



# Maximize Instrument Efficiency Through Automation of Method Development and Data Analysis

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# Introduction

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- LC method development is a constant need for analytical labs for several reasons:
  - new products or new product compositions
  - changes in specification, registration
  - new requirements regarding speed, resolution or availability of column types/chemistries
- Manual method development can be laborious...
  - often does not get beyond applying C<sub>8</sub>/C<sub>18</sub> columns
  - starts with either Acetonitrile/buffer or Methanol/buffer
  - can take many weeks before a final method is obtained
- Automation of the method development process is required

# Agilent Method Development Solution

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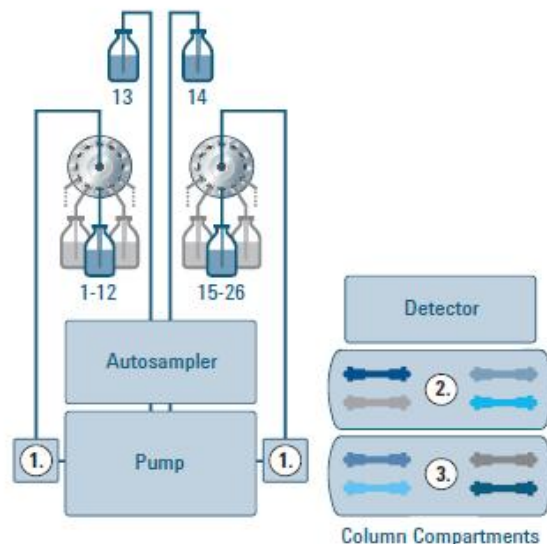


- Provides semi-automated method development
  - screening of several LC columns (up to 8)
  - screening of solvents (organic modifiers / buffers)
  - screening of gradient conditions and temperatures
  - enhanced data analysis / reporting tools
- Runs with “Method Scouting Wizard” in Chemstation/OpenLAB
- Does not automatically fine-tune separation conditions -> needs user input
- Can use other SW in addition such as Chromsword or ACD to fully automate method development

# 1200 Infinity Series Method Development Solution



Classic configuration



up to 1200 bar



Classic configuration  
Infinity Series

Valve located in

Comment

1 x 8pos/9port valve (2)

1 x 1290 TCC (G1316C)

1 x 8pos/9port valve (3)

1 x 1290 TCC (G1316C)

For up to 8 columns (column selection)

1 x 12pos/13port valve (1)

1 x 1290 ext valve drive (G1170A)

e.g., for **organic modifiers**

1 x 12pos/13port valve (1)

1 x 1290 ext valve drive (G1170A)

e.g., for **aqueous buffers**

# Method Scouting Wizard



- **Define project**

Choose scouting combinations and base method.

- **Select columns**

All installed columns are shown automatically.

- **Select solvents**

Pump types and valves are automatically detected.

- **Define gradients**

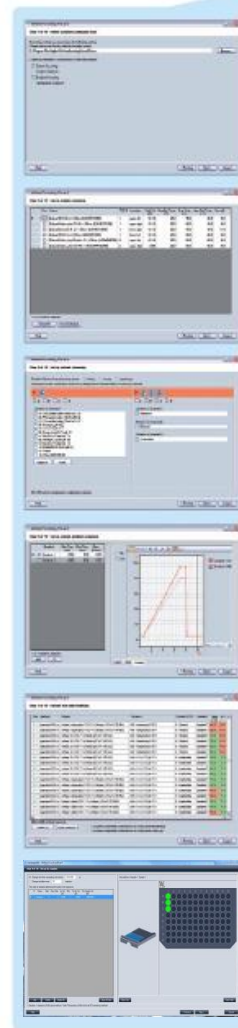
Select between different gradients and temperatures.

- **Review and select screening methods**

Check for incompatible combinations.

- **Set up samples**

Define injection volumes and number of repetitions.



G2196AA

→ Facilitating setup of screening sequences

# Method Scouting Wizard



Automation of sequence (campaign) setup

- Settings for solvent and column selection

- Time and solvent usage estimation

**Configure Columns : Instrument 1 (LC)**

Installed Columns

Valve Pos.	Color Code	Column ID	Controlled	StandBy Temp. [°C]
1	Red	Eclipse Plus C18, 4.6x50mm, 1.8µm	<input checked="" type="checkbox"/>	40.0
2	Blue	Eclipse XDB-C18, 4.6x50mm, 1.8µm [USWDY...	<input checked="" type="checkbox"/>	40.0
3	Green	Eclipse Plus Phenyl Hexyl, 4.6x50mm, 1.8µm	<input checked="" type="checkbox"/>	40.0
4	Black	Bonus RP, 4.6x50mm, 1.8µm	<input checked="" type="checkbox"/>	40.0
5	Black	Ascentis Express C18, 4.6x150mm, 2.7µm [#4]	<input checked="" type="checkbox"/>	40.0
6	Black	Ascentis Express RP-Amide, 4.6x150mm, 2.7µm	<input checked="" type="checkbox"/>	40.0

Temperature of P... 20.0

**Method Scouting Wizard**

Step 6 of 9: Review and select methods

#	Use	Method	Column	Gradient	Temp [°C]	pH
1	<input checked="" type="checkbox"/>	Injection0001.m	Eclipse Plus C18, 4.6x50mm, 1.8µm	Normal	40.0	N/A
2	<input checked="" type="checkbox"/>	Injection0002.m	Eclipse Plus C18, 4.6x50mm, 1.8µm	High-res.	40.0	N/A
3	<input checked="" type="checkbox"/>	Injection0003.m	Eclipse Plus C18, 4.6x50mm, 1.8µm	Normal	50.0	N/A
4	<input checked="" type="checkbox"/>	Injection0004.m	Eclipse Plus C18, 4.6x50mm, 1.8µm	High-res.	50.0	N/A
5	<input checked="" type="checkbox"/>	Injection0005.m	Eclipse XDB-C18, 4.6x50mm, 1.8µm [USWDY08235]	Normal	40.0	N/A
6	<input type="checkbox"/>	Injection0006.m	Eclipse XDB-C18, 4.6x50mm, 1.8µm [USWDY08235]	High-res.	40.0	N/A
7	<input type="checkbox"/>	Injection0007.m	Eclipse XDB-C18, 4.6x50mm, 1.8µm [USWDY08235]	Normal	50.0	N/A
8	<input checked="" type="checkbox"/>	Injection0008.m	Eclipse XDB-C18, 4.6x50mm, 1.8µm [USWDY08235]	High-res.	50.0	N/A

**Method Scouting Wizard**

Step 9 of 9: Summary

You have set up method screening campaign "Dowcomplex002" as summarized:

Description **Sequence** Solvent Usage

#	Sample	Inj	Method	Type	Flow [ml/min]	Run Time [min]	Post Time [min]	Vial	Column
1			FlushWaste0001.m	Flush	1.00	3.00	0.00		Waste
2			FlushWaste0002.m	Flush	1.00	1.00	0.00		Waste
3			Equilibration0001.m	Equilibration	0.40	10.00	0.00		Eclipse Plus C18.
4	S6	1	Injection0001.m	Injection	0.40	15.00	0.00	P1-F-02	Eclipse Plus C18.
5	S6 spiked	1	Injection0001.m	Injection	0.40	15.00	0.00	P1-F-03	Eclipse Plus C18.
6	S8	1	Injection0001.m	Injection	0.40	15.00	0.00	P1-F-04	Eclipse Plus C18.
7	100ppm Target compounds	1	Injection0001.m	Injection	0.40	15.00	0.00	P1-F-05	Eclipse Plus C18.
8	S6	1	Injection0002.m	Injection	0.40	19.00	0.00	P1-F-02	Eclipse Plus C18.
9	S6 spiked	1	Injection0002.m	Injection	0.40	19.00	0.00	P1-F-03	Eclipse Plus C18.
10	S8	1	Injection0002.m	Injection	0.40	19.00	0.00	P1-F-04	Eclipse Plus C18.
11	100ppm Target compounds	1	Injection0002.m	Injection	0.40	19.00	0.00	P1-F-05	Eclipse Plus C18.

61 lines. 44 injections. Estimated total run time: 18 hours 23 minutes.  
 9 equilibration-, 2 flush- and 6 column storage runs.  
 5 column-, 0 solvent- and 0 simultaneous column and solvent changes.

Press 'Finish' to create the methods in the campaign folder and exit

Help Print < Previous Finish Cancel

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## Example 1: Herbicide impurity separation

- Two isocratic LC methods were used, one column no longer available – needed to upgrade the method
- 50x4.6 mm columns, 1.8  $\mu$ m particle size
- Screening of methanol/buffer and acetonitrile/buffer gradients at 1.5 ml/min

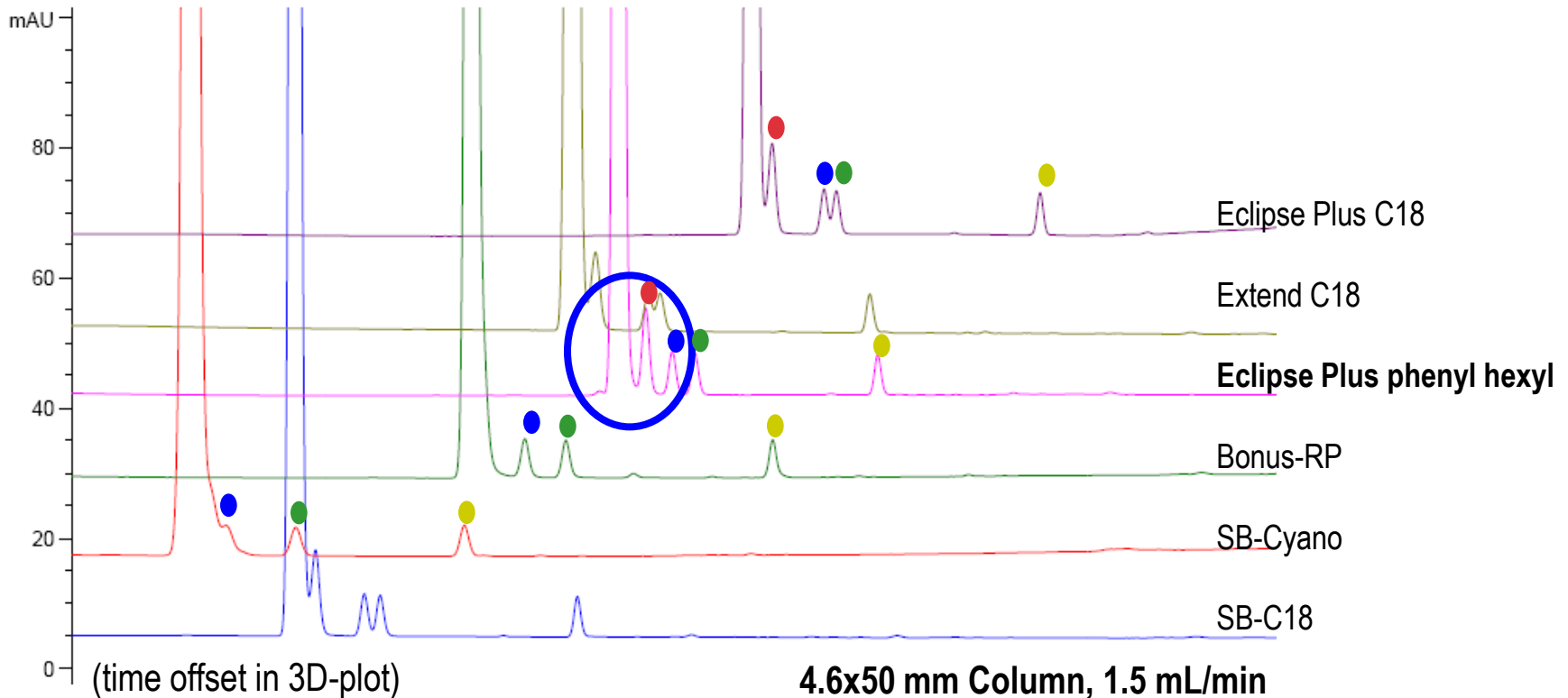
# Method Development Solution



## Herbicide impurities – Acetonitrile/buffer gradient

Separation of impurity standard

H<sub>2</sub>O:ACN (0.05% TFA) 10 to 95% in 10 minutes.





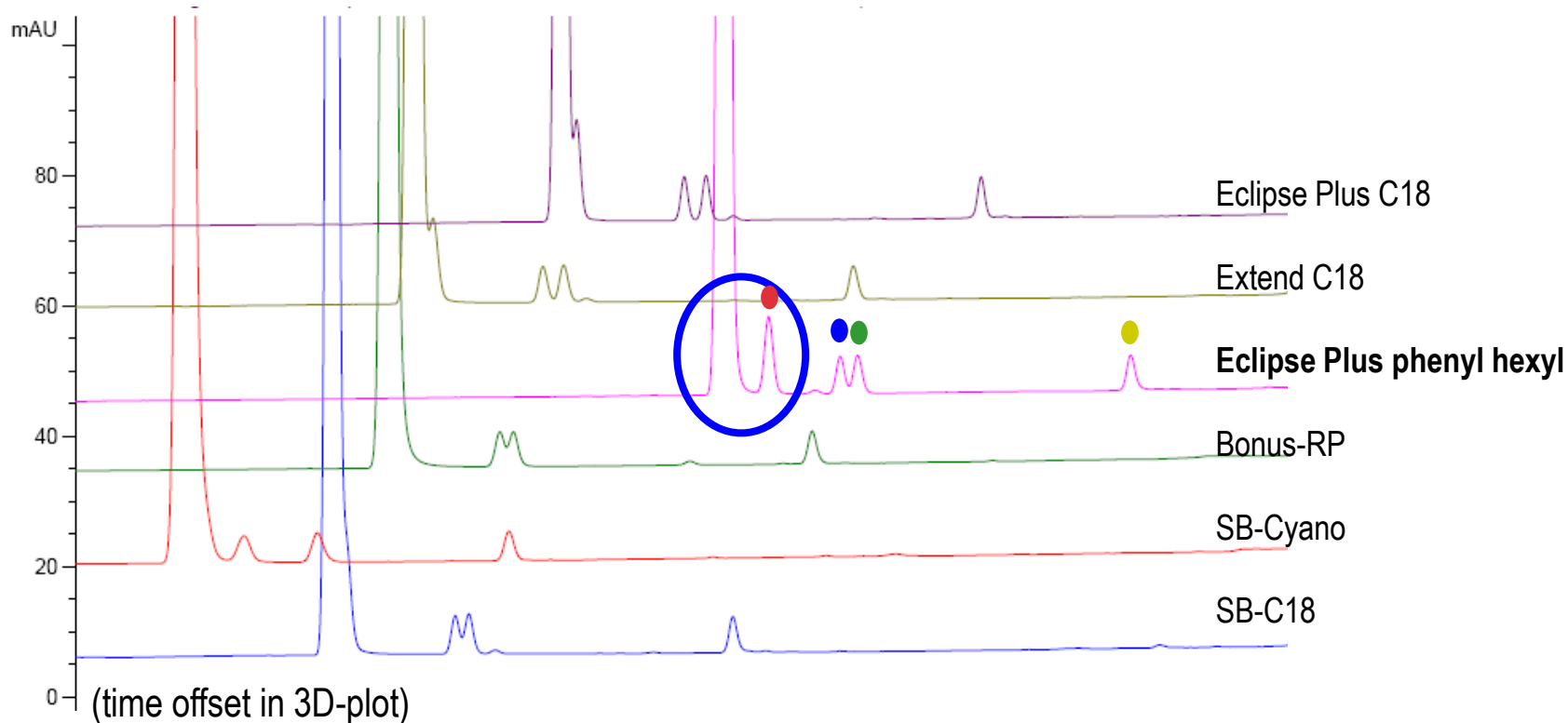
# Method Development Solution



## Herbicide impurities – Methanol/buffer gradient

Separation of impurity standard

H<sub>2</sub>O:MeOH (0.05% TFA) 10 to 95% in 10 minutes.



# Conclusions - Example 1

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- Overnight screening of 6 columns with two different solvent combinations
- Successful column and mobile phase conditions for impurity analysis was rapidly identified
- Additional gradient fine-tuning for final method with phenyl hexyl stationary phase – confirmation of compound identities by LC-MSD
- Current method is using a mixture of ACN/MeOH
- Method is used in QC lab

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## Example 2: Aromatic amine oligomers

- “Manual” method development with C<sub>18</sub> column
- Automated screening of 50x4.6 mm columns, 1.8 um particle size
- Screening of methanol/buffer and acetonitrile/buffer gradients at 1.5 ml/min

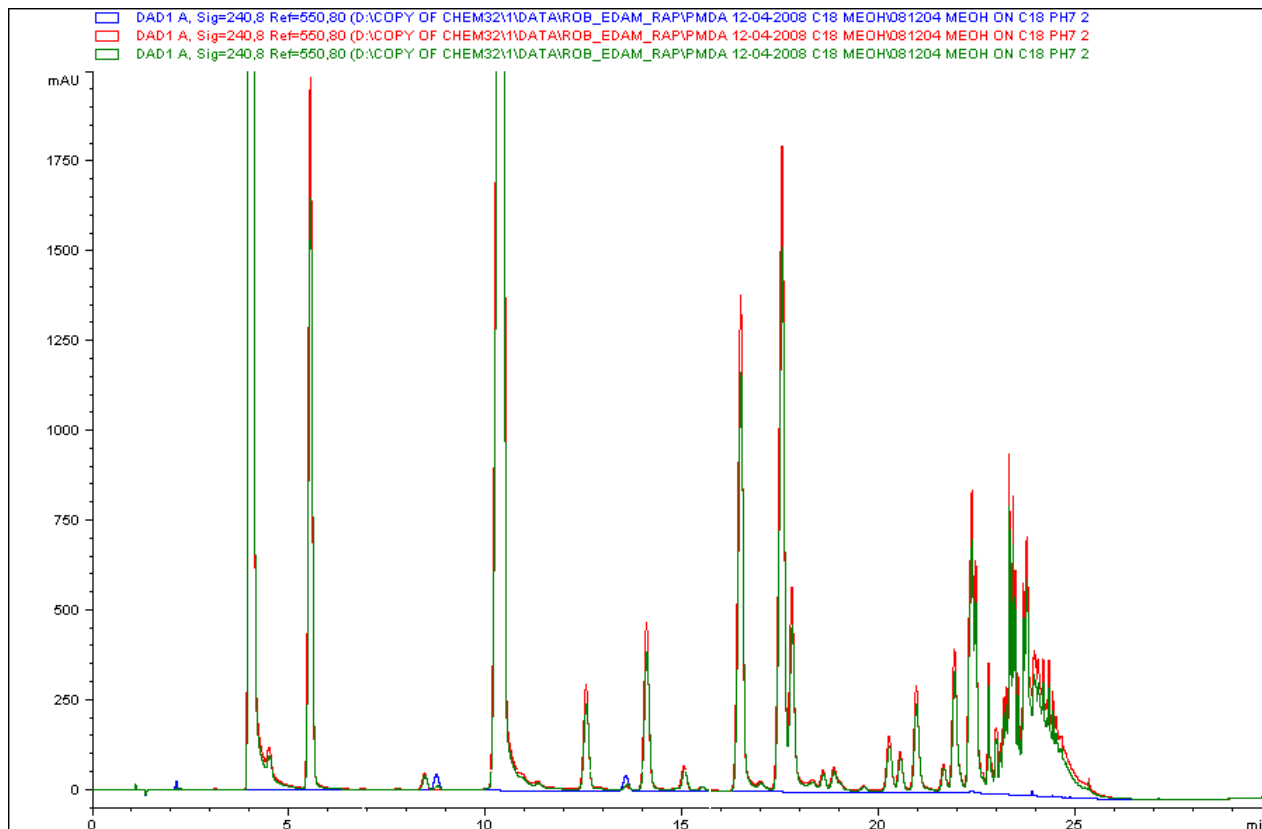
# Method Development Solution



## Aromatic amine oligomers (process sample)

Column: Ascentis Express C18 (4.6x150mm 2.7 $\mu$ m)

Gradient: 0 min 20% MeOH; 5 min 35% MeOH; 20 min 50% MeOH



# Method Development Solution

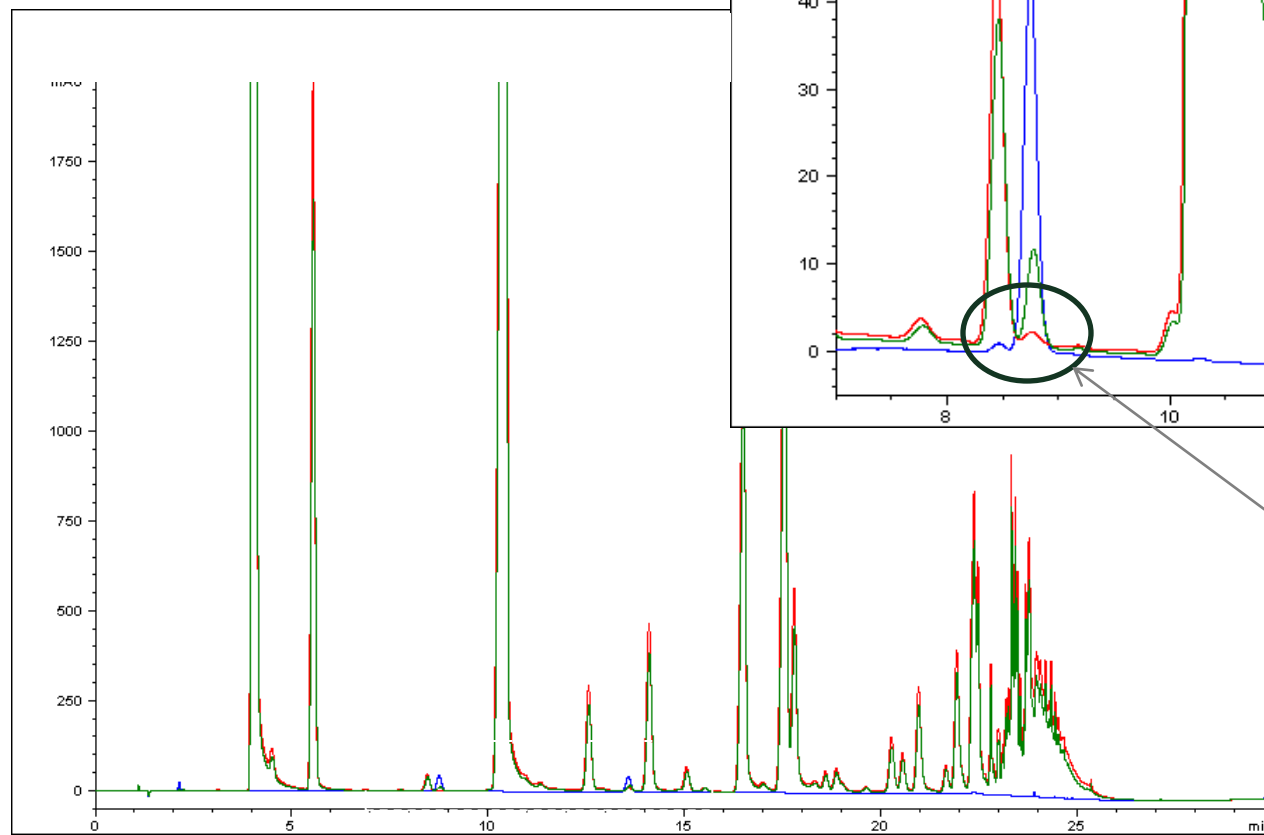
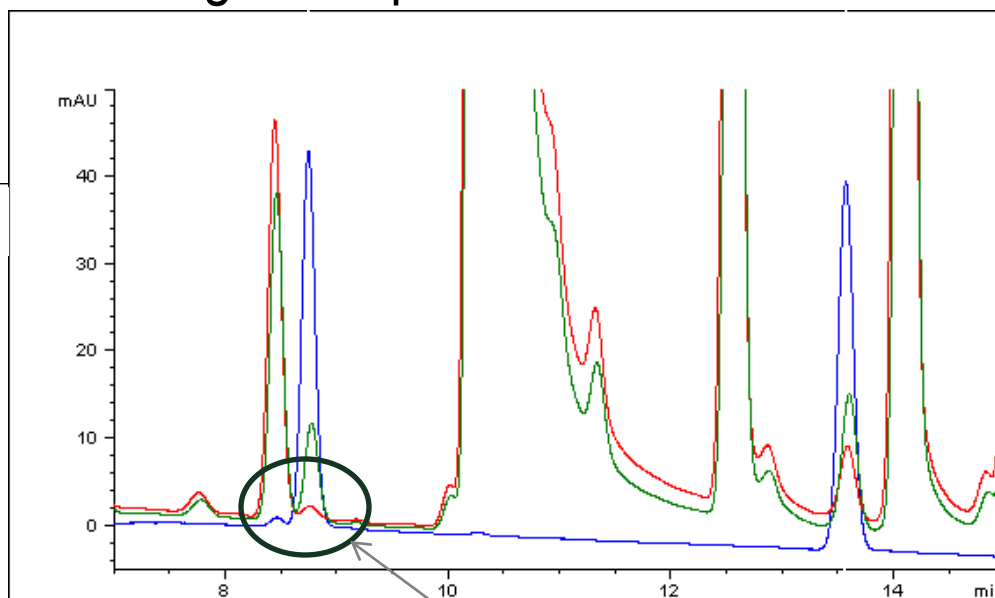


Separation after ~3 weeks of “manual” method development

Blue: standard, Red: sample,  
Green: spiked sample

Target compound TC#1

TC#2

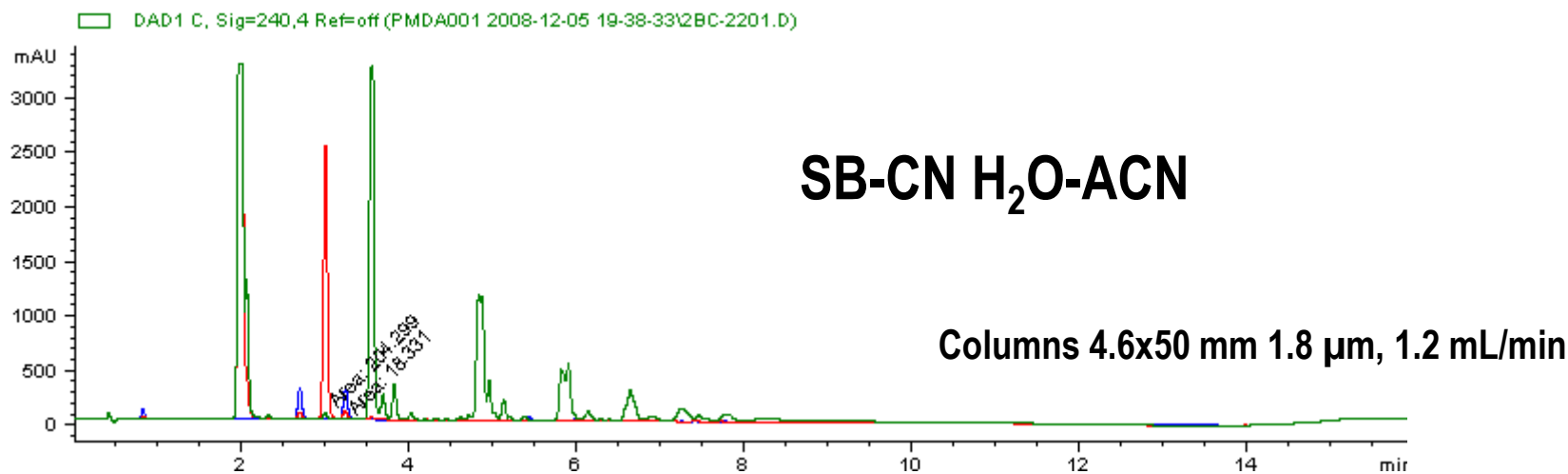
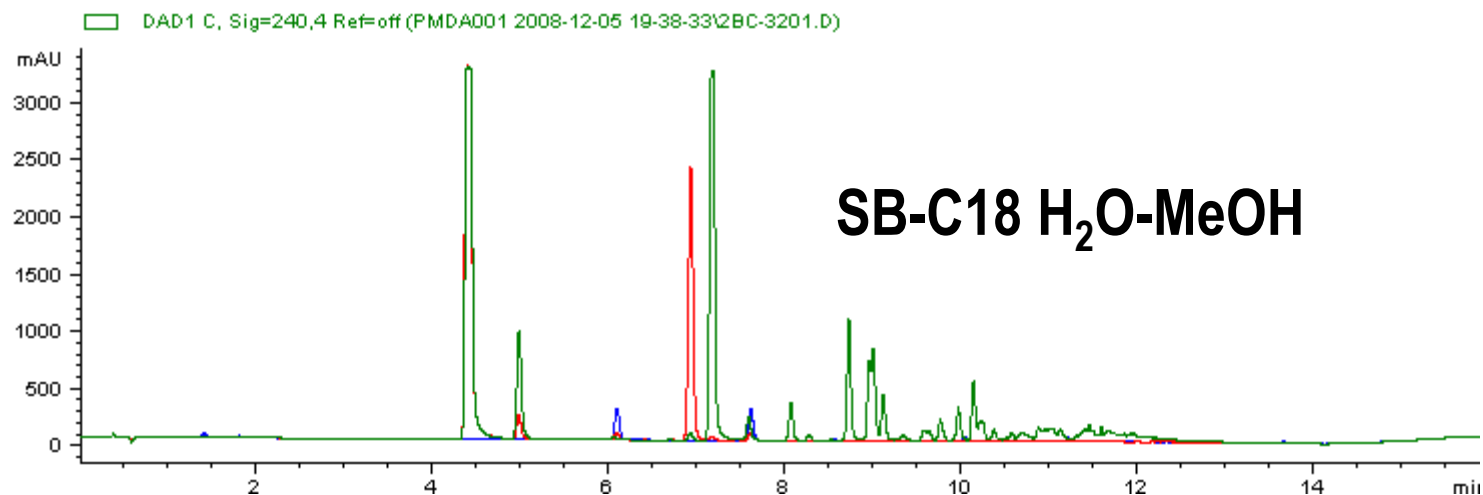


Separation not ideal

# Method Development Solution



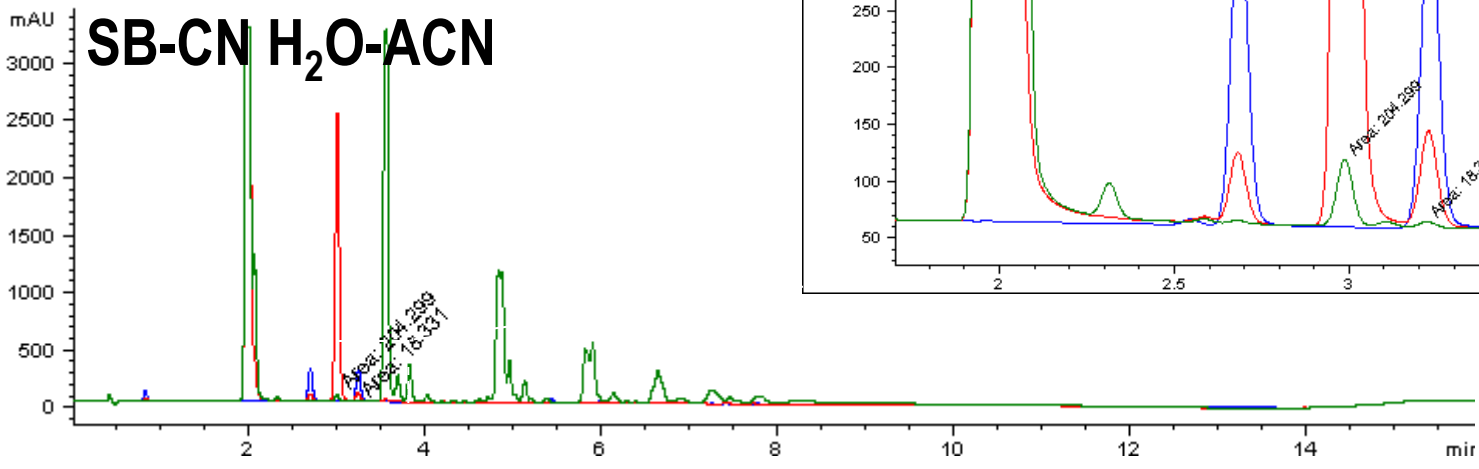
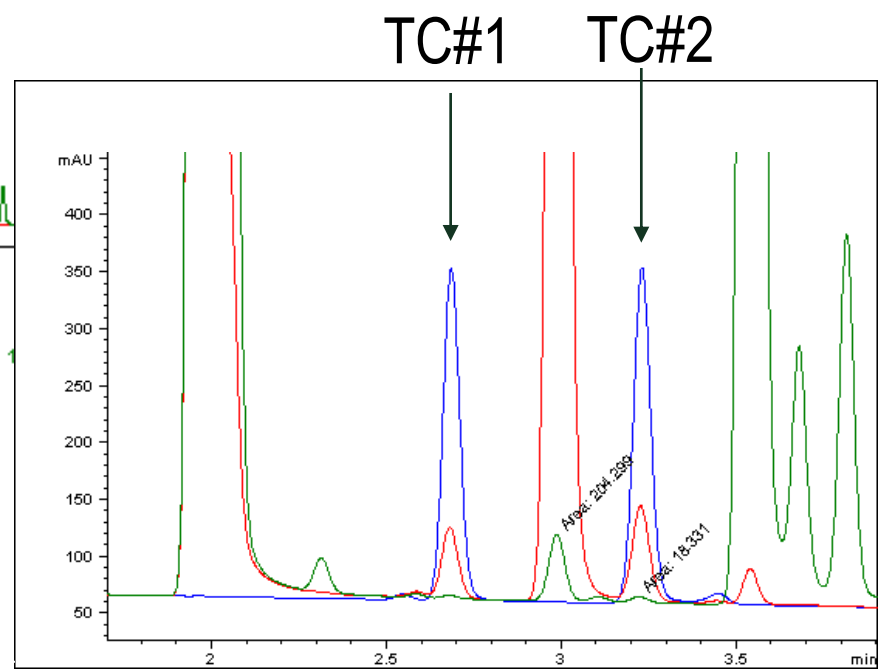
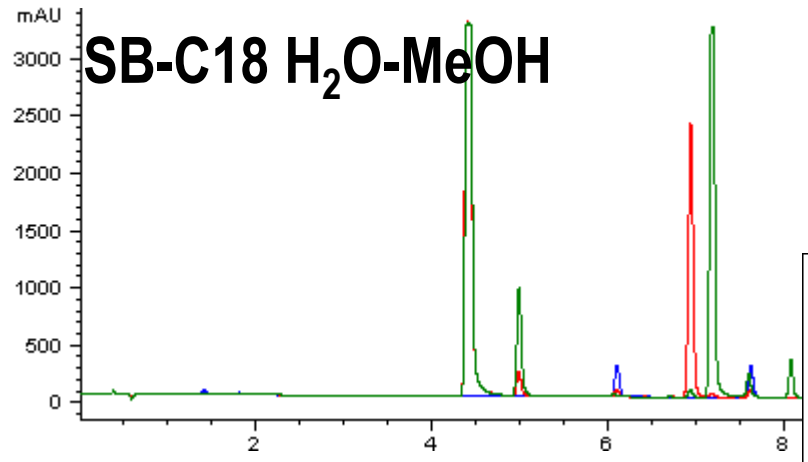
## Column/Solvent screening:



# Method Development Solution



Blue: standard, Red: spiked sample (addtl. spike components), Green: sample

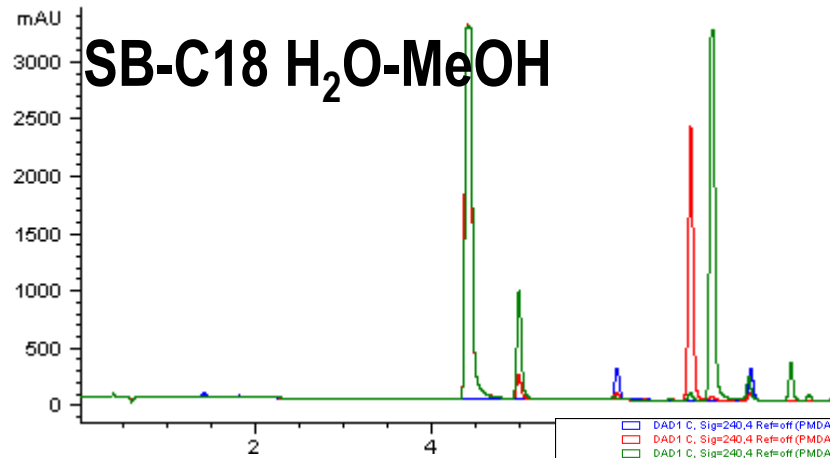


# Method Development Solution

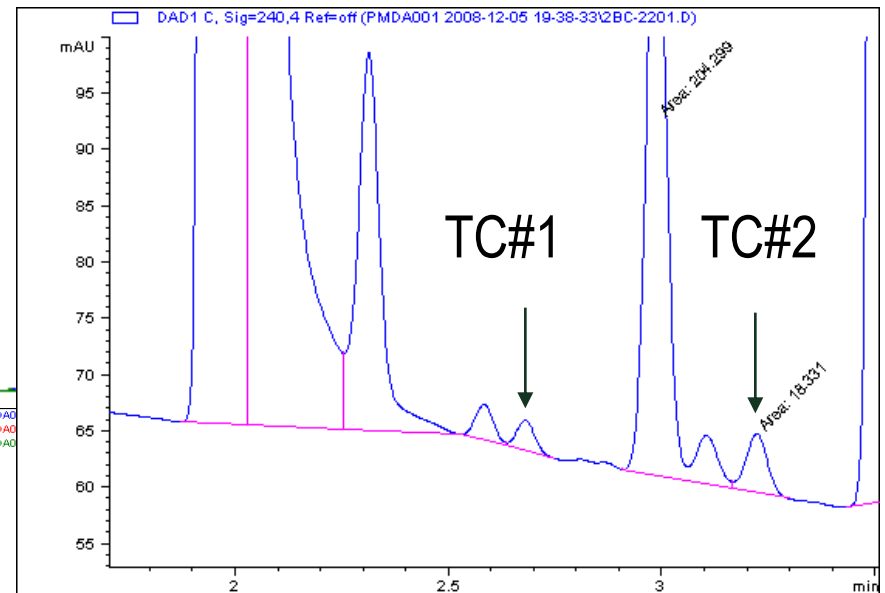


Real sample:

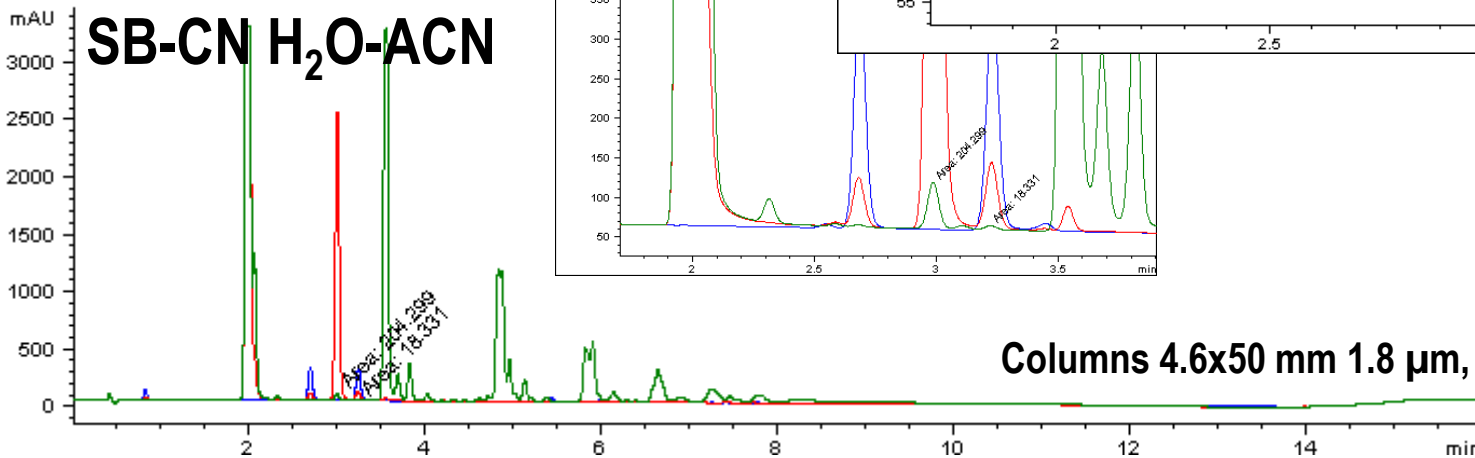
DAD1 C, Sig=240,4 Ref=off (PMDA001 2008-12-05 19-38-33\2BC-3201.D)



SB-C18 H<sub>2</sub>O-MeOH



DAD1 C, Sig=240,4 Ref=off (PMDA001 2008-12-05 19-38-33\2BC-3201.D)



SB-CN H<sub>2</sub>O-ACN

Columns 4.6x50 mm 1.8  $\mu$ m, 1.2 mL/min



# Conclusions Example 2

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- Overnight screening of 6 columns with two different solvent combinations (MeOH/water, Acetonitrile/water)
- SB-Cyano column showed excellent selectivity for 2 target compounds
- Automated screening provided better and much faster results with different stationary phase (cyano propyl) compared to manual method development using a high resolution C<sub>18</sub> column

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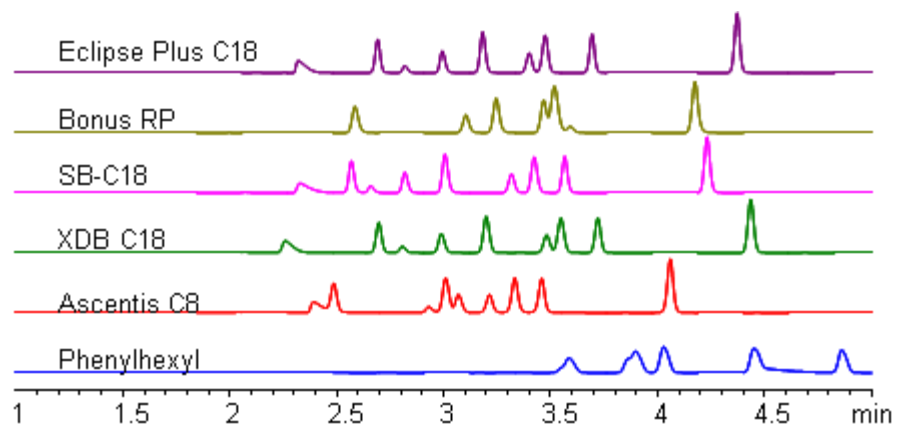
## Example 3: Nitro aromatics analysis

- Client wanted to replace MeCN by other organic modifier
- Separation of 9 components
- 50x4.6 mm columns, 1.8  $\mu\text{m}$  and 2.7  $\mu\text{m}$  particle size
- Screening of six columns and four organic mobile phases

# Fast screening



MeOH

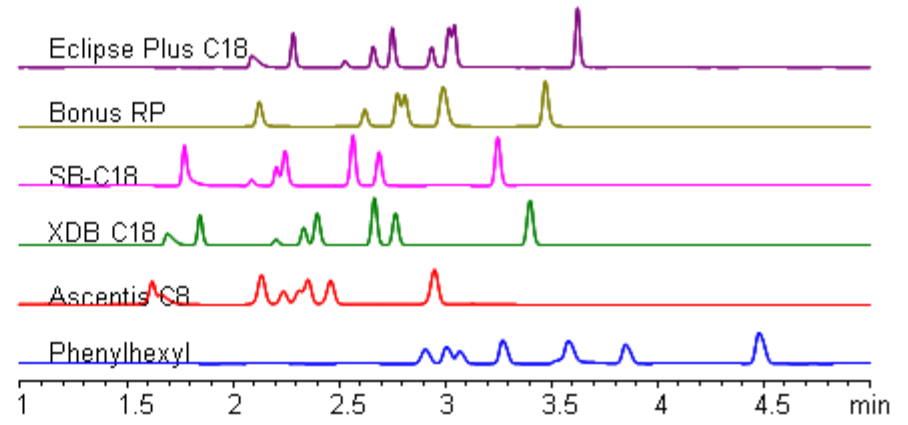
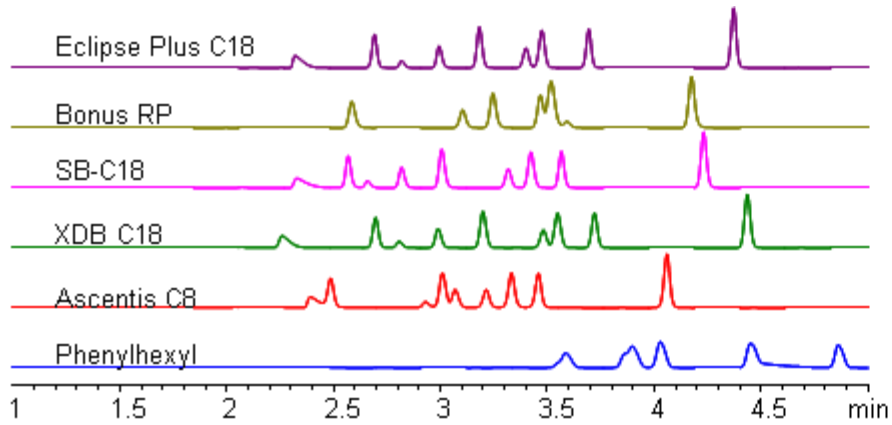


# Fast screening



MeOH

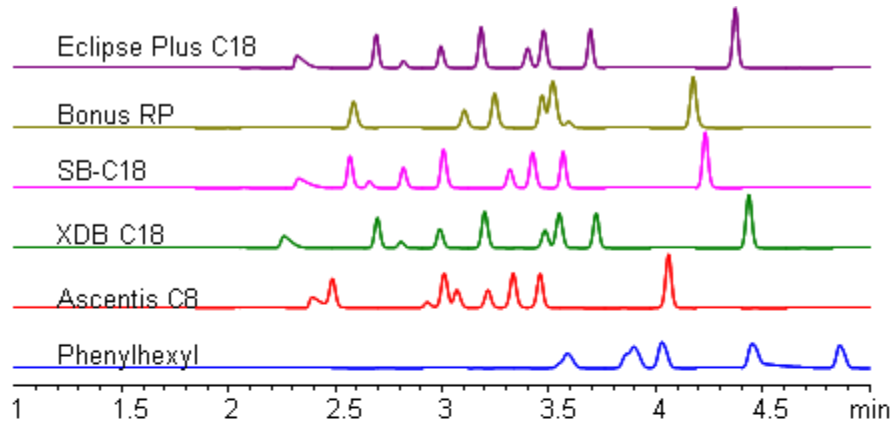
EtOH



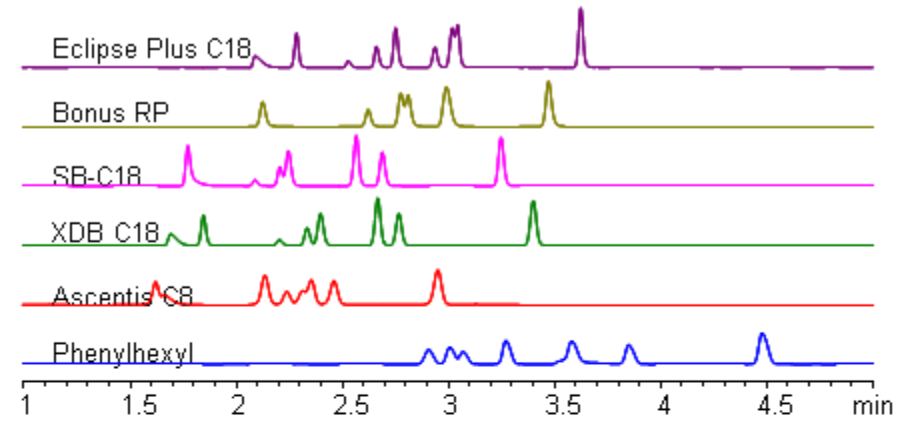
# Fast screening



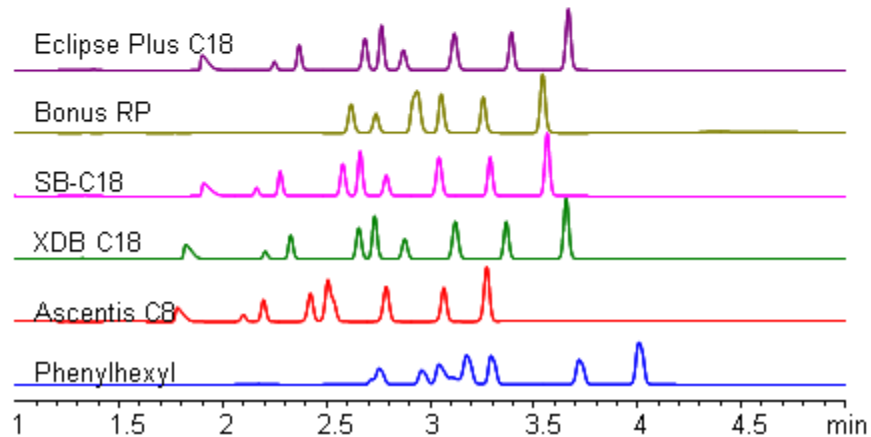
MeOH



EtOH



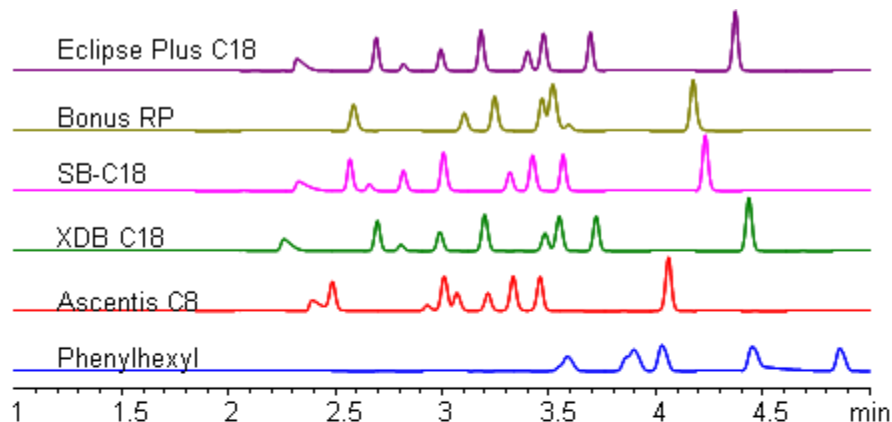
MeCN



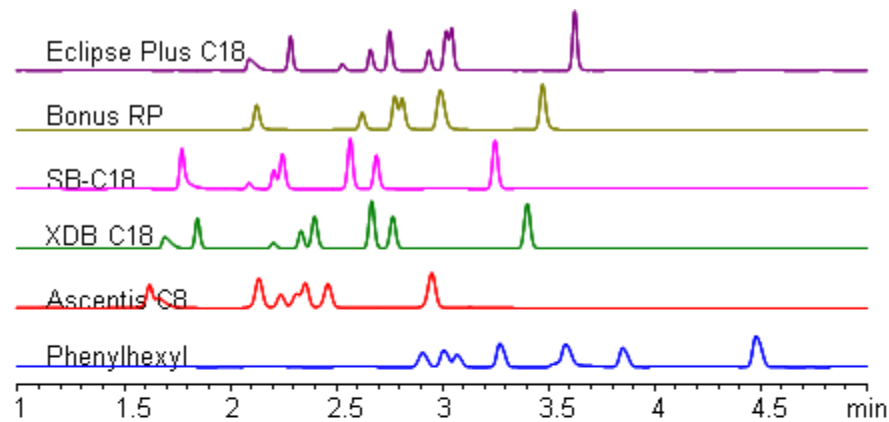
# Fast screening



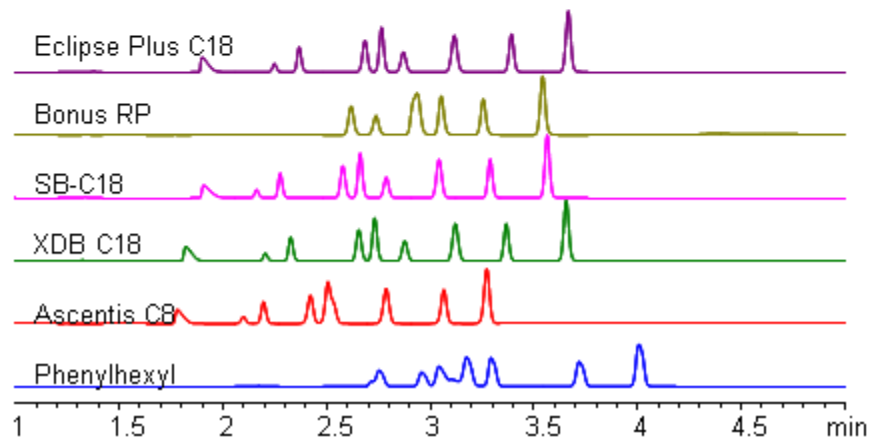
MeOH



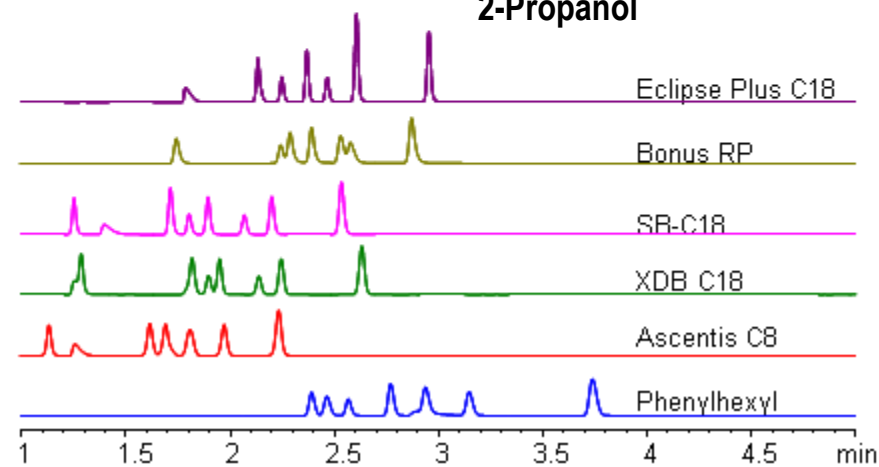
EtOH



MeCN



2-Propanol

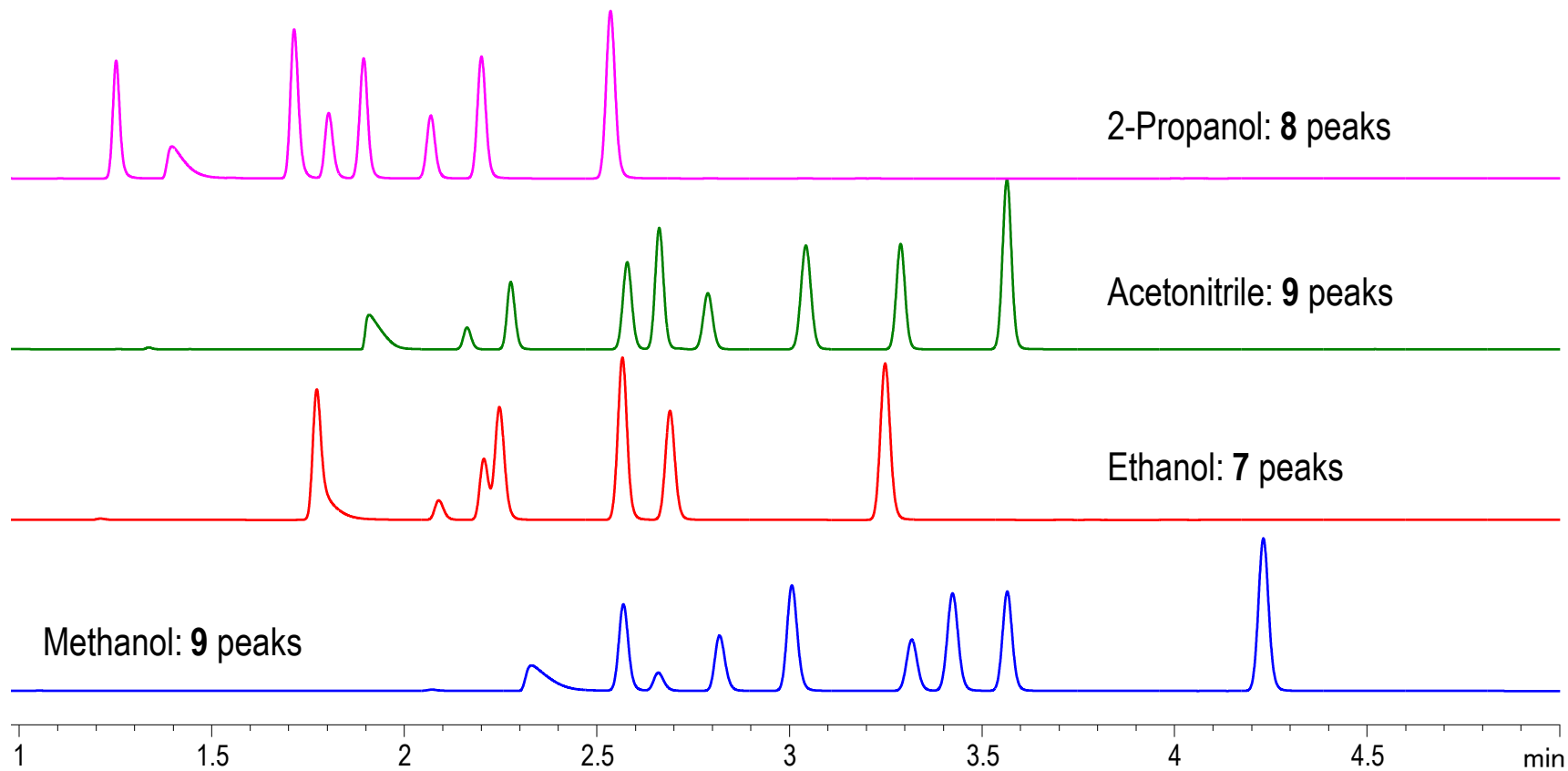


24 chromatograms in less than 6 hours run time (includes blank gradient)

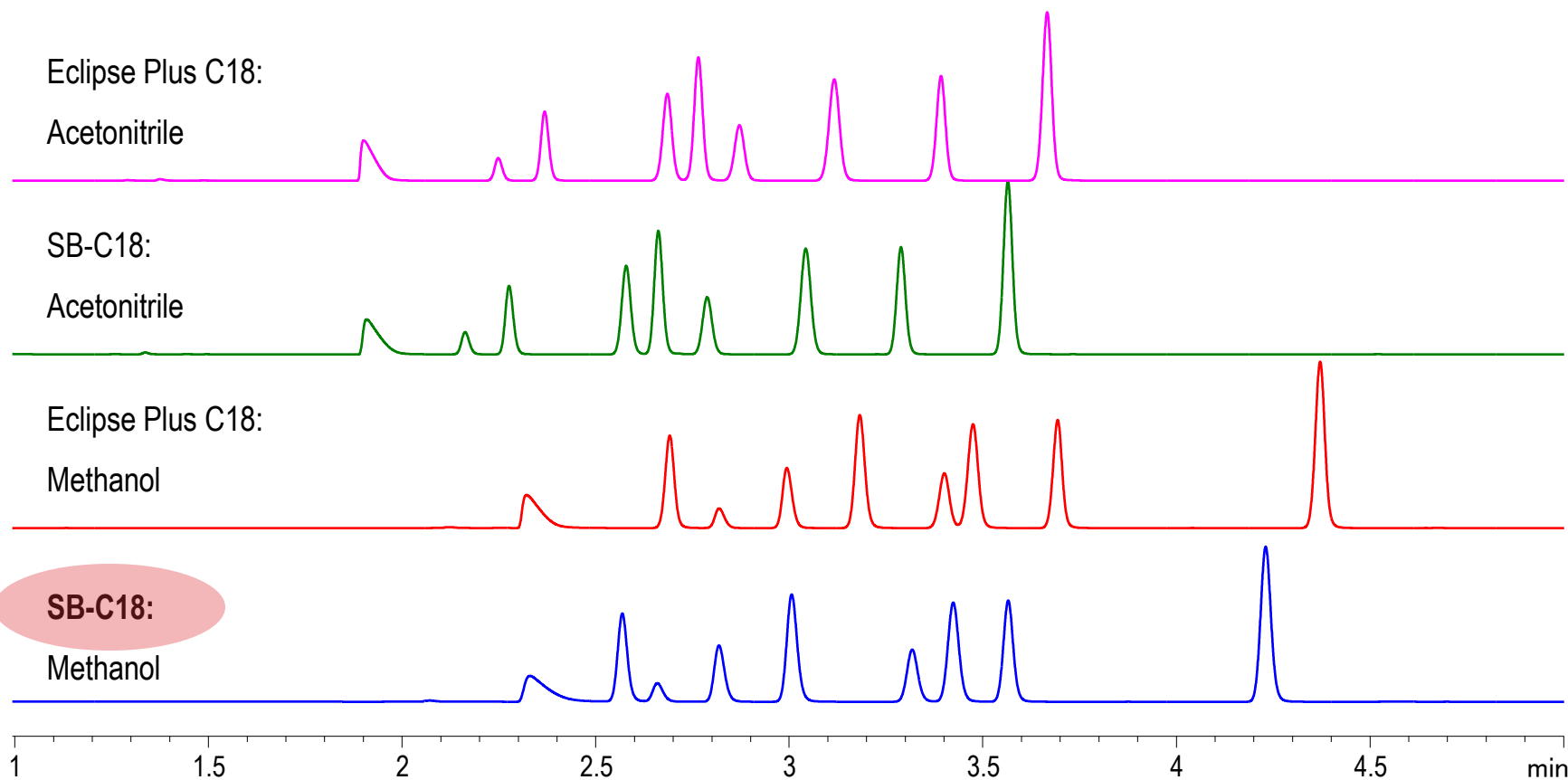
# Nitro aromatics – solvent screening



Zorbax SB-C18 column:



# Best nitro aromatics separations



Several options of solvents and columns available for optimal separation

SB-C18 showing best selectivity at applied conditions



# Summary I

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- Automated column and solvent/buffer screening is very powerful for method development
- Provides significant productivity improvement.
  - In several cases, a nearly “ready-to-go” method was obtained by overnight screening
  - In other cases it took another 1-2 days until the final method was obtained
- For some separations “unexpected” stationary phases provided the best separations (e.g., phenyl hexyl or cyano propyl).

# Summary II

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- Recommend to vary only few variables (columns, solvents, T, gradients, buffers) in one screening campaign
  - use broad section of stationary phase chemistries: C8/C18/C30, polar embedded C18, Phenyl hexyl/phenyl/biphenyl, cyano propyl, fluorinated phase, etc.
  - start with column screening, one gradient and two organic modifiers (e.g., MeOH/buffer and MeCN/buffer)
  - start with very broad gradient – e.g., 10% B to 90% B, applicable to all columns of choice
  - example: 6 columns, two gradients (two organic modifiers), four injections per gradient per column (standard, standard, sample, spiked sample) = **48** chromatograms
  - considering 5 min run time and additional flush steps ~ **5-6 h** total analysis time
  - based on initial screening, perform additional fine tuning on gradient shape, temperature and/or buffers in subsequent campaign(s)

# Acknowledgments

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- Helmut Schulenberg-Schell (Agilent)



# Maximize Instrument Efficiency Through Automation of Sample Preparation, Method Development, and Data Analysis

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