# P O P L C®

## Phase Optimized Liquid Chromatography

***The New Way***
***to Speed Up H P L C***

*Yuri Kalambet, Yuri Kozmin,Klaus Bischoff, Stefan Lamotte, Rainer Brindle*

BISCHOFF CHROMATOGRAPHY

# Outline

- Motivation
- Method Development in HPLC
- **P**hase **OP**timized **L**iquid **C**hromatography (**POPLC®**)
- The POPLC® Optimizer Software
- Examples
- Summary

# RP-HPLC

## Situation

- more than 800 RP packings are commercially available today
- How to find the right packing for my separation?

# Properties of RP Packings

- **Hydrophobicity**
- **Silanophilic Activity**
- **Molecular Planarity Recognition (“Shape Selectivity”)**
- **Polar Selectivity**
- **Metal Content**

# Selectivity in RP HPLC

- all modern classical bonded RP packings are looking the same in terms of selectivity
- the stationary phases are optimized to solve as much applications as possible and are suited for about 80% of all applications today
- new stationary phases with other selectivities are needed to solve the remaining separation problems

# Method Development in HPLC

## Procedure

- rough choice of the column (C18, polar embedded C18, Phenyl, etc.)

- Optimization of the mobile phase (pH, solvent strength, if necessary gradient, type of organic solvent, buffer)

- Optimization of temperature

# What is POPLC®?

**P**hase **O** **P**timized **L**iquid **C**hromatography®

**P**ersonal

**P**erformance

# Why POPLC®?

- POPLC® leads to the ultimate best column for each separation

  Benefit: no longer „Trial & Error“

- In many cases no gradient elution is required

  Benefit: possible use of all detectors (Conductivity, RI, Electrochemical Detector), constant Ionization in LC/MS, easy method transfer

- modular POPLink® - column system decreases follow up costs

  Benefit: economical and ecological

- POPLC® Optimizer Software is an excellent tool for Optimization, understanding and documentation of HPLC separations

  Benefit: adaptation of separation task is easy

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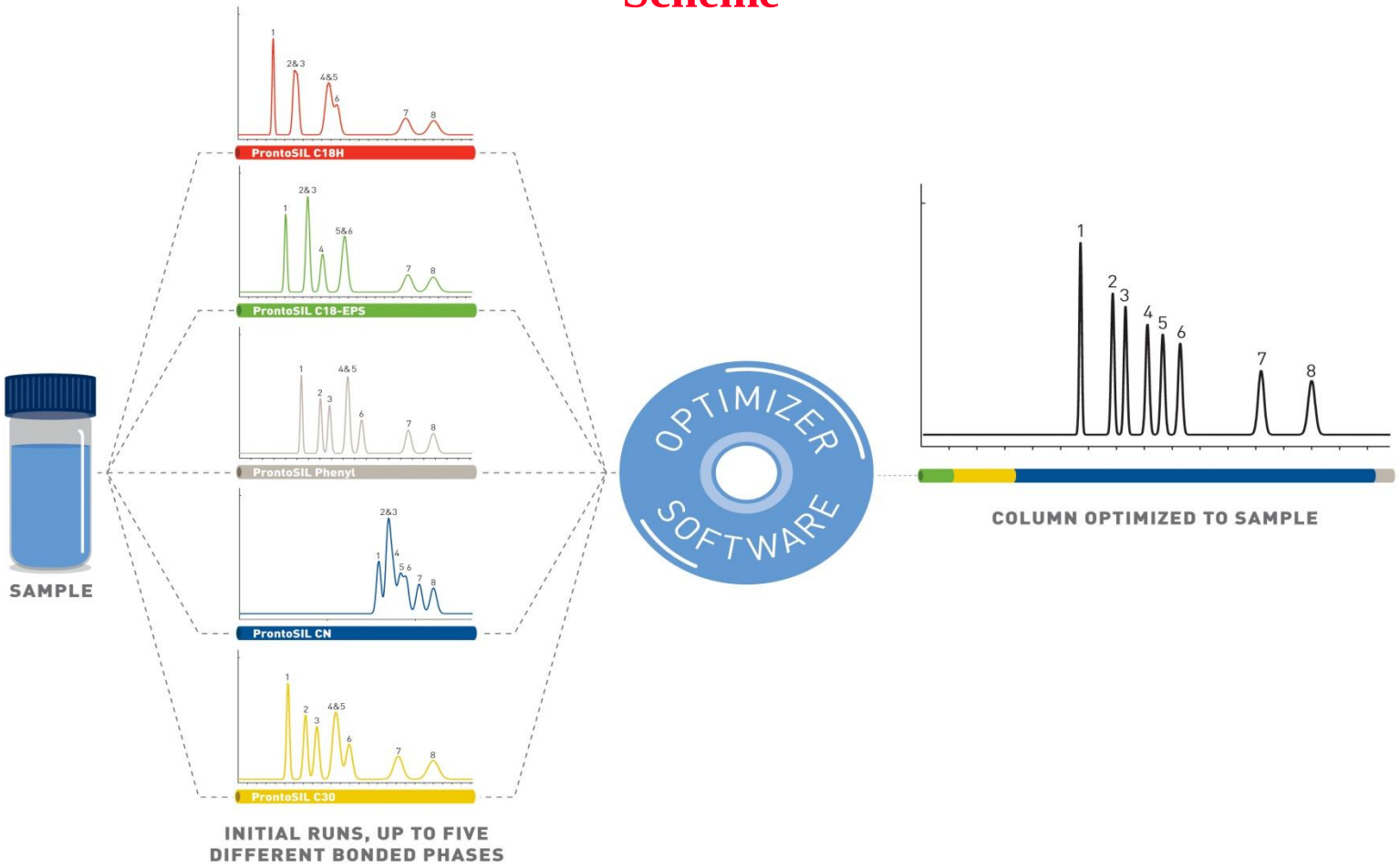
# Method Development in POPLC®

## Scheme

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# Method Development in POPLC®

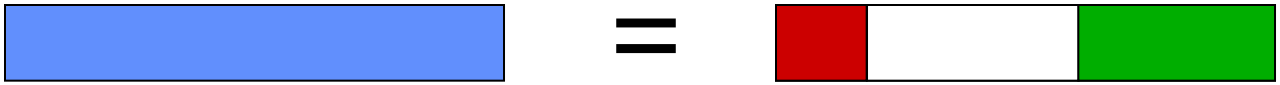
## Procedure

- rough choice of mobile phase (% organic, type, pH)
- one base measurement on each of n (often 3 to 5) different stationary phases
- Determination (optimization) of the ideal stationary phase via computer software

# Simple Principal of POPLC®

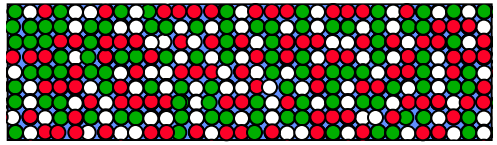
**"Retention times are additive !!!"**

$$t_{R_{total}} = t_{R_A} + t_{R_B} + t_{R_C}$$



# Possibilities of Realization for POPLC®

1. **Mixing of Stationary Phases**



# Possibilities of Realization for POPLC®

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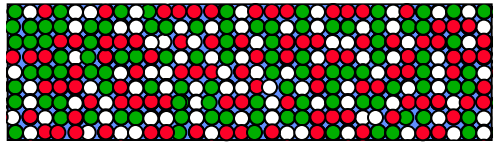
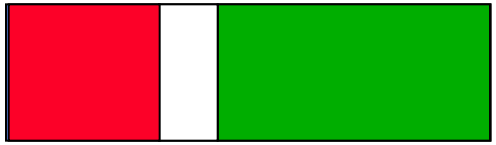


2. **Combination of Differnent Column Lengths**



# Available Column Cartridges

**Dimensions:** **ID 3.0 mm**

**Lengths: 10, 20, 40, 60, 80 mm**

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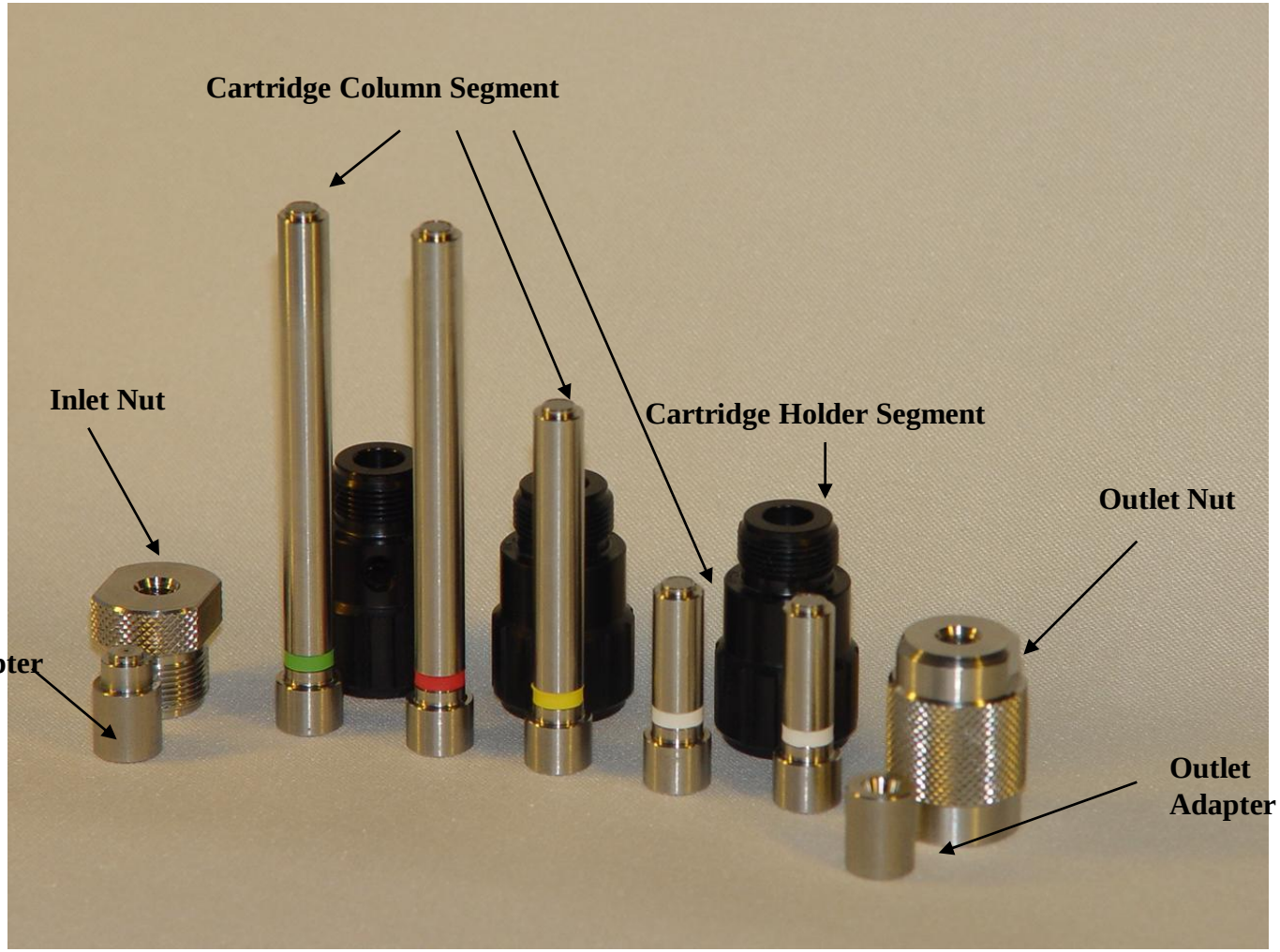


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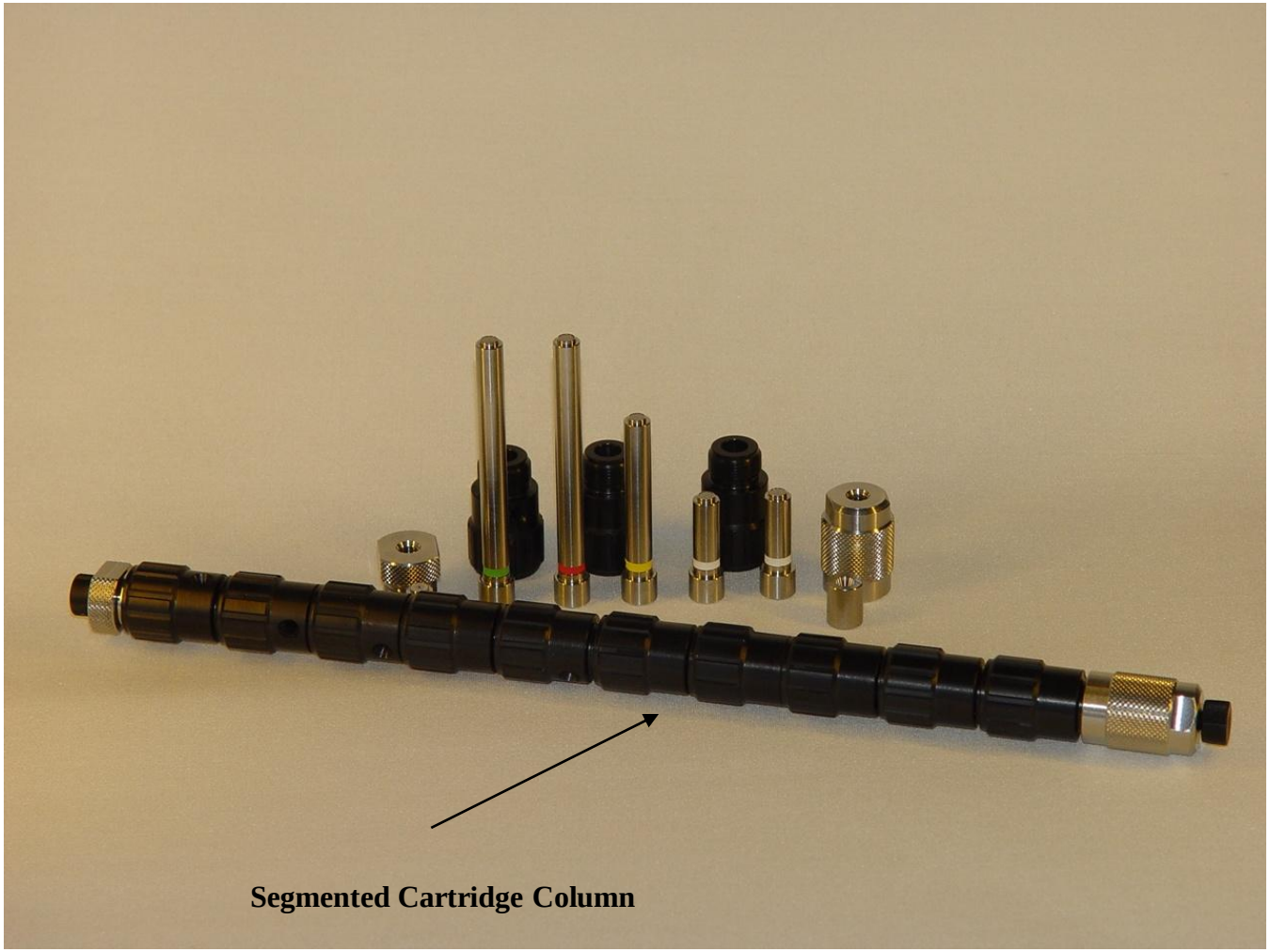

**Segmented Cartridge Column**

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# Colour coded Column cartridges

# Segmented Cartridge Column

## One Column Fits All

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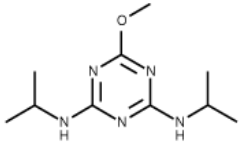
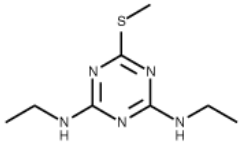
# Triazine Pesticides

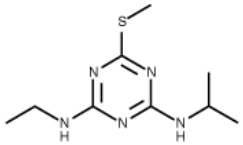
## Chemical structures



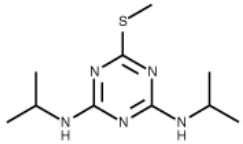
**(1) Prometon**



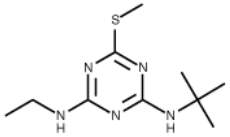
**(2) Simetryn**



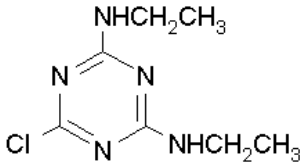
**(3) Ametryn**



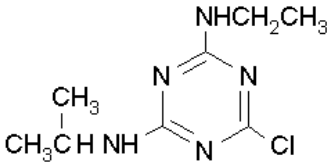
**(4) Prometryn**



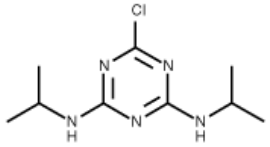
**(5) Terbutryn**




**(6) Simazin**

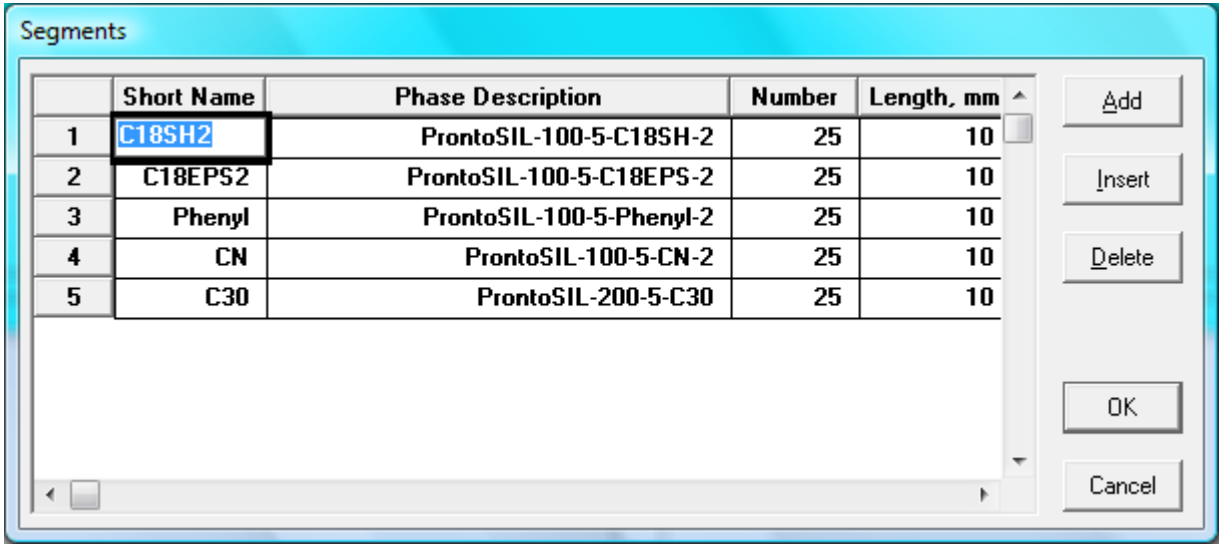


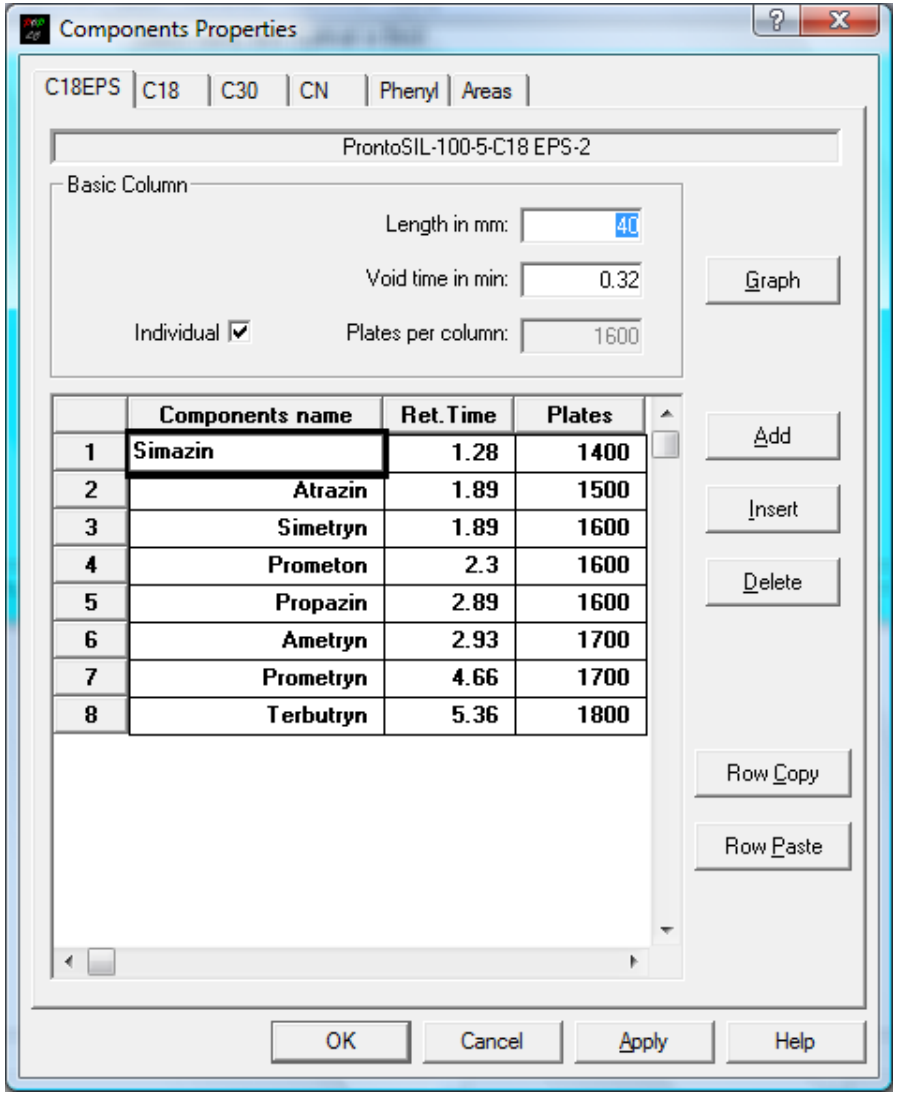

**(7) Atrazine**



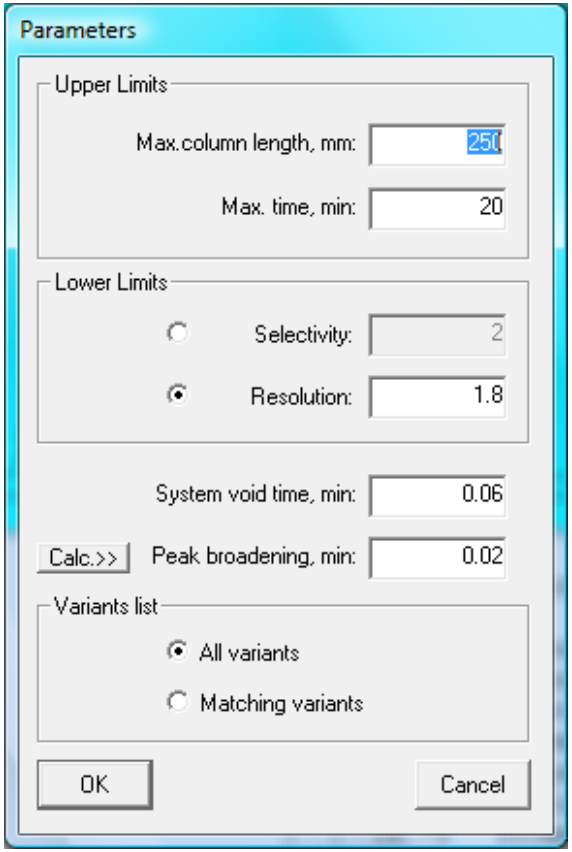

**(8) Propazine**

# List of column segments





# Optimization parameters

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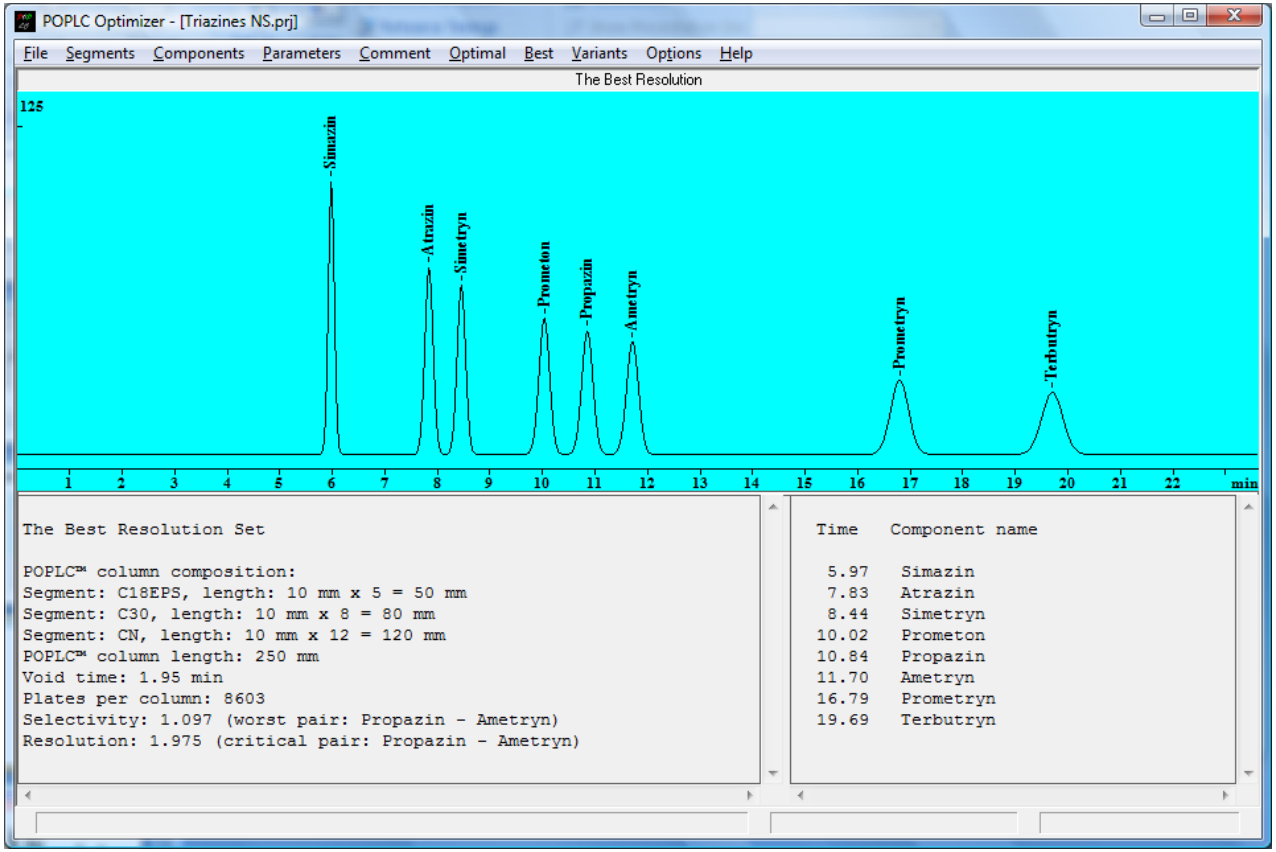
# Best variant

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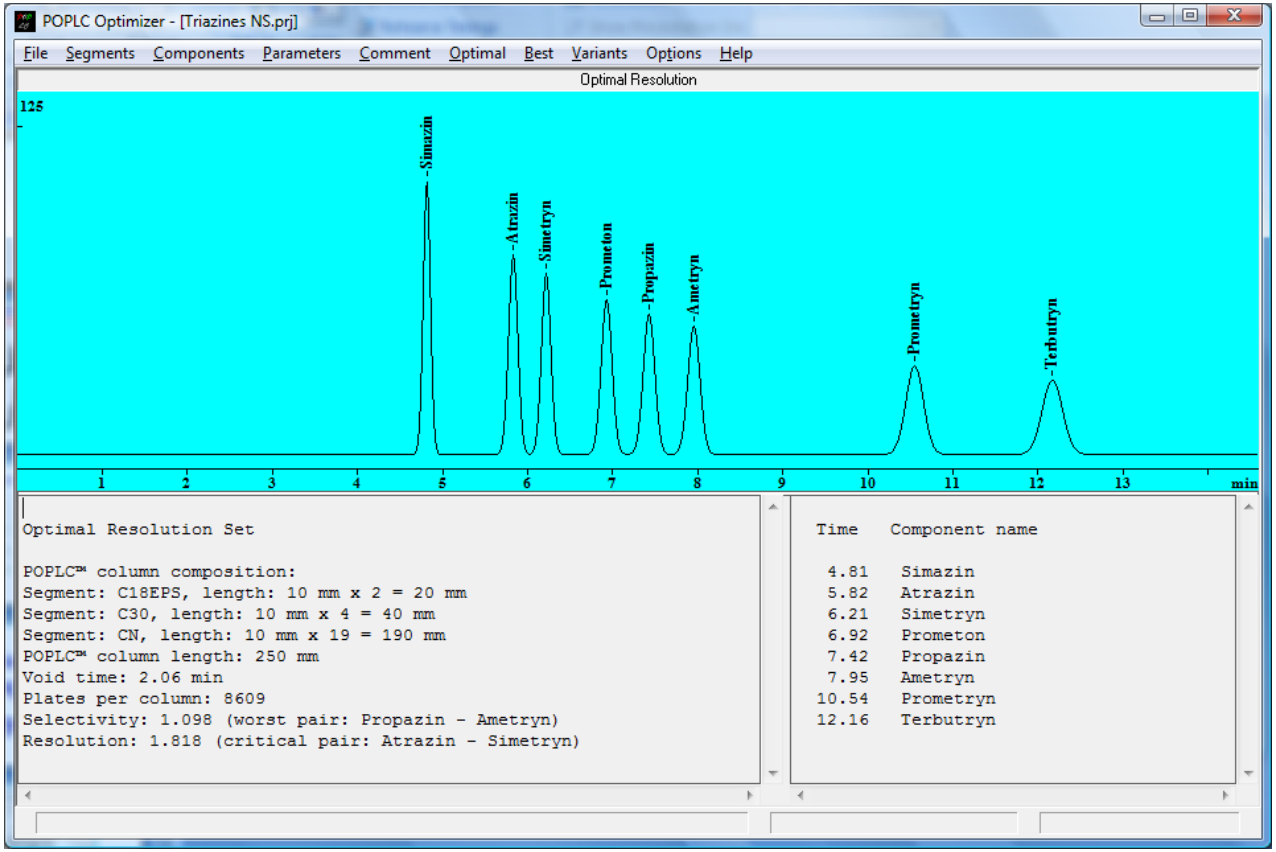
# Fastest (Optimal) variant

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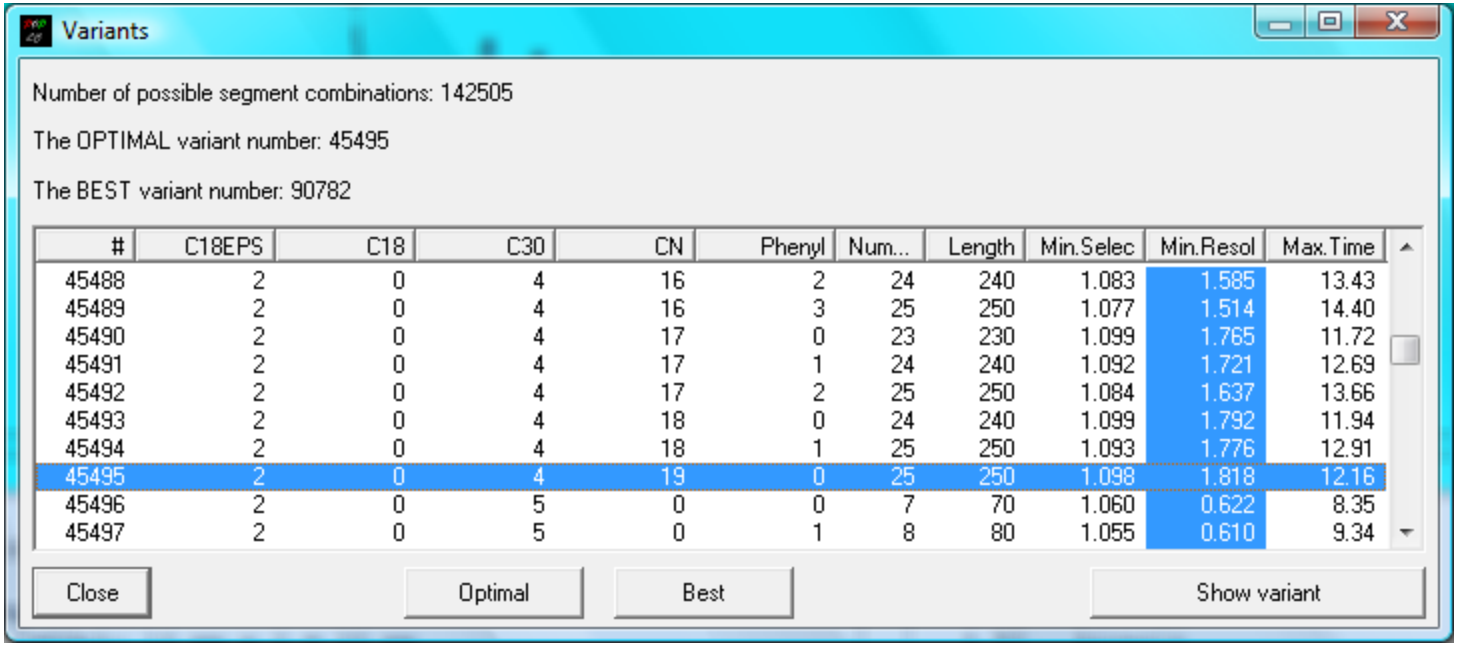
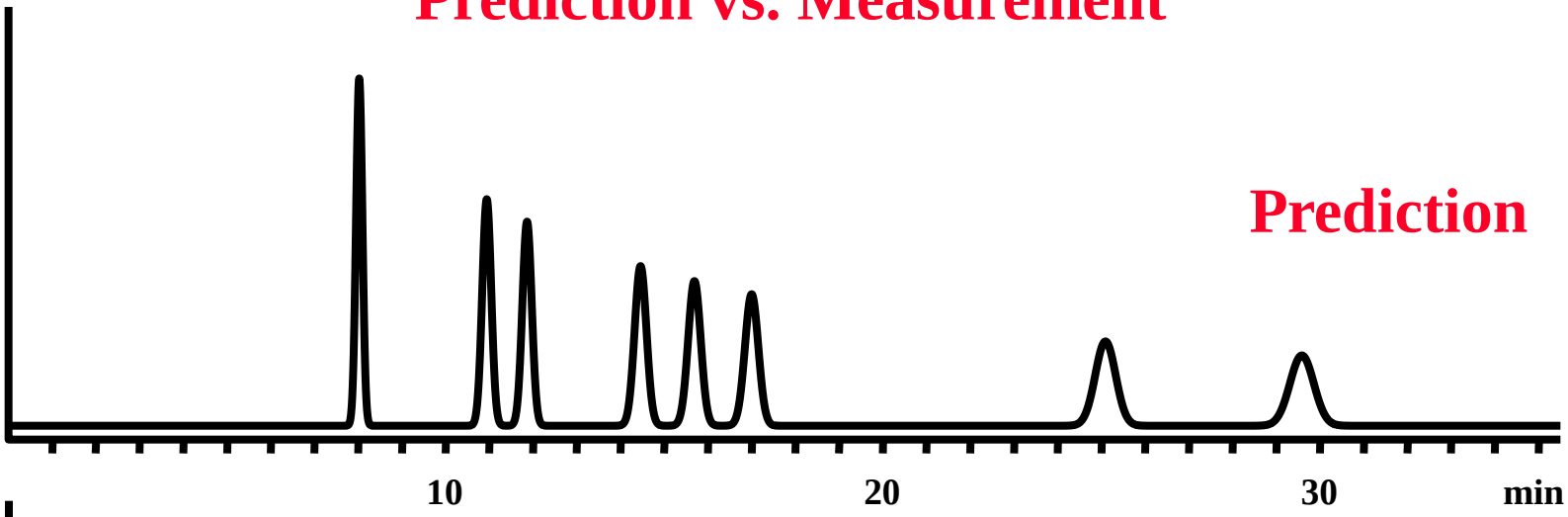
# Variants list

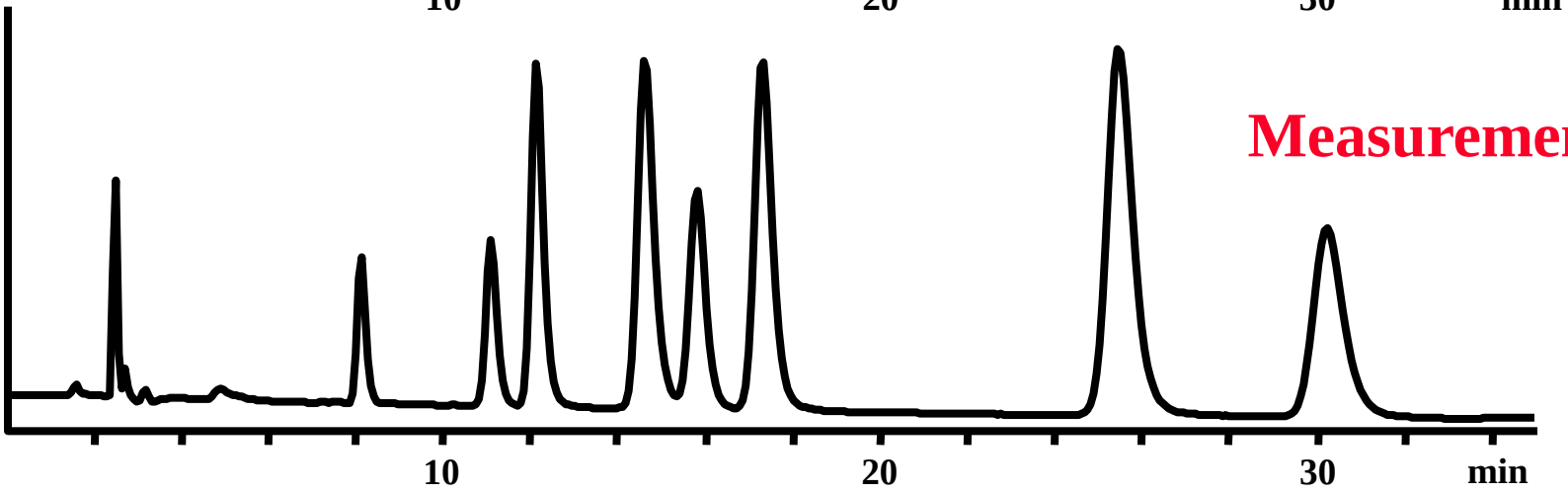



| # | C18EPS | C18 | C30 | CN | Phenyl | Num... | Length | Min.Selec | Min.Resol | Max.Time |
|---|---|---|---|---|---|---|---|---|---|---|
| 45488 | 2 | 0 | 4 | 16 | 2 | 24 | 240 | 1.083 | 1.585 | 13.43 |
| 45489 | 2 | 0 | 4 | 16 | 3 | 25 | 250 | 1.077 | 1.514 | 14.40 |
| 45490 | 2 | 0 | 4 | 17 | 0 | 23 | 230 | 1.099 | 1.765 | 11.72 |
| 45491 | 2 | 0 | 4 | 17 | 1 | 24 | 240 | 1.092 | 1.721 | 12.69 |
| 45492 | 2 | 0 | 4 | 17 | 2 | 25 | 250 | 1.084 | 1.637 | 13.66 |
| 45493 | 2 | 0 | 4 | 18 | 0 | 24 | 240 | 1.099 | 1.792 | 11.94 |
| 45494 | 2 | 0 | 4 | 18 | 1 | 25 | 250 | 1.093 | 1.776 | 12.91 |
| 45495 | 2 | 0 | 4 | 19 | 0 | 25 | 250 | 1.098 | 1.818 | 12.16 |
| 45496 | 2 | 0 | 5 | 0 | 0 | 7 | 70 | 1.060 | 0.622 | 8.35 |
| 45497 | 2 | 0 | 5 | 0 | 1 | 8 | 80 | 1.055 | 0.610 | 9.34 |

# Separation of Triazine Pestizides

## Prediction vs. Measurement

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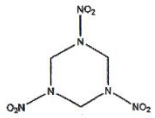
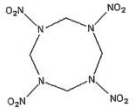
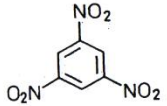
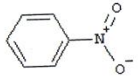
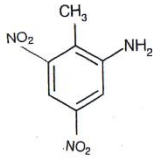
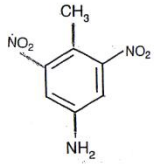
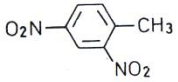
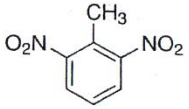
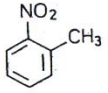
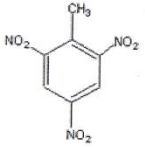
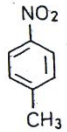
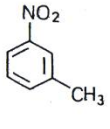
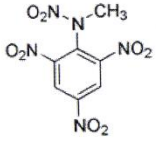
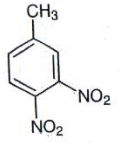
# Explosives according EPA 8330

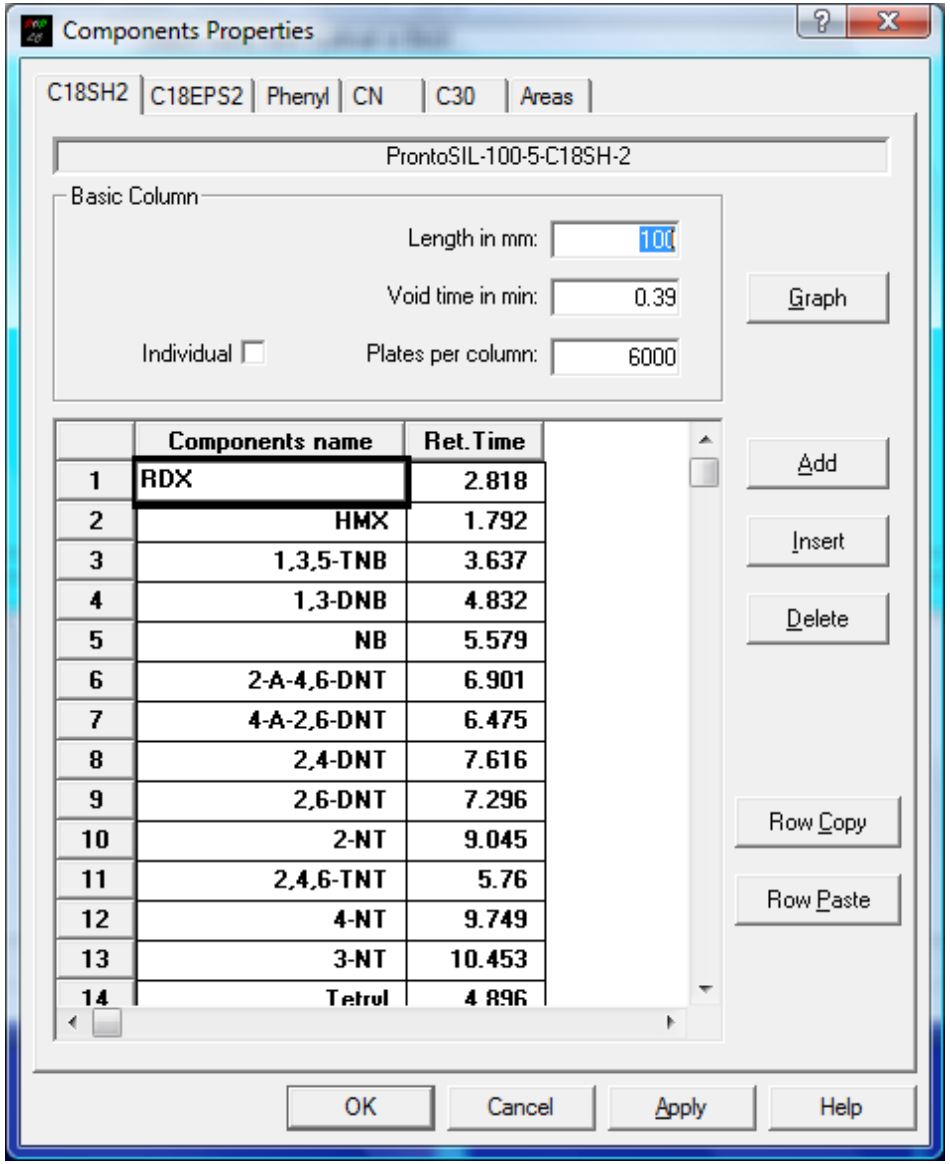




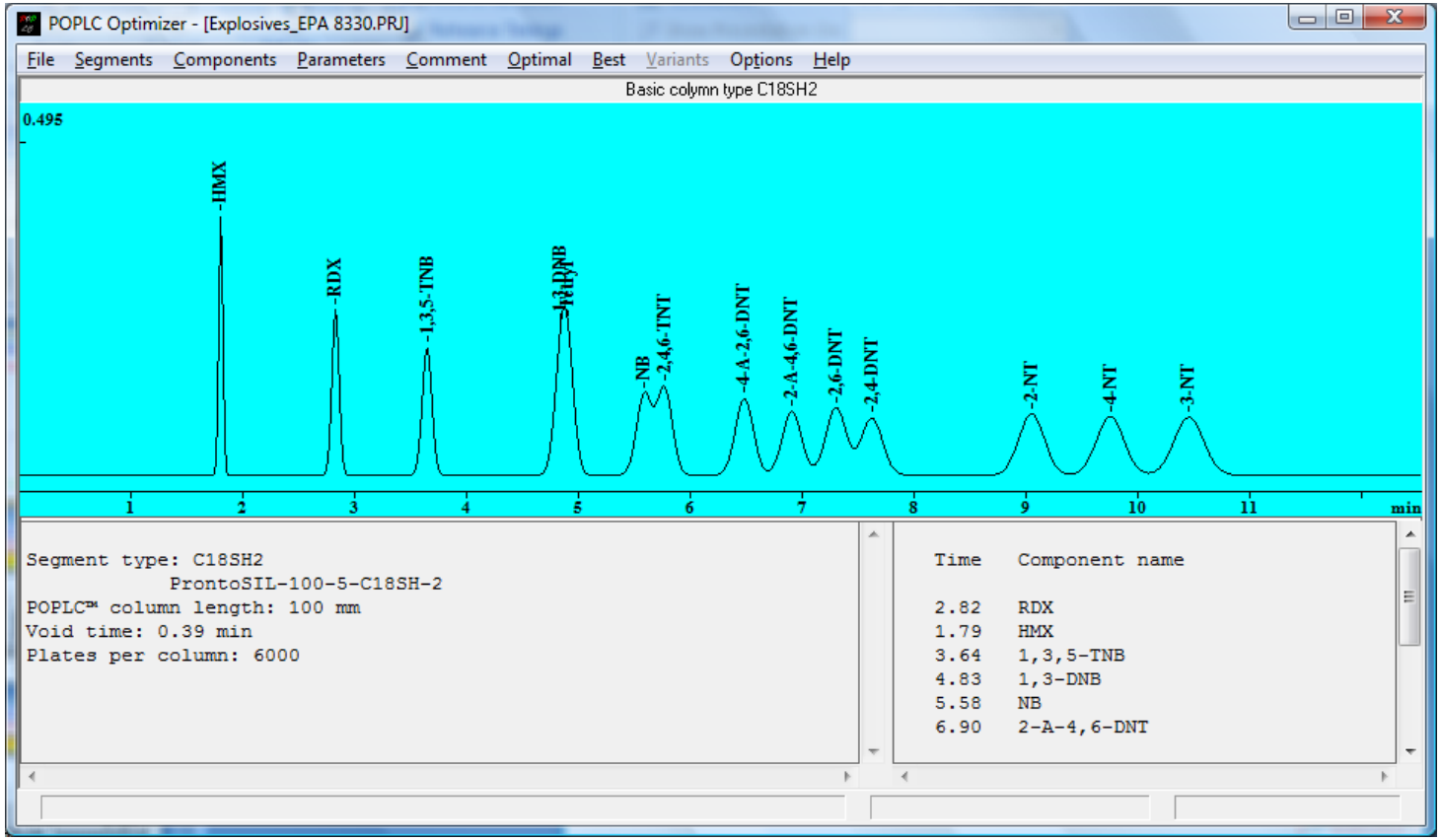
**1.** Hexogen (RDX) **2.** Octogen (HMX) **3.** 1,3,5-TNB **4.** 1,3-DNB **5.** Nitro-benzen **8.** 2-A-4,6-DNT **7.** 4-A-2,6-DNT

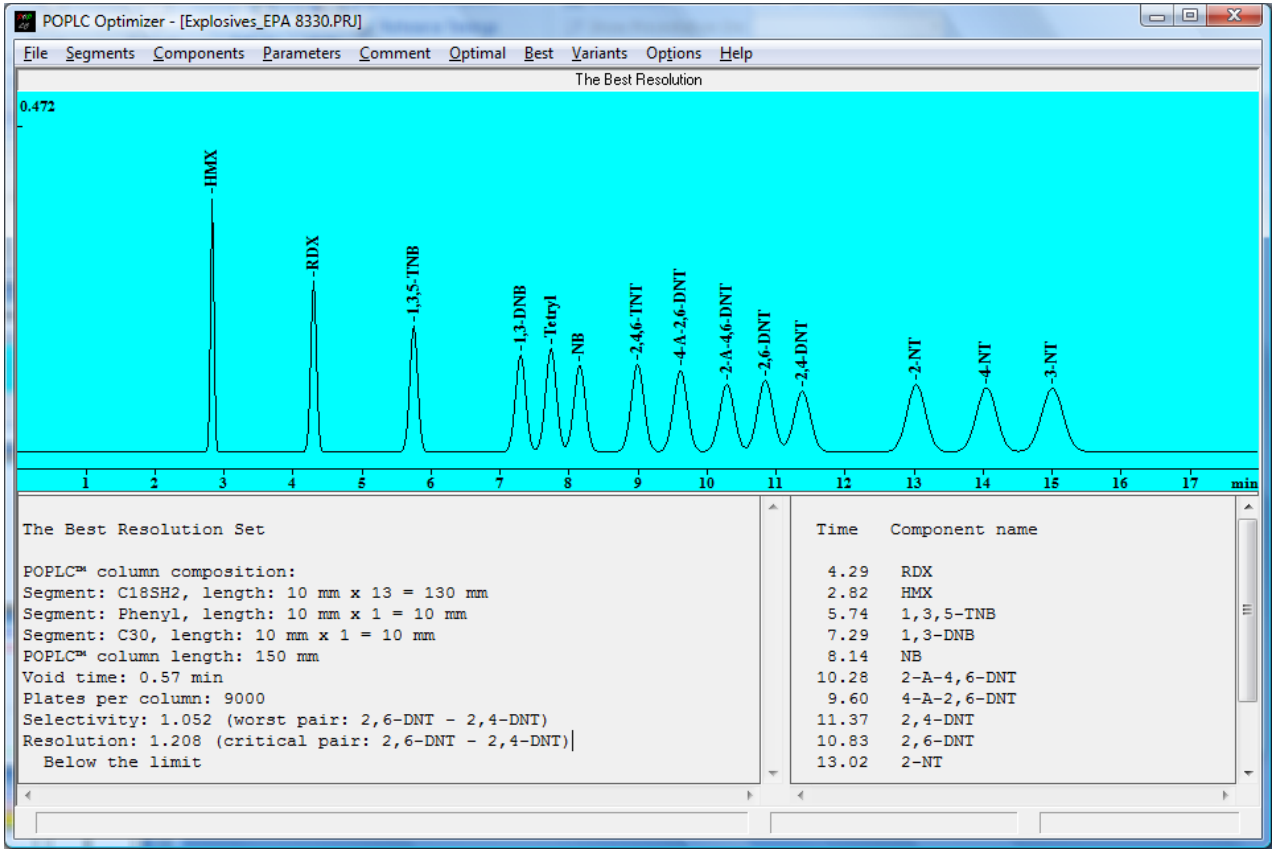
**10.** 2,4-DNT **9.** 2,6-DNT **11.** 2-NT **6.** 2,4,6-TNT **12.** 4-NT **13.** 3-NT **14.** Tetryl



# Emulated test run



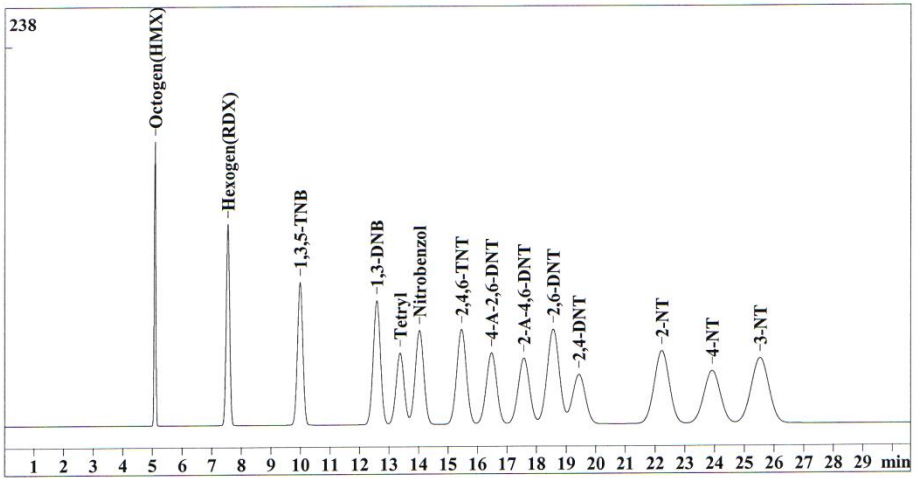
# Best resolution variant



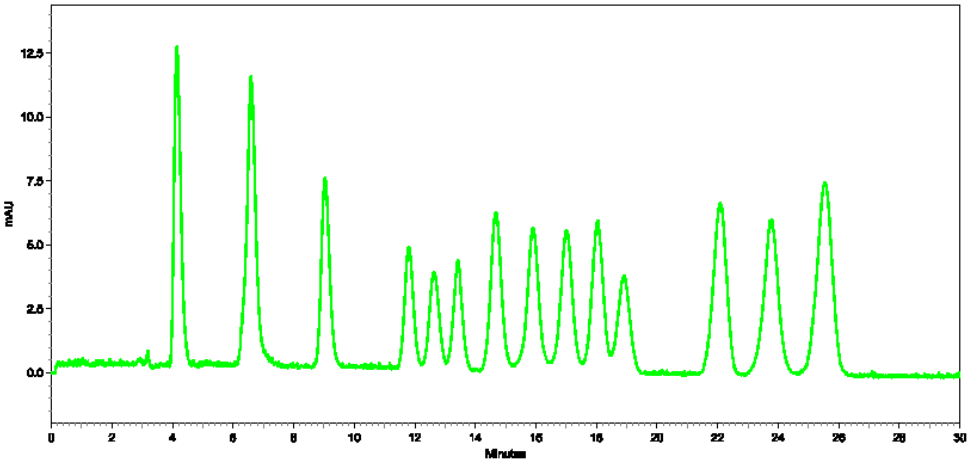
# Separation of Explosives according EPA 8330

## Prediction vs. Measurement

**Prediction**




**Measurement**



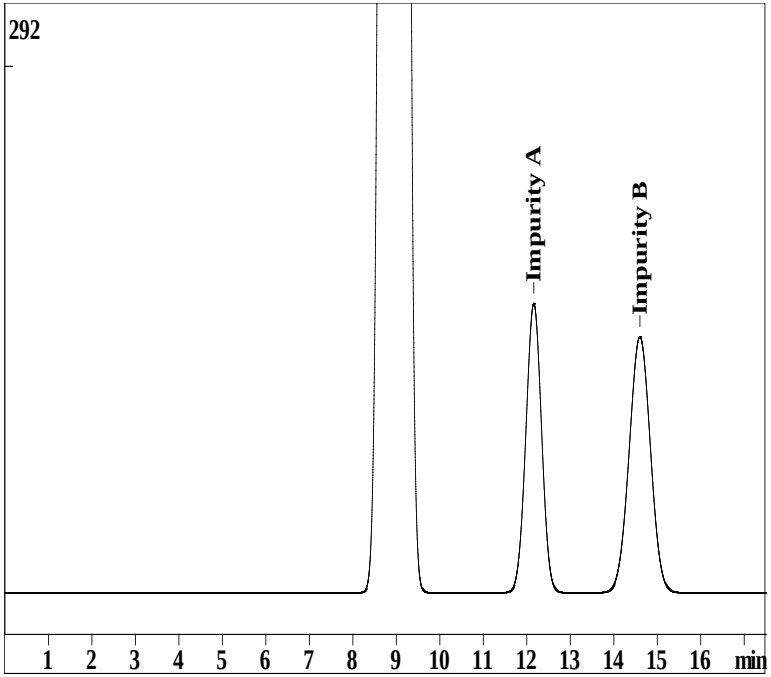

**Prof. W. Engewald,**
**Dr. F.-M. Matysik,**
**U. Schuman,**
**Uni Leipzig**

# POPLC® Method Development

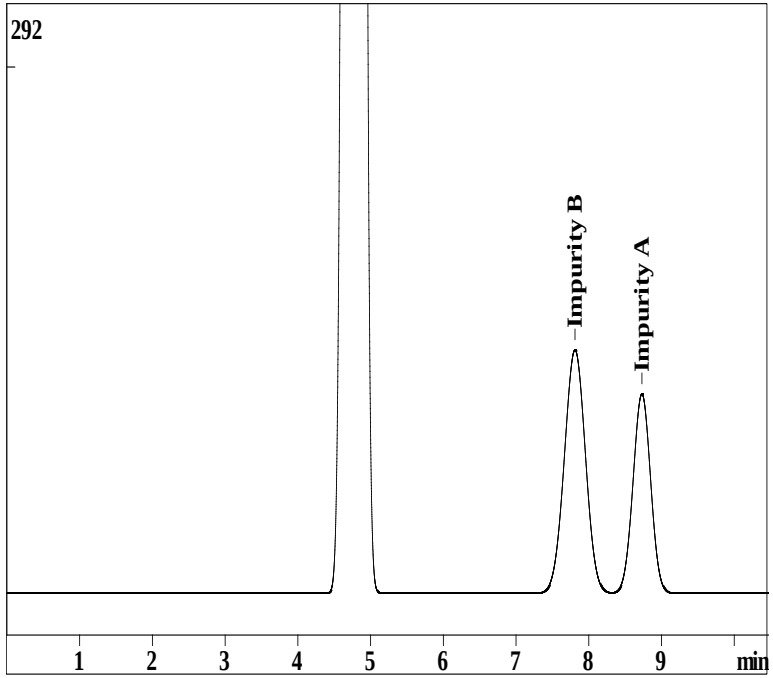
## In Pharmaceutical Industry

### Basic Runs on Different Stationary Phases

**ProntoSIL 100-5 C18 SH 2**
**80 x 3.0 mm**




**ProntoSIL 100-5 C18 EPS 2**
**80 x 3.0 mm**




| | |
|---|---|
| Mobile Phase: | Acetonitrile/20 mM Phosphate Buffer pH 3 30:70 (v/v) |
| Flow rate: | 0,5 ml/min |
| Detection: | UV @ 270 nm |

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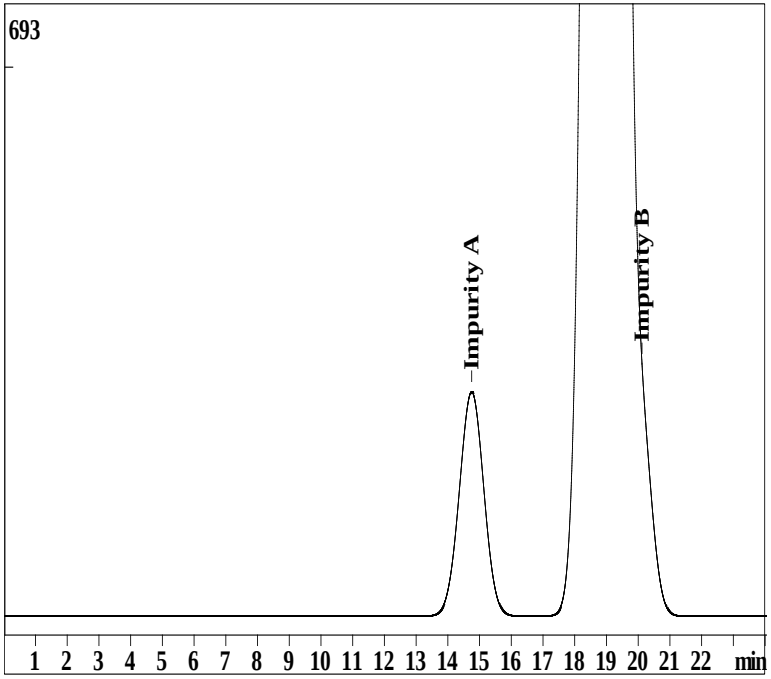
# POPLC® Method Development

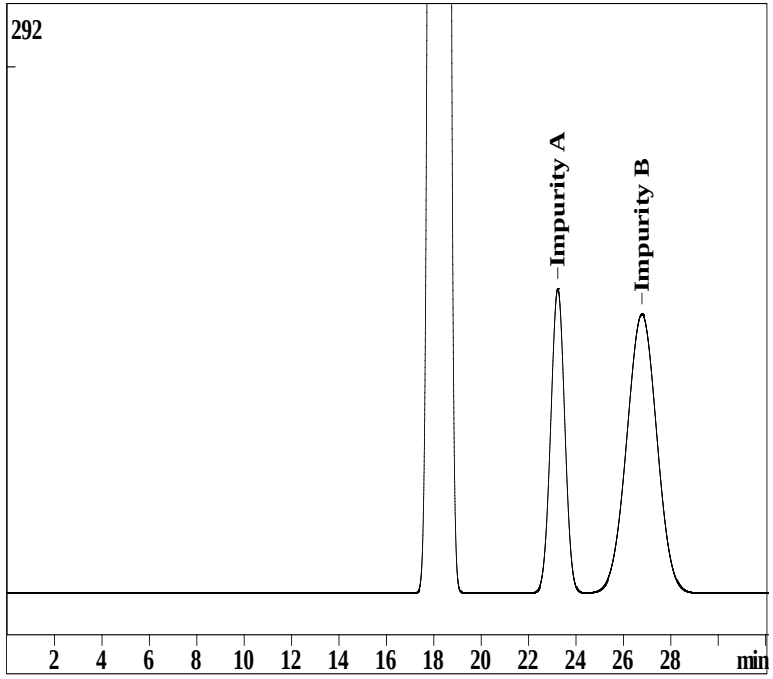
## In Pharmaceutical Industry

### Basic Runs on Different Stationary Phases

**ProntoSIL 100-5 Phenyl 2**
**120 x 3.0 mm**




**ProntoSIL 100-5 CN 2**
**240 x 3.0 mm**




| | |
|---|---|
| Mobile Phase: | Acetonitrile/20 mM Phosphate Buffer pH 3 30:70 (v/v) |
| Flow rate: | 0,5 ml/min |
| Detection: | UV @ 270 nm |

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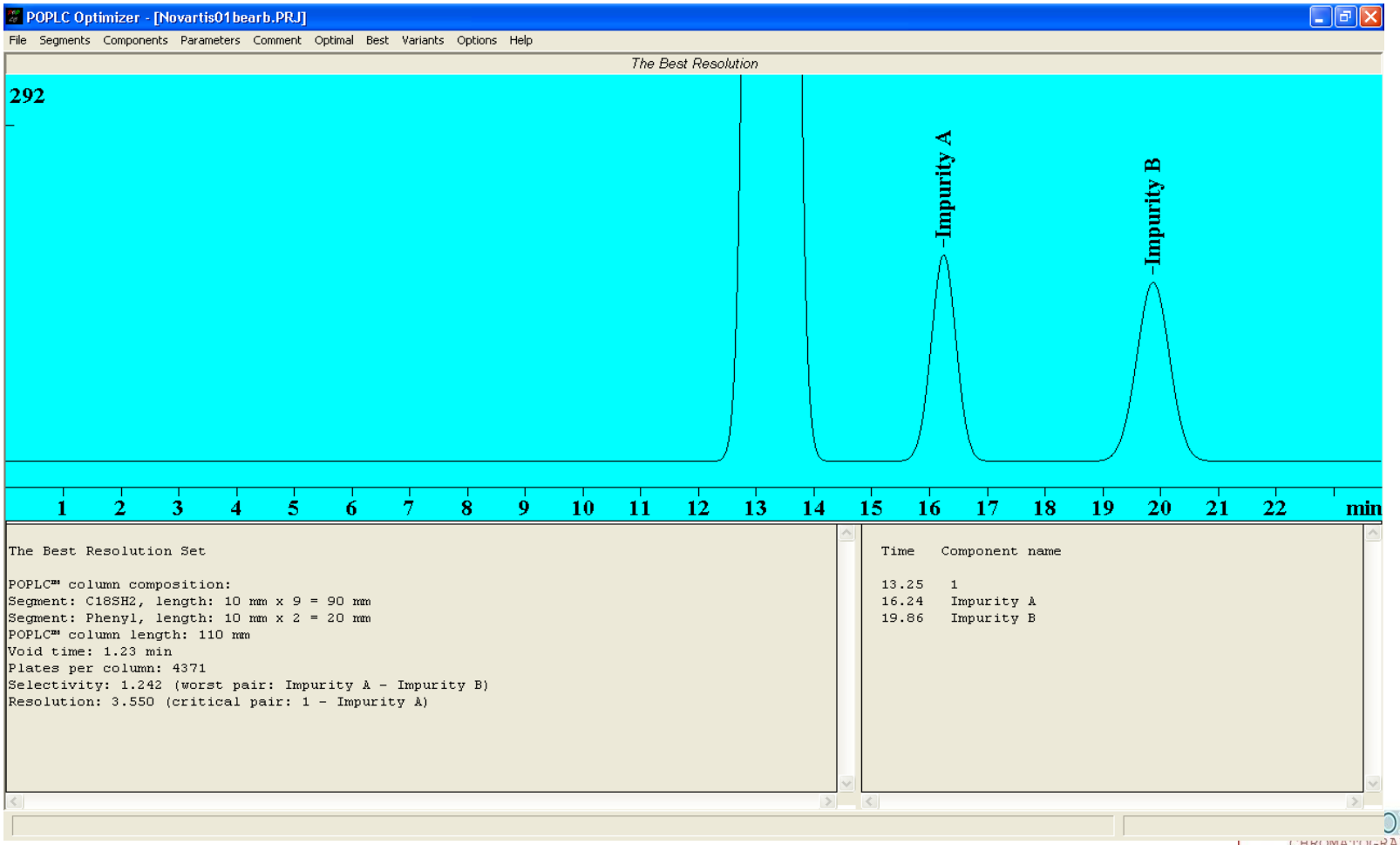
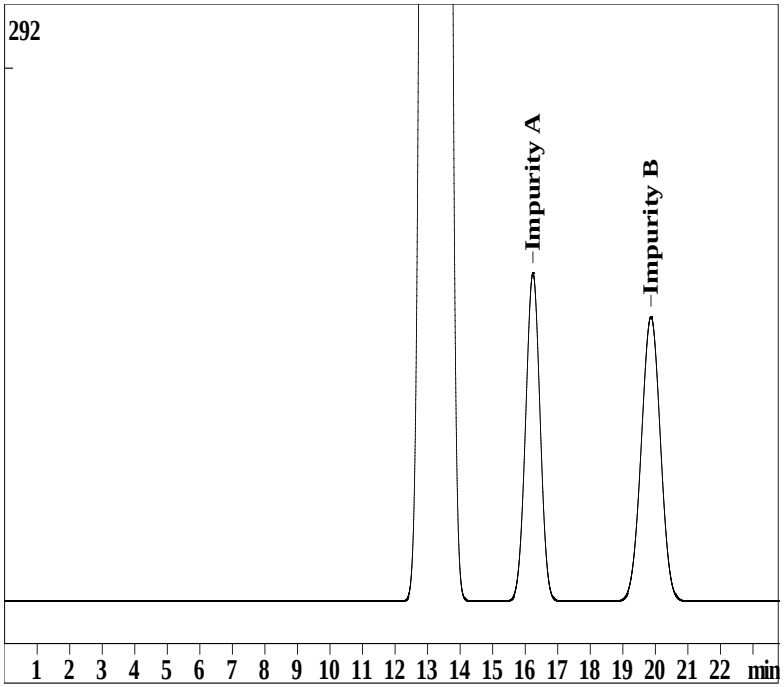
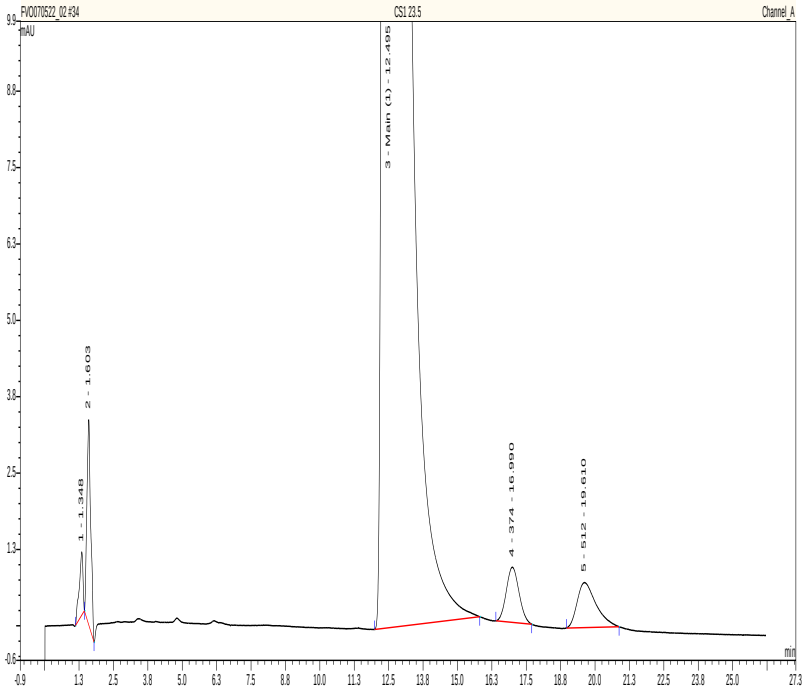
# POPLC® Method Development
## In Pharmaceutical Industry
### Best Separation within 20 Minutes



# POPLC® Method Development
## In Pharmaceutical Industry
### Best Separation within 20 Minutes




| | |
|---|---|
| Column: | 90 mm ProntoSIL 100-5-C18 SH2 and 20 mm ProntoSIL 100-5-Phenyl 2 |
| Column Dimension: | 110 x 3.0 mm |
| Mobile Phase: | Acetonitrile/20 mM Phosphate Buffer pH 3 35:65 (v/v) |
| Flow rate: | 0,5 ml/min |
| Detection: | UV @ 270 nm |

BISCHOFF CHROMATOGRAPHY

# POPLC® Method Development
## In Pharmaceutical Industry
### Best Separation within 20 Minutes

**Results Table: Optimized Column**

The Best Resolution Set

POPLC™ column composition:
Segment: C18SH2, length: 10 mm x 9 = 90 mm
Segment: Phenyl, length: 10 mm x 2 = 20 mm
POPLC™ column length: 110 mm
Void time: 1.23 min
Plates per column: 4371
Selectivity: 1.242 (worst pair: Impurity A - Impurity B)
Resolution: 3.550 (critical pair: 1 - Impurity A)

**Results Table: Measured Resolution**

Resolution: 3.5 (critical pair: 1 - Impurity A)

**Results Table: Predicted Retention Times**

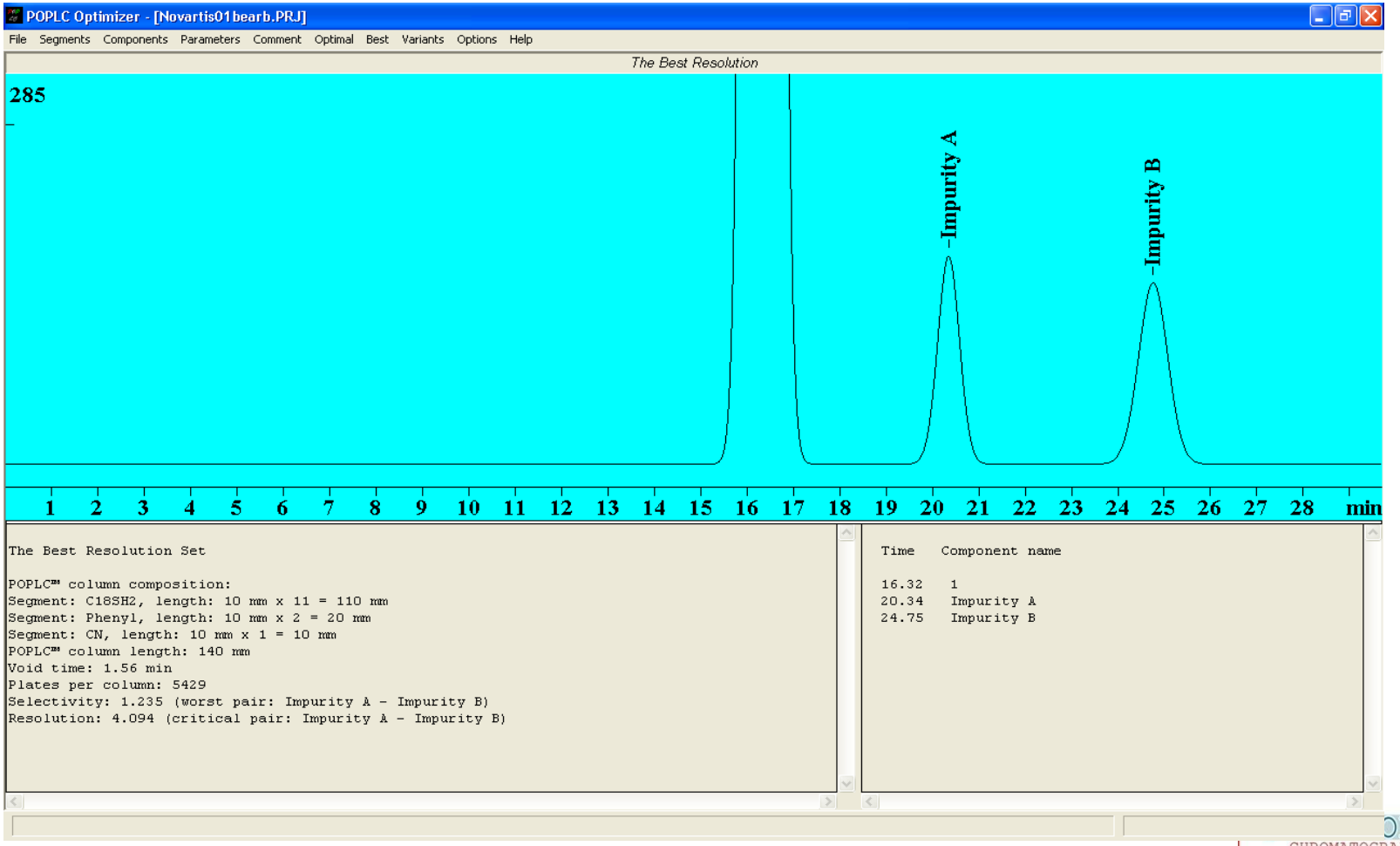
| Time | Component name |
|---|---|
| 13.25 | 1 |
| 16.24 | Impurity A |
| 19.86 | Impurity B |

**Results Table: Measured Retention Times**

| Time | Component name |
|---|---|
| 12.4 | 1 |
| 16.9 | Impurity A |
| 19.6 | Impurity B |

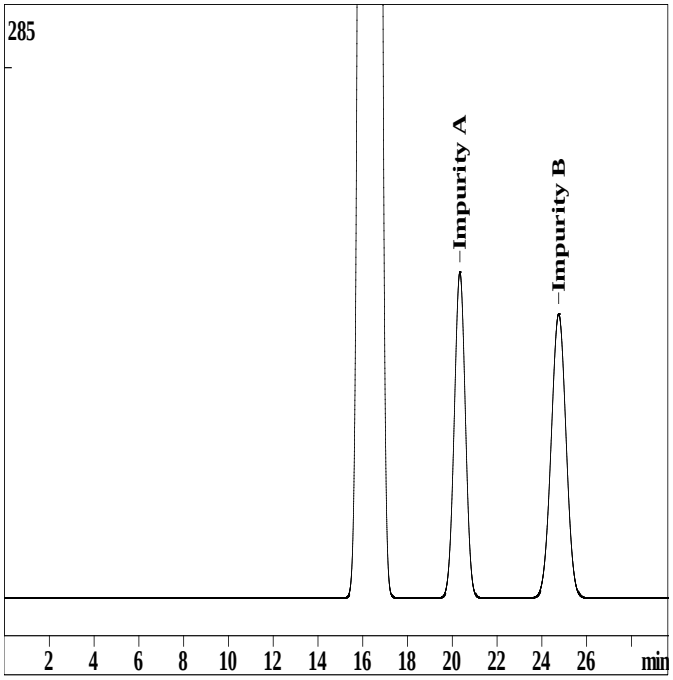
BISCHOFF CHROMATOGRAPHY

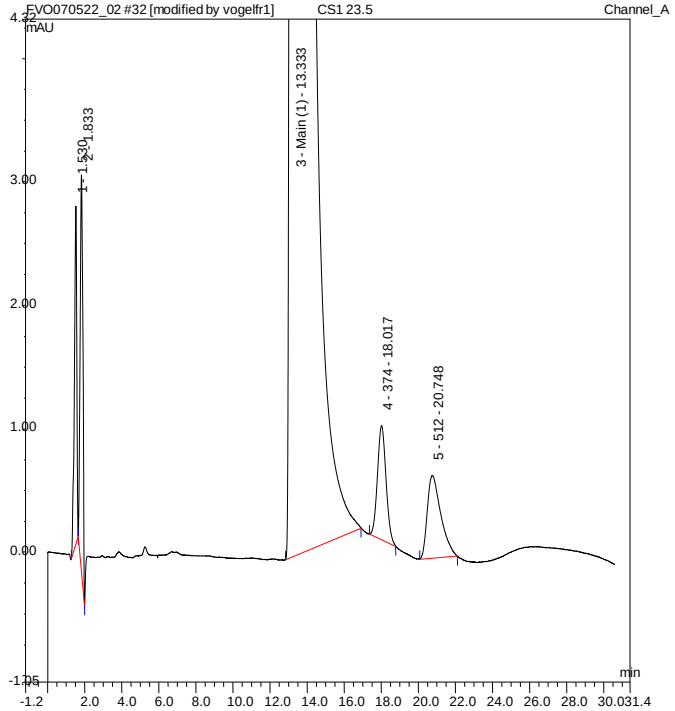
# POPLC® Method Development
## In Pharmaceutical Industry
### Best Separation within 25 Minutes



# POPLC® Method Development
## In Pharmaceutical Industry

### Best Separation within 25 Minutes




| | |
|---|---|
| Column: | 110 mm ProntoSIL 100-5-C18 SH2 and 20 mm ProntoSIL 100-5-Phenyl 2 and 10 mm ProntoSIL 100-5-CN 2 |
| Column Dimension: | 140 x 3.0 mm |
| Mobile Phase: | Acetonitrile/20 mM Phosphate Buffer pH 3 35:65 (v/v) |
| Flow rate: | 0,5 ml/min |
| Detection: | UV @ 270 nm |

BISCHOFF CHROMATOGRAPHY

# POPLC® Method Development
## In Pharmaceutical Industry

### Best Separation within 25 Minutes

### Results Table: Optimized Column

The Best Resolution Set

POPLC™ column composition:
Segment: C18SH2, length: 10 mm x 11 = 110 mm
Segment: Phenyl, length: 10 mm x 2 = 20 mm
Segment: CN, length: 10 mm x 1 = 10 mm
POPLC™ column length: 140 mm
Void time: 1.56 min
Plates per column: 5429
Selectivity: 1.235 (worst pair: Impurity A - Impurity B)
Resolution: 4.094 (critical pair: Impurity A - Impurity B)

### Results Table: Measured Resolution

Resolution: 4.1 (critical pair: 1 - Impurity A)

### Results Table: Predicted Retention Times

| Time | Component name |
|---|---|
| 16.32 | 1 |
| 20.34 | Impurity A |
| 24.75 | Impurity B |

### Results Table: Measured Retention Times

| Time | Component name |
|---|---|
| 13.3 | 1 |
| 18.0 | Impurity A |
| 20. 7 | Impurity B |

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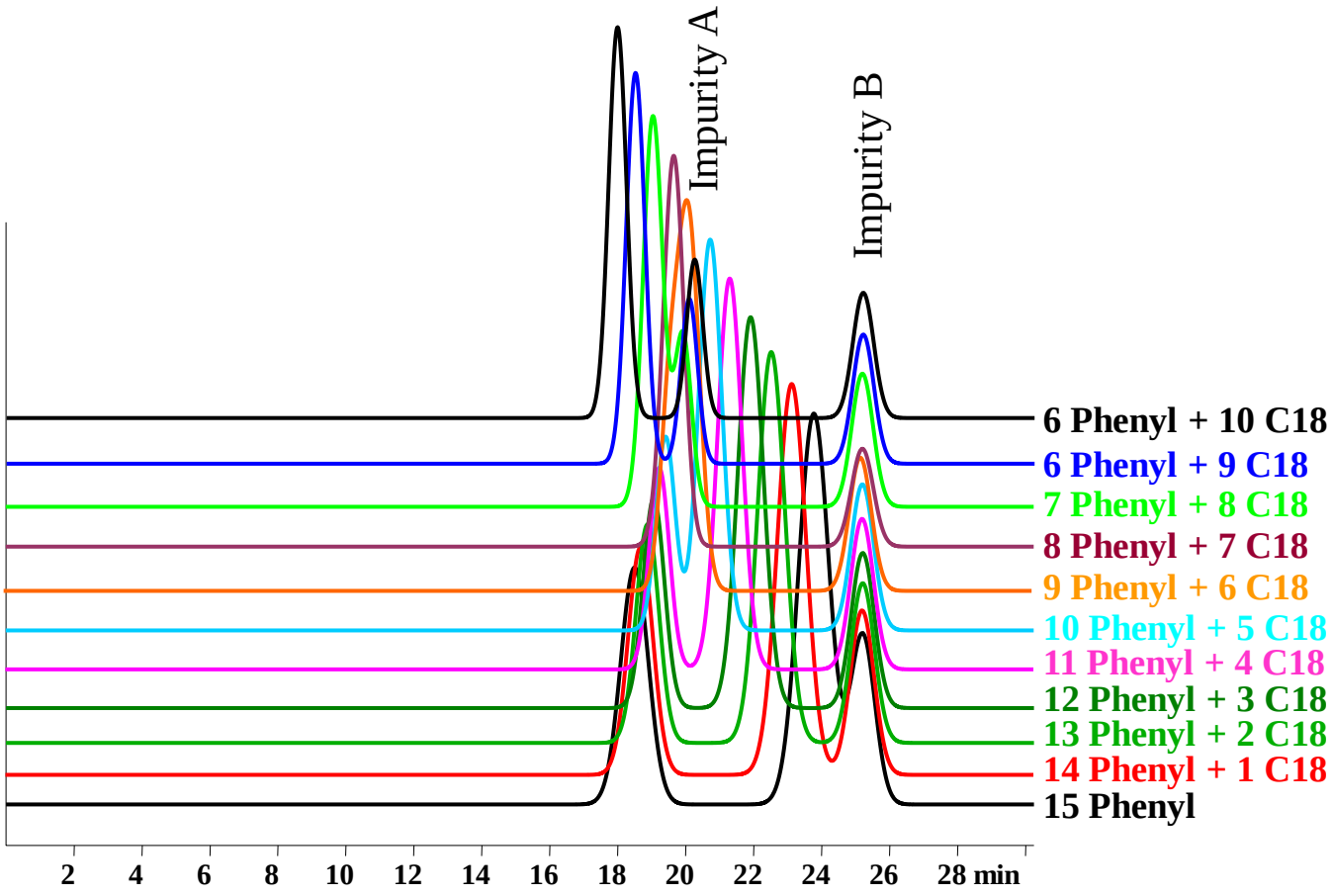
# POPLC® Method Development
## In Pharmaceutical Industry

### Monitoring of the Retention Behavior

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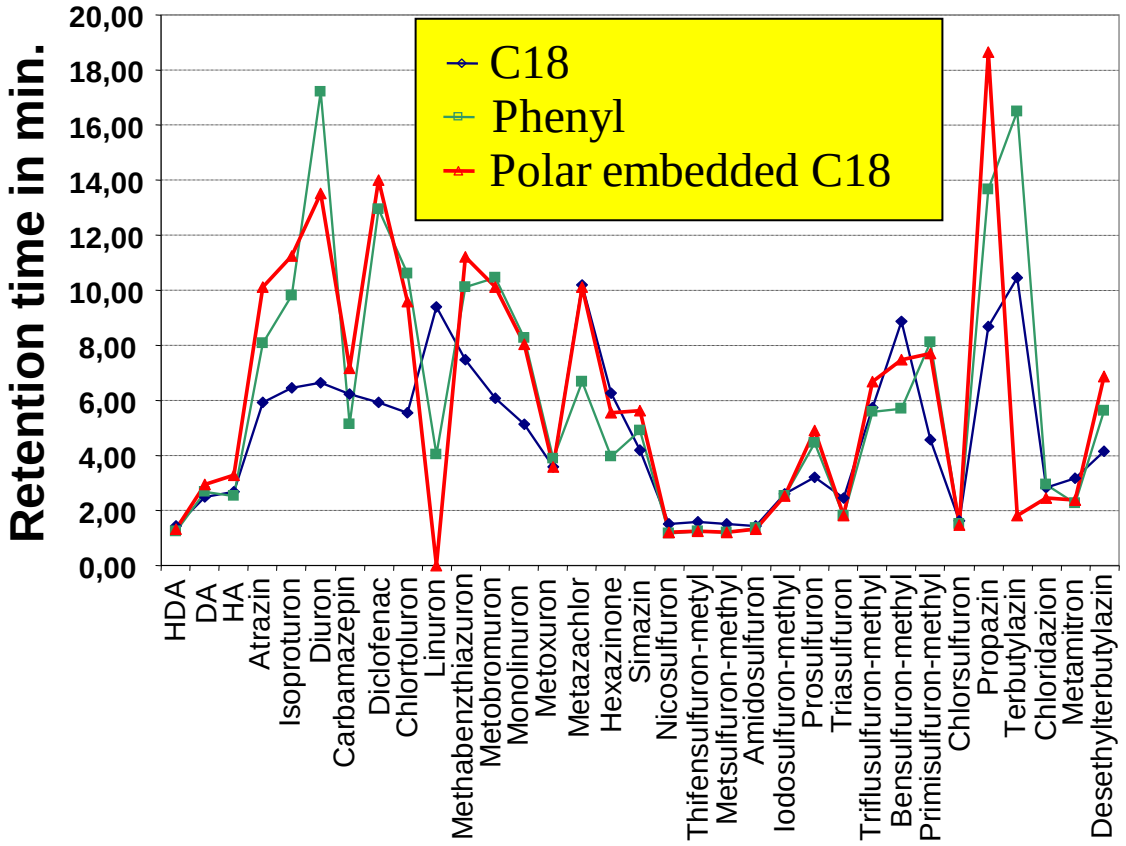
# Retention Behaviour of 33 Compounds in Municipal Waste Water on Different Stationary Phases

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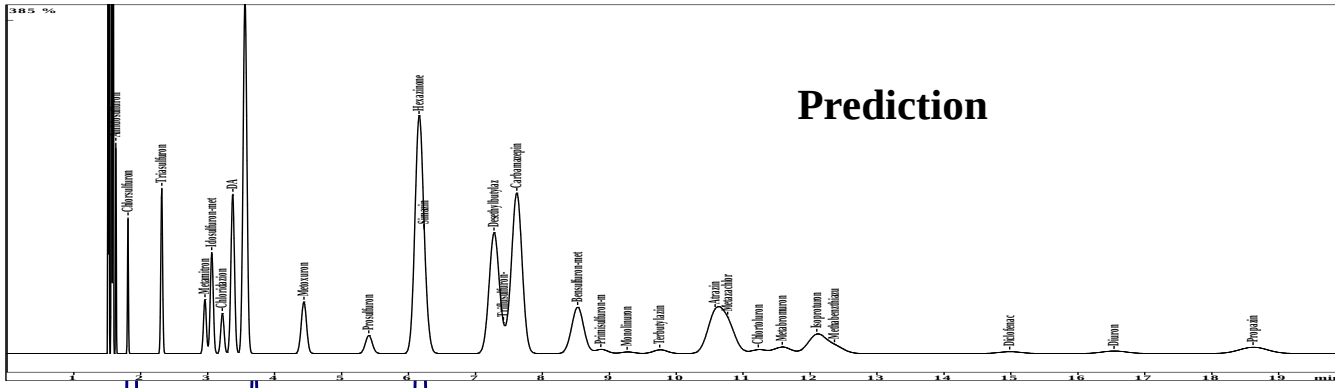
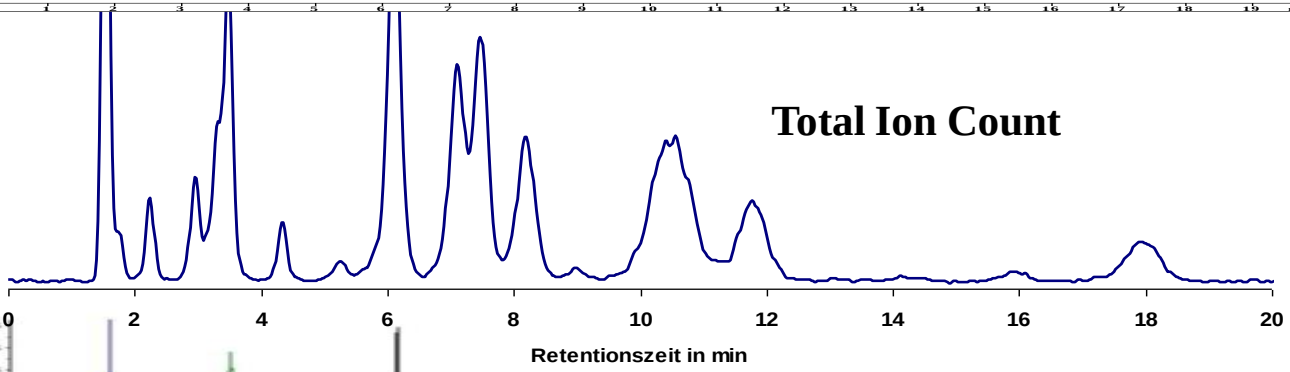
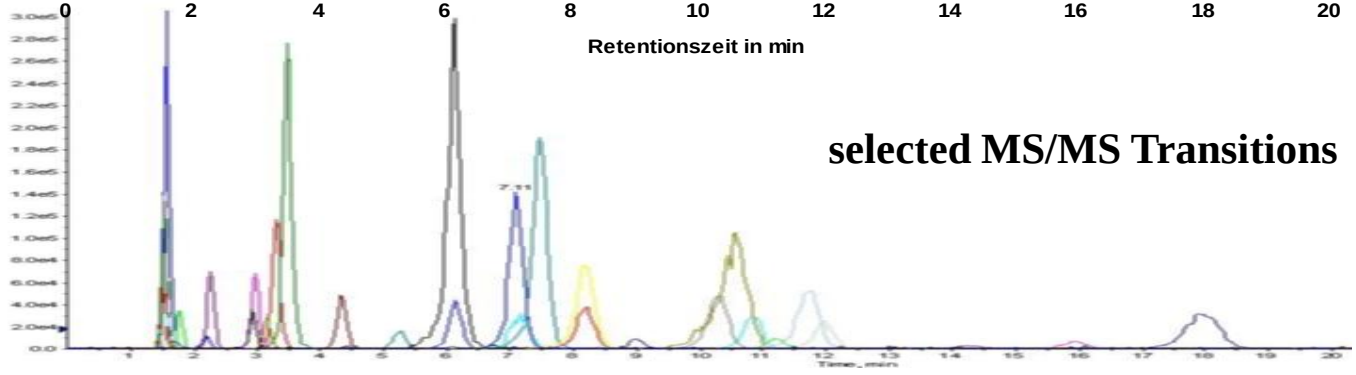
# Separation of 33 Compounds in LC/MS/MS

## Optimized POPLC® Column




BISCHOFF CHROMATOGRAPHY

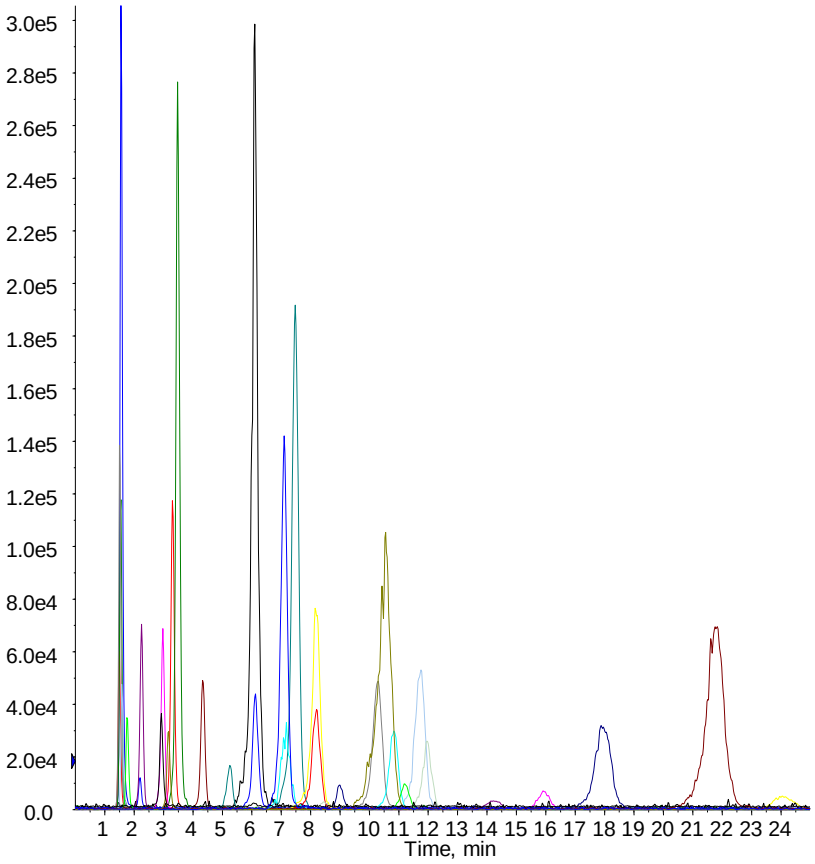
# Separation of 33 Compounds in LC/MS/MS

## Prediction vs. Reality

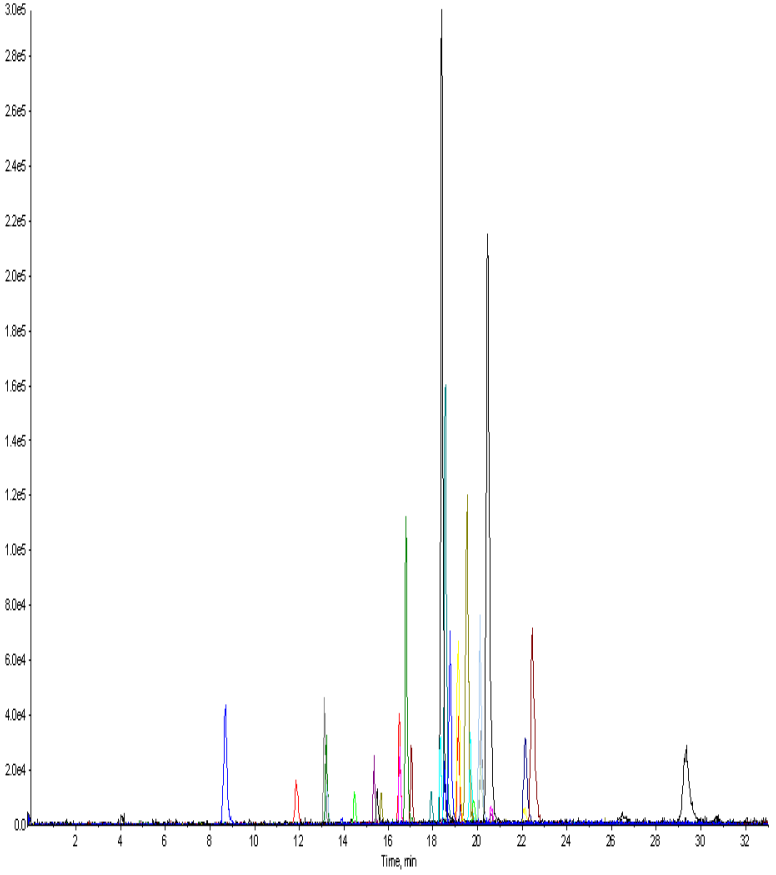
| Analyte | Retention time predicted | Retention time meassured | % Deviation |
|---|---|---|---|
| HDA | **1,580** | **1,56** | 1,58 |
| DA | **3,37** | **3,29** | 2,37 |
| HA | **3,55** | **3,46** | 2,68 |
| Atrazin | **10,57** | **10,25** | 3,03 |
| Isoproturon | **12,09** | **11,65** | 3,64 |
| Diuron | **16,53** | **15,80** | 4,42 |
| Carbamazepin | **7,61** | **7,43** | 2,43 |
| Diclofenac | **14,97** | **14,10** | 5,81 |
| Chlortoluron | **11,22** | **10,75** | 4,19 |
| Methabenzthiazuron | **12,36** | **11,85** | 4,13 |
| Metobromuron | **11,58** | **11,10** | 4,15 |
| Monolinuron | **9,26** | **8,92** | 3,73 |
| Metoxuron | **4,43** | **4,30** | 3,05 |
| Metazachlor | **10,76** | **10,45** | 2,88 |
| Hexazinone | **6,15** | **6,05** | 1,71 |
| Simazin | **6,23** | **6,07** | 2,57 |
| Nicosulfuron | **1,5** | **1,49** | 1,00 |
| Thifensulfuron-metyl | **1,55** | **1,54** | 0,97 |
| Metsulfuron-methyl | **1,52** | **1,51** | 0,99 |
| Amidosulfuron | **1,62** | **1,58** | 2,47 |
| Iodosulfuron-methyl | **3,06** | **2,95** | 3,76 |
| Prosulfuron | **5,4** | **5,18** | 4,07 |
| Triasulfuron | **2,31** | **2,23** | 3,46 |
| Triflusulfuron-methyl | **7,41** | **7,10** | 4,25 |
| Bensulfuron-methyl | **8,52** | **8,11** | 4,87 |
| Primisulfuron-methyl | **8,87** | **8,40** | 5,30 |
| Chlorsulfuron | **1,8** | **1,76** | 2,50 |
| Propazin | **18,61** | **17,80** | 4,35 |
| Chloridazion | **3,21** | **3,17** | 1,25 |
| Metamitron | **2,95** | **2,91** | 1,53 |
| Desethylterbutylazin | **7,27** | **7,06** | 2,96 |

# Separation of 33 Compounds in LC/MS/MS

## Isocratic Separation




## Gradient Separation



# Isocratic POPLC® Separation

**Column:** 120 x 3.0 mm

ProntoSIL 120-5-C18 SH : ProntoSIL 120-5-C18 ace-EPS : ProntoSIL 120-5-Phenyl 1:3:2

**Flow Rate:** 0.6 ml/min

**Mobile Phase:**

50 % Eluent A: 5 mM $NH_4OAc$

50 % Eluent B: MeOH + 5 mM NH4OAc

**Injection:** 10 µl

**Temperature:** 25°C

# Gradient HPLC Separation

**Column:** 250 x 4.6 mm Luna C18 (2)

**Mobile Phase:**

Eluent A: 5 mM $NH_4OAc$

Eluent B: MeOH + 5 mM NH4OAc

**Flow Rate:** 0.6 ml/min

**Injection:** 10 µl

**Temperature:** 25°C

**Gradient:**

4 min: 35% B

12 min: 80% B

25 min: 80% B

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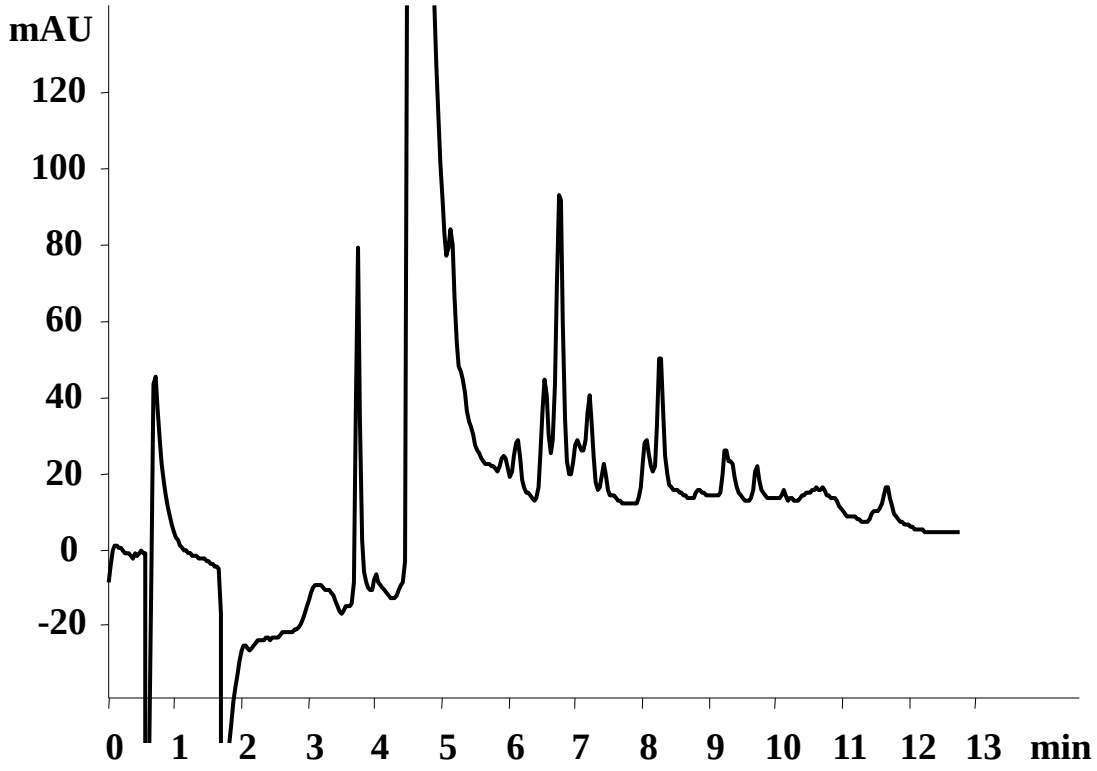
# POPLC® Method Development
## of a complex unknown mixture

### „Scouting Gradient“

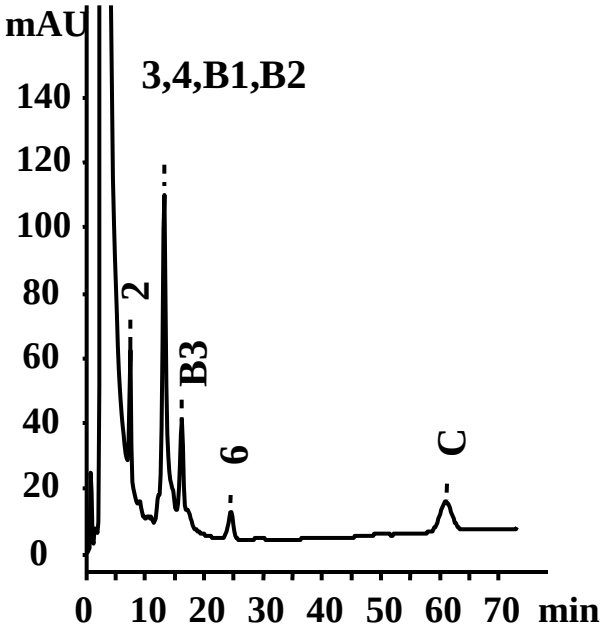



Column: POPLink® column segment ProntoSIL 100-5-C18 SH2, 40 x 3.0 mm
Eluent: A: H3PO4 1 ml/l in H2O; B: ACN; Gradient: 0 – 100% in 10 min.
Flow rate: 0.5 ml/min; Detection: UV @ 210 nm

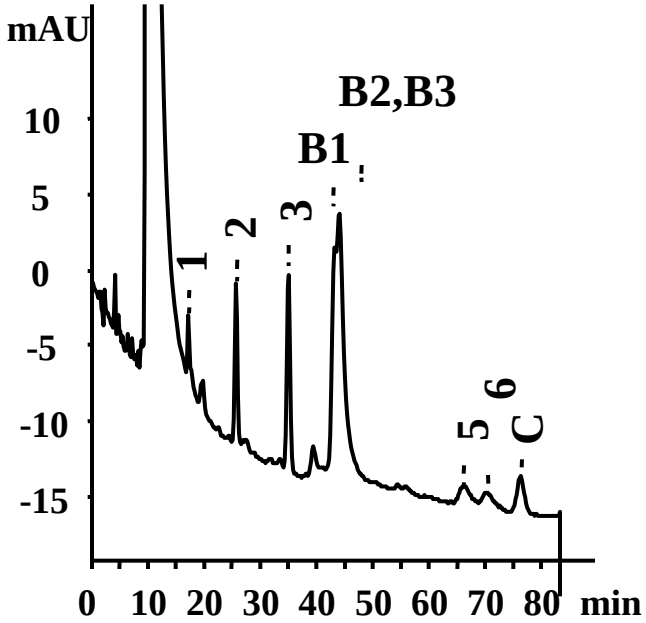
BISCHOFF CHROMATOGRAPHY

# POPLC® Method Development
## of a complex unknown mixture
### Basic Runs on Different Stationary Phases

**ProntoSIL 100-5 C18 SH 2**
**120 x 3.0 mm**




**ProntoSIL 100-5 C18 EPS 2**
**250 x 3.0 mm**

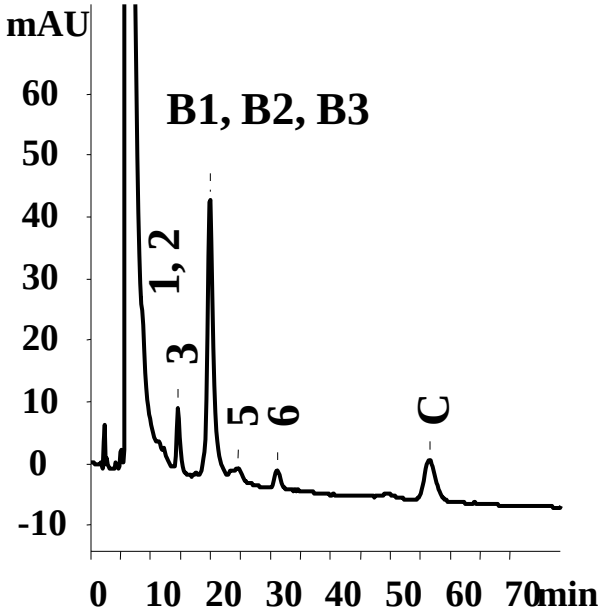



Mobile Phase: Acetonitrile/0.1% $H_3PO_4$ 40:60 (v/v)
Flow rate: 0,5 ml/min
Detection: UV @ 210 nm

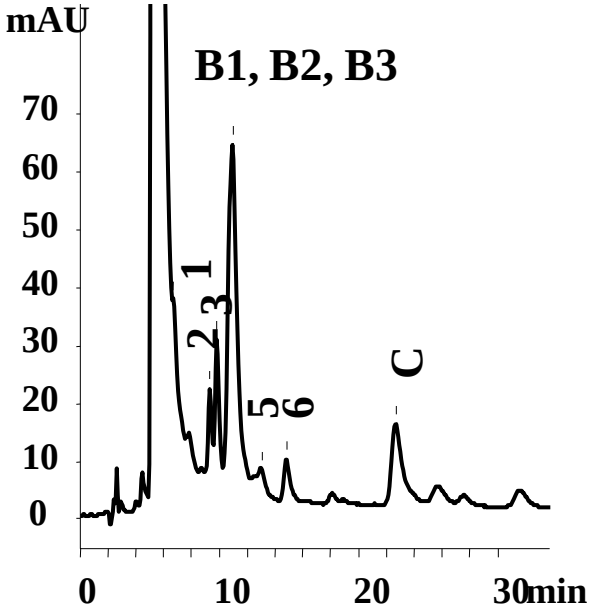
BISCHOFF CHROMATOGRAPHY

# POPLC® Method Development
## of a complex unknown mixture
### Basic Runs on Different Stationary Phases

**ProntoSIL 100-5 Phenyl 2**
**250 x 3.0 mm**




**ProntoSIL 100-5 CN 2**
**250 x 3.0 mm**

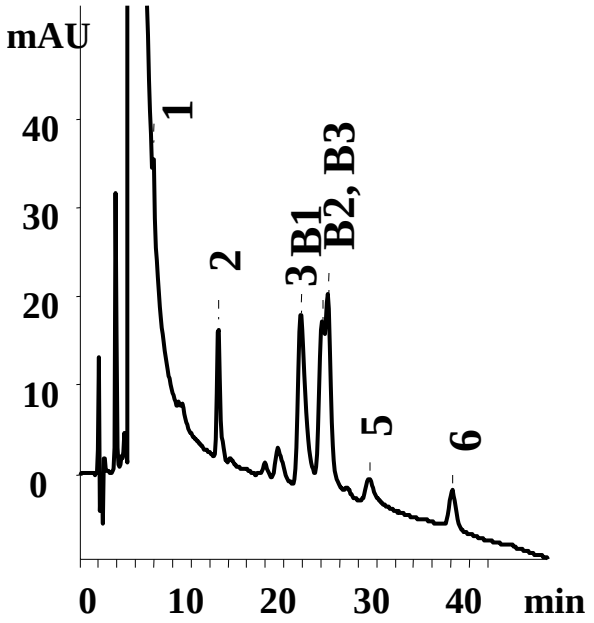



| | |
|---|---|
| Mobile Phase: | Acetonitrile/0.1% $H_3PO_4$ 40:60 (v/v) |
| Flow rate: | 0,5 ml/min |
| Detection: | UV @ 210 nm |

BISCHOFF CHROMATOGRAPHY

# POPLC® Method Development
## of a complex unknown mixture
### Basic Runs on Different Stationary Phases

**ProntoSIL 200-5 C30**
**250 x 3.0 mm**

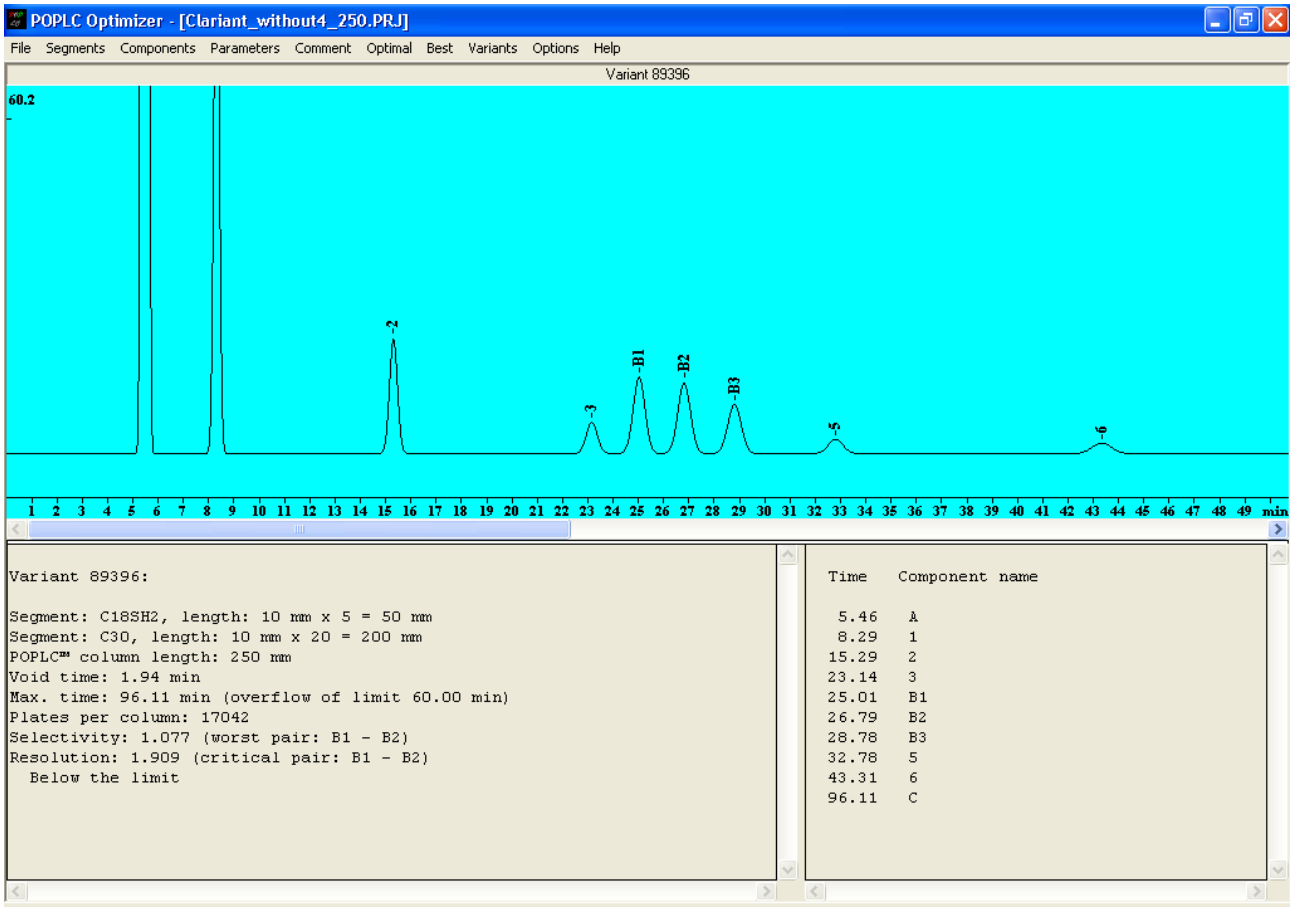



| | |
|---|---|
| Mobile Phase: | Acetonitrile/0.1% $H_3PO_4$ 40:60 (v/v) |
| Flow rate: | 0,5 ml/min |
| Detection: | UV @ 210 nm |

BISCHOFF CHROMATOGRAPHY

# POPLC® Method Development
## of a complex unknown mixture

## Prediction of POPLC® Optimizer Software

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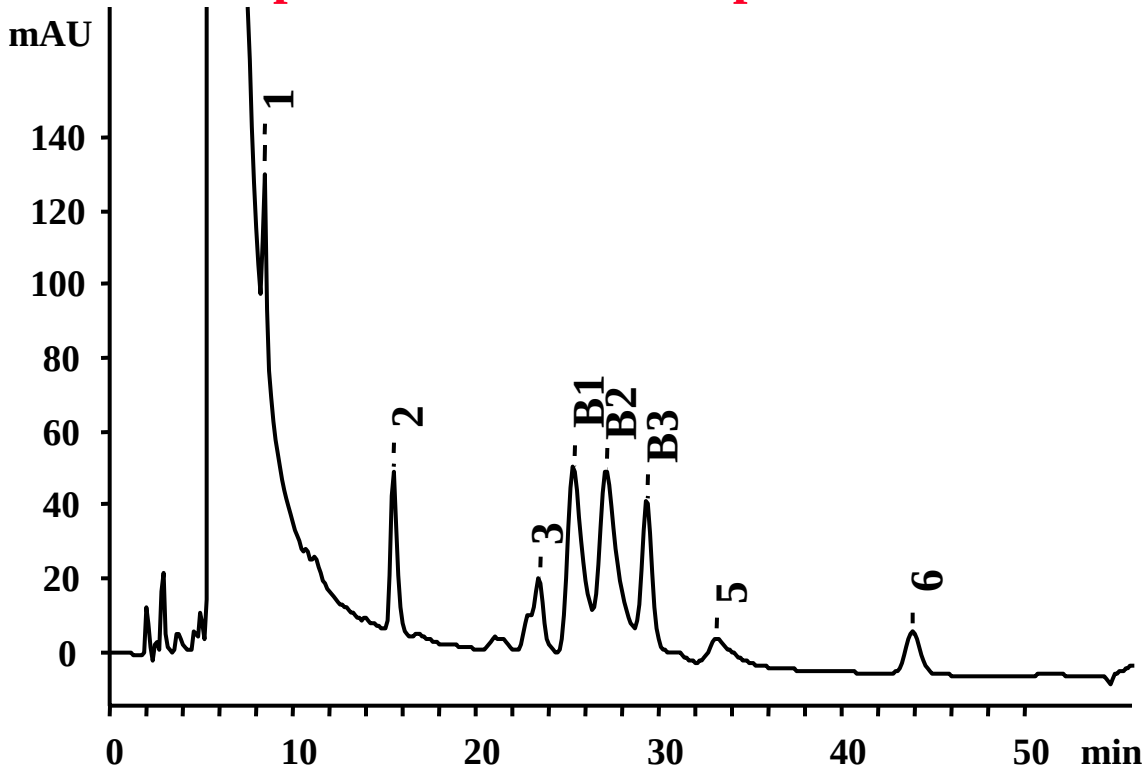
# POPLC® Method Development
## of a complex unknown mixture

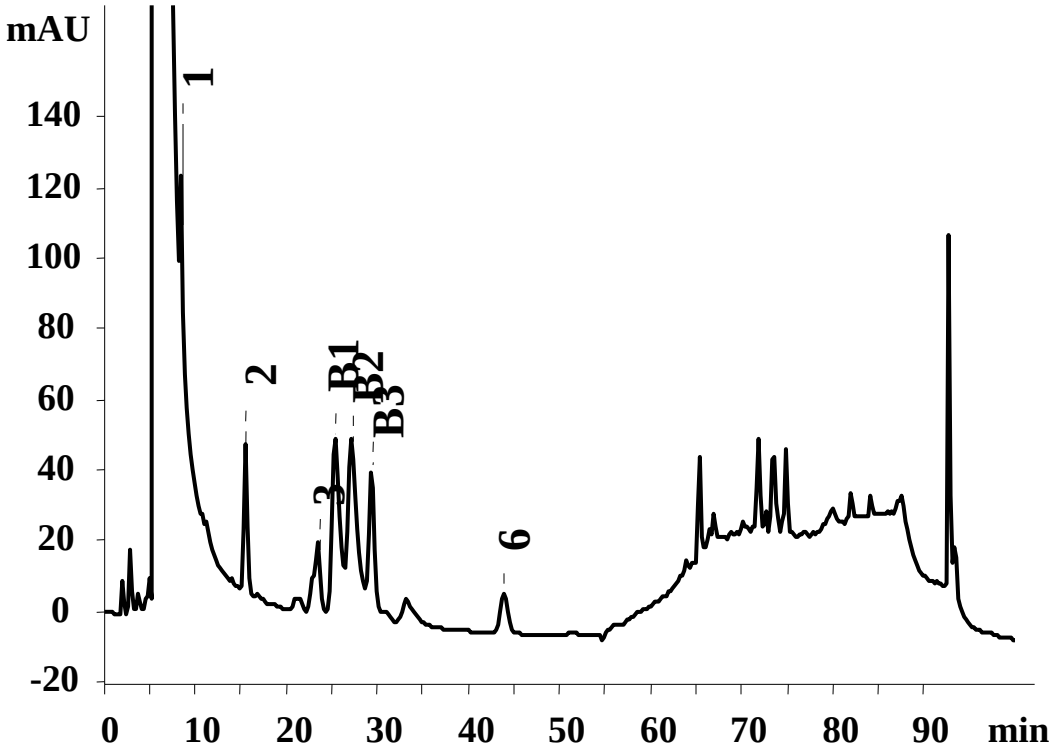
### Optimized isocratic separation




Column: 50 mm ProntoSIL 100-5-C18 SH 2 and
200 mm ProntoSIL 200-5- C30
Eluent: A: H3PO4 1 ml/l in H2O; B: ACN; 40/60 (v/v)
Flow rate: 0.5 ml/min Detection: UV @ 210 nm

# POPLC® Method Development
## of a complex unknown mixture

### Optimized Gradient Elution




Column: ProntoSIL 100-5-C18 SH2 / ProntoSIL 200-5-C30 50:200, 250 x 3.0 mm
Eluent: A: H3PO4 1 ml/l in H2O; B: ACN
Gradient: 40% B 50 min.; 40% - 100% B in 85 min.
Flow Rate: 0.5 ml/min Detection: UV @ 210 nm

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# Advantages of POPLC®

- **in many cases no gradient elution required**
  - ☺ **constant detector background**
  - ☺ **less requirements for HPLC devices**
  - ☺ **no reequilibration required (faster analysis)**
  - ☺ **reusable mobile phase**
  - ☺ **detectors like RI, EC and conductivity are possible**
- **easy method development via software**
- **easy exchange of column parts (POPLink® column hardware)**
- **every HPLC column selectivity can be simulated**
- **This method can be applied in all areas of chromatography**

  **from micro to prep LC, in GC, TLC, SPE und Flash Chromatography**

# Summary

- Selectivity is the most important tool in HPLC
- The column is the most important choice
- The optimization strategy is important
- POPLC® offers a simple possibility for future method development

# We did not invent HPLC but we make it

# POP

**More information: http://www.POPLC.de**

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