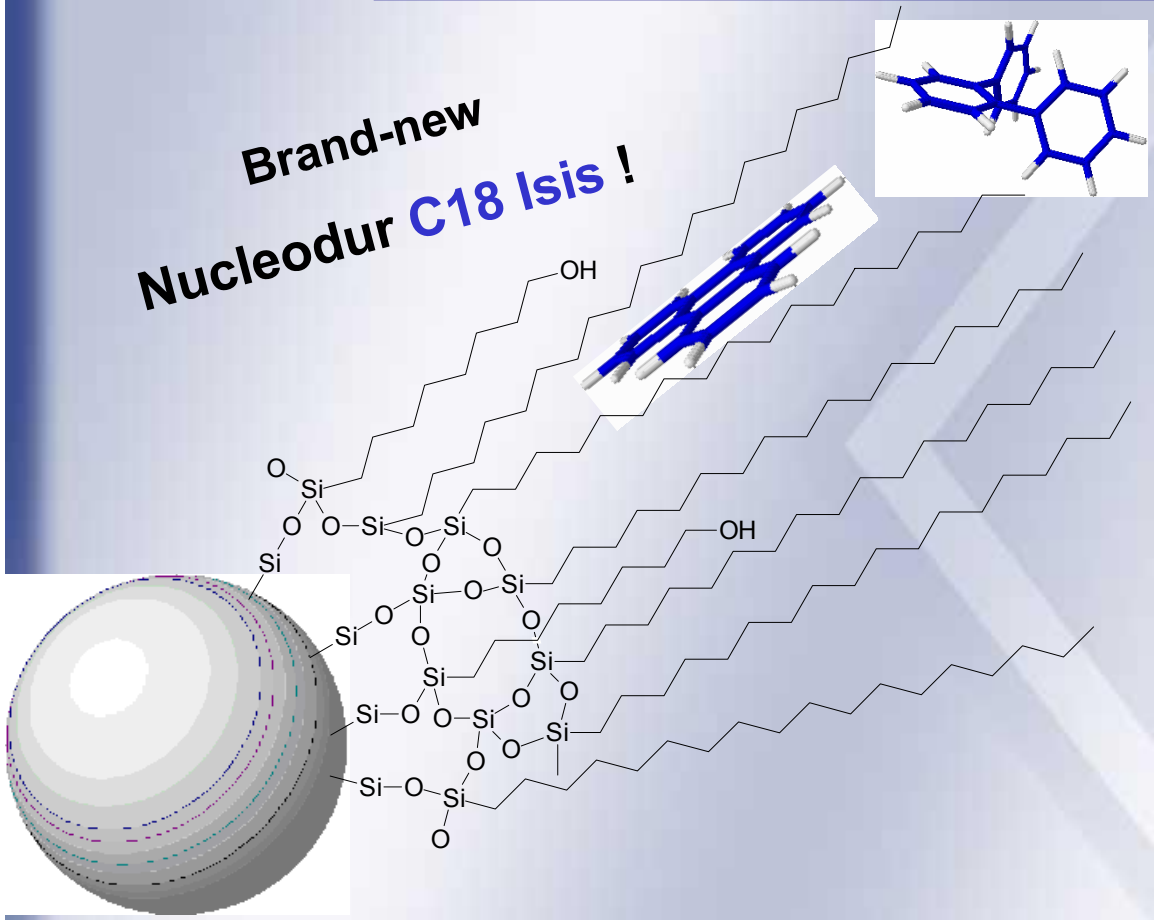
aliphatic compounds with rigid structure (double bonding)

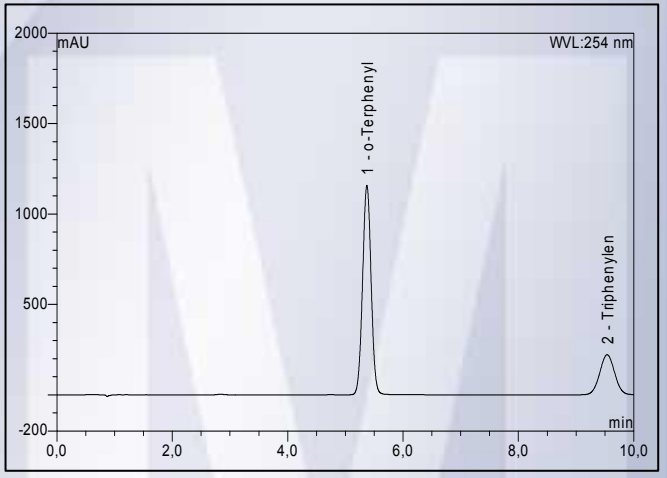


C18 phase with steric selectivity

Brand-new

Nucleodur C18 Isis !



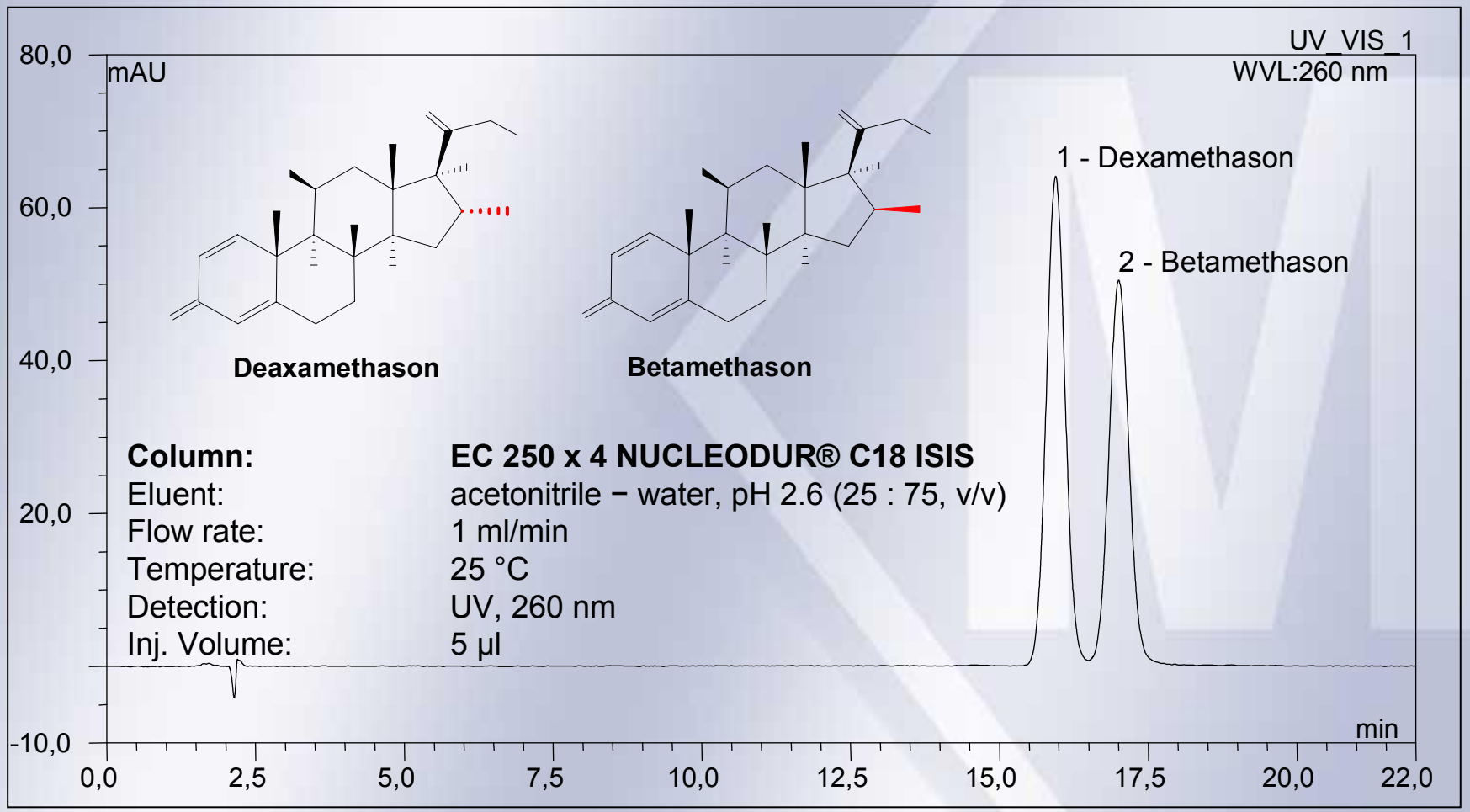


$\alpha_{Triphenylene/o\text{-}Terphenyl}$ **= 1,93 !**

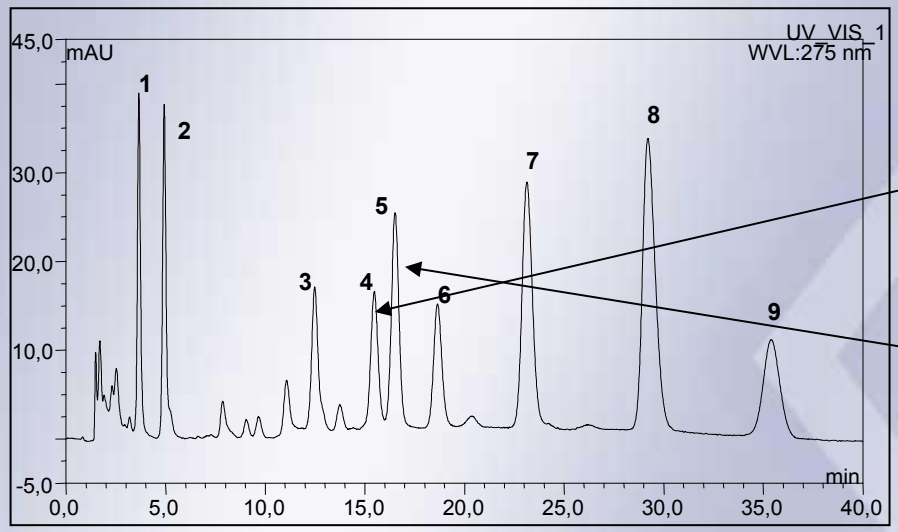
column: 125 x 4 NUCLEODUR **C18 Isis**
eluent: methanol / water (80:20)
flow rate: 1 ml/min
temp.: 40 °C
detection: UV, 254 nm

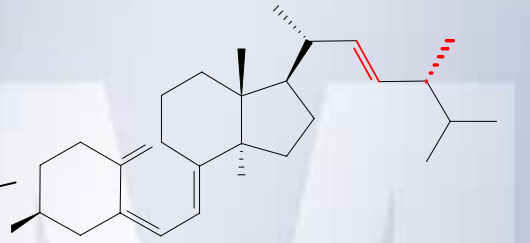
For <u>standard</u> RP 18 phases:
$\alpha_{Triphenylene/o\text{-}Terphenyl}$ **= 1,0 - 1,2**

Dexamethason / Betamethason

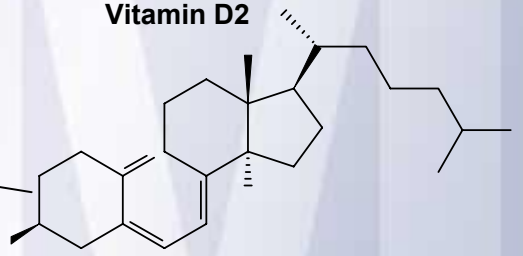


Fat-soluble vitamins





Vitamin D2



Vitamin D3

Column:	**EC 125 x 2 mm NUCLEODUR® C18 ISIS**
Eluent:	acetonitrile / water (100:5, v/v)
Flow rate:	0.2 ml/min
Temperature:	25 °C
Detection:	UV, 275 nm
Inj. Volume:	5 µl

1) **Vitamin A**
2) **Vitamin A acetate**
3) **Vitamin K2**
4) **Vitamin D2**
5) **Vitamin D3**
6) **γ-Toccopherol**
7) **α-Toccopherol**
8) **α-Toccopherol acetate**
9) **Vitamin K1**

For more information and applications: