Rapid Automatic HPLC Method Development With Different Column Types

Sergey Galushko
www.chromsword.de

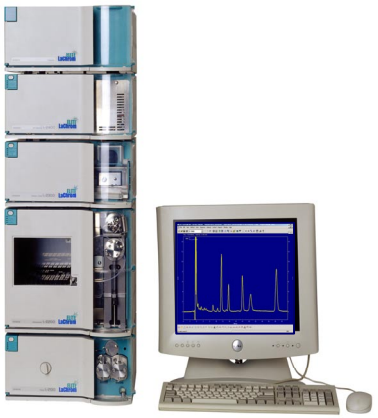
Automation of Optimisation Procedure

Systems Control

On-line Expert system

ChemStation

EZChrom



Empower

Processes retention data

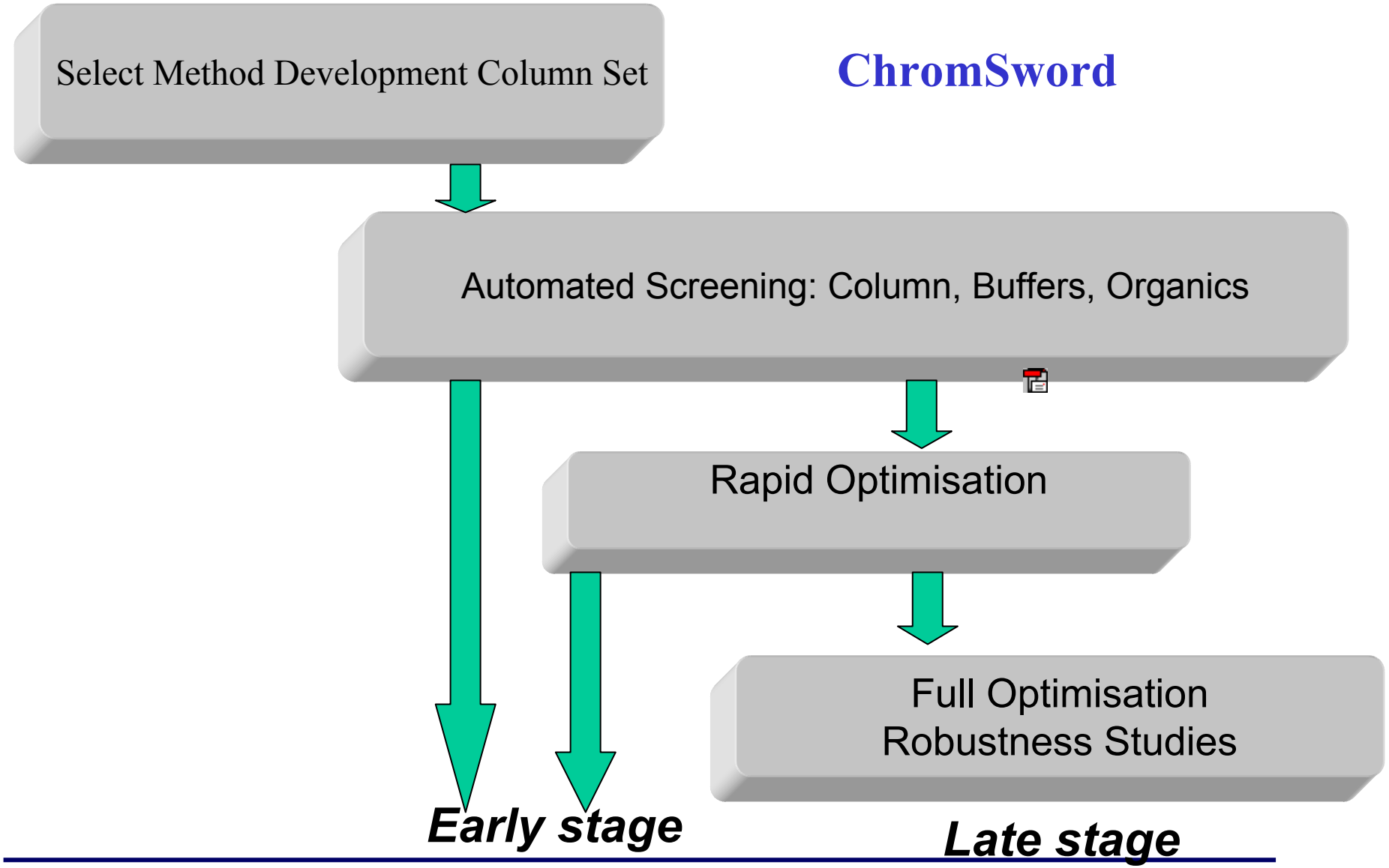
Unattended Method Development

ChromSword

Fully Automated steps:

1. Start a first method
2. Get results
3. Evaluate a chromatogram
4. Update models and start the next method
5. Repeat steps 1-4 to find an optimum
6. Generate reports

One Software Platform for Automatic Method Development with ChromSword



Column Properties – terms and definitions

- ***Polarity***
- ***Polar Selectivity***
- ***Size (hydrophobic) selectivity***
- ***Concentration of high energy sites –***

(The sites normally should be suppressed for good peak shapes)

Solvatic Retention Model in RP LC to describe column characteristics

$$\ln k = a(S) + b(\Delta G_{,H_2O}) + c$$

S - partial molecular surface in water

$\Delta G_{,H_2O}$ - energy of interaction with water

a, b, c - column and eluent parameters

S.V. Galushko, et.al J. Chromatogr. 660, 47-59, (1994)

Polar Selectivity

$$\ln k = a(S) + b(\Delta G_{,H_2O}) + c$$

$$S_1 = S_2$$

$$b = \ln \alpha / (\Delta G_{,H_2O,\,1} - \Delta G_{,H_2O,\,2})$$

Polar selectivity is a capability of a column under specified conditions to separate solutes that differ in their polarity and have no differences in the size.

Size Selectivity

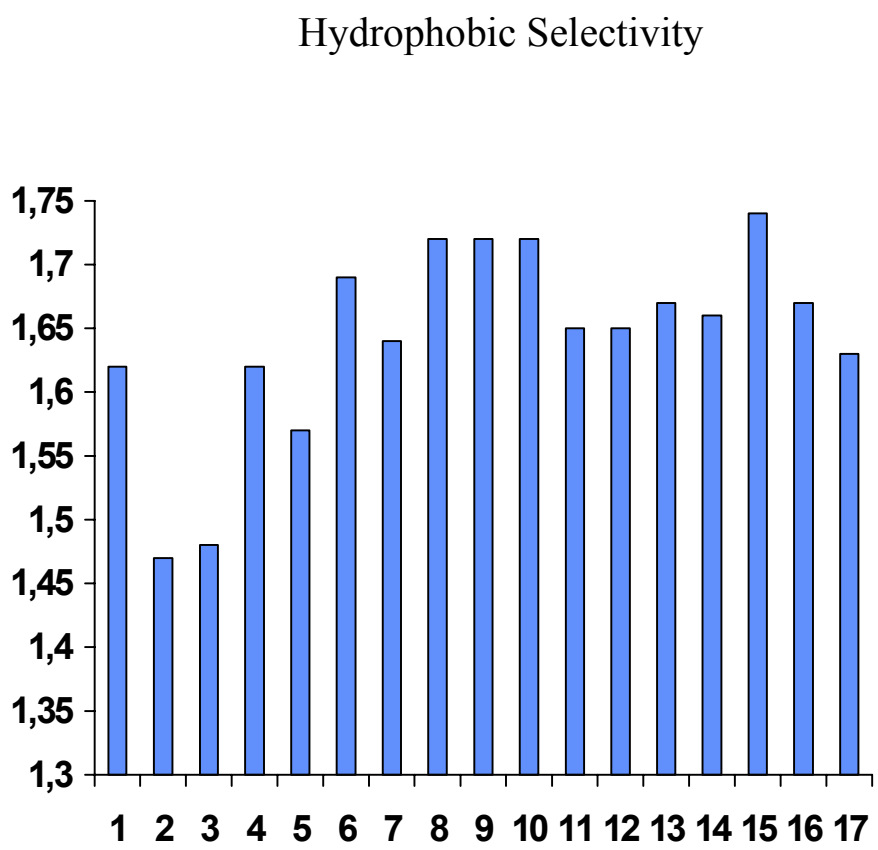
$$\ln k = a(S) + b(\Delta G_{,H_2O}) + c$$

$$(\Delta G_{,H_2O})_1 = (\Delta G_{,H_2O})_2$$

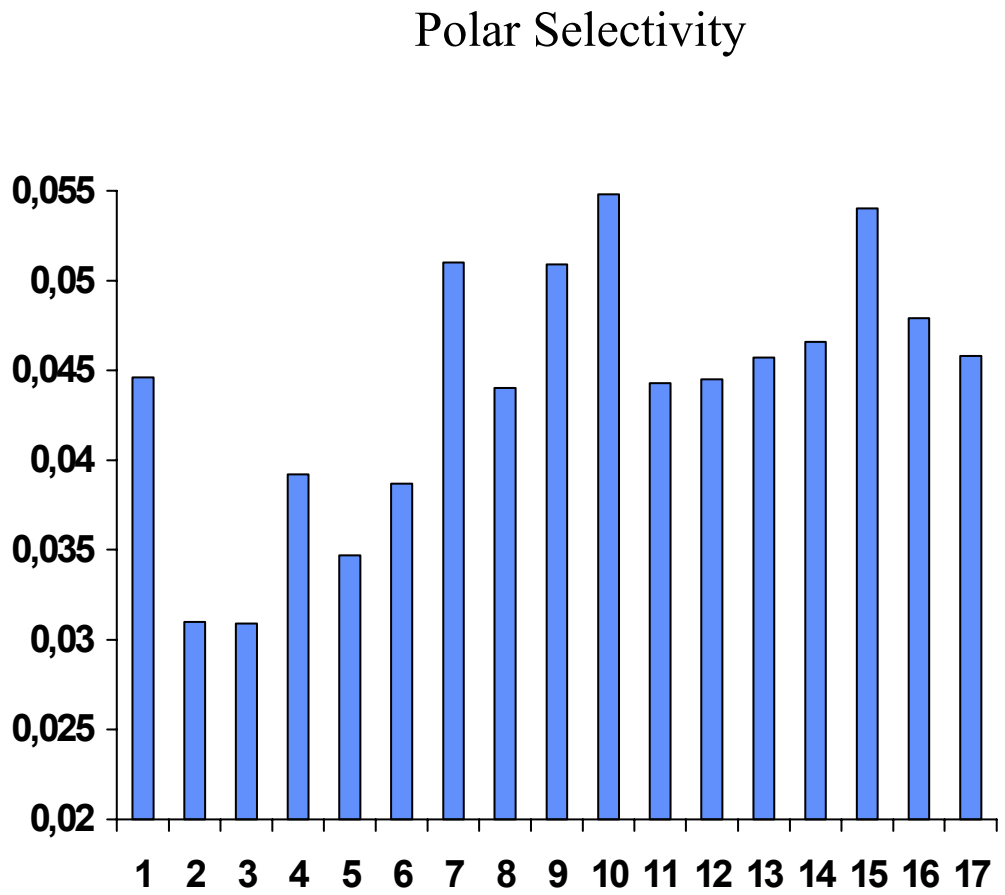
$$a = \ln \alpha / (S_1 - S_2)$$

Size selectivity is a capability of a column under specified conditions to separate solutes that differ in their size and have no differences in the polarity.

Hydrophobic Selectivity for different columns.



Polar Selectivity for different columns.

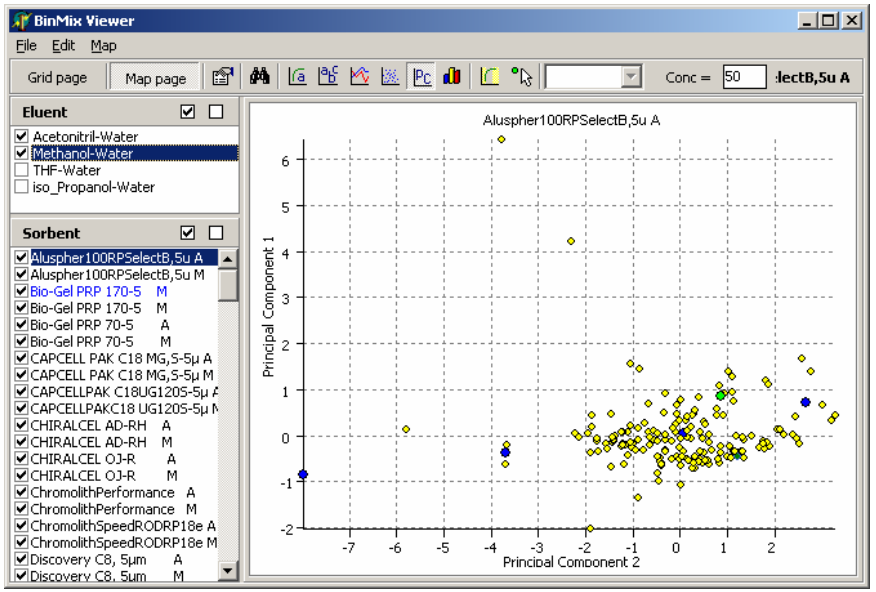


ChromSword Columns Data Base

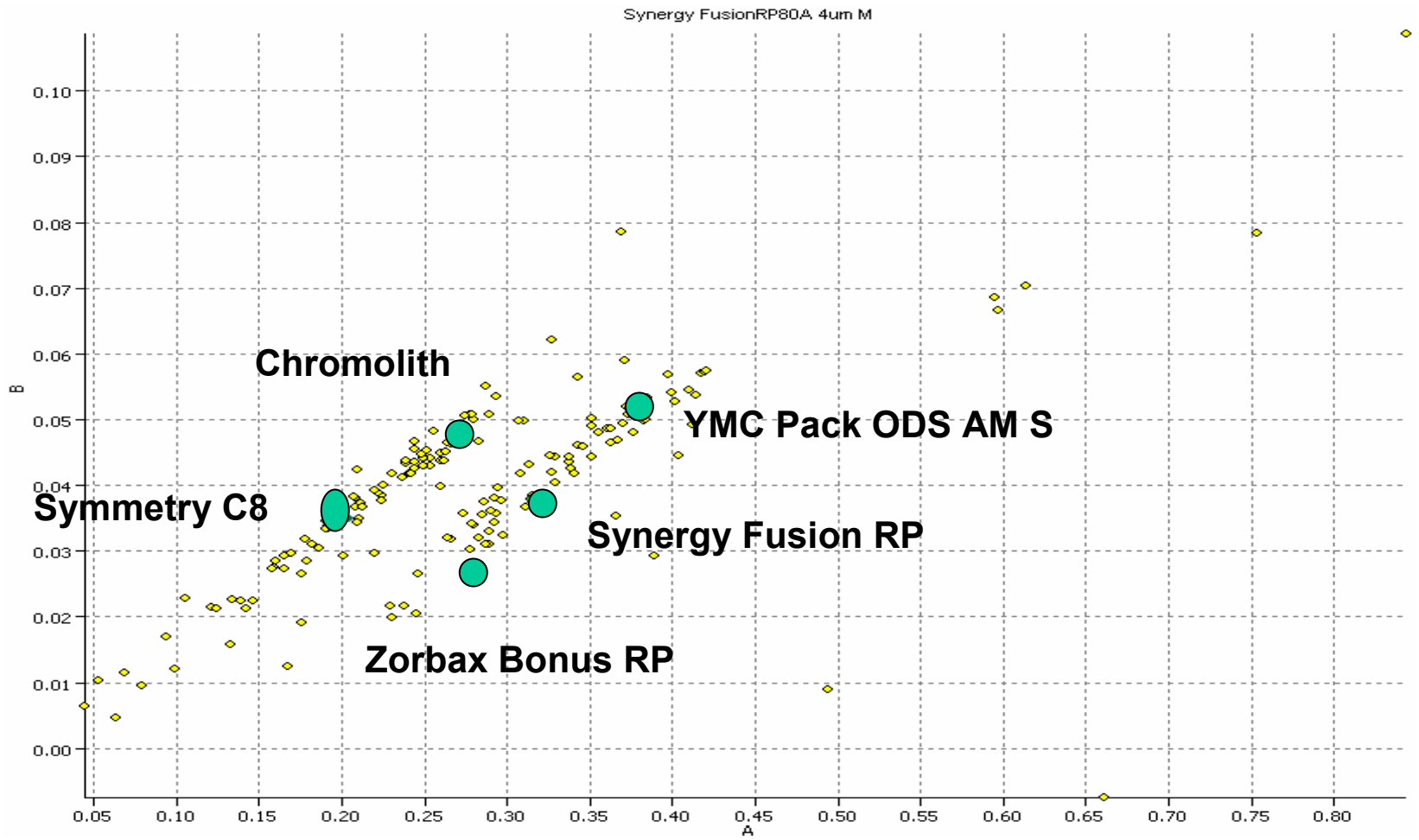
1. 90 commercially available RP columns.

2. Regular updates

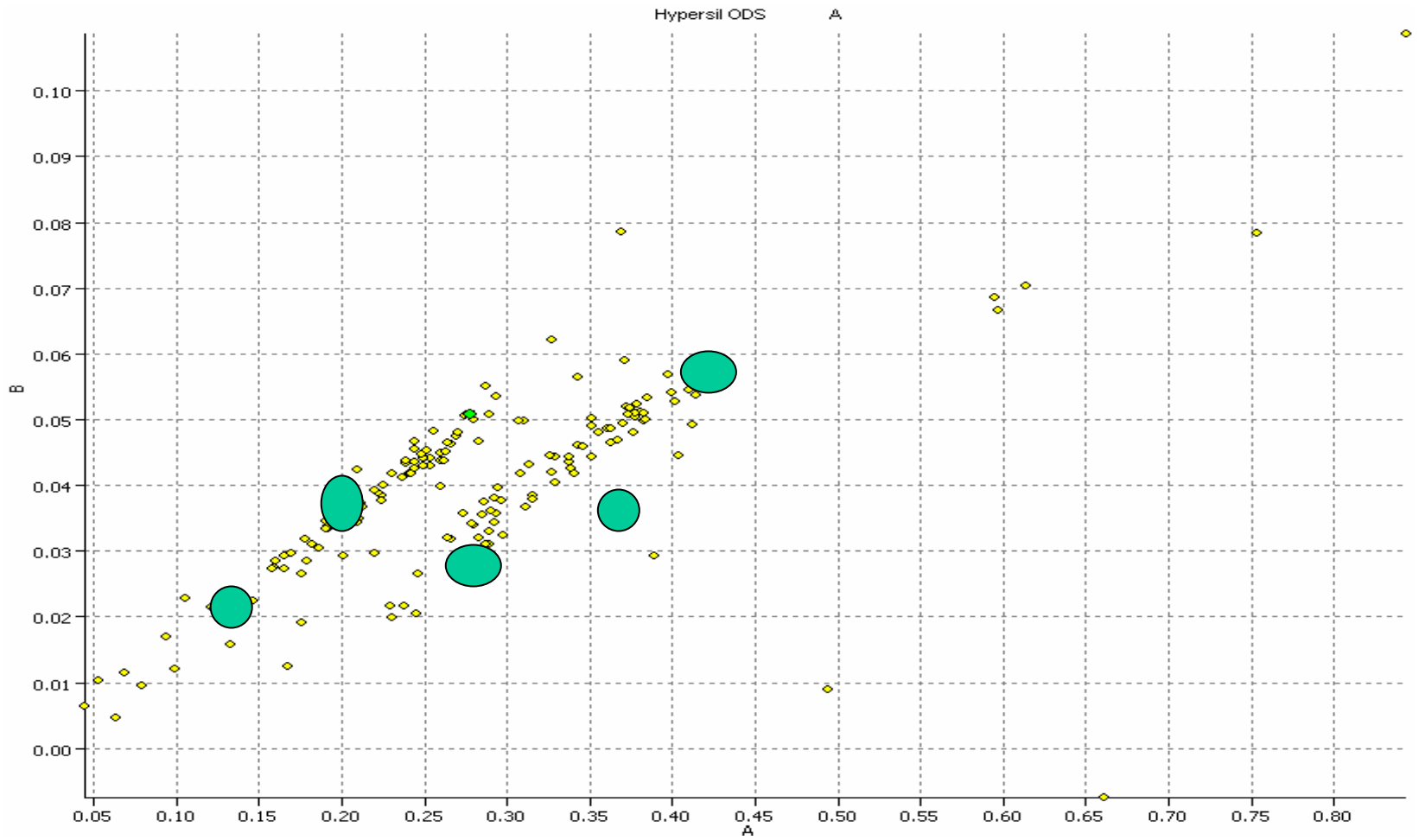
3. Retention data from 10.800 experiments processed to build column maps for any concentration in range of 10-90% of MeOH or ACN in a MP



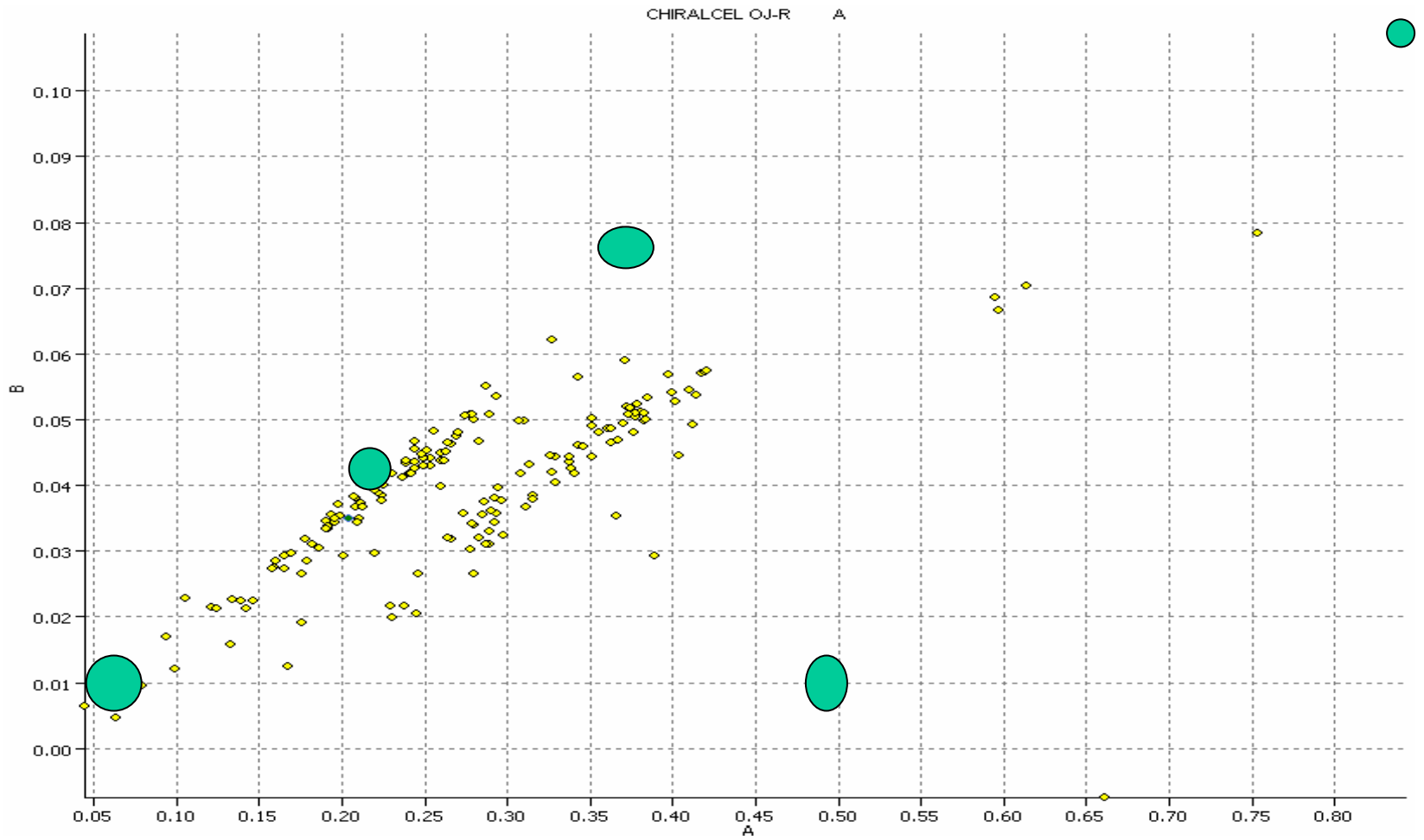
Selection of Columns with Different Selectivity
Set 1 - initial



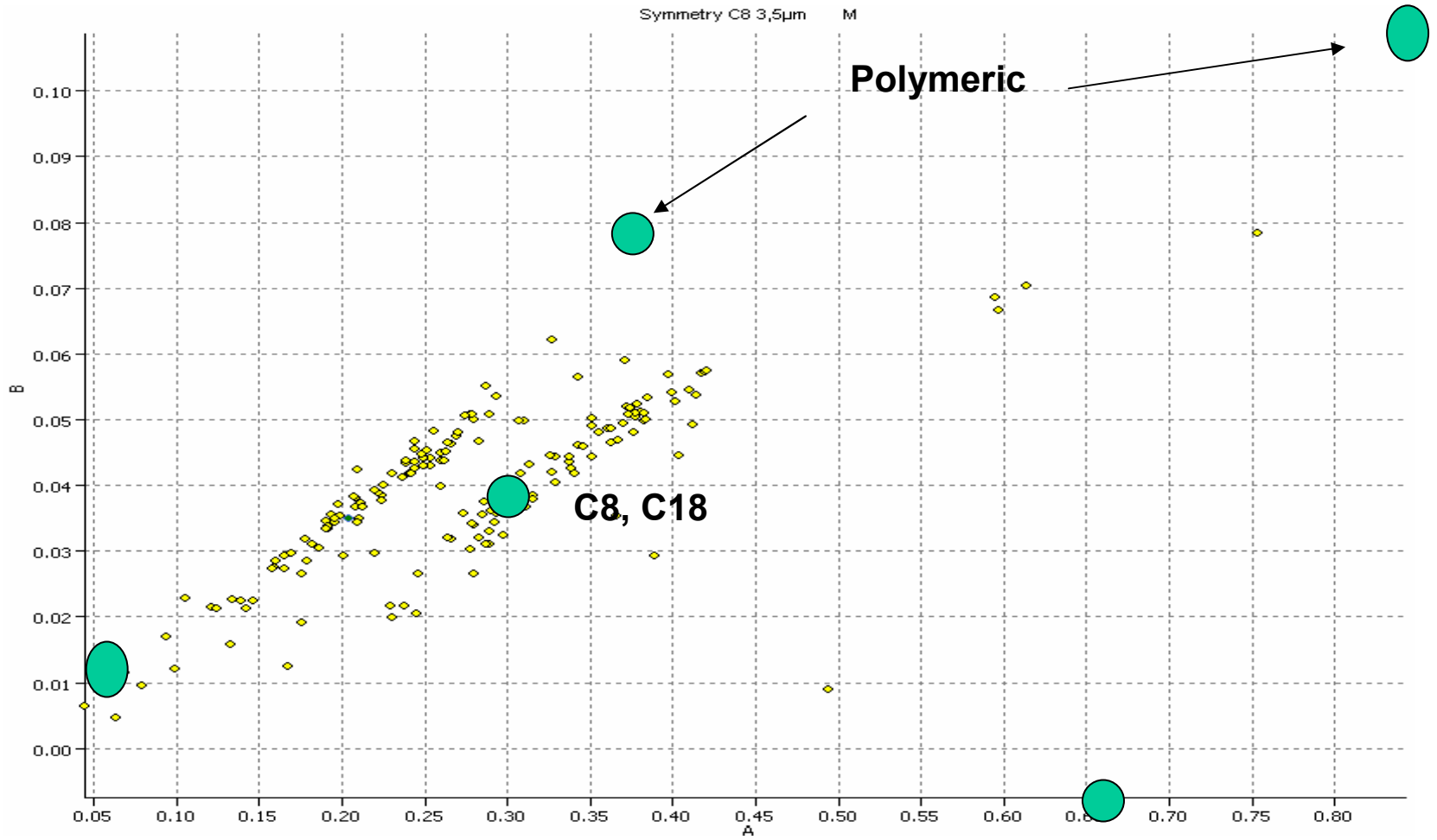
Selection of Columns with Different Selectivity.
Set 2 - alternative



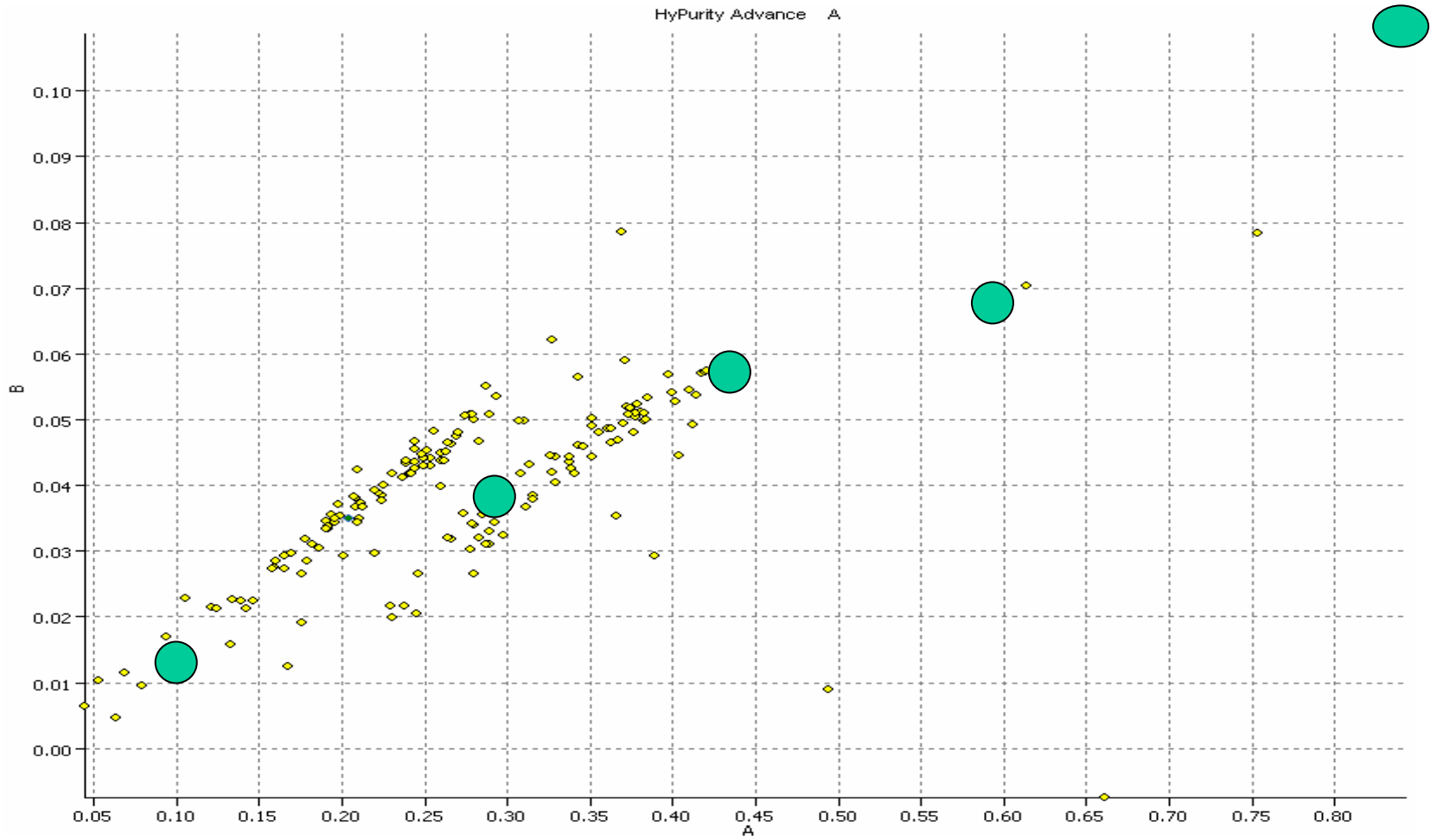
Selection of Columns with Different Selectivity
Set 3 – for higher difference



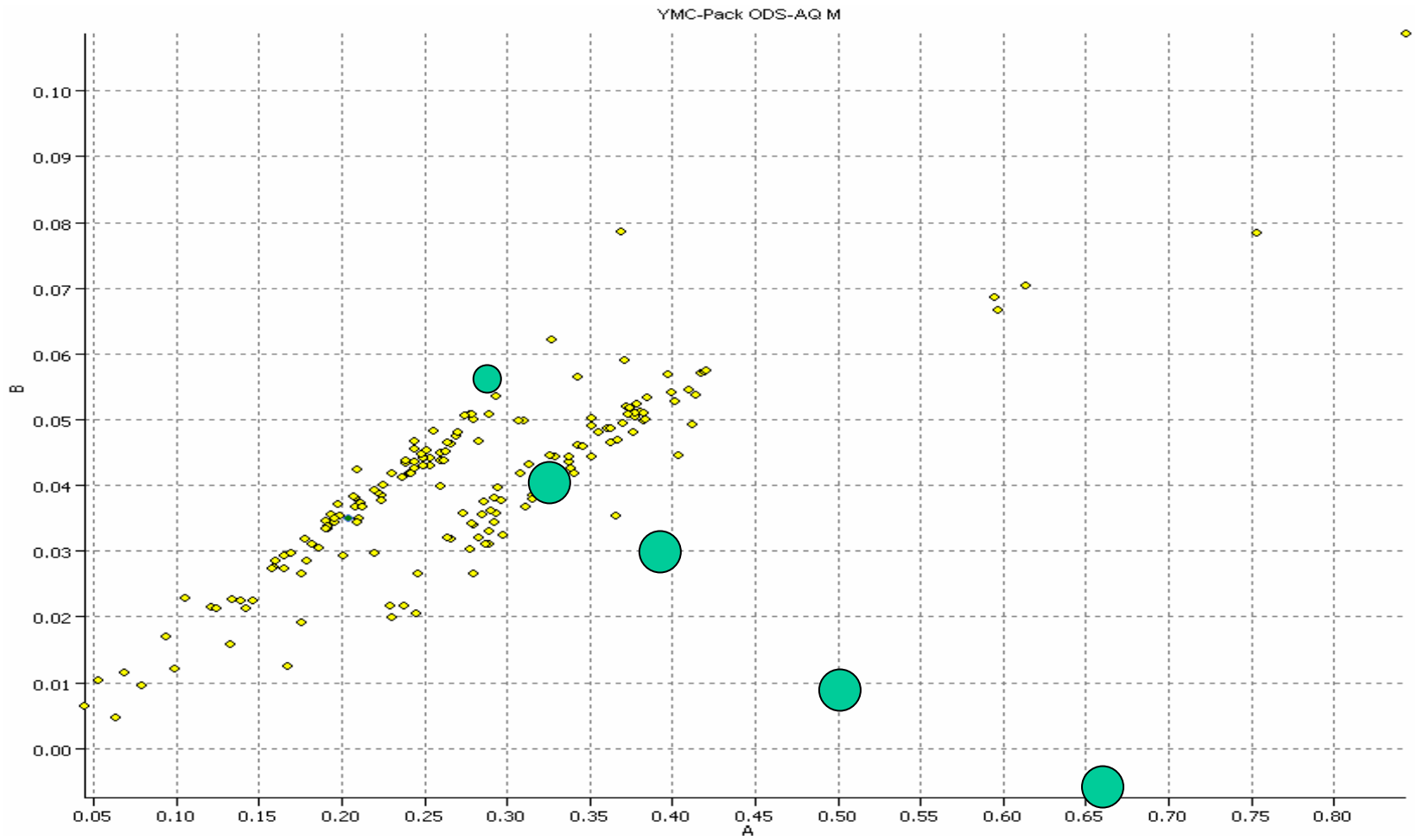
Selection of Columns with Different Selectivity
Set 4 – with different column types



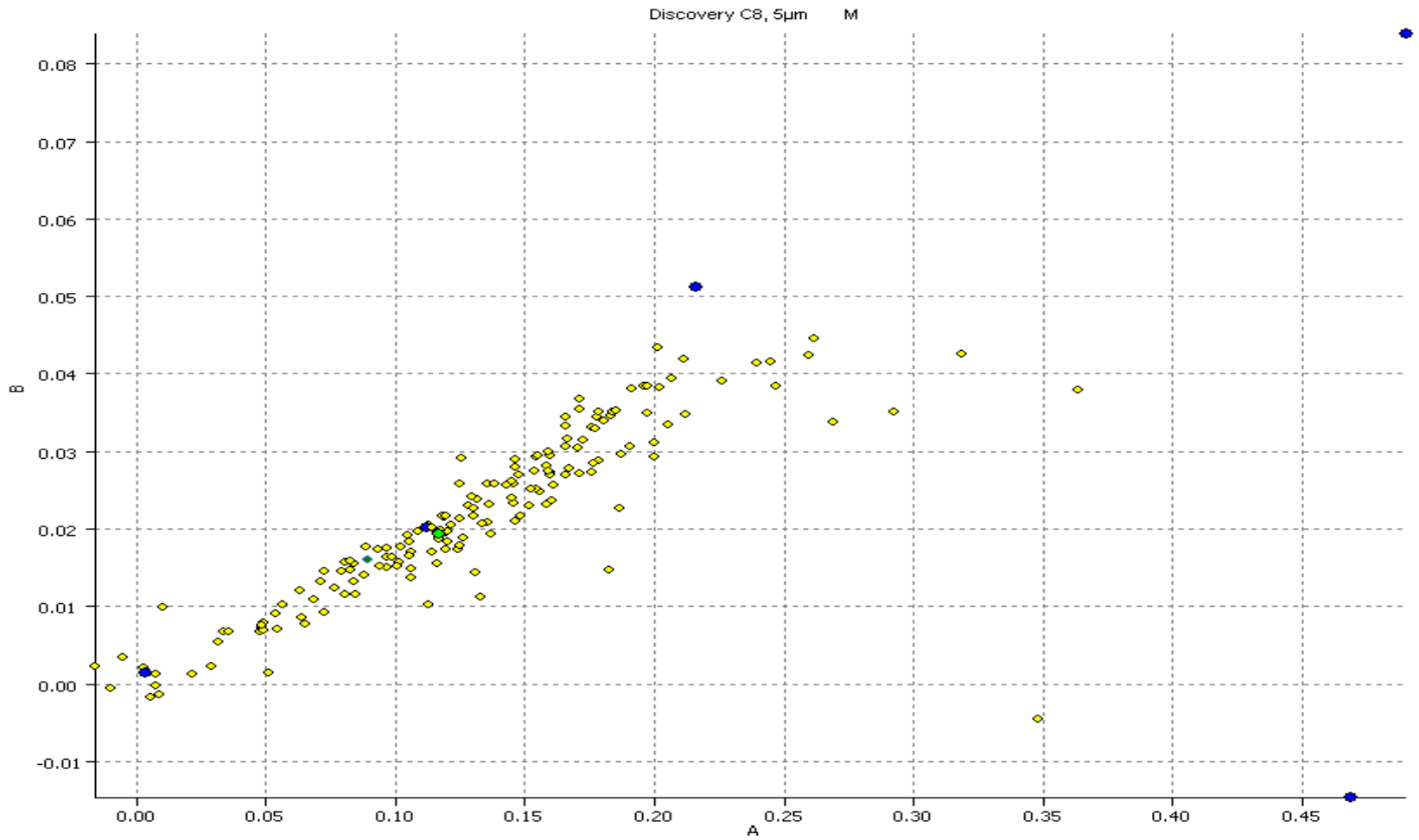
Selection of Columns with Different Selectivity
Set 5 – for fine tuning selectivity



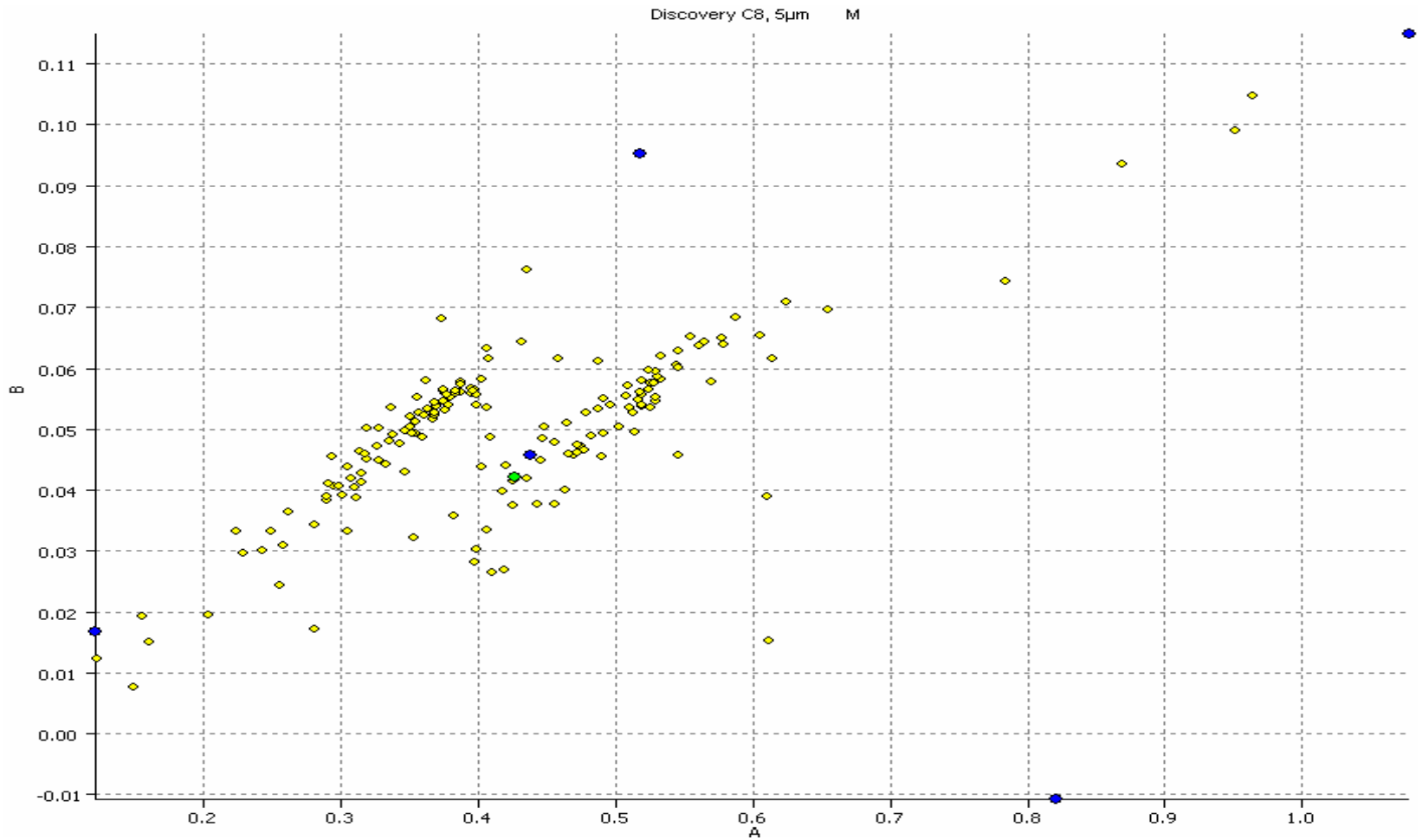
Selection of Columns with Different Selectivity
Set 6 - for fine tuning selectivity



Effect of a solvent concentration in a MP on column characteristics. 80% of AcN



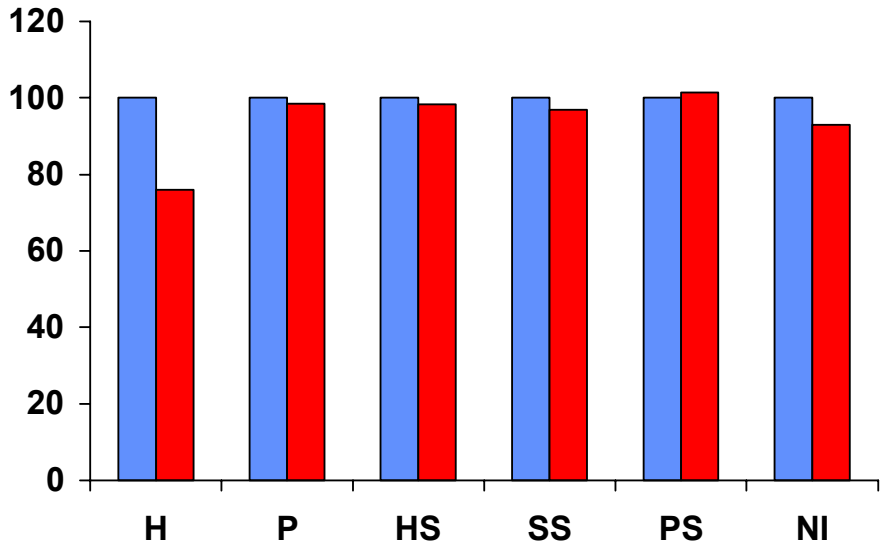
Effect of a solvent concentration on a MP on column characteristics. 30% of AcN



Column Characteristics for Practice:

Find columns with similar properties

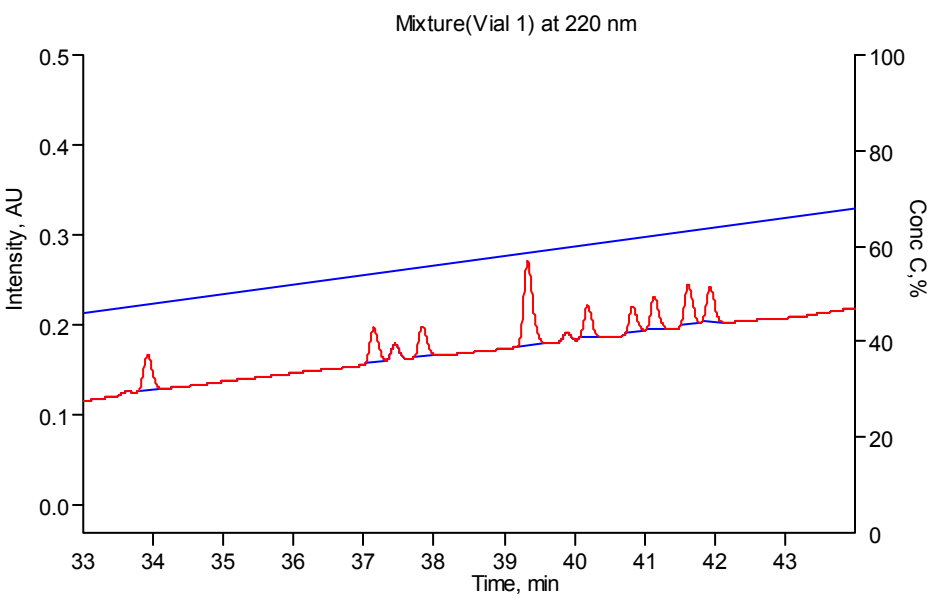
Comparison of PurospherRP18 and Zorbax ODS
(DCV=0,075)

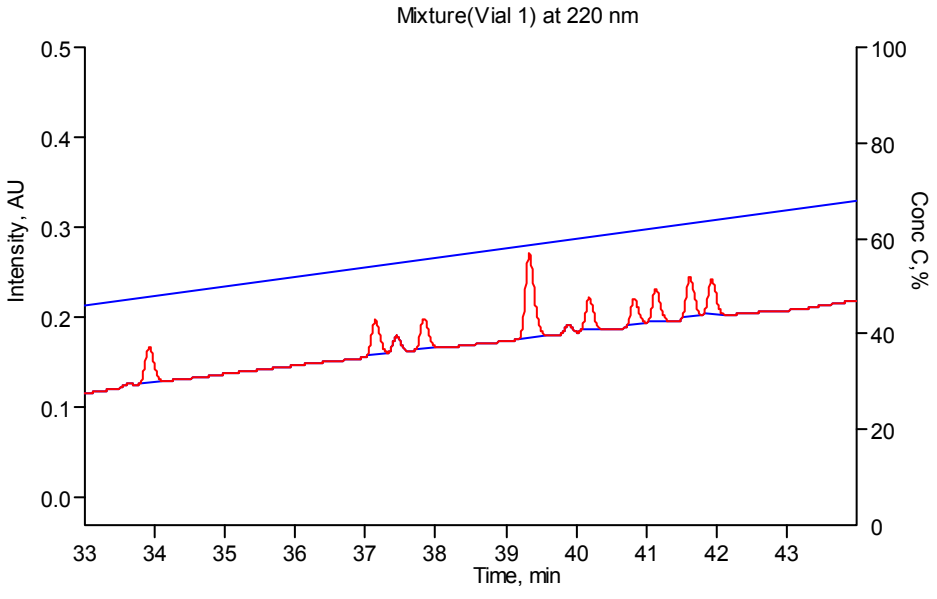


Column Characteristics for Practice:

Find columns with similar properties

- Zorbax Extend C18
- 2. PurospherStar RP18e

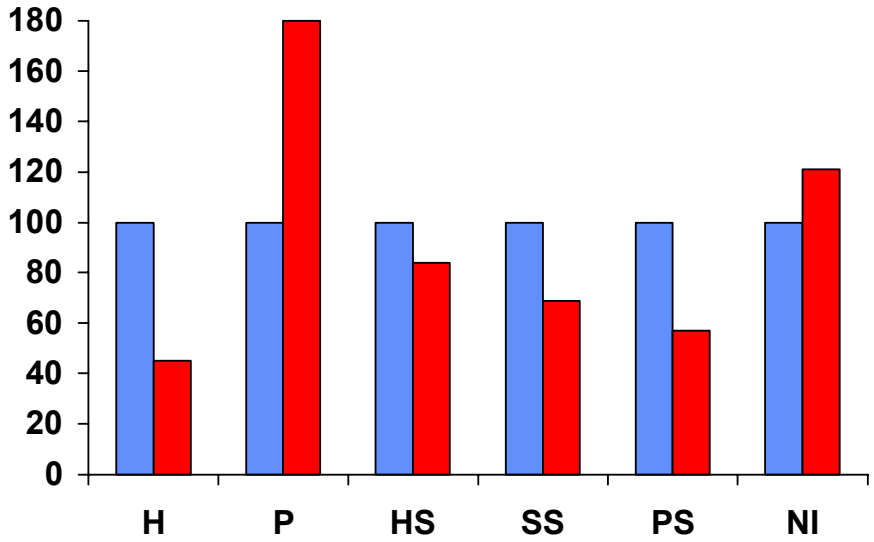




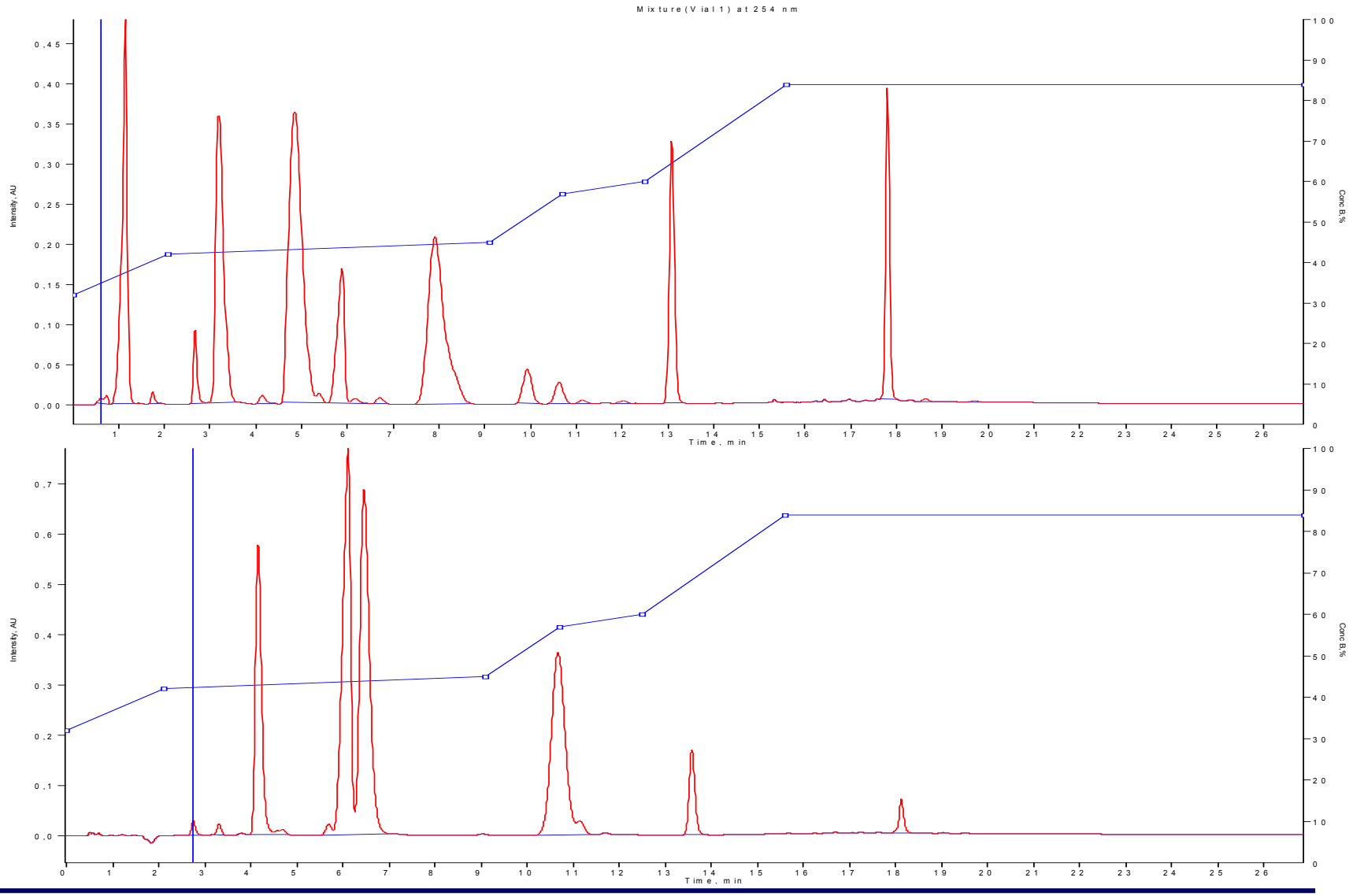
Column Characteristics for Practice:

Find columns with different selectivities

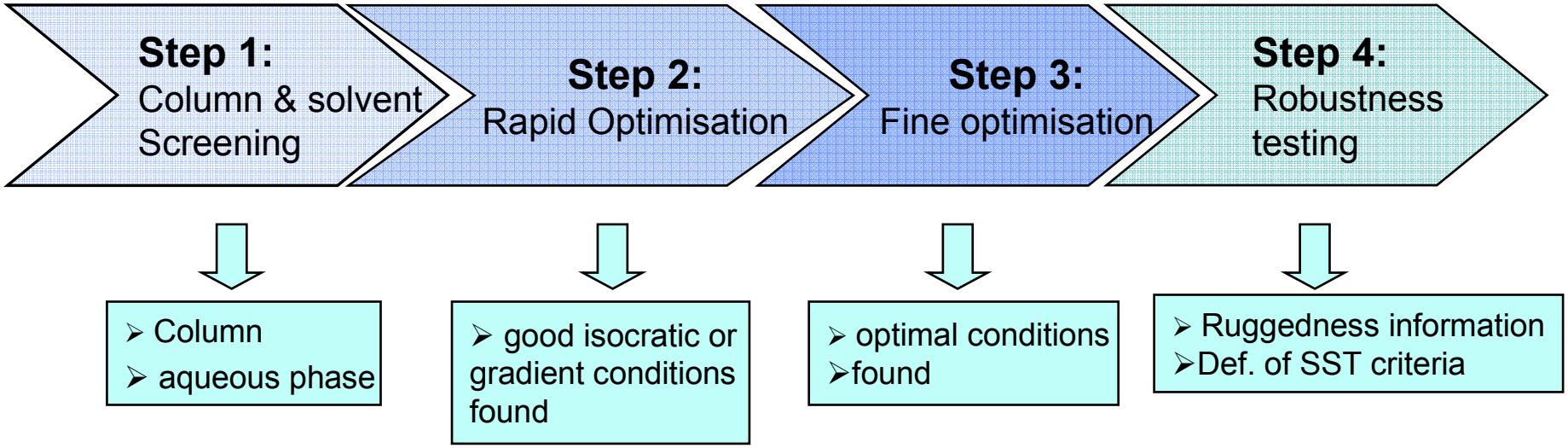
Comparison of PurospherRP18 and
LiChrospher60RPSelectB (DCV=0.403)



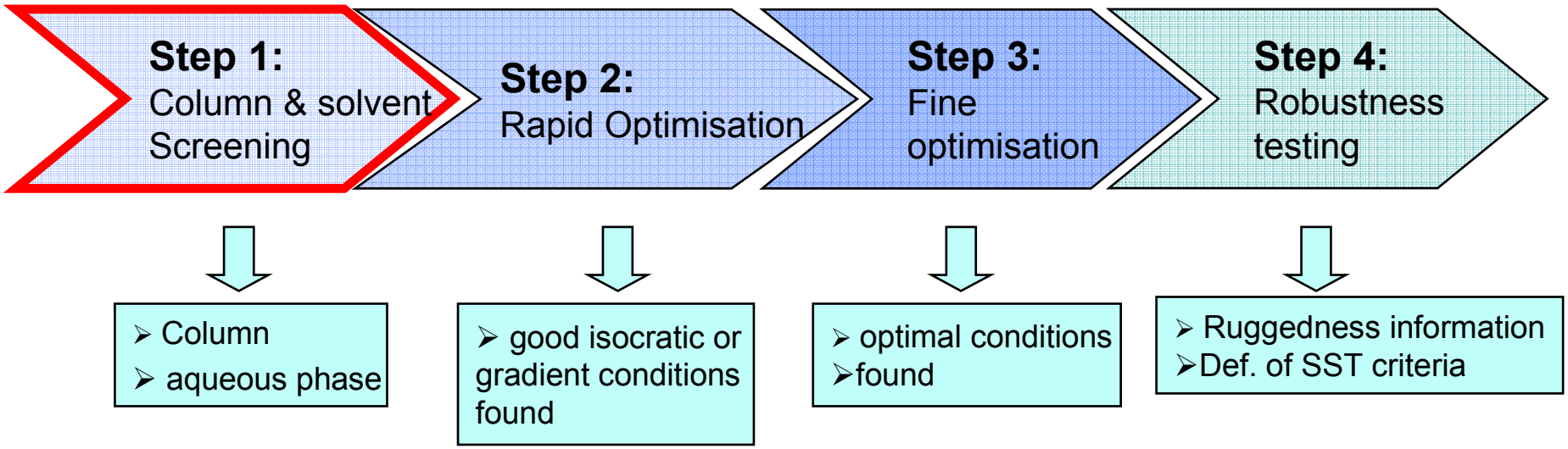
Column Characteristics for Practice:



Automatic Method Development Process: Overview



Method Development Process: Step 1



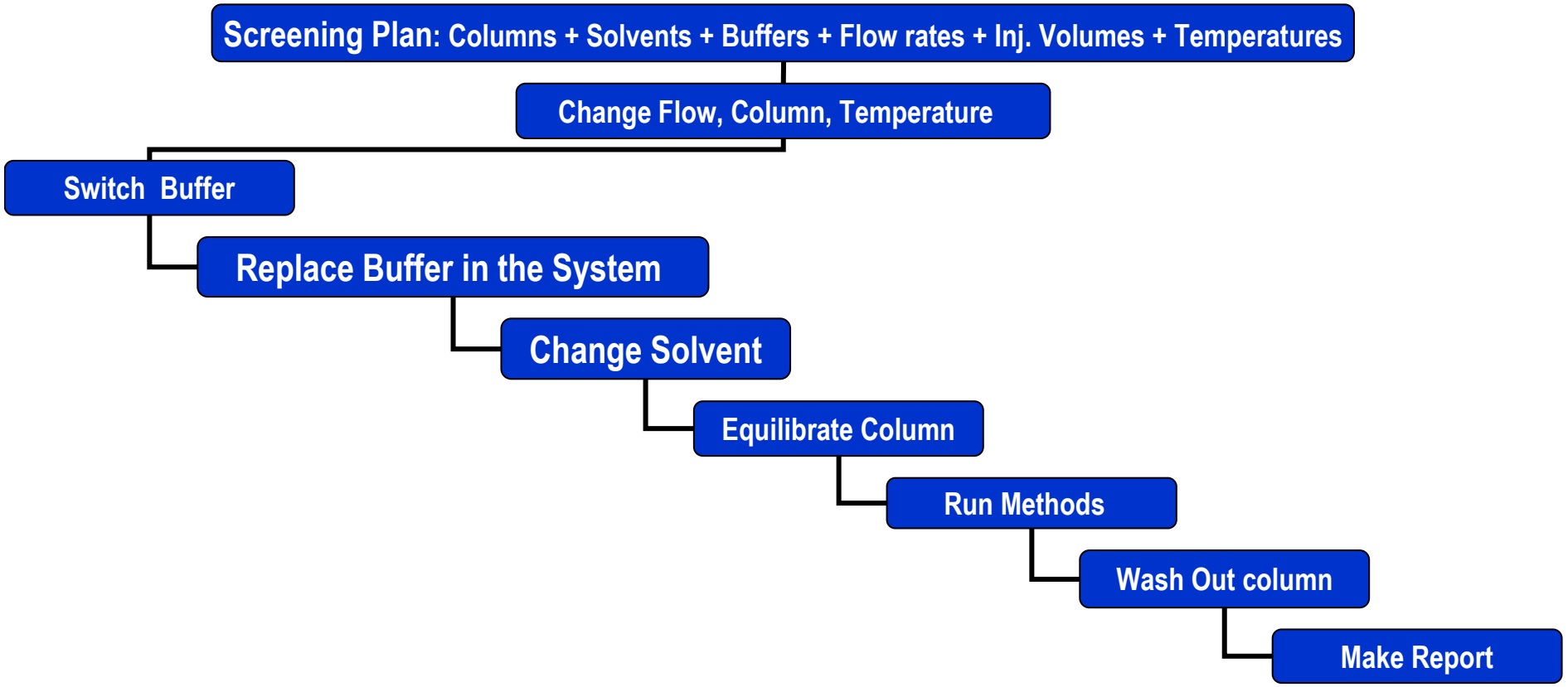
Metods Screening

Intelligent column and solving switching procedures are fully supported in order to try a variety of column/solvent combinations completely automatically.

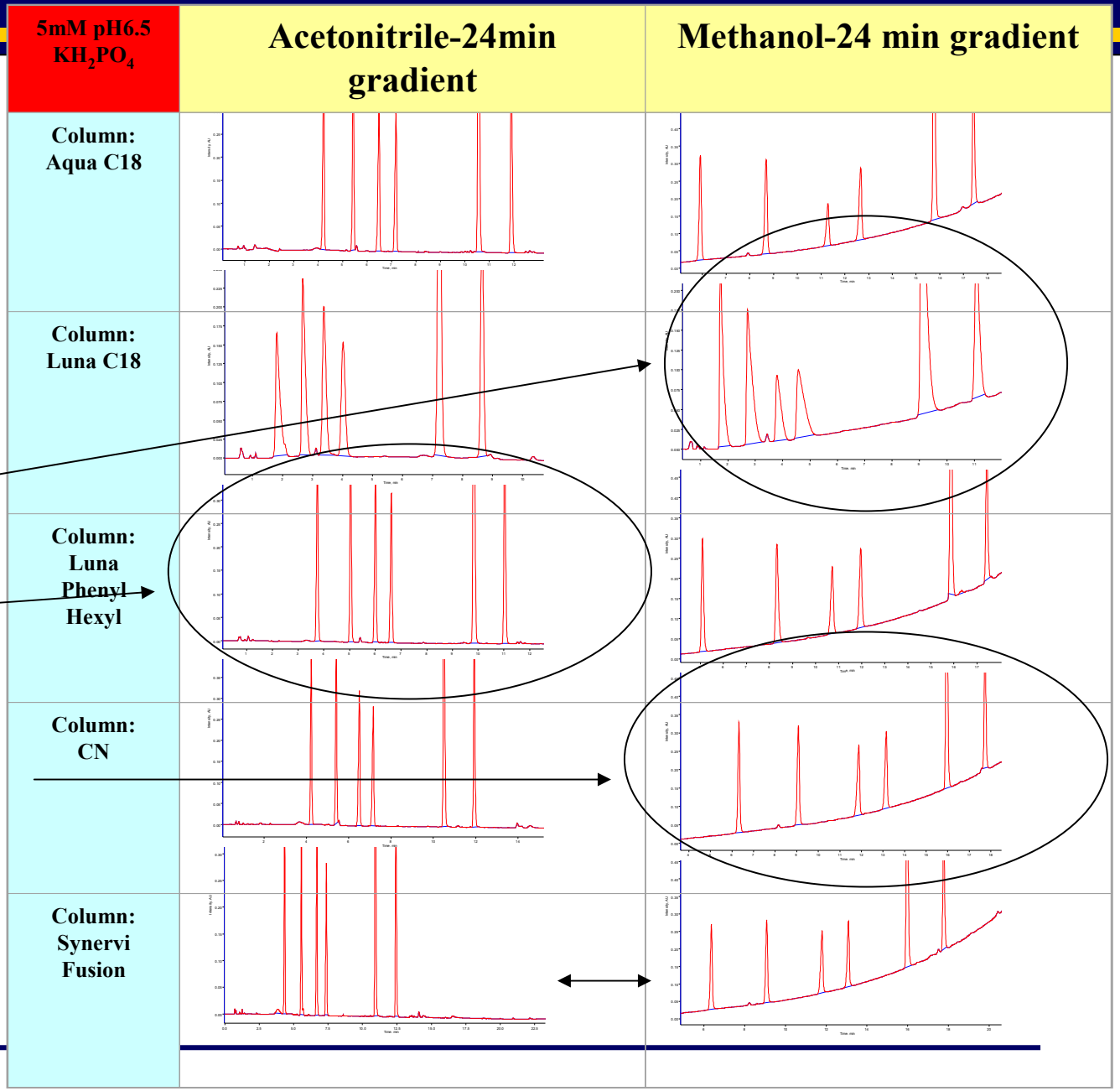
This is applied in method development laboratories for rapid screening of columns and solvents, with the goal to quickly find a suitable separation system.

The resulting best separation system is then subjected to rapid and fine optimisations.

ChromSwordAuto Intelligent Screening.
One step = 8 methods in a normal sequence



Screening Results

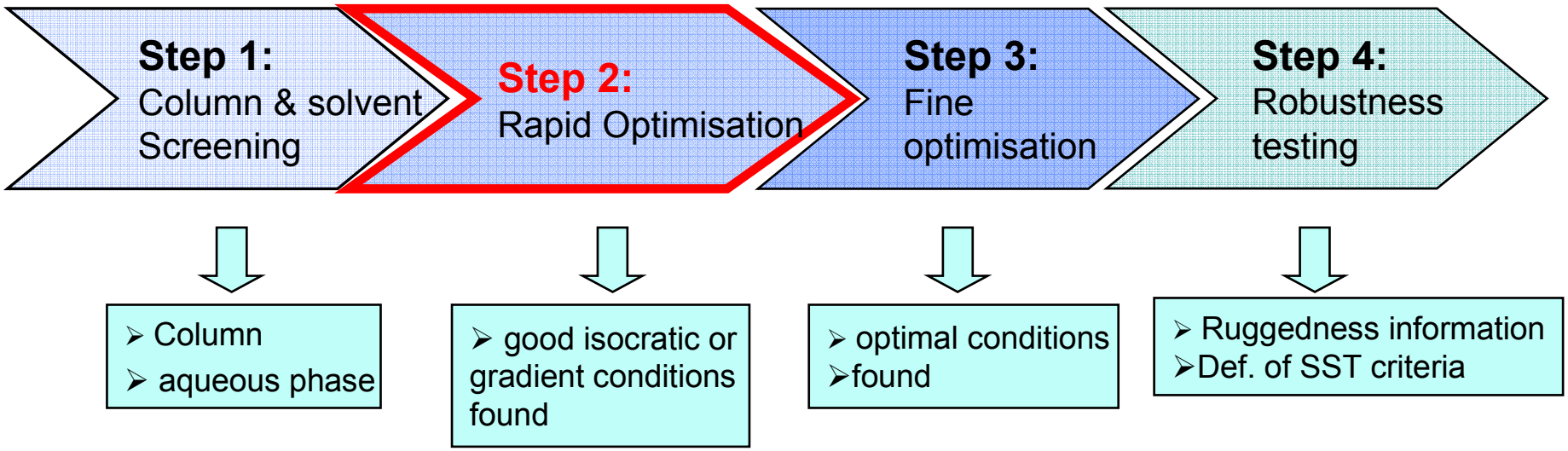


Bad Peak Shape and resolution

Good Peak Shape and resolution

Highest Resolution for prep. isolation

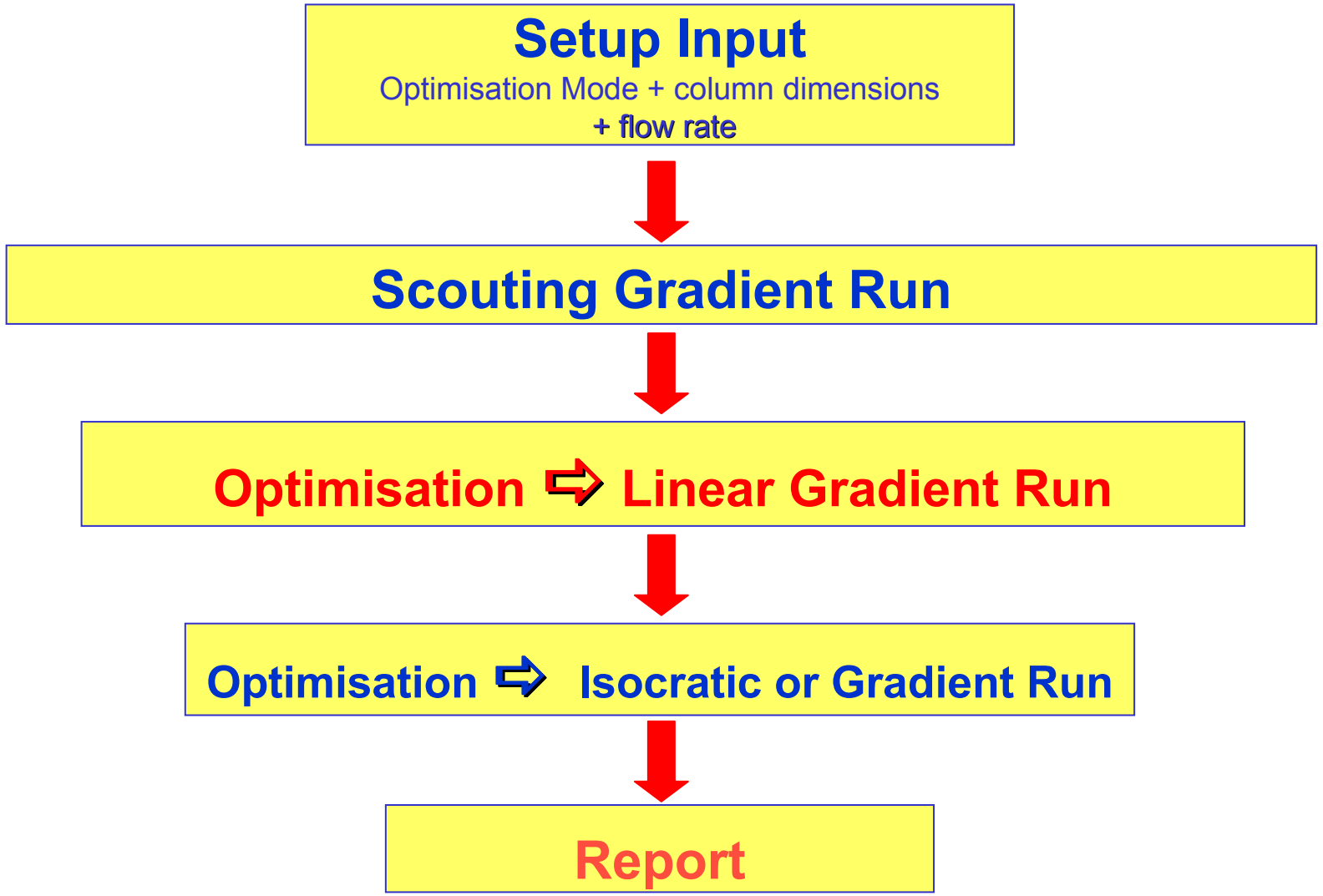
Method Development Process: Step 2



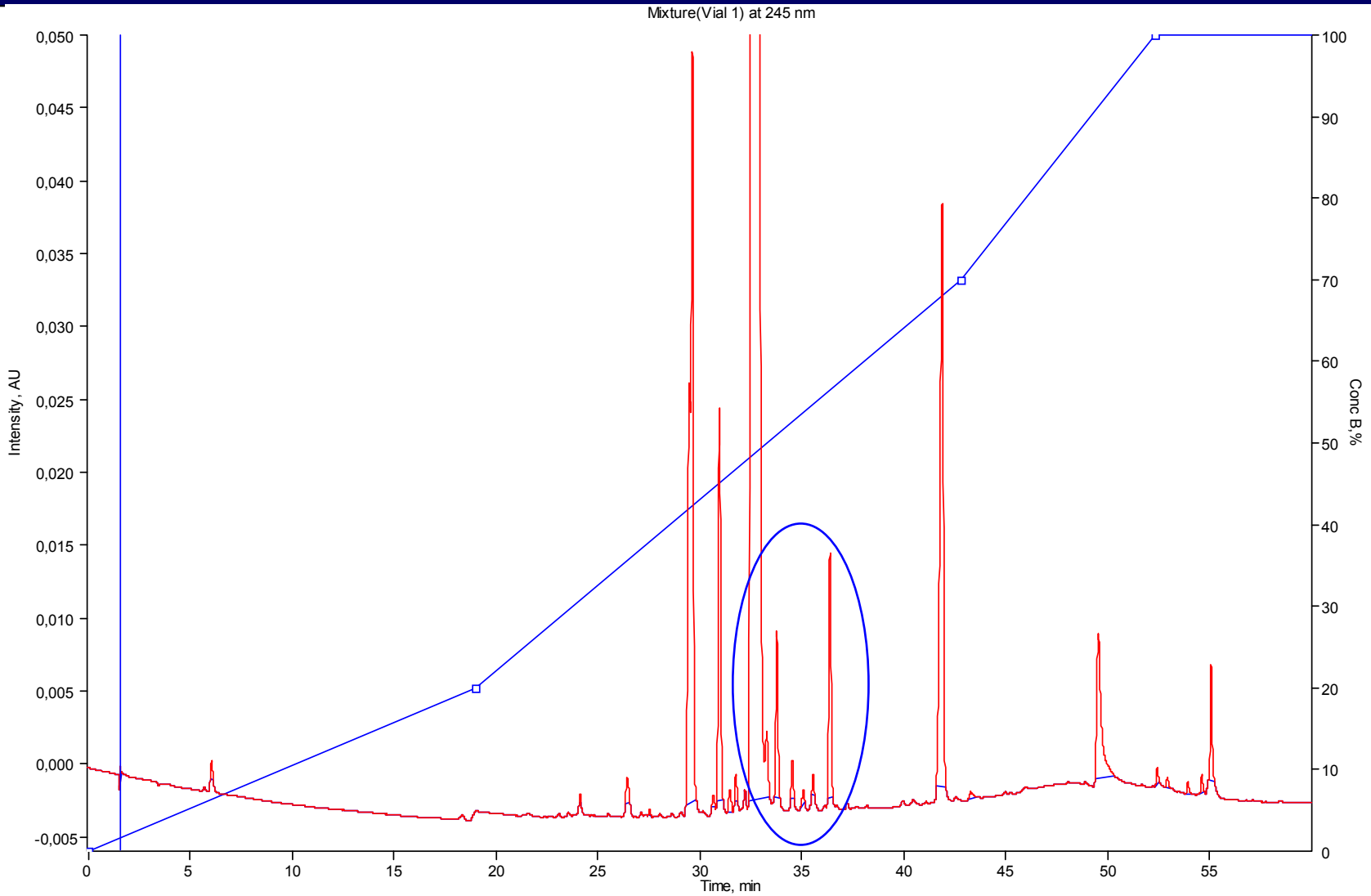
Rapid Optimisation

To find rapidly good conditions ChromSword® Auto executes 3 automatically performed chromatographic runs necessary to build retention models and to find optimal conditions for a column/solvent combination selected.

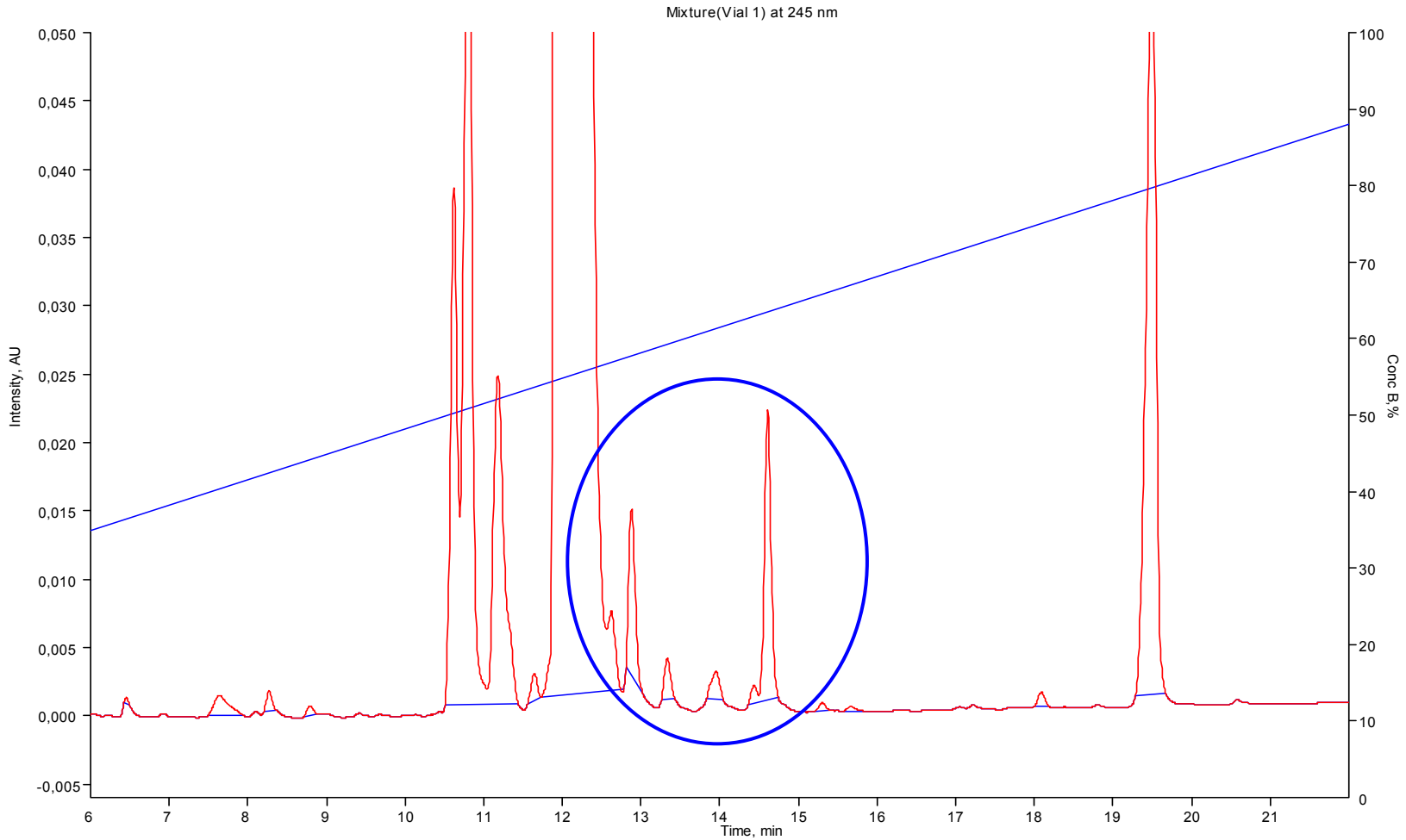
ChromSwordAuto. Rapid Optimisation Procedure



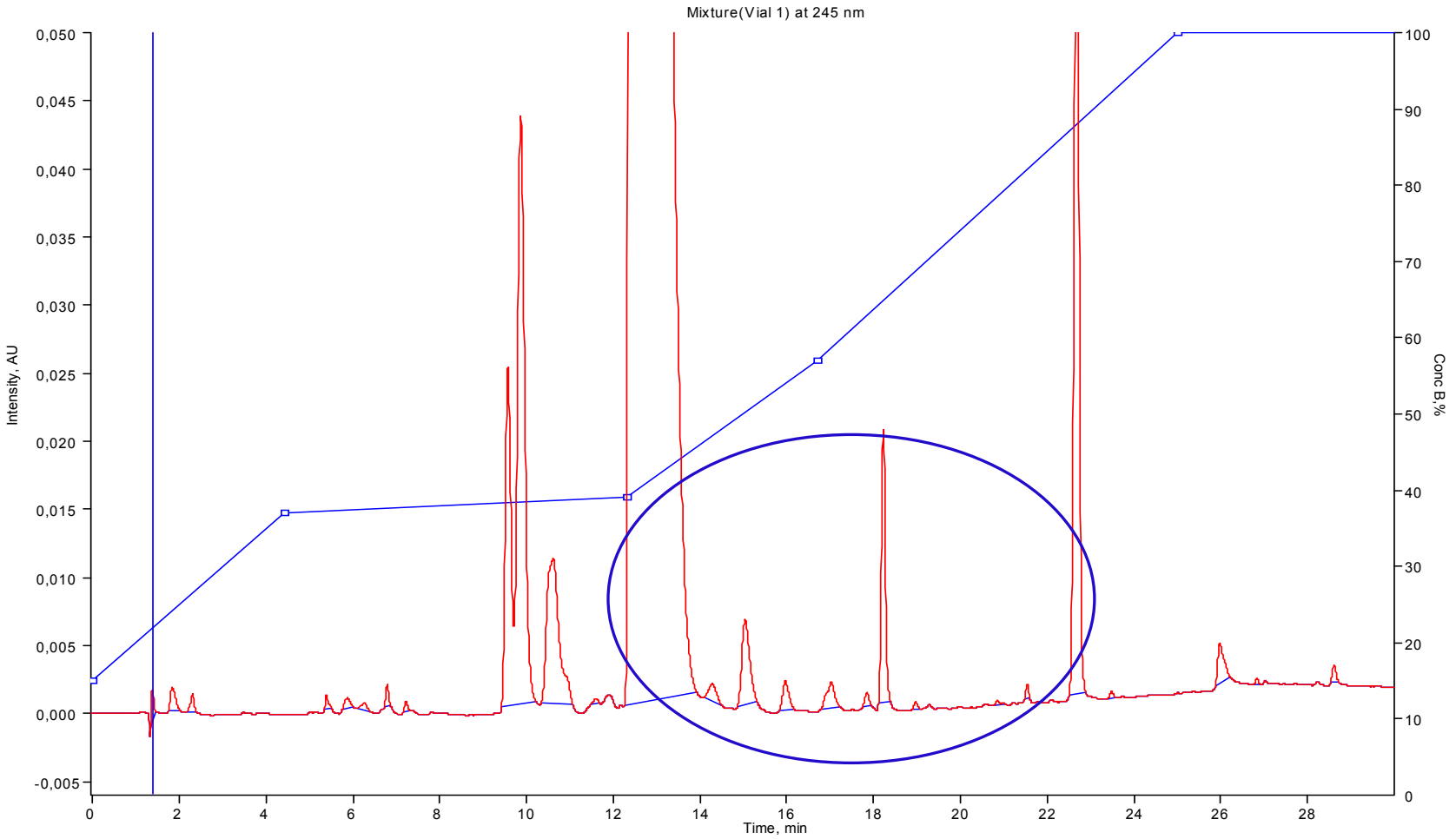
Rapid Optimisation. Gradient 1



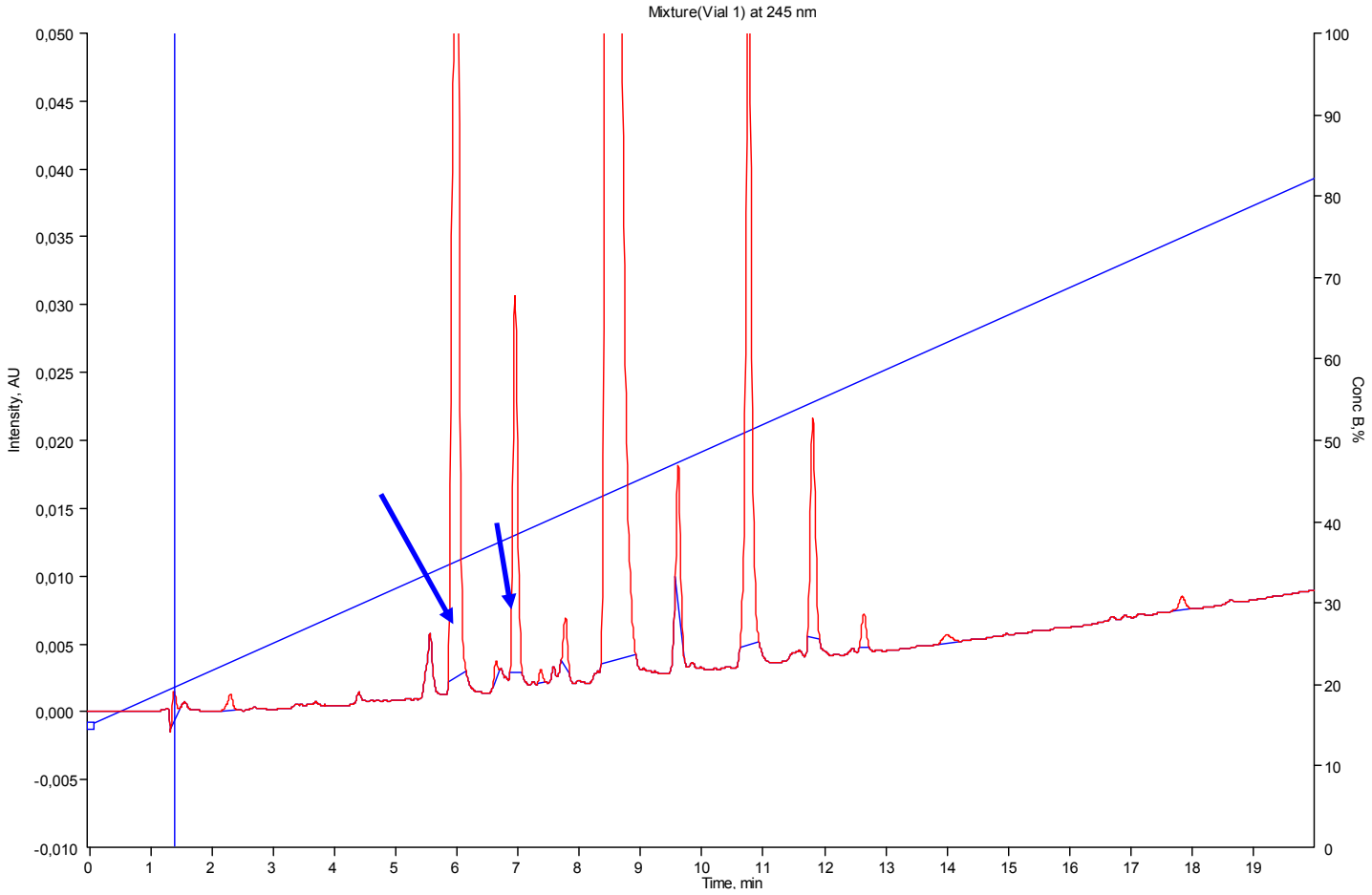
Rapid Optimisation. Gradient 2



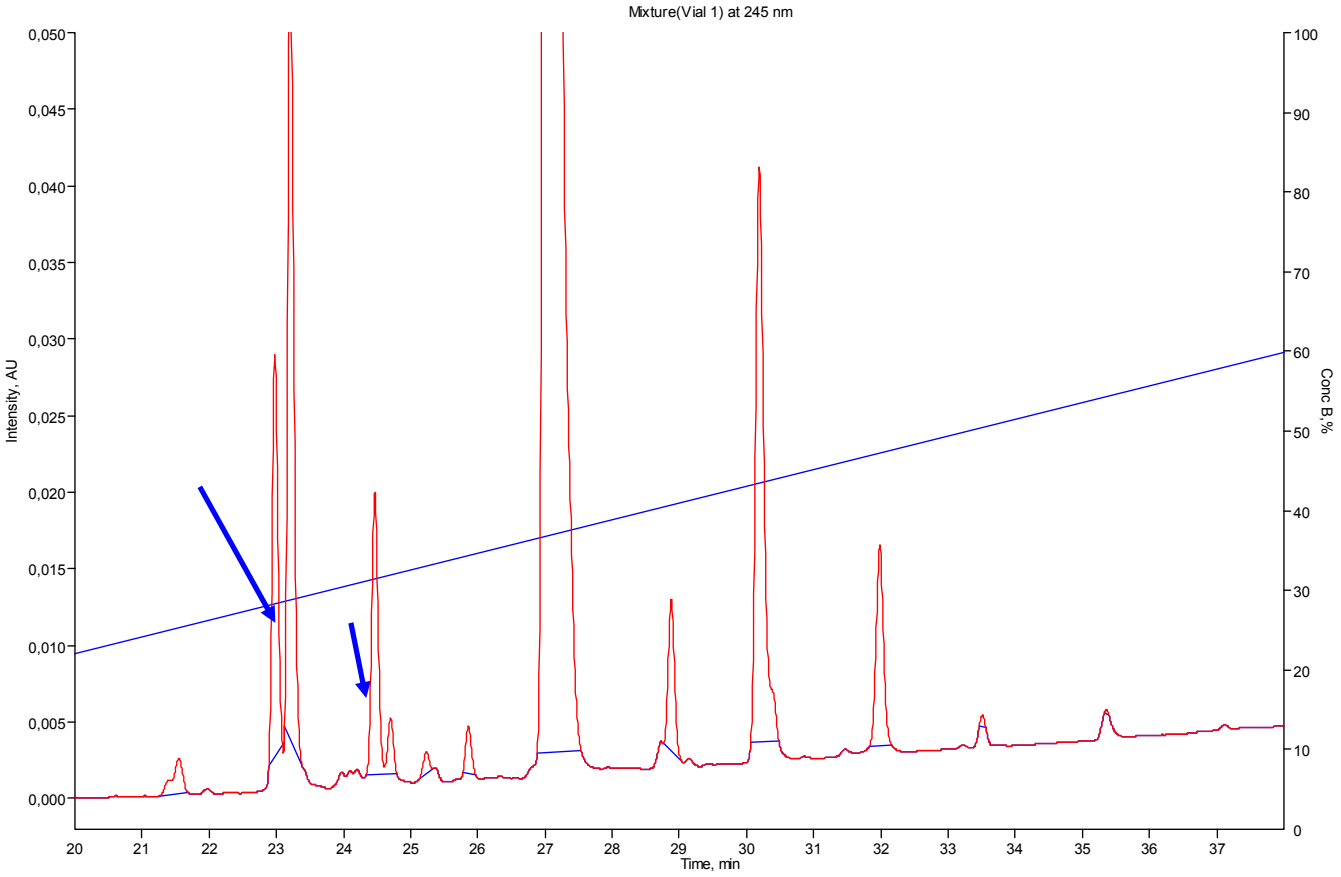
Rapid Optimisation. Gradient 3



Automatic Rapid Optimisation. Gradient 1

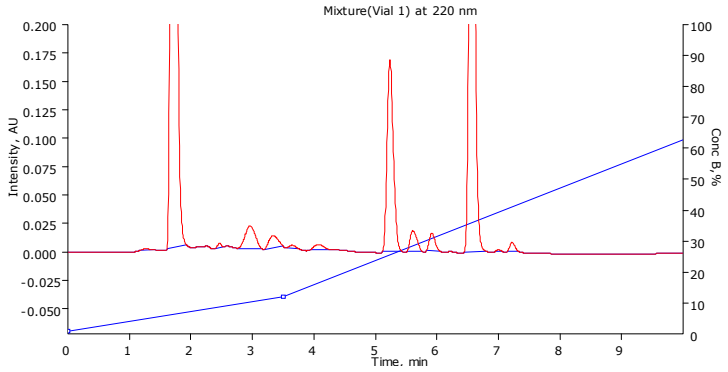


Step 1. Automatic Rapid Optimisation. Gradient 2

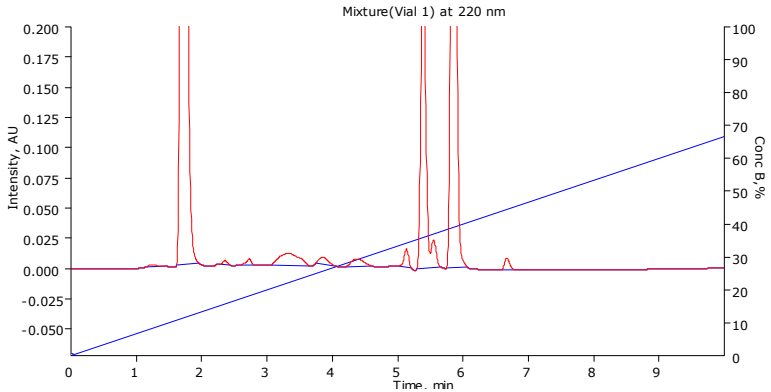


Automated Rapid Optimisation.

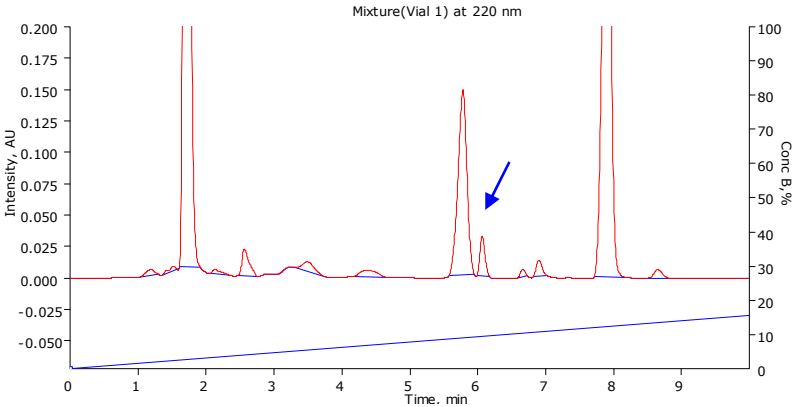
Gradient 1



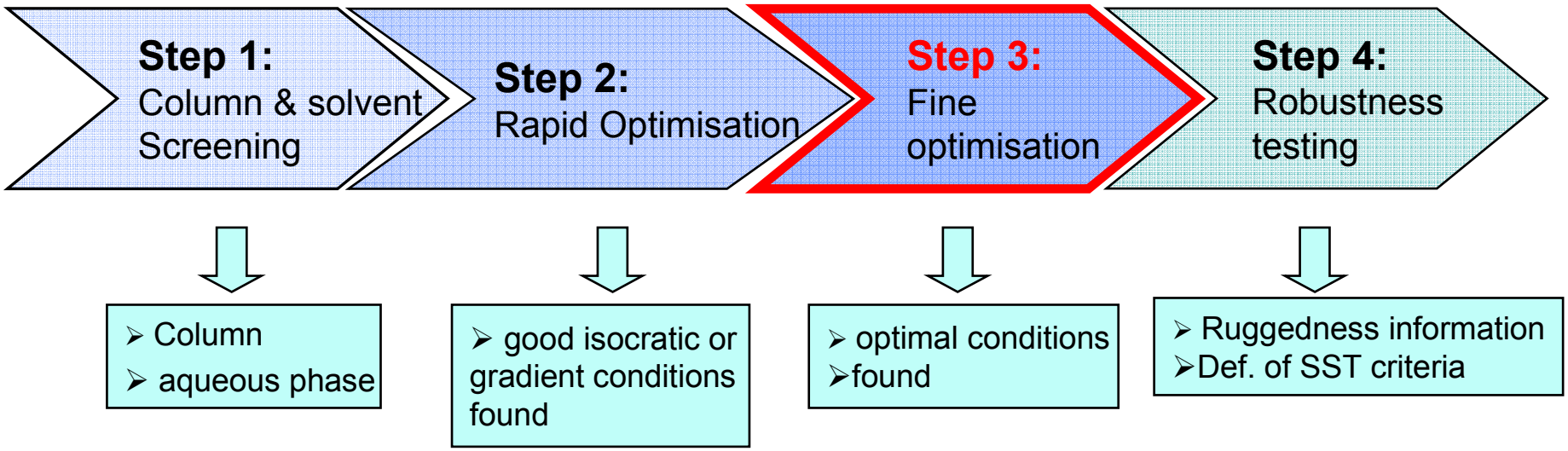
Gradient 2



Gradient 3



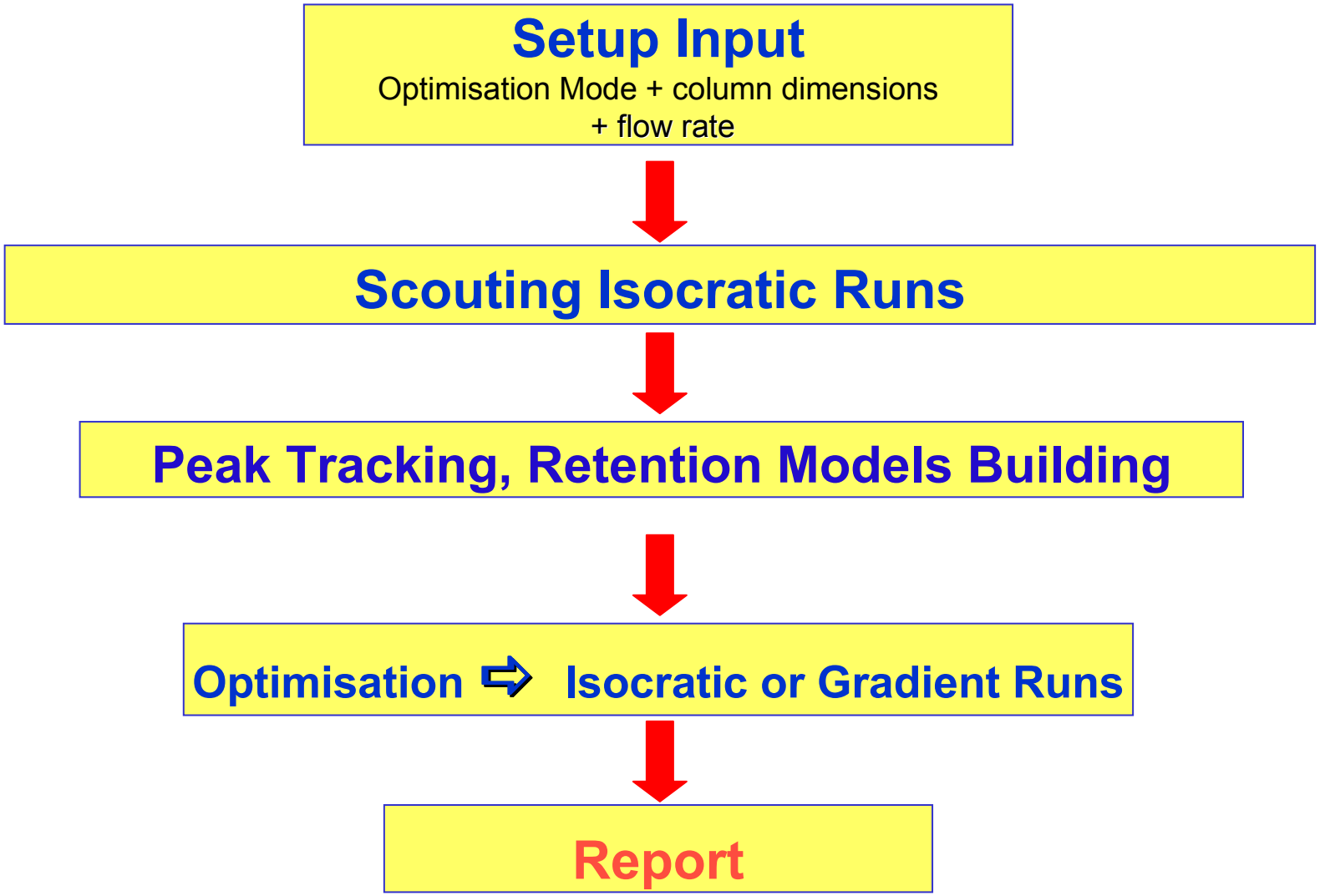
Method Development Process: Step 3



Fine Optimisation

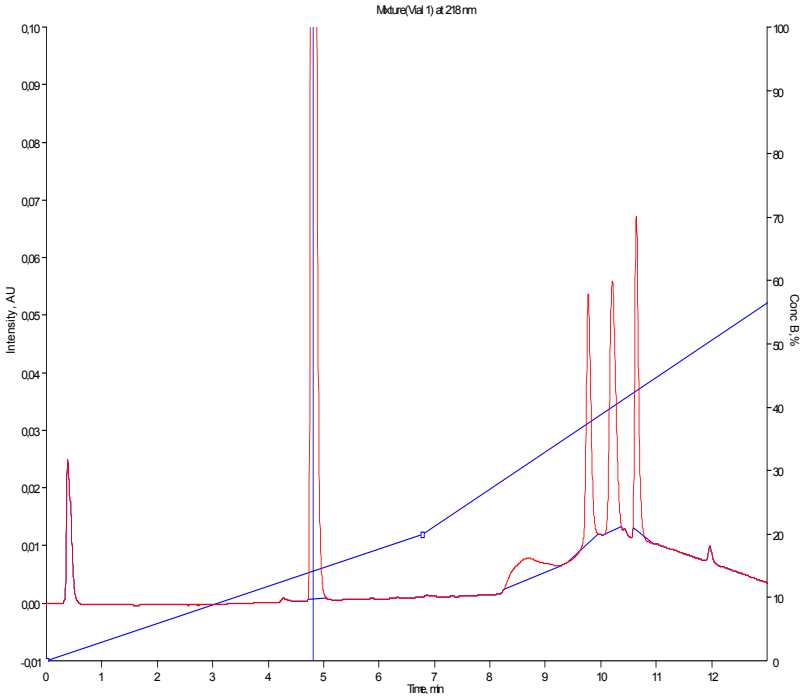
During fine optimisation ChromSword® Auto acquires and analyses more data for being able to fine tune its retention models and to predict and execute a variety of final optimum conditions.

Fine Optimisation Procedure

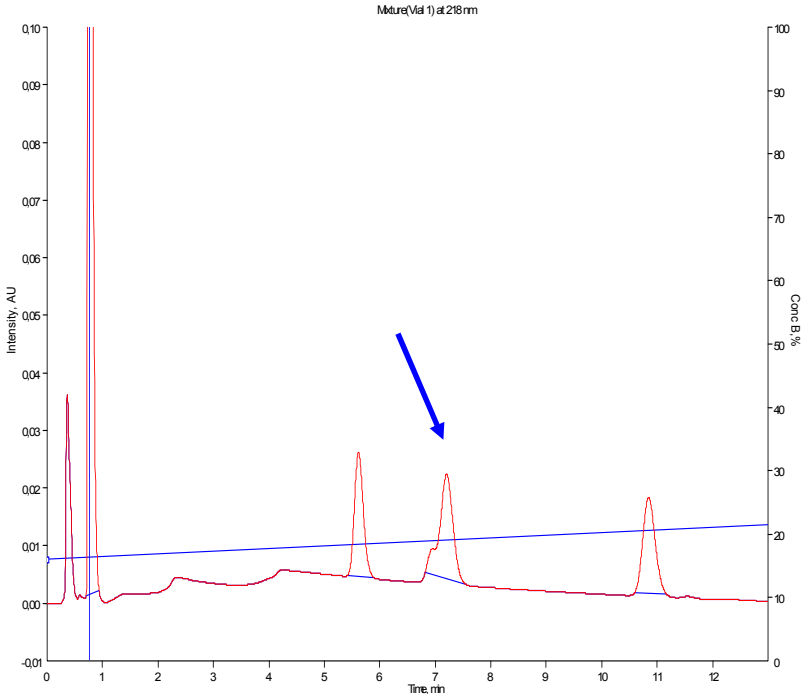


Step 2. Automated Rapid Optimisation.

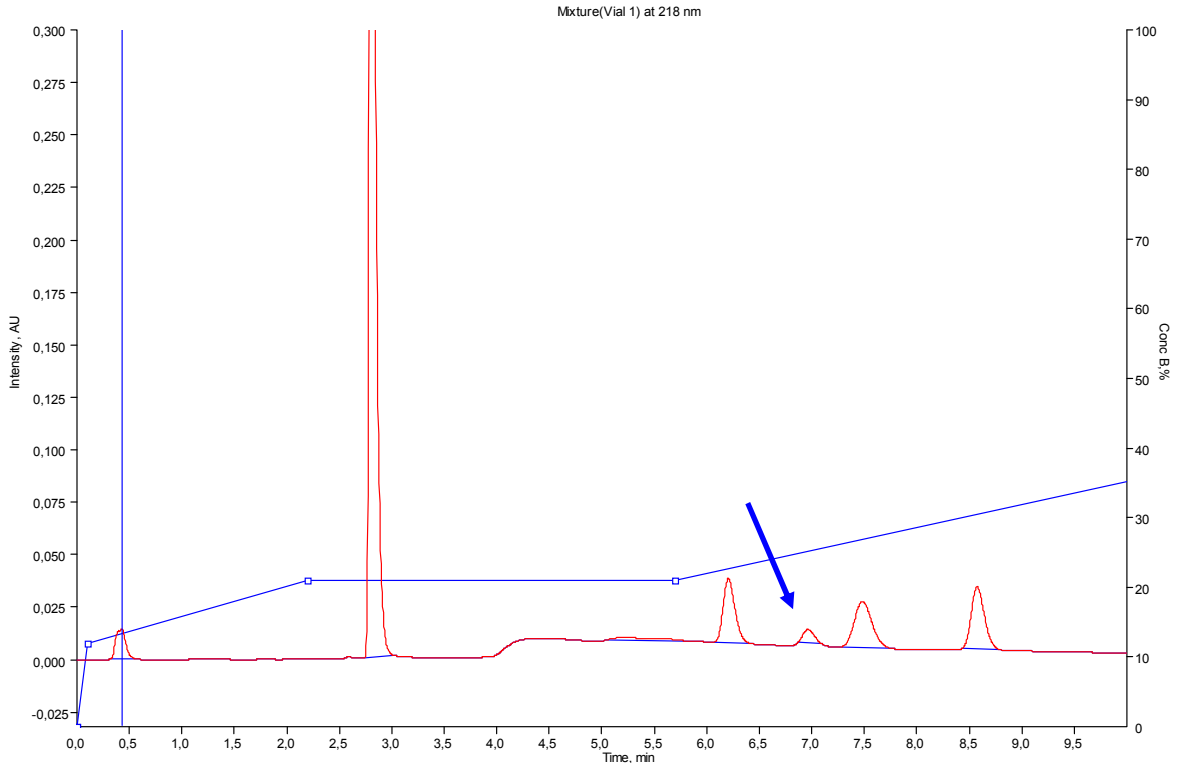
Gradient 1



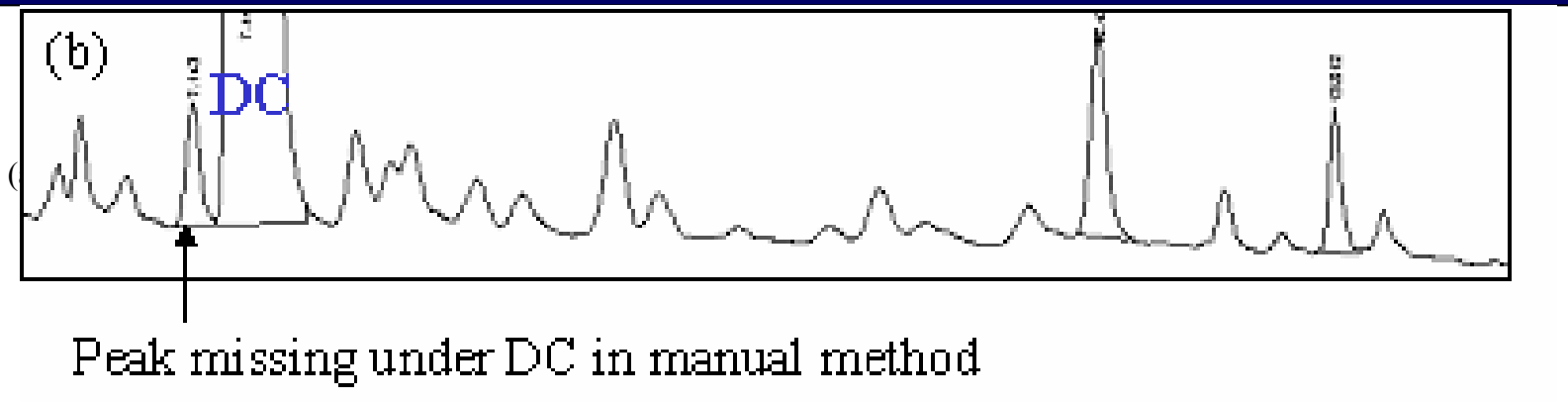
Gradient 2

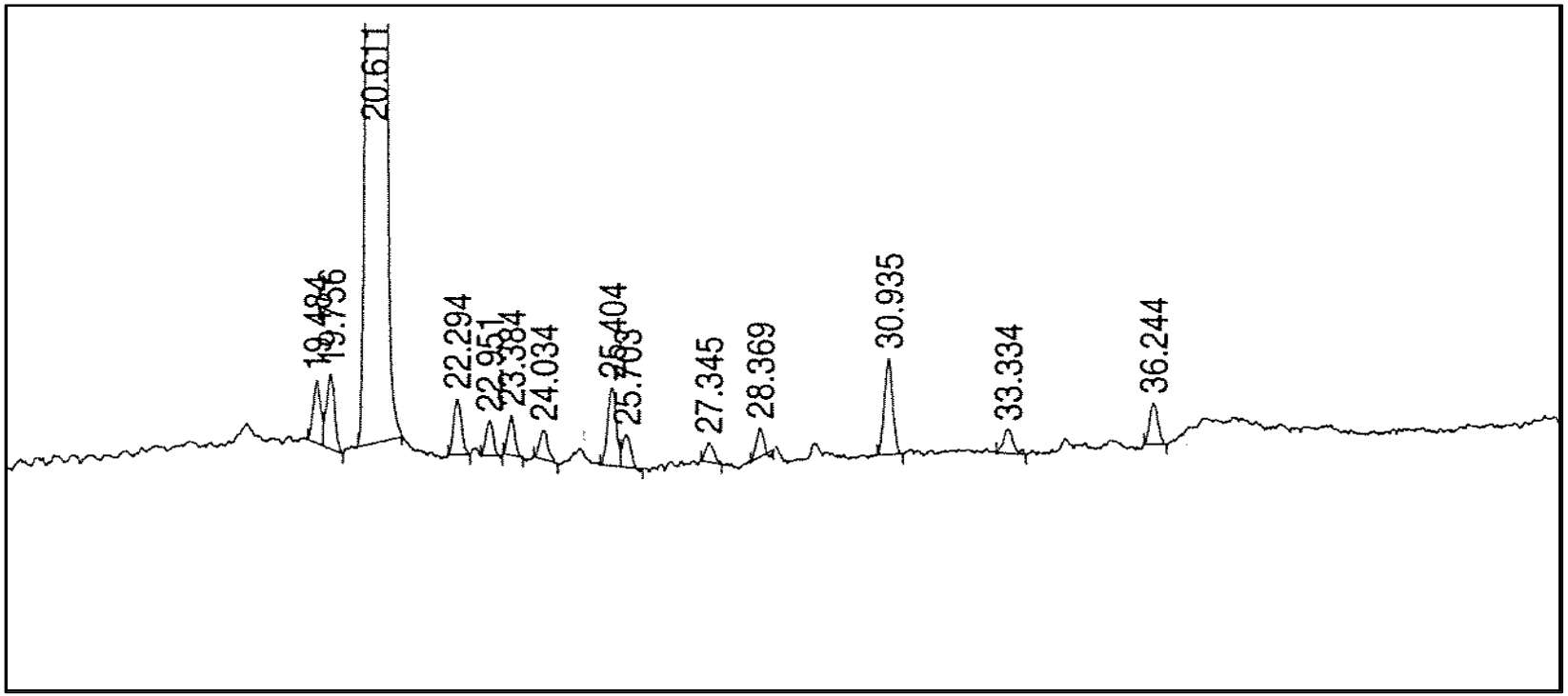


Step 3. Automated Fine Optimisation.
Result: more impurities found and better separation.



Comparison of Manual and Automated Method





Components to Implement Automatic Method Development and Screening

- Agilent 1100/1200 LC or LC-MS; Hitachi LaChrom or LaChromElite LC System; Waters Alliance;

+

- Agilent (Reodine), Waters(Reodine) or VICI/VALCO column or (and) solvent selectors

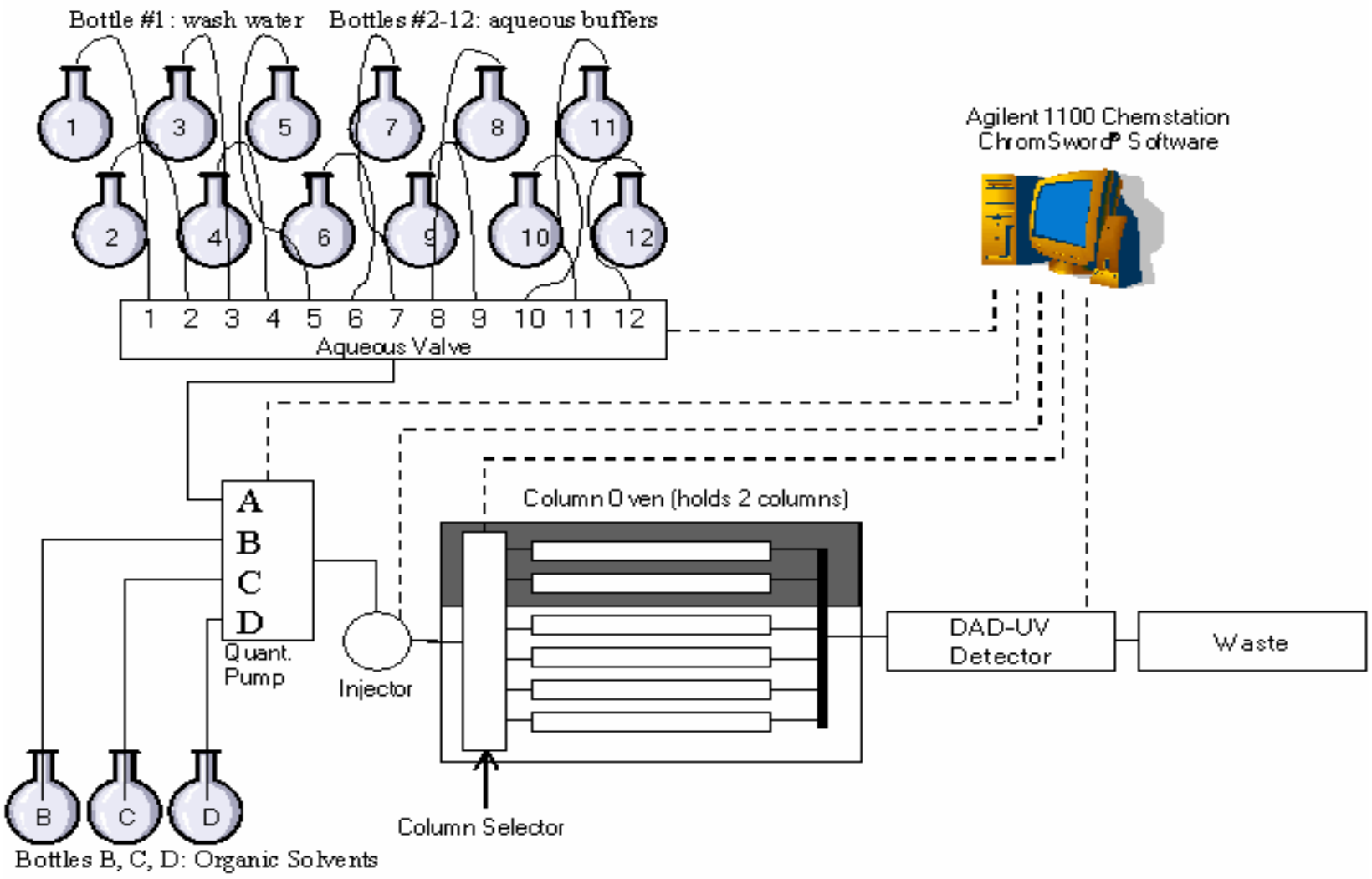
+

- Agilent ChemStation, Hitachi HSM, Agilent EZChrom, Waters Millennium or Empower HPLC software

+

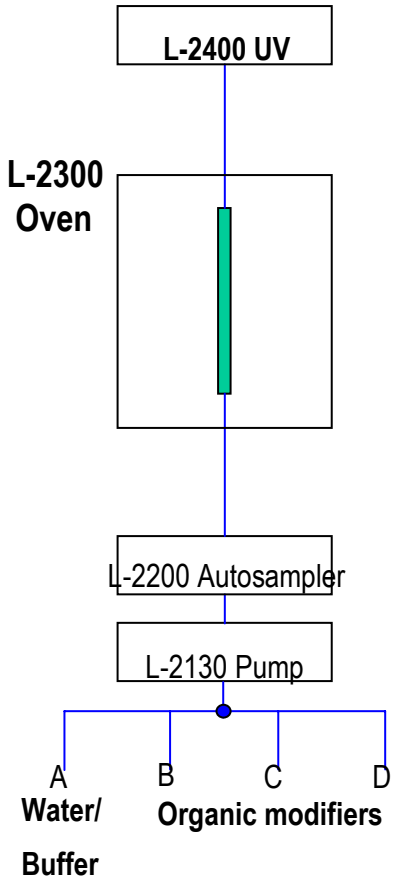
- ChromSword, ChromSwordAuto Method Development Software

Schematic of Column/Solvent Screening and Optimisation System: ChromSword with Agilent1100/1200 -

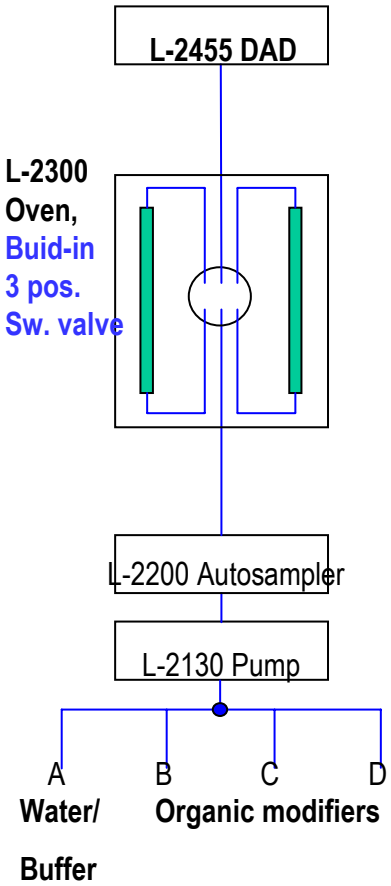


Suitable HPLC System Configurations for ChromSword with HITACHI LaChromElite HPLC Method Development Systems

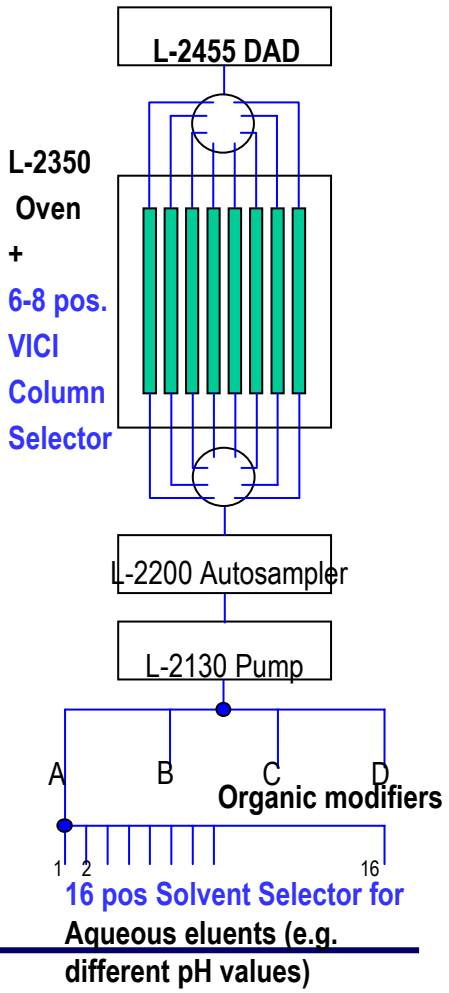
Economic System with 1 column



Standard System with 2-3 columns



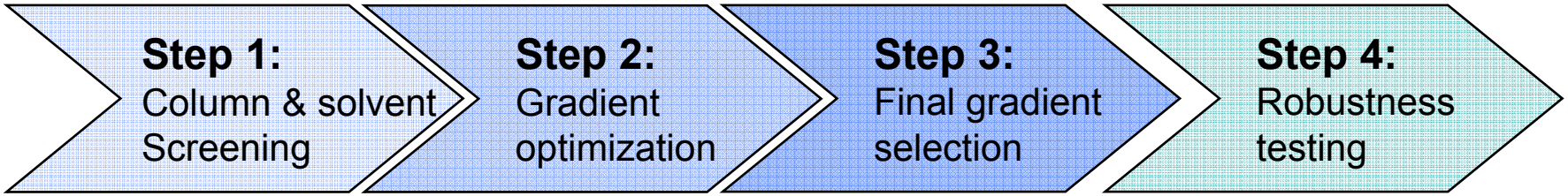
Professional System with 6-8 columns + 16 aqueous solvents



Typical Procedures and Time Norms for Automated Method Development

- **Columns, solvents, buffers screening**
  5 min - 60 min/column/solvent/buffer
- **Rapid optimisation with 1 column and 1 organic solvent**
  1 - 3 hours
- **Rapid optimisation with 5 columns, 2 solvents and 2 buffers**
  20 – 60 hours
- **Fine optimisation with 1 column and 1 organic solvent**
  10 – 36 hours
- **Robustness tests**
  10 – 36 hours

Automated method development: time contributions



Step 1	Step 2	Step 3	Step 4
CS: **over night**	CS: ≤ **30 hours**		CS: **over night**
A: **2 hours**	A: **2 hours**	A: **1 hour**	A: **1 hour**

CS = ChromSword

A = Analyst

ChromSword: 90%
Analytic: 10%

Conclusions

- **A seamless work flow is suggested for chromatographic method development.**
- **The use of a generic procedure is recommended instead of a generic method**
- **The method development process involves selection of columns and a screening step followed by optimization steps.**
- **Method development strategy proposed follows a staged-approach based on stage of drug development**
- **Computer powered automatic optimisation detects more impurities than manual method development !!!**

www.chromsword.de

- *E.Hewitt, P.Lukulay, S. Galushko. J.Chromatogr. A, 1107 (2006) 79*