Automated LC Method Development and Robustness Tests with ChromSwordAuto® 5 and the Agilent 1290 Infinity II LC using the Agilent Instrument Control Framework

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The Agilent Instrument Control Framework (ICF) enables other providers of LC data acquisition and processing software to simplify the development of the control of Agilent LC instrumentation. In this article we demonstrate how the combination of Agilent ICF and ChromSwordAuto® software provides a fully automated method development and optimisation with the Agilent 1290 Infinity II UHPLC system.

The Agilent instrument control framework (ICF) is a software component that makes it easier and faster for software providers to control Agilent LC equipment in their chromatographic data systems or workstations. ChromSwordAuto® is a software suite for automated HPLC method development. The earlier versions of the software were designed to control Agilent LC instruments through Agilent ChemStation and other chromatography data systems (CDS). This configuration was utilised for automated method development of different pharmaceutical samples [1–4]. ICF substantially extended functionality of ChromSwordAuto® 5, which operates with Agilent LC and SFC instruments as an independent method development CDS [5,6]. ChromSwordAuto® 5 controls LC instruments, executes a sequence of runs, and acquires data. The user can predefine a sequence of runs - this is a scouting approach to screen different stationary (SP) or mobile phases (MP). Alternatively, users can choose a statistical design of experiments (DoE) to study the effect of method variables on separation. This method is defined as the robotic process automation. Another approach is intelligent automation. This automates nonroutine tasks like multistep gradient optimisation involving complex data processing and reasoning. In combination with ICF, ChromSwordAuto® supports both types of automation to assist chromatographers with routine and intelligent method development workflow.

In this Technical Overview we demonstrate:

- Which prerequisites must be fulfilled to ensure seamless interaction of an Agilent 1290 Infinity II UHPLC system with ChromSwordAuto® software and ICF software
- Which modules and instrument features are supported
- Which method development tasks and workflows are supported by using ChromSwordAuto® software
- That the performance of the 1290 Infinity II UHPLC system fulfils expectations using ChromSwordAuto® data acquisition, processing, and method optimisation tools

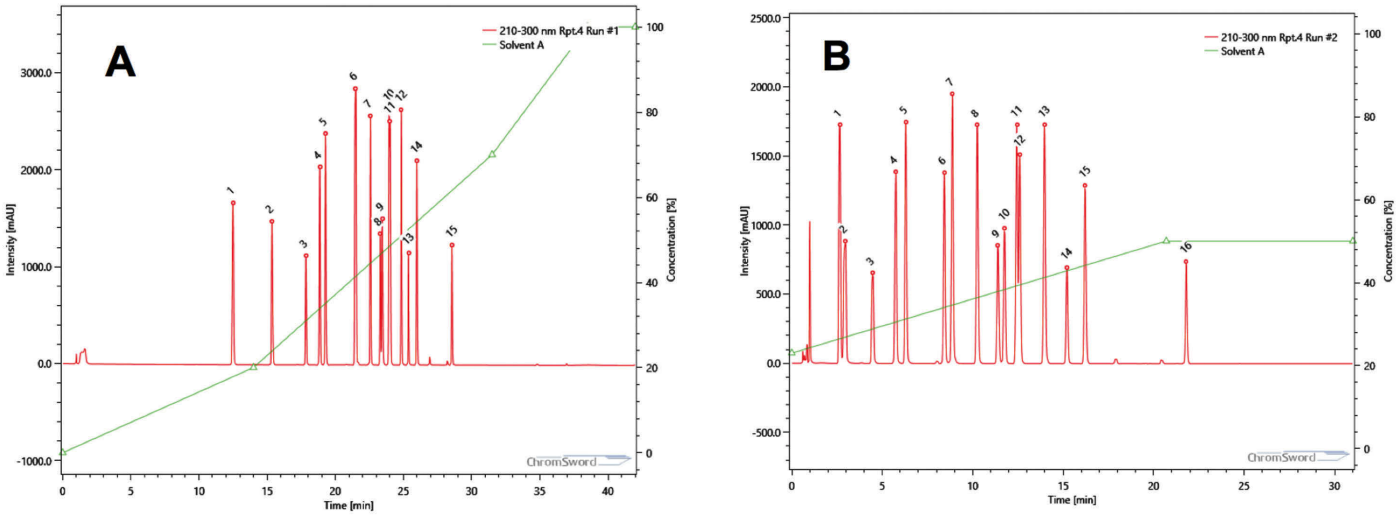

Figure 1: Rapid optimisation of the separation of 16 pesticides. A) Run 1. B) Run 2

- The development of a method that is capable of separating a complex multicomponent sample

Experimental

Instrumentation

An Agilent 1290 Infinity II UHPLC system with the following modules was used for the automated method development:

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Two Agilent 1290 Infinity Valve Drives (G1170A) with an Agilent InfinityLab Quick Change 12-Position/13-Port Bio-Inert Valve (G4235A)
- Agilent 1290 Infinity II Multicolumn Thermostat (MCT) G7116B with a valve drive (option number 058) equipped with an Agilent InfinityLab Quick Change 8-Position/18-Port Valve (G4239C) including an Agilent InfinityLab capillary kit (option number 005)
- Agilent 1290 Infinity II Diode Array Detector (DAD) (G7117B)
- Agilent 1290 Infinity II Multisampler (G7167B)

Software

- ICF A.02.05 package with LC drivers A.02.18
- ChromSwordAuto® 5.1 chromatography method development data system

The ChromSwordAuto® 5.1 package contains ChromSwordAuto® Scout, Developer, AutoRobust, and ReportViewer applications, which support different tasks for automated HPLC method development:

- ChromSwordAuto® Scout - method screening
- ChromSwordAuto® Developer - rapid and fine method optimisation for small and large molecules
- ChromSword AutoRobust - robustness studies
- ReportViewer - data browsing, processing, and projects management

ChromSwordAuto® incorporates automation of routine operations: column equilibration, column washout methods, system purging, column, and solvent switching sequences.

Prerequisites for the combination of ChromSwordAuto® 5.1 and ICF:

- ICF and the Agilent LC driver package must first be installed on the PC.
- All Agilent LC modules must have firmware version A.06.50, B.06.75, D.06.75, or higher.

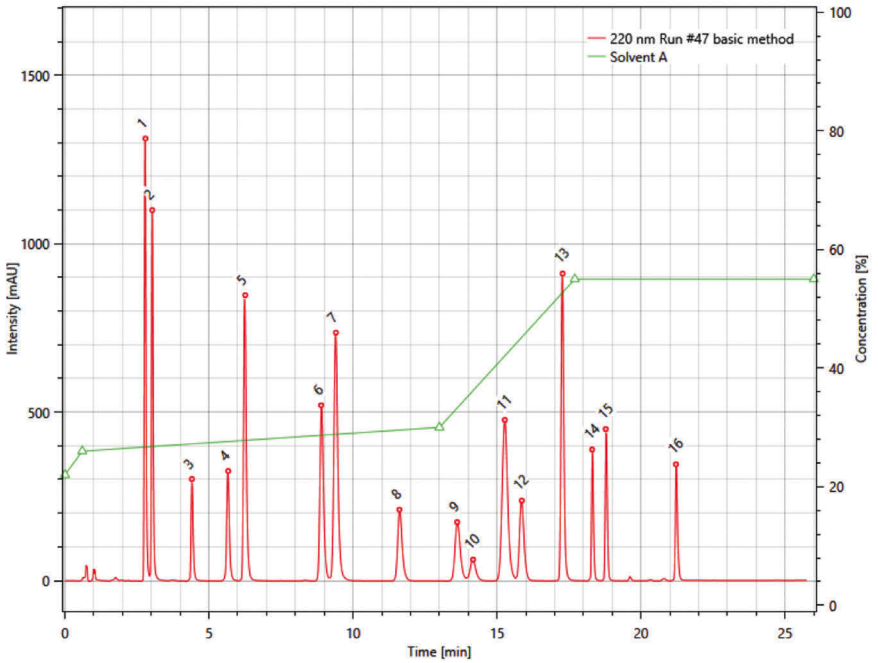

Figure 2: Finally optimised gradient and separation which was achieved in the Rapid Optimization

- The individual Agilent modules should be connected via CAN. Connect the whole instrument to the PC via LAN, use the LAN card in the Agilent module that produces the largest amount of data (DAD > FLD > MWD > VWD). Where DAD, FLD, MWD and VWD – are diode-array, fluorescence, multi wave and variable wavelength detector correspondently.

Columns:

1.Agilent ZORBAX Bonus-RP, 100 mm × 2.1 mm, 1.8 µm (part number 858768-901)

2.Agilent ZORBAX RRHD StableBond C18, 100 mm × 2.1 mm, 1.8 µm (part number 858700-902)

3.Agilent ZORBAX StableBond C8, 100 mm × 2.1 mm, 1.8 µm (part number 858700-906)

4.Agilent ZORBAX Eclipse Plus, 100 mm × 2.1 mm, 1.8 µm (part number 959758-902)

Final method

Solvents: A) acetonitrile; B) water + 0.1% phosphoric acid, pH = 2.4

Flow rate: 0.3 mL/min

Gradient: 0 min: 22% A; 0.6 min: 26% A; 13 min: 30% A; 17.7 min: 55% A

Stop time: 25 min

Column: ZORBAX Bonus-RP, 100 mm × 2.1 mm, 1.8 µm

Column temperature: 30 °C

Sample: 2 µL

DAD: 220 nm; data rate: 5 Hz

Sample

Agilent 2D-LC checkout standard, containing 16 pesticide compounds at a concentration of 1 mg/mL each in acetonitrile/acetone (4:1). The identities of the constituent compounds can be found in the information accompanying the sample (part number 5190-6895).

Sample preparation: dilute 1:10 with acetonitrile and use the dilution in experimentation.

Solvents

All solvents were purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

Results and Discussion

Method development study

The following method development strategy was applied for this application:

- For the first rapid optimisation with different types of reverse phase columns and different organic solvents, an instrument time of about 48 hours and about one hour of manual analyst work was applied.
- For the data browsing and selection of the most promising column, one hour of analyst work was applied.
- The final fine optimisation was done with the best column/solvent combination with an instrument time of about 16 hours and 30 minutes of manual work.

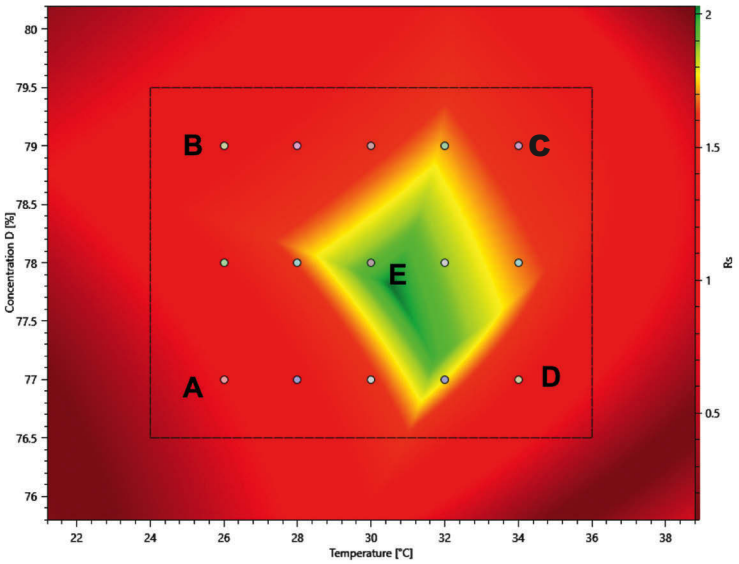

Figure 3: Two-dimensional resolution map for the effect of concentration of solvent A and column temperature on compound resolution at the optimal flow rate. The optimum space is shown in green. The circles indicate the conditions of test runs). The measured points marked A to E are shown figure 4, all available chromatograms and resolutions between these points are calculated

In the *Rapid Optimization* mode, the software performs three to four runs for each possible column, solvent, and temperature combination to optimise the gradient profile.

The best results obtained in the rapid optimisation study were achieved with the ZORBAX Bonus-RP column.

The initially applied gradient started at a very low percentage of organic solvent and increased to nearly 100 % organic solvent in 40 minutes. The complete set of compounds eluted in the middle of the run between 12 and 30 minutes, with insufficient resolution (Rs < 1.5) between some compounds (Figure 1A). To improve the resolution, the software raised the initial content of organic solvent up to 20%, which moved the elution pattern to the beginning of the run. The applied gradient was shallower than that in the first experiment, to achieve the necessary resolution over the complete run time (Figure 1B). However, in two cases, the resolution was still not sufficient, and was improved by an automated adaption of the gradient, which was applied in the third experiment. In this case, the peak pairs 9/10 and 11/12 were clearly separated with sufficient resolution (Figure 2).

Following this optimisation, all compounds were separated with maximised resolution (Rs = 1.6) in less than 22 minutes. In the Fine Optimization mode, the software could perform detailed sample profiling, peak tracking, and optimisation.

Robustness study and method improvement

Robustness of a method is extremely important for providing method transfer to other laboratories and instruments. ChromSword® AutoRobust is a specialised application for automatic evaluation of robustness of HPLC methods and method improvement. For robustness tests different operation factors and levels of method variables can be considered and specified. The factor levels of variables to be tested should be set around the nominal values specified in the operating (basic) method. The interval chosen between the extreme values represents the limits between which the factors are expected to vary when a method is transferred. To define the factor levels for the temperature, concentration and time of gradient steps it is recommended to study the effect of these variables in more details. Such studies can be specified in ChromSwordAuto® and AutoRobust and performed automatically.

For robustness studies, AutoRobust supports different design of experiments (DoE) to test effect of method variables:

- One parameter at a time
- Full factorial design
- Statistical Plakett-Burman design, working with up to seven method variables simultaneously

In this study, we applied the full factorial design, which tested all possible

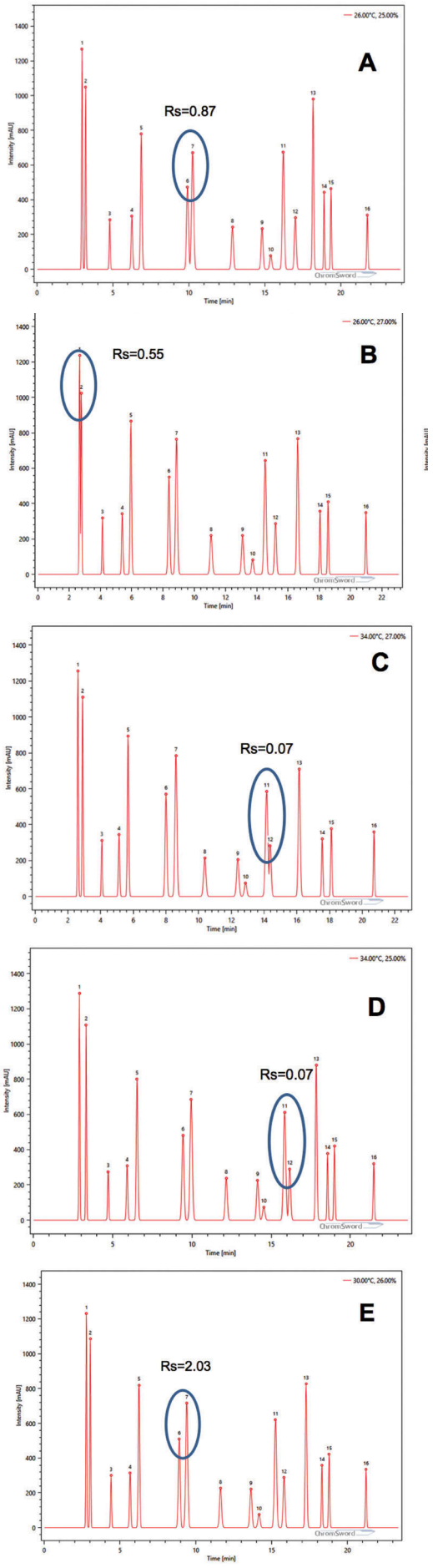

Figure 4: Measured chromatograms A to E as indicated in the two-dimensional resolutions pace shown in figure 3. The minimum resolution under the given separation conditions are marked and indicated. Chromatogram E is showing the chromatogram which obtained under the optimum condition

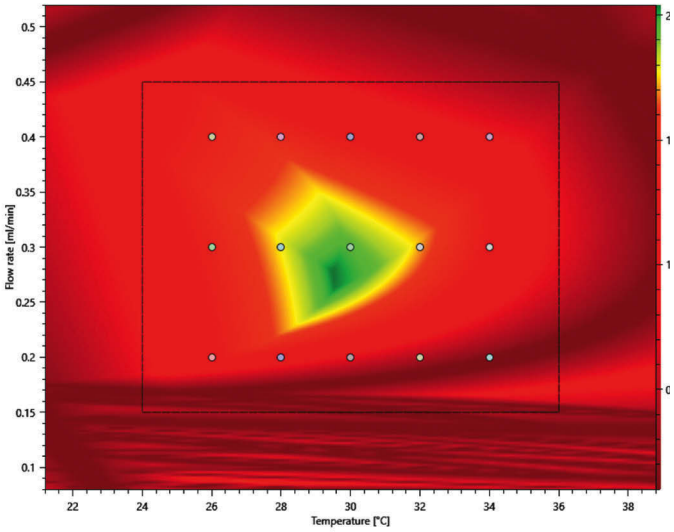

Figure 5: Resolution map for effect of flow rate and temperature indicating an optimum range between 0.25 and 0.35 mL/min and 28 to 32 °C

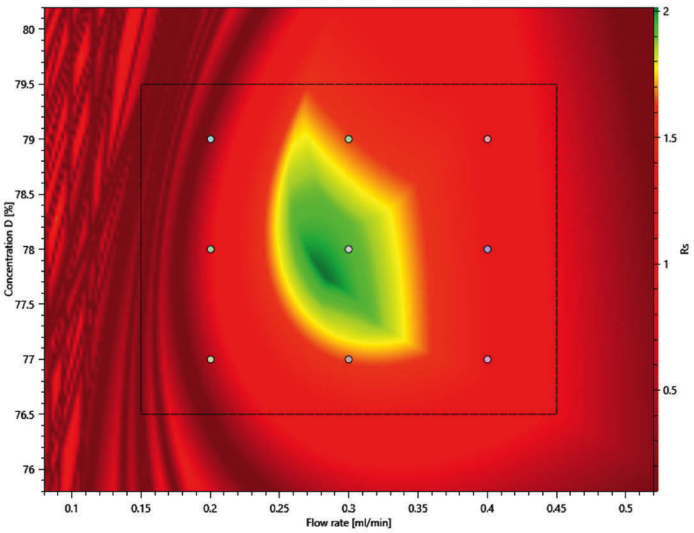

Figure 6: Resolution map showing the effect of flow rate and concentration of a solvent in a gradient mode

combinations of flow rate, temperature, and concentration of solvent A [%]. The applied gradients were exactly parallel, with one percent distance between them.

AutoRobust automatically creates the selected DoE and executes every run of the design. After performing the tests, the ReportViewer analyses the results and reports the critical analytical parameters by building two- and three-dimensional (2D and 3D) design spaces for a tested method.

The robustness tests took about 18 hours of instrument time and about 30 minutes of analyst work including the generation of a report.

The achieved 2D resolution map for the effect of concentration of solvent A and temperature shows a space between 26 and 34°C column temperature and an initial concentration between 25 and 27% of organic solvent percentage in the gradient (Figure 3). The real experiments appearing in the 2D space are indicated by circles. The centre point, which is the basic method, is marked by a square. The flow rate, which is the third dimension, was 0.3 mL/min for the basic method. Additional runs under these concentration and temperature conditions were performed at 0.2 mL/min and 0.4 mL/min. The indicated green area is the optimum resolution range, where measured resolution is above 2.0. The data points, which are indicated by A to E, are shown in Figure 3. The chromatogram shown for point A has its lowest resolution of Rs = 0.78 for the critical pair of peaks 6 and 7. By moving to point B, the situation changes, and the lowest resolution (Rs = 0.55) is obtained for the critical pair of peaks 1 and 2 at the corresponding gradient concentration. By changing the temperature for the gradients, the situation changes to points C and D (Figure 3). All available chromatograms in this 2D space are calculated from those obtained experimentally. By moving through the third dimension, the applied flow rate, the optimised condition could be identified at 0.28 mL/min (Figure 4, Chromatogram E). Together with a temperature at 29.5°C and an initial concentration of organic solvent of 25.9%, the critical peak pair resolution remained above 2.03. For flow rates below 0.23 mL/min and above 0.33 mL/min, the space for this optimum resolution completely disappeared. Effects of combination of method parameters on 2D resolution maps are shown in figures 5-8. Using such maps it is possible to determine optimal operational conditions and limits of method variables which provide the required resolution.

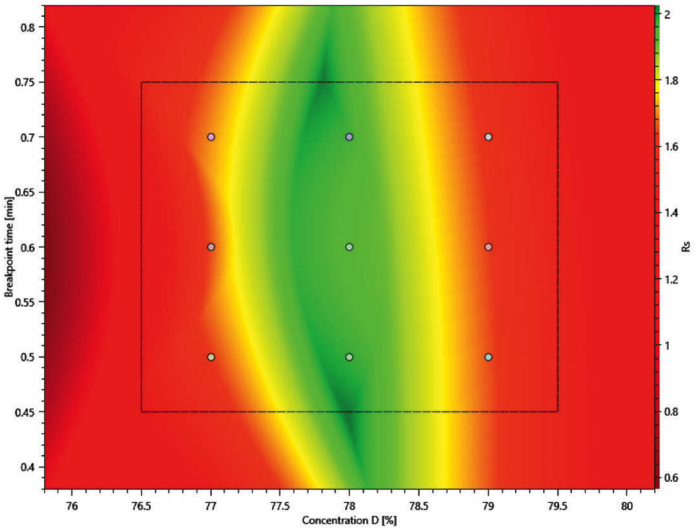

Figure 7: Resolution map showing the effect of concentration of acetonitrile and gradient breakpoint time

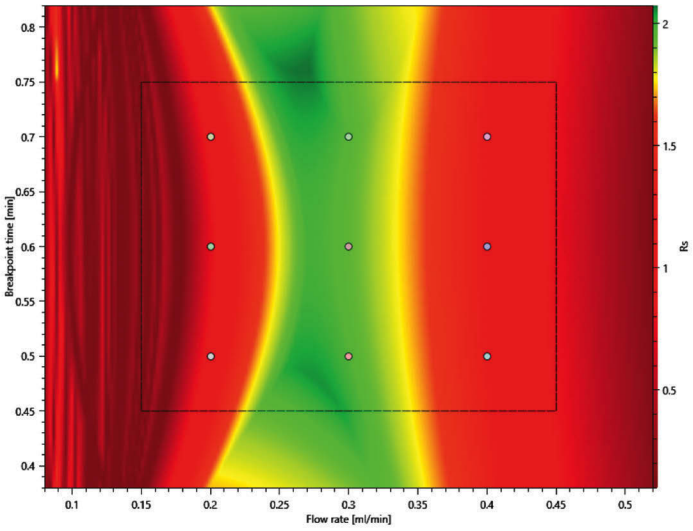

Figure 8: Resolution map showing the effect of gradient time and flow rate

Conclusion

Automated method development with the Agilent 1290 Infinity II LC and ChromSwordAuto® software is an effective means to find optimal conditions for separation of complex mixtures in a short time.

References

1. Galushko, S.; Tanchuk, V.; Shishkina, I.; Pylypchenko, O.; Beinert, W. D. ChromSword® Software for Automated and Computer-Assisted Development of HPLC Methods. In HPLC Made to Measure: A Practical Handbook for Optimization. Kromidas, S., Ed.; Wiley-VCH Verlag, Weinheim, 2006; pp 557–570.

2. Hewitt, E. F.; Lukulay, P.; Galushko, S. Implementation of a Rapid and Automated High Performance Liquid Chromatography Method Development Strategy for Pharmaceutical Drug Candidates. J. Chromatogr. A 2006, 1107(1–2), pp 79–87.

3. Xiao, K. P.; Xiong, Y.; Liu, F. Z.; Rustum, A. M. Efficient Method Development Strategy for Challenging Separation of Pharmaceutical Molecules Using Advanced Chromatographic Technologies. J. Chromatogr. A 2007, 1163(1–2), pp 145–156.

4. Vogel, F.; Galushko, S. Application of ChromSword® Software for Automatic HPLC Method Development and Robustness Studies. Separation of Terbinafine and Impurities. Chromatography Today 2013, pp 3–6.

5. Zhuang, J.; Kumar, S.; Rustum, A. Development and Validation of a Normal Phase Chiral HPLC Method for Analysis of Afoxolaner Using a Chiralpak® AD-3 Column. J. Chromatogr. Sci. 2016, 54(10), pp. 1813–1819. https://doi.org/10.1093/chromsci/bmw162.

6. http://www.chromsword.com/