Automated method development with sub 2-µm particle columns for LC separation of chemical and agricultural samples

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Introduction

Liquid chromatography is used for R&D, quality control and troubleshooting of many chemical, agricultural and pharmaceutical products or intermediates. Often a wide range of columns, solvents and other experimental parameters are used to develop a separation based on chemistry of different molecules[1].

In recent years, a lot of developments have occurred which allow users to take advantage of higher separation efficiencies per column length. This has been an important milestone for fast or high-resolution separations. However not only efficiency N, but selectivity α and retention k are also required to obtain separation *(1)*.

$$R_s = 0.25\, N^{0.5}\, (\alpha\text{-}1/\alpha)\, (k_2/1+k_2) \qquad (1)$$

Improving the selectivity α is often less straight-forward than improving the number of plates N or retention k. With the increasing availability of new stationary phase chemistries a potentially significant improvement in resolution for many separations is therefore left unused, unless a series of columns/solvents can be screened comprehensively.

Figure 1: Agilent 1200 Rapid Resolution LC Method Development Solution at Dow Rhine Center Germany

Challenging separations of chemical and agricultural samples were subject to method scouting. Many columns, solvent gradients and separation conditions have been screened using Agilent's 1200 LC series method development solution *(Figure 1)*. 50 mm Rapid Resolution High Throughput (RRHT) columns with 1.8 µm particle technology were used with pressures up to 600 bar to facilitate fast screening. An overview of alternate separation conditions, resulting in different selectivity, is presented.

Matrix interference with target compounds

Changes in herbicide formulation did result in failure of the current LC separation for quantification of active compounds. Screening of six columns and two organic solvents was performed overnight to scout for improved conditions. Good results were obtained with phenyl and cyano functionalized column phases. Changing to a cyano stationary phase and methanol gradient solvent provided the best selectivity between new formulation aromatic solvent and active compounds *(Figure 2)*.

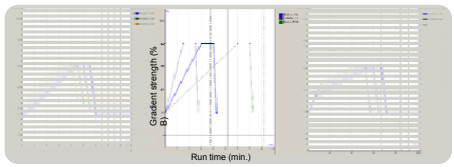

Figure 3: Graphical set-up of gradient optimization experiments

Further improvement could be made by optimization of the gradient program. Screening for optimal initial conditions and gradient slope was set-up using the Method Scouting Wizard *(Figure 3)*. In the final separation, the gradient has been optimized for different regions of the chromatogram *(Figure 4)*.

Herbicide impurities for quality control

Column and solvent screening was applied for impurity profiling in herbicides. Changes in selectivity were readily obtained for columns with different surface functionalization *(Figure 5)*. The EclipsePlus phenyl-hexyl column was identified as the stationary phase providing best selectivity with both acetonitrile and methanol gradients *(Figure 6)*. Separation of all reported impurities was achieved on a 50 mm column under conditions optimized for selectivity *(Figure 7)*.

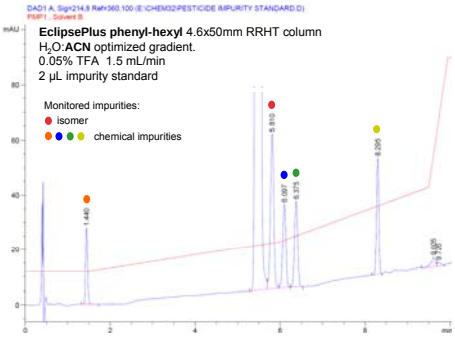

Figure 7: Herbicide impurities after gradient optimization

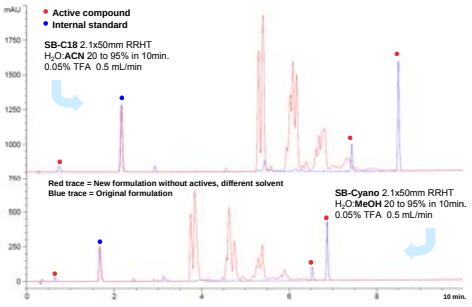

Figure 2: Herbicide formulations initial and optimized separation

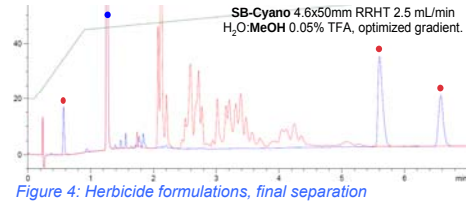

Figure 4: Herbicide formulations, final separation

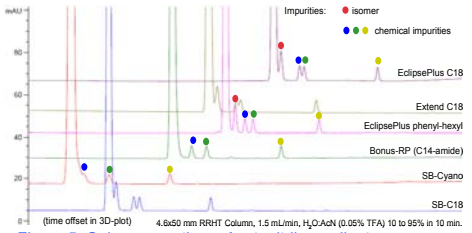

Figure 5: Column scouting – Acetonitrile gradient

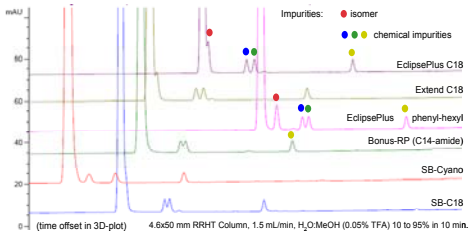

Figure 6: Column scouting – Methanol gradient

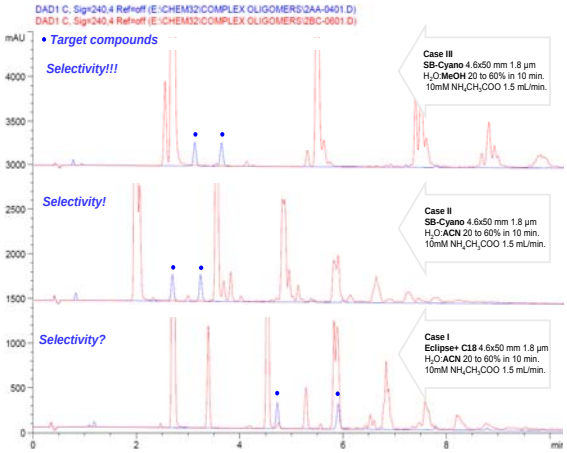

Figure 8: Target compounds in complex samples

Target compounds in complex samples

Separation of highly complex and concentrated samples is required for determination of target compounds down to 10ppm. Target compounds at high concentration and a typical sample matrix were used to investigate the selectivity changes for different columns and solvent combinations *(Figure 8)*. In **Case I** the separation using a C18 stationary phase is presented. Target compounds co-elute with the late-eluting matrix that has an increase number of isomers and prevents robust integration settings. **Case II** uses a cyano column under identical conditions. Target compounds elute in a favourable region of the chromatogram and the matrix compounds are clustered more together. Changing acetonitrile to methanol as the organic modifier improves this selectivity advantage even further in **Case III**.

Conclusions

Selectivity is an important parameter in the optimization of chromatographic separation. Separation of complex samples and rapid screening of different selectivity modifiers (*e.g.* columns, solvents, gradient conditions and temperature) can be achieved using short columns with sub 2-µm particles.

Column and solvent scouting often resulted in significant improvement or complete separation, while not taking more than a single overnight sequence. Screening for improved selectivity has become an economically attractive option due to the time savings by semi-automated systems. Highly efficient (selective) separations on shorter columns with sub 2-µm particles can also be performed using wide-spread equipment that provides up to 400 bar, thus providing higher compatibility with other labs, while saving solvent.

Column phases with functionalities other than the most commonly used C8 or C18 may offer a better starting point for further method development. Unexpected column surface functionality / solvent combinations were found to perform well in several instances. Their successes can be reasoned by their contribution to π- π interactions with the analytes[2].

Bianca Lurk (Dow) and Uwe Freisler (Agilent) are acknowledged for their contributions to the work presented on this poster.

[1] P.J. Schoenmakers, Optimization of chromatographic selectivity, J. Chrom. Lib. – Vol. 35, Elsevier, Amsterdam, The Netherlands, 1986.
[2] D.H. Marchand, K. Croes, J.W. Dolan, L.R. Snyder, R.A. Henry, K.M.R. Kallury, S. Waite, P.W. Carr, J. Chrom. A 1062 (2005) 65.

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