

GUIDE FOR RUNNING CHROMATOGRAPHIC EXPERIMENTS AS INPUT DATA FOR DRYLAB® MODELS

to establish the Design Space for T and tG, incl. gradient form and flowrate influences

We recommend first optimizing gradient time and temperature. In this case, 4 experiments at different temperatures and gradient times must be run:

1. run with gradient time tG = 30 min, Temp.= 40°C
2. run with gradient time tG = 90 min, Temp.= 40°C
3. run with gradient time tG = 30 min, Temp.= 70°C
4. run with gradient time tG = 90 min, Temp.= 70°C

For a gradient from 5 to 95 %B and 30 min gradient time this means the gradient:

<u>time(min)</u>	<u>%B</u>
0	5
30	95

Other conditions could be (adjust if necessary to the needs of your sample):

Eluent A: 50 mMol/L phosphate buffer, pH 2.3
Eluent B: acetonitrile
Gradient: 5->95% B
Flowrate: 1.0 mL/min

If short columns are used (around 5 cm for example) the gradient times could be reduced to 10 and 30 min.

The used gradient (start- and end-%B) must ensure that all peaks elute in the increasing part of the gradient. Pre- and posteluting peaks are not accepted by DryLab®.

Preelution can be avoided by lowering the start-%B value.

Postelution can be avoided by increasing the gradient time and/or increasing the end-%B.

For all four input experiments the same sample and the same injection volume [µL] should be used.

All other parameters which can influence the chromatogram (gradient start- and end-%B, pH of the aqueous eluent, separation column, buffer conc., etc.) must be the same in all four experiments.

All changes in the four chromatograms should be caused only by the different gradient times and temperatures.

The DryLab® model of such a separation yields optimum values for T and tG. It also shows how the selectivity changes under changing experimental conditions. Moreover, an optimum linear gradient or manually a step gradient with up to 10 steps can be developed. The influence of flow rate, particle dimension and column dimensions (length and diameter) can be simulated, too.

To calculate the DryLab® model, some additional information is required:

The dimensions of the used column: length and inner diameter, particle size, flow rate and the dwell volume of the apparatus.

Please integrate the chromatograms so that the baseline includes all peaks of interest.

For the transfer of chromatographic data to the Molnar-Institut we recommend to export the data as *.cdf files (AIA/AnDI format, see under „export“). The AIA/AnDI format is supported by nearly all chromatographic data systems. If necessary, consult the documentation of your integration software. The AIA/AnDI format is vendor independent and therefore very suitable for the exchange of chromatographic data.

Rename your chromatograms so that the names show the conditions under which the chromatogram has been acquired, for example:

```
"Aspirin_40°C_30 min.cdf",  
"Aspirin_40°C_90min.cdf",  
"Aspirin_70°C_30min.cdf",  
"Aspirin_70°C_90min.cdf".
```

We can open and view chromatograms in this format in Winword, Excel or Powerpoint. With PeakMatch, we can use these files to support the peak tracking.

After we got all data, you will receive from us a DryLab® model (.dlb file), which can be evaluated by yourself with a demo version of DryLab®. You will be able to develop a method which exactly meets your needs using the DryLab® model you will receive from us.

After optimum values of T and tG have been found, additional parameters can be optimized:

- pH of the aqueous eluent A with +/- 0.6 pH units
- Type of the organic eluent, ion strength, etc.
- Ternary eluents methanol-acetonitrile or THF-acetonitrile
- Buffer concentration (factor 0.5 or factor 2.0)
- Different separation materials (for example columns of type B (Basic), columns of type A (Acidic), Polar Bonded Phase materials, Zirconium-C18, etc.)

PLEASE SEND THE FOLLOWING DATA TO THE MOLNAR-INSTITUT:

Chromatograms:

Four reproducible chromatograms as described on page 1 in „AIA/AnDI“ format (file extension .cdf)

Experimental conditions:

Temperature 1:

Temperature 2:

Gradient time 1:

Gradient time 2:

Column:

Name:

Length (cm):

Inner diameter(cm):

Particle size (μm):

Flowrate:

Gradient:

Start-%B:

End-%B:

Dwell volume of the HPLC apparatus (mL):

to:

Molnar-Institute for applied chromatography

Schneegloeckchenstrasse 47

10407 Berlin, Germany

Fax: +49 30 421 559 99

info@molnar-institute.com