Chemistry Screening – The Analytical Quality by Design Approach to LC Method Development

By Dr Robert Lott

Read any chromatography textbook or indeed Wikipedia and you will see that resolution equation consists of three, largely independent constituent parts:

$$R_s = [N^{1/2}/4]\ [k_2'/(1+k_2')]\ [(\alpha-1)/\alpha]$$

The first part $[N^{1/2}/4]$ is the Column or Efficiency Factor where N is the number of theoretical plates. As the name suggests it is largely influenced by the physical characteristics of the column, namely length, diameter, and particle size.

The second part $[k_2'/(1+k_2')]$ is the Retention Factor where K' is the K Prime/Capacity Factor and represents the time spend on the columns stationary phase relative to the mobile phase. It is principally modified by changes in solvent strength and is where many chromatographers (and solvophobic equation-based software products) concentrate their efforts.

The final part $[(\alpha-1)/\alpha]$ is the Separation or Thermodynamic Factor where α is the Selectivity Term. Changing α is the most effective way of modifying resolution and is determined by the column stationary phase, pH, and strong solvent type (the "chemistry system").

An analytical quality by design (AQbD) approach to method development would dictate that major factors are accessed first. This means a systematic approach is required to first determine the correct selectivity term (α). However, adopting AQbD can be a difficult, error prone, and a laborious task if the right tools are not adopted also.

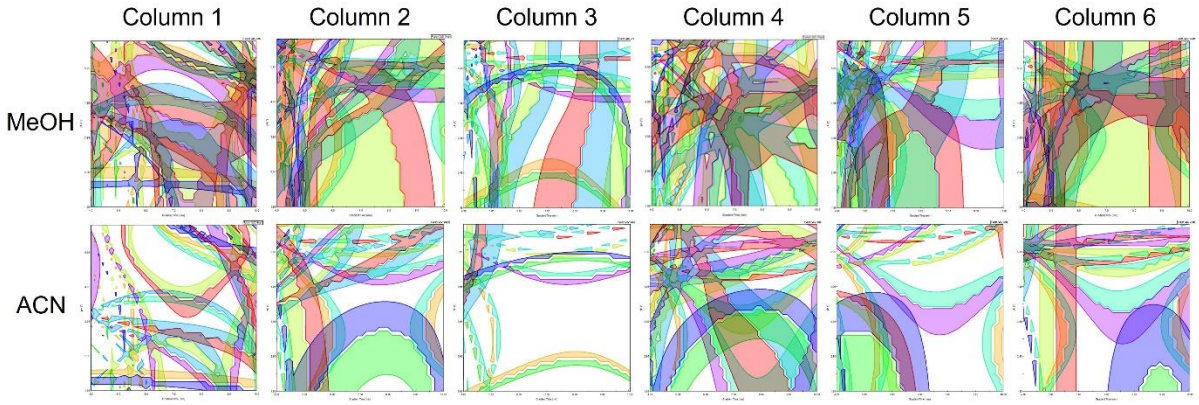
- Difficult, because few chromatographers are statisticians, or have any wish to be so.
- Error prone, because poor design choice, simple transcription errors during method creation or result transcription, and data analysis errors can result in poorly predicting models.
- Laborious, because AQbD is a data driven methodology, which without the right tools takes time to plan, generate, collate, and analyse.

Fortunately, Fusion QbD's LC Method Development platform supports Chemistry Screening experiments to first determine the best selectivity for your method, as well as Optimisation experiments to determine the optimum Retention Factor(s) to achieve the mean performance and robustness goals stipulated in your Analytical Target Profile (ATP).

Chemistry Screening experiments are supported by automation tools that allow execution in a highly efficient manner. These tools include:

- Automated design creation (no statistical/DoE knowledge required)
- Automated column switching
- Automated mobile phase pH preparation
- Automated strong solvent selection
- Automated generation of all CDS methods and sequence(s) ready for execution in the CDS
- Automated and validated result import (no transcription necessary)
- Trend Response metrics negate the need to track peaks altogether.
- If tracking is desired, Fusion's automated PeakTracker negates the need to track peaks in the CDS.
- Automated statistical Data Analysis
- Automated search for "Best Chemistry System" to adopt for Optimization.

The image below is a graphical representation of the knowledge spaces obtained from a single fully automated chemistry screening study. The study comprised six columns, two strong solvents, pH accessed at 6 different levels between 2 – 6.5, and gradient time accessed at 5 different levels between 4 – 10 minutes (starting at 5% strong solvent rising to 95% in a single linear gradient). The sample comprised of 10 peaks with detection by UV diode array. The design comprised a total of 74 experiments out of a potential 360 different combinations.



Each column/strong solvent combination are represented by a knowledge space of gradient time vs pH. Shaded areas indicate peak co-elution with different colours assigned to different peaks. White areas indicate no co-elution and potential scope for method optimisation.

It can be clearly seen that Column 3 in combination with ACN and a pH centred around 4 affords the best selectivity (α) and is the chemistry system that should be promoted to optimisation.

To conclude, don't start your method development activities in the dark (shaded areas). Let there be light (white space)!

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