Maximize Instrument Efficiency Through Automation of Method Development and Data Analysis

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Introduction

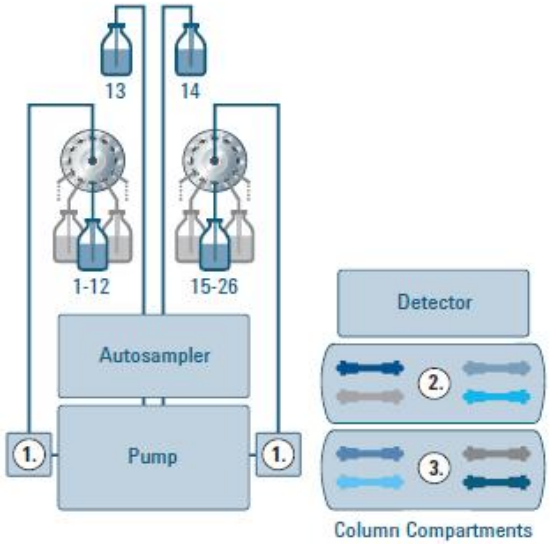
- LC method development is a constant need for analytical labs for several reasons:
  - new products or new product compositions
  - changes in specification, registration
  - new requirements regarding speed, resolution or availability of column types/chemistries
- Manual method development can be laborious…
  - often does not get beyond applying C_8/C_{18} 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

- 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**

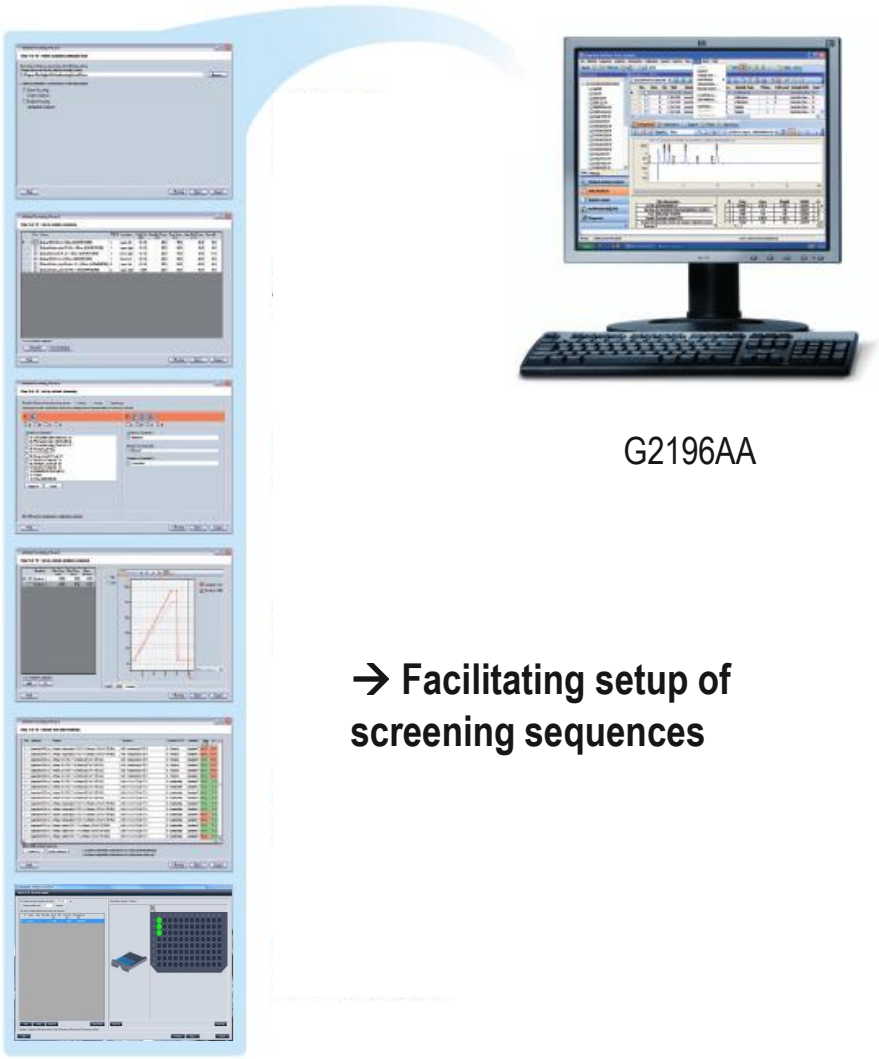
Select between different gradients and temperatures.

- **Review and select screening methods**

Check for incompatible combinations.

- **Set up samples**

Define injection volumes and number of repetitions.



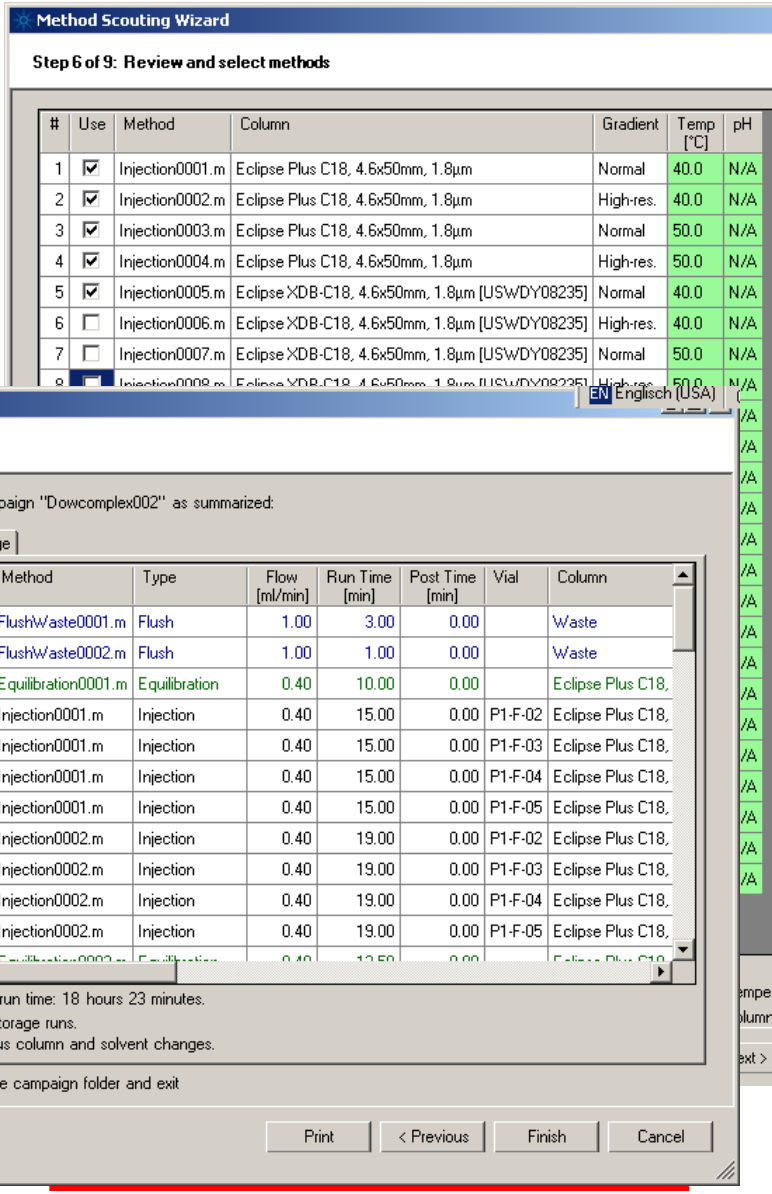
G2196AA

→ Facilitating setup of screening sequences

Method Scouting Wizard

Automation of sequence (campaign) setup

- Settings for solvent and column selection
- Time and solvent usage estimation



Example 1: Herbicide impurity separation

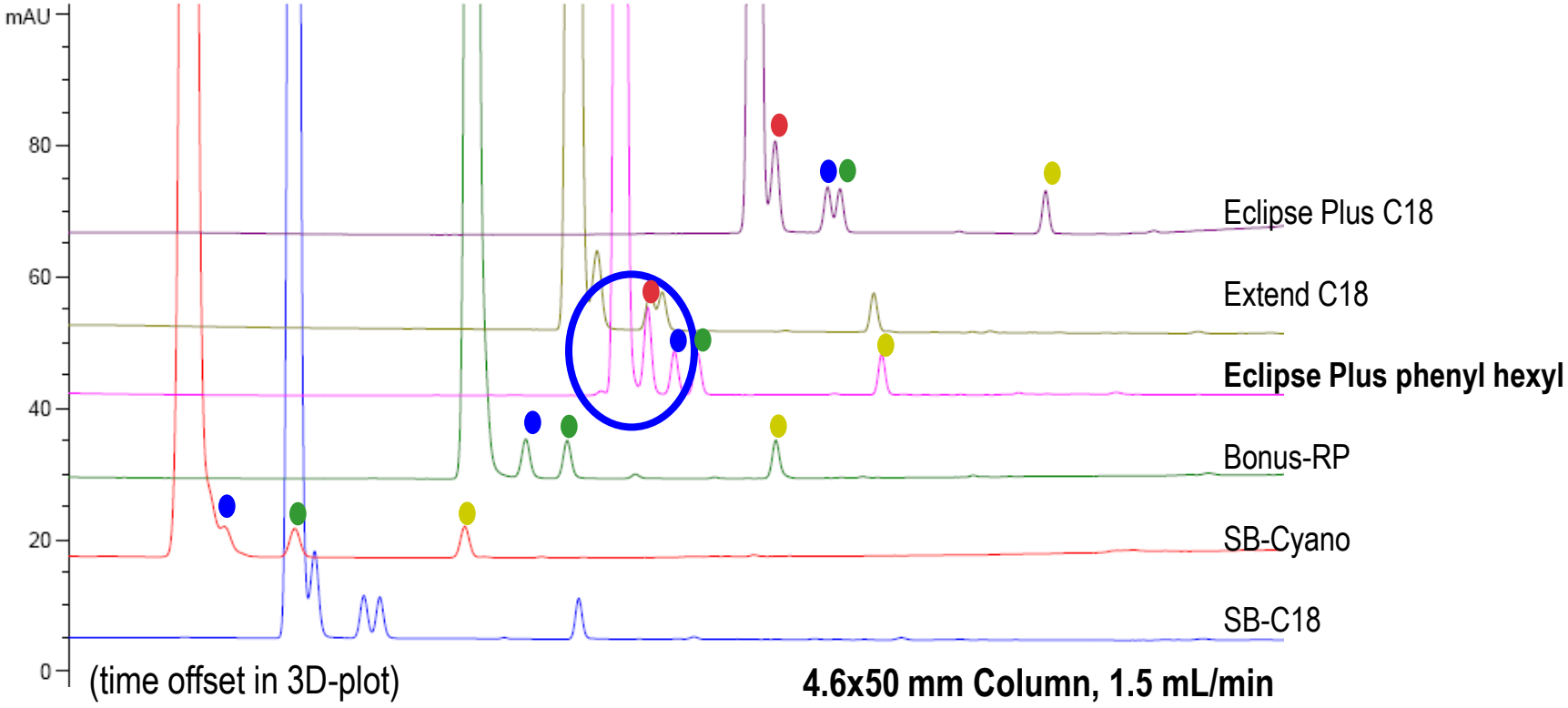
- Two isocratic LC methods were used, one column no longer available – needed to upgrade the method
- 50x4.6 mm columns, 1.8 um particle size
- Screening of methanol/buffer and acetonitrile/buffer gradients at 1.5 ml/min

Method Development Solution

Herbicide impurities – Acetonitrile/buffer gradient

Separation of impurity standard

H_2O:ACN (0.05% TFA) 10 to 95% in 10 minutes.

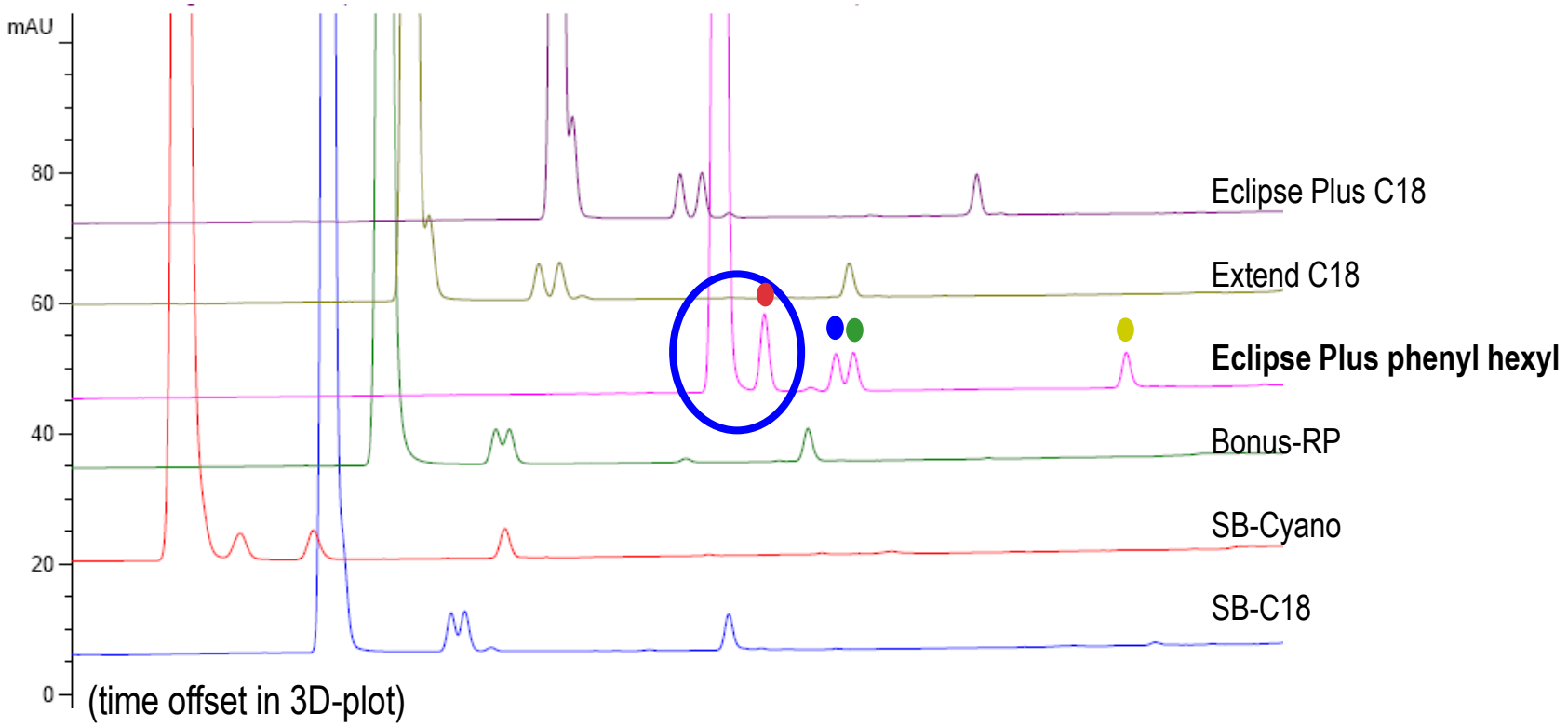


Method Development Solution

Herbicide impurities – Methanol/buffer gradient

Separation of impurity standard

H_2O:MeOH (0.05% TFA) 10 to 95% in 10 minutes.



Conclusions - Example 1

- 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

Example 2: Aromatic amine oligomers

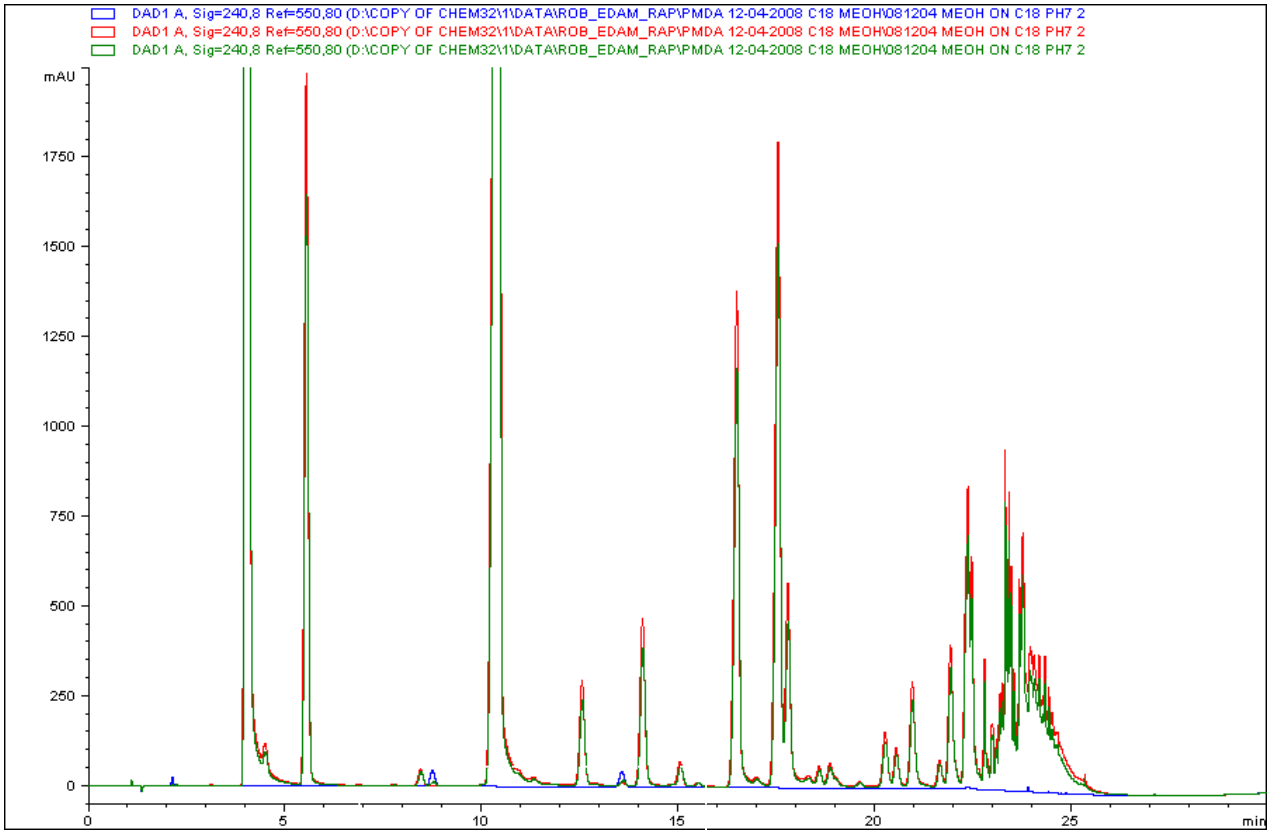
- "Manual" method development with C_{18} column
- Automated screening of 50x4.6 mm columns, 1.8 um particle size
- Screening of methanol/buffer and acetonitrile/buffer gradients at 1.5 ml/min

Method Development Solution

Aromatic amine oligomers (process sample)

Column: Ascentis Express C18 (4.6x150mm 2.7µm)

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



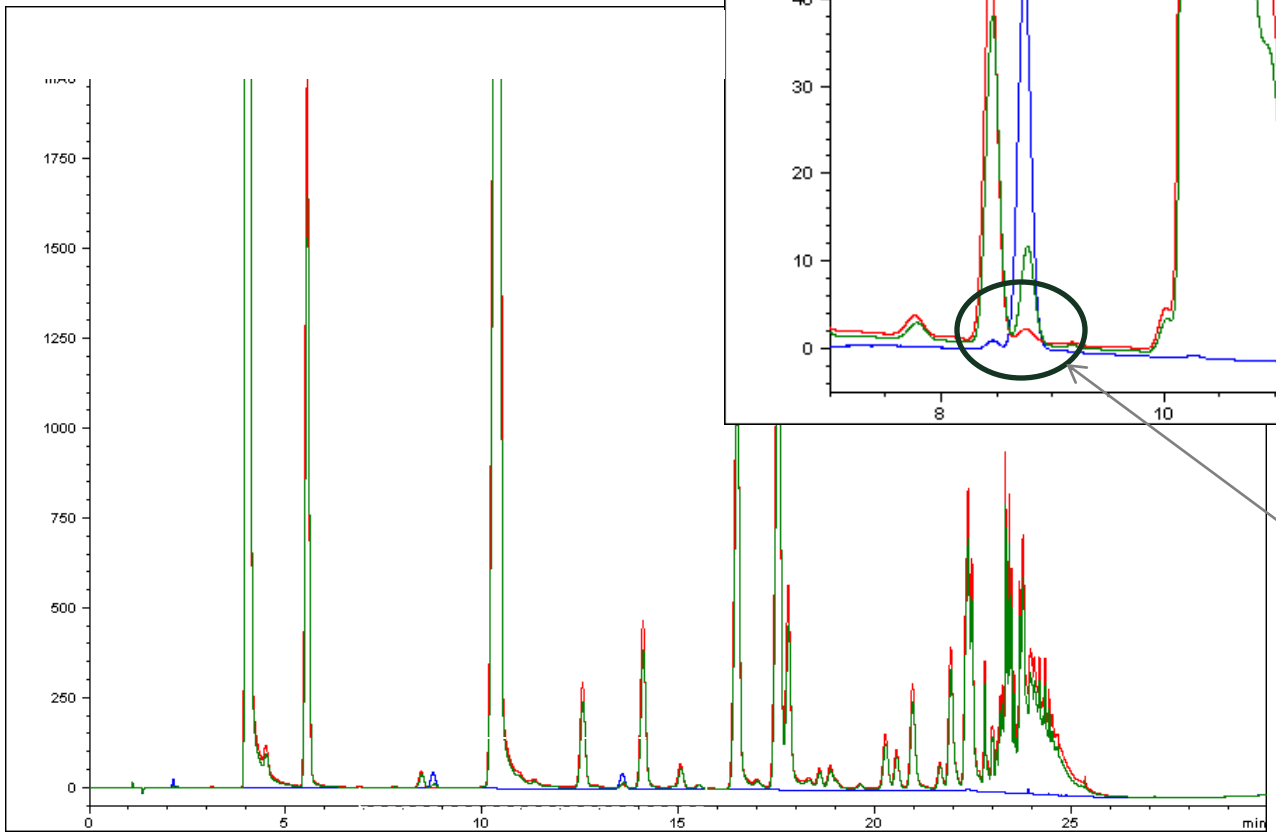
Method Development Solution

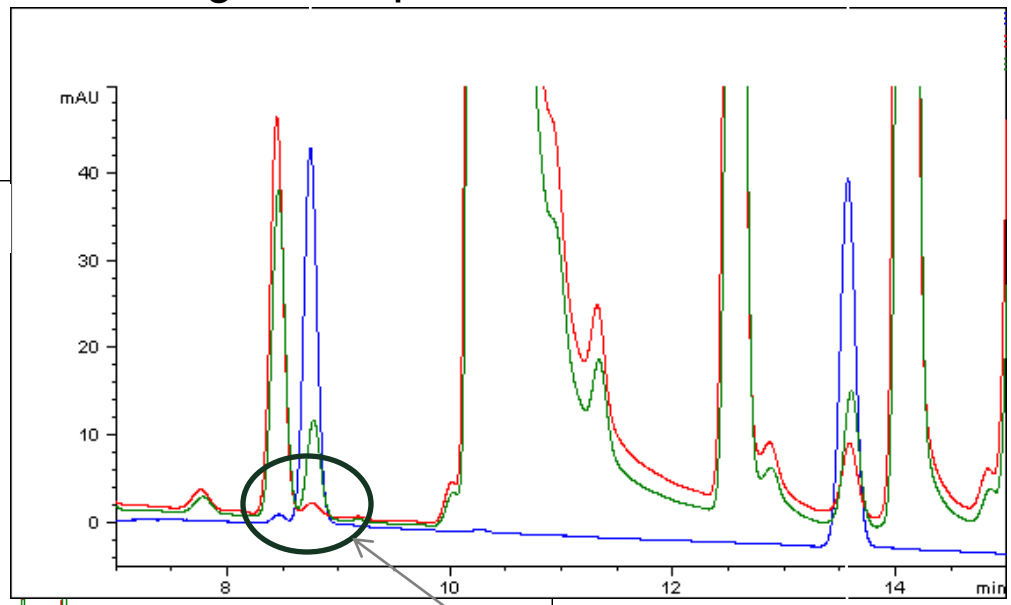
Separation after ~3 weeks of "manual" method development

Blue: standard, Red: sample,

Green: spiked sample

Target compound TC#1 TC#2

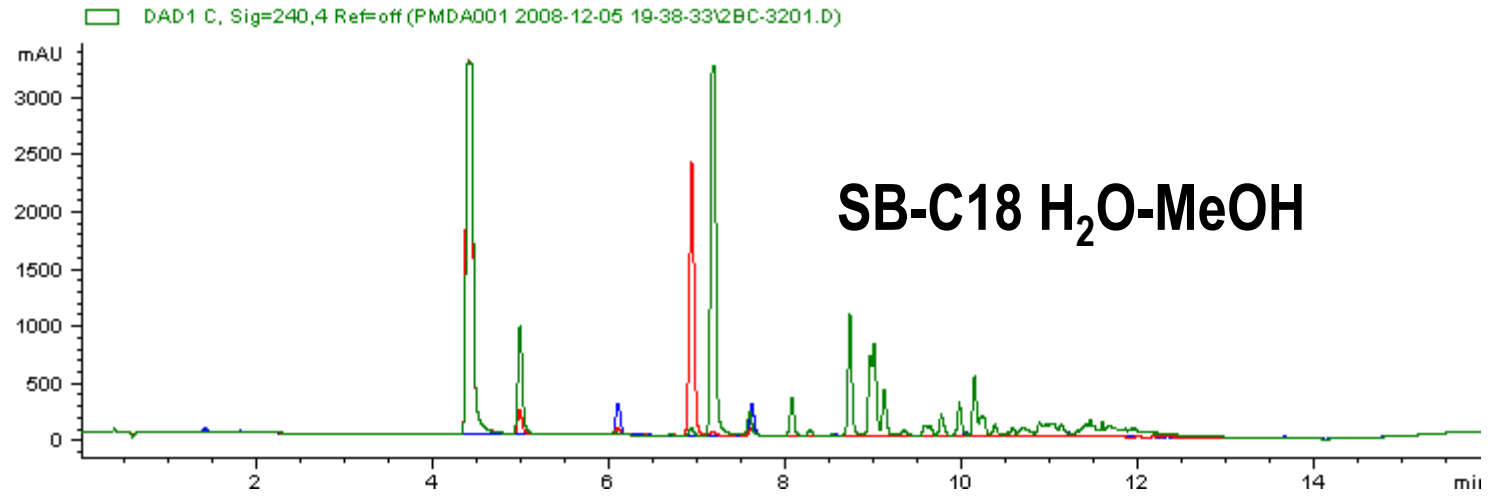


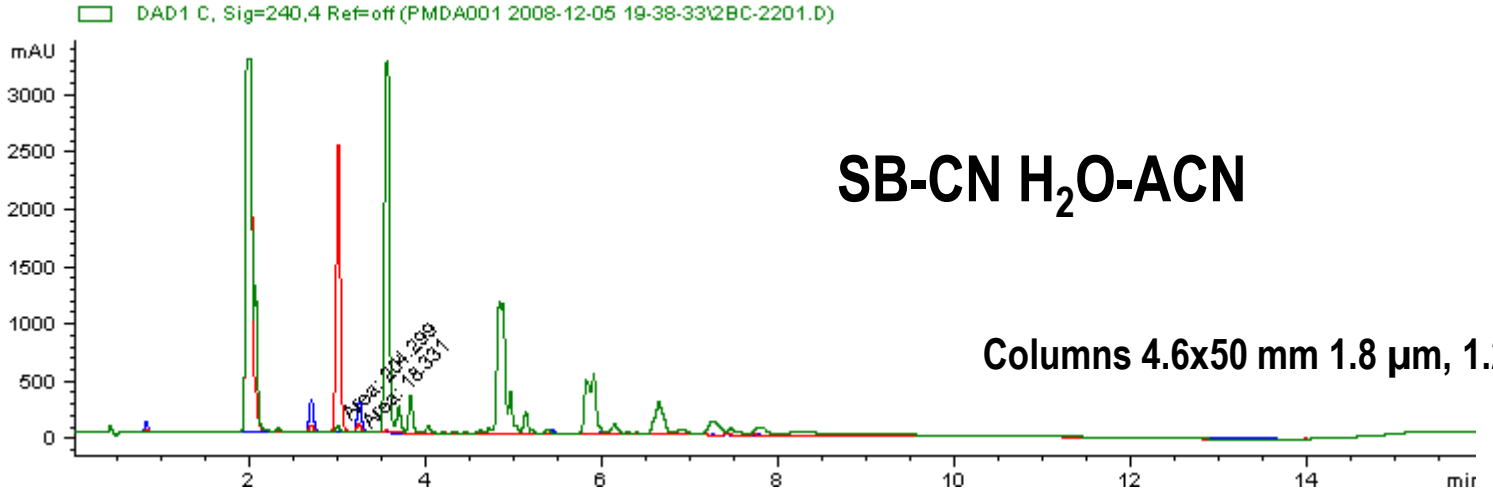


Separation not ideal

Method Development Solution

Column/Solvent screening:

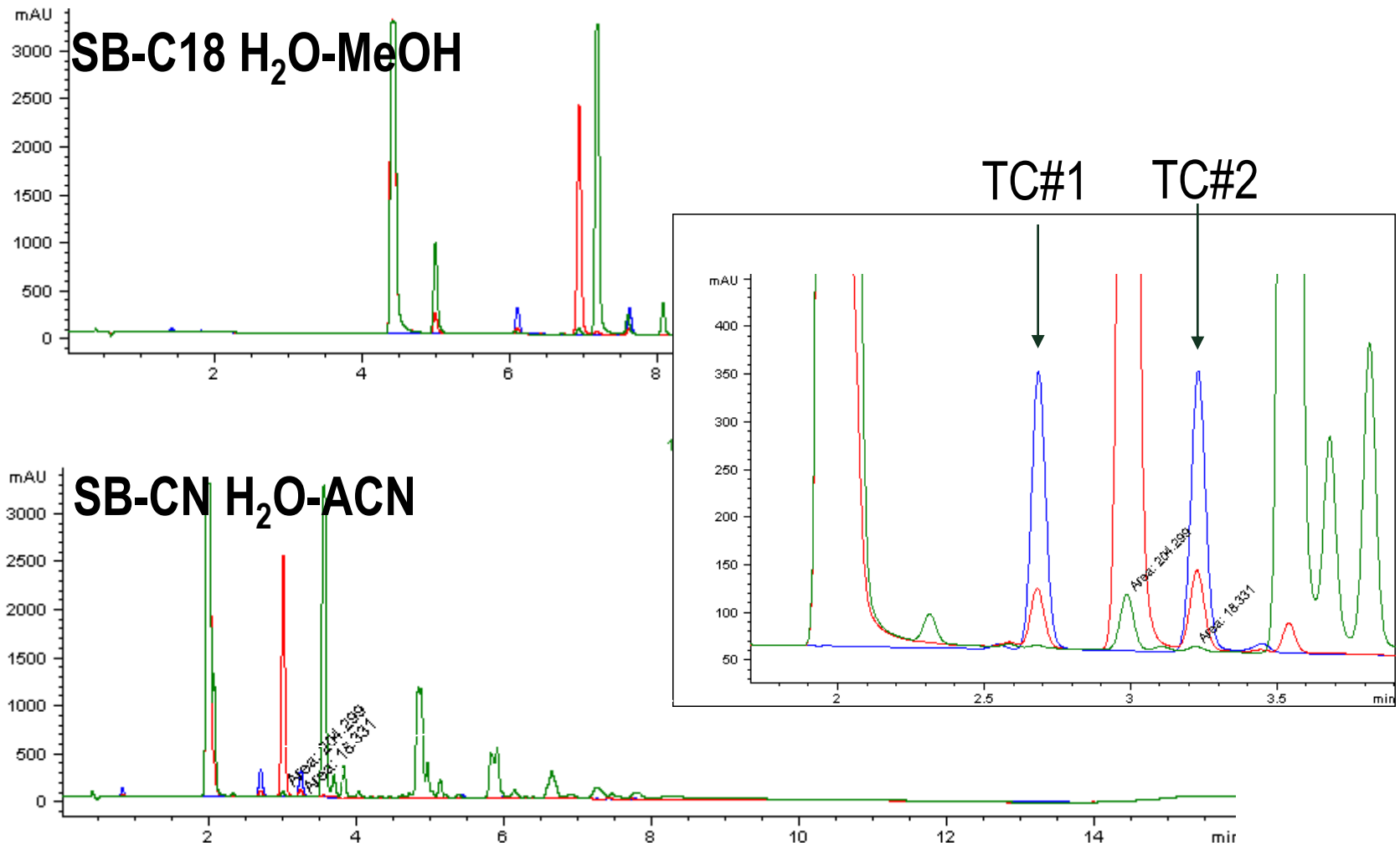




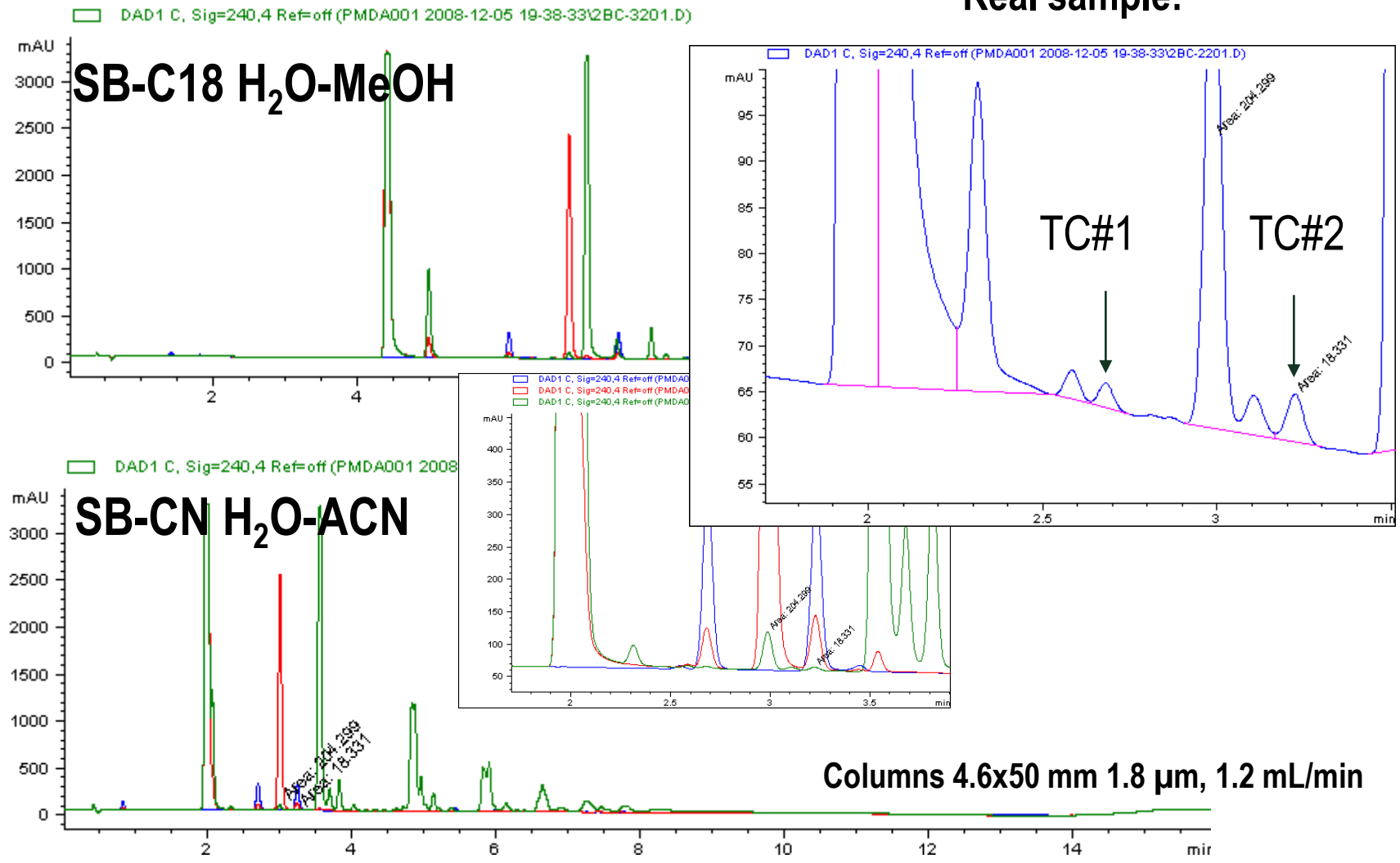
Columns 4.6x50 mm 1.8 µm, 1.2 mL/min

Method Development Solution

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



Method Development Solution



Conclusions Example 2

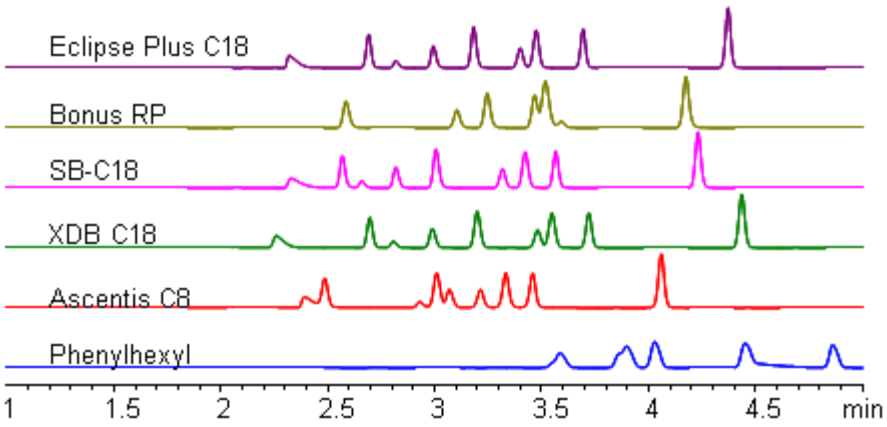
- Overnight screening of 6 columns with two different solvent combinations (MeOH/water, Acetonitrile/water)
- SB-Cyano column showed excellent selectivity for 2 target compounds
- Automated screening provided better and much faster results with different stationary phase (cyano propyl) compared to manual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

Example 3: Nitro aromatics analysis

- Client wanted to replace MeCN by other organic modifier
- Separation of 9 components
- 50x4.6 mm columns, 1.8 um and 2.7 um particle size
- Screening of six columns and four organic mobile phases

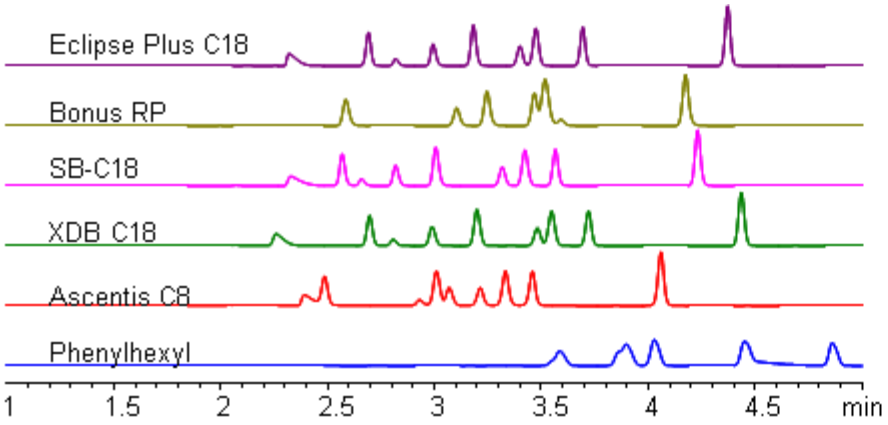
Fast screening

MeOH

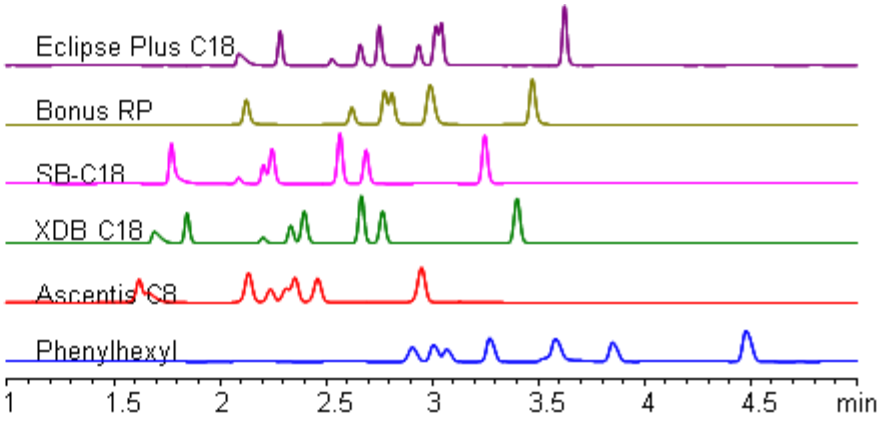


Fast screening

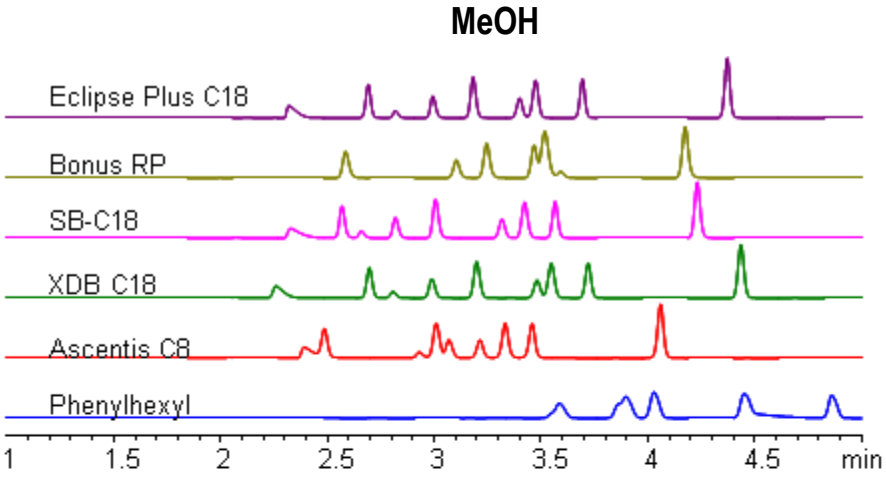
MeOH

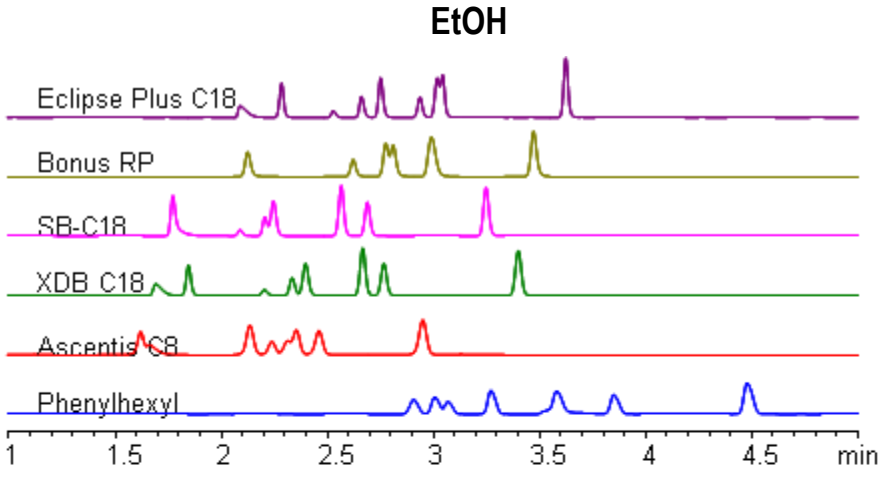


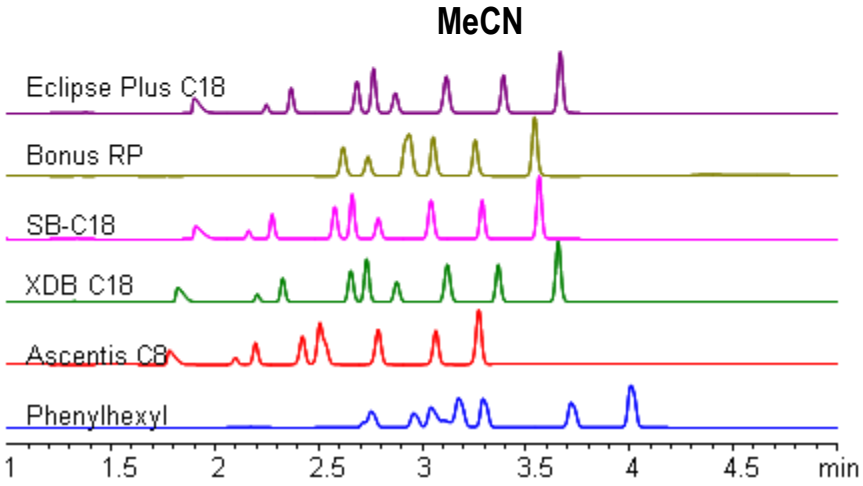
EtOH



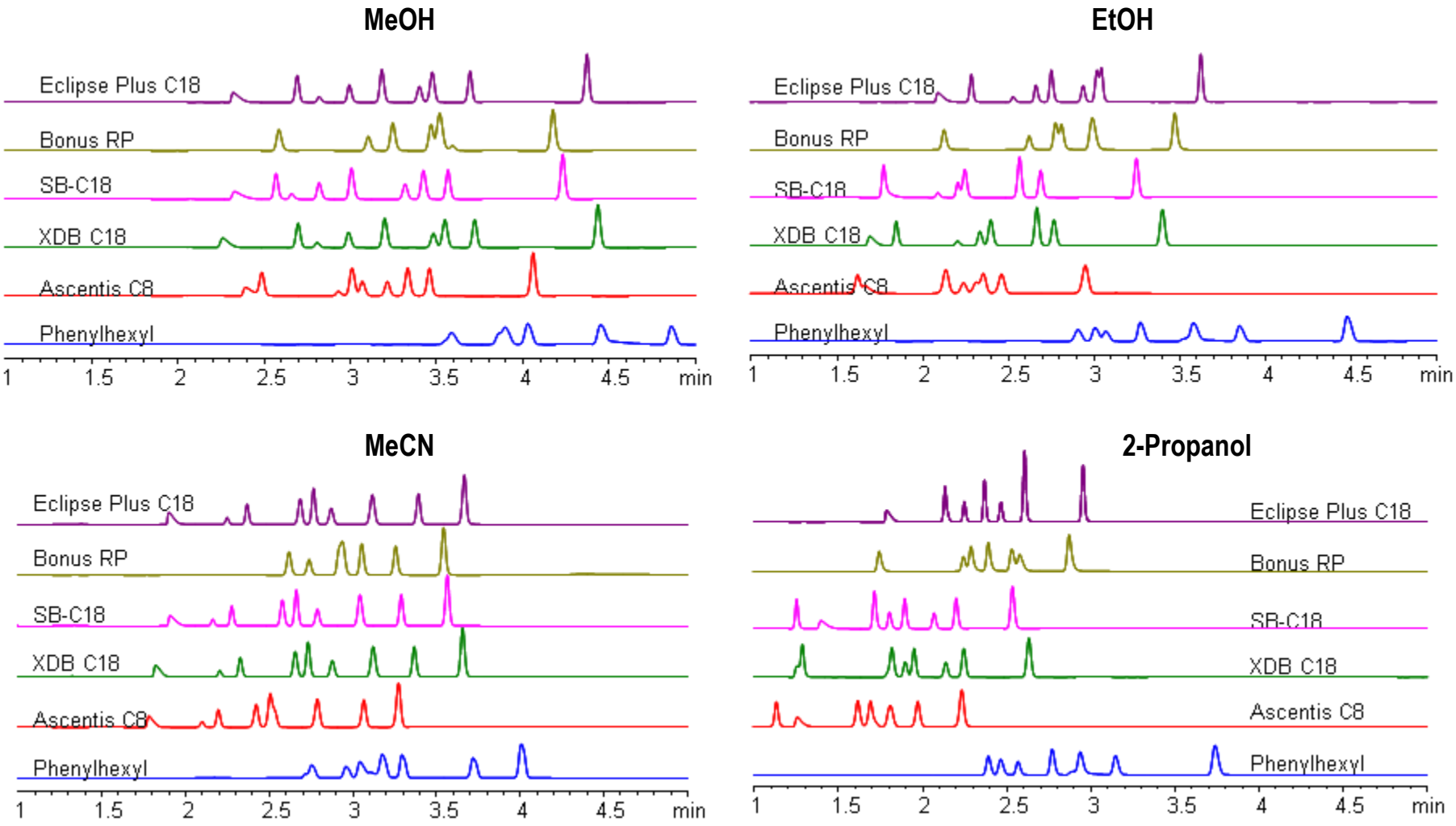
Fast screening







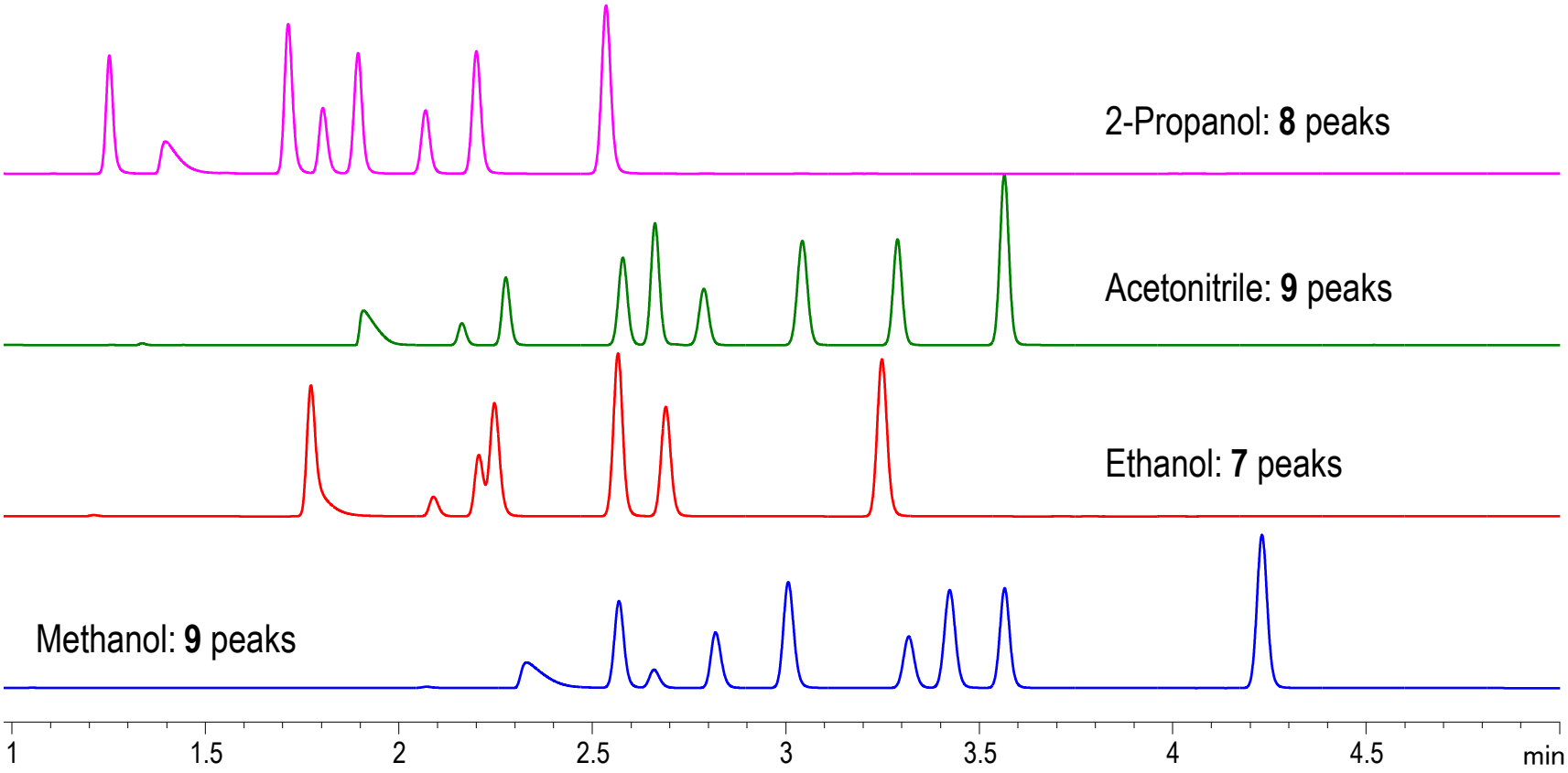
Fast screening



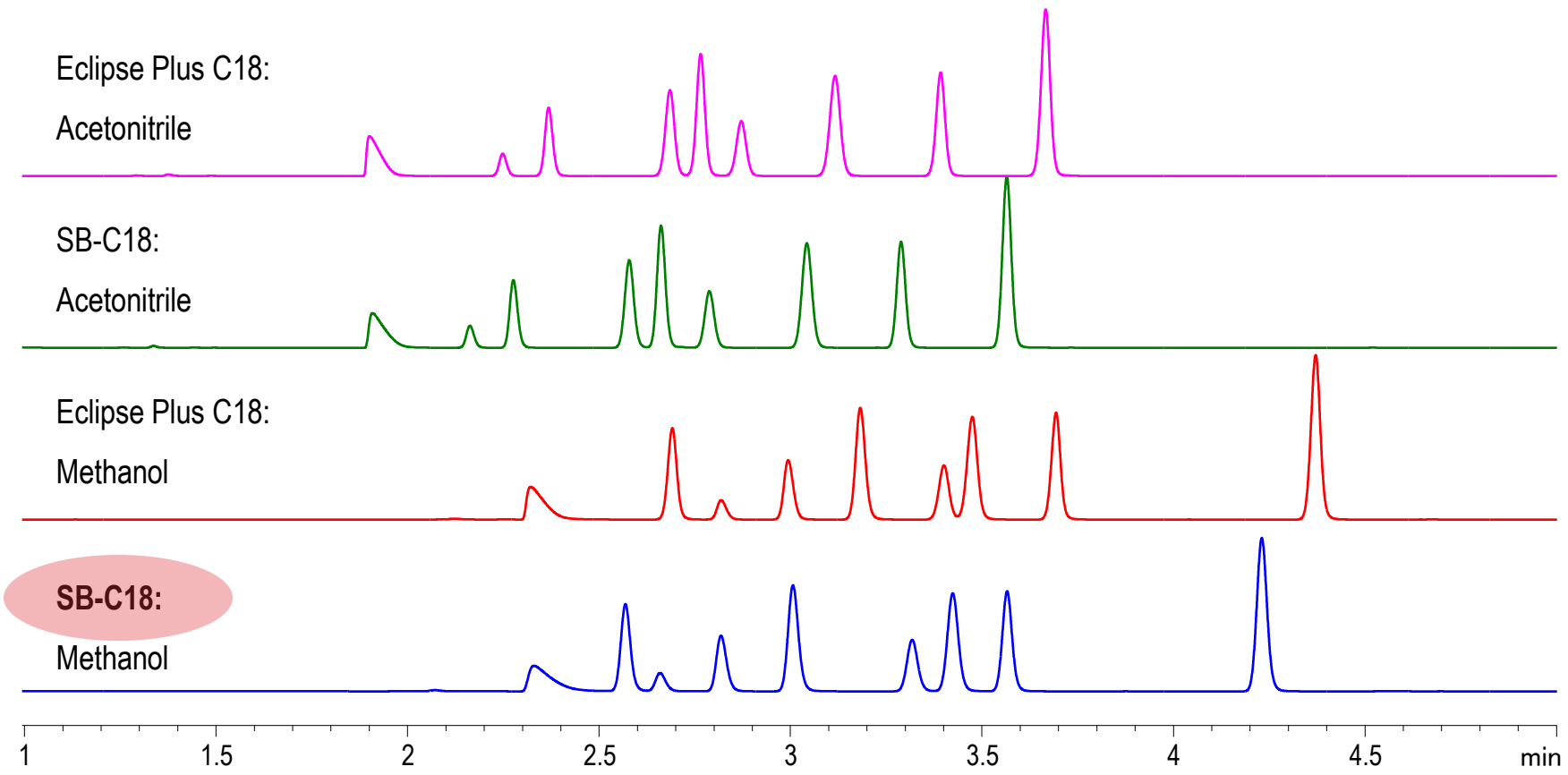
24 chromatograms in less than 6 hours run time (includes blank gradient)

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

- 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

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

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