

Suitable for Agilent  
1290 Infinity III LC

# Automated LC Method Development and Robustness Tests

## ChromSwordAuto 5 and the Agilent 1290 Infinity II LC using the Agilent Instrument Control Framework

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### Introduction

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 (CDSs). This configuration was used for automated method development for different pharmaceutical samples<sup>1-4</sup>.

ICF substantially extended the functionality of ChromSwordAuto 5, which operates with Agilent LC and SFC instruments as an independent method development CDS<sup>5,6</sup>. 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 robotic process automation. Another approach is intelligent automation. This automates nonroutine tasks like multistep gradient optimization 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 workflows.

This Technical Overview demonstrates:

- Which prerequisites must be fulfilled to ensure seamless interaction between an Agilent 1290 Infinity II UHPLC system and ChromSwordAuto and ICF software
- Which modules and instrument features are supported
- Which method development tasks and workflows are supported using ChromSwordAuto software
- That the performance of the 1290 Infinity II UHPLC system fulfills expectations using ChromSwordAuto data acquisition, processing, and method optimization tools
- 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 optimization for small and large molecules
- **ChromSword AutoRobust:** Robustness studies and method improvement
- **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.
- The individual Agilent modules should be connected using CAN. Connect the whole instrument to the PC through LAN, use the LAN card in the Agilent module that produces the largest amount of data (DAD > FLD > MWD > VWD).

### Columns

- Agilent ZORBAX Bonus-RP, 100 mm × 2.1 mm, 1.8 μm (p/n 858768-901)
- Agilent ZORBAX RRHD StableBond C18, 100 mm × 2.1 mm, 1.8 μm (p/n 858700-902)
- Agilent ZORBAX StableBond C8, 100 mm × 2.1 mm, 1.8 μm (p/n 858700-906)
- Agilent ZORBAX Eclipse Plus, 100 mm × 2.1 mm, 1.8 μm (p/n 959758-902)

### Final method

Parameter	Value
Solvents	A) Acetonitrile B) Water + 0.1 % phosphoric acid, pH = 2.4
Flow rate	0.3 mL/min
Gradient	0 minutes: 22% A; 0.6 minutes: 26 %A; 13 minutes: 30 %A; 17.7 minutes: 55 %A
Stop time	25 minutes
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 (p/n 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

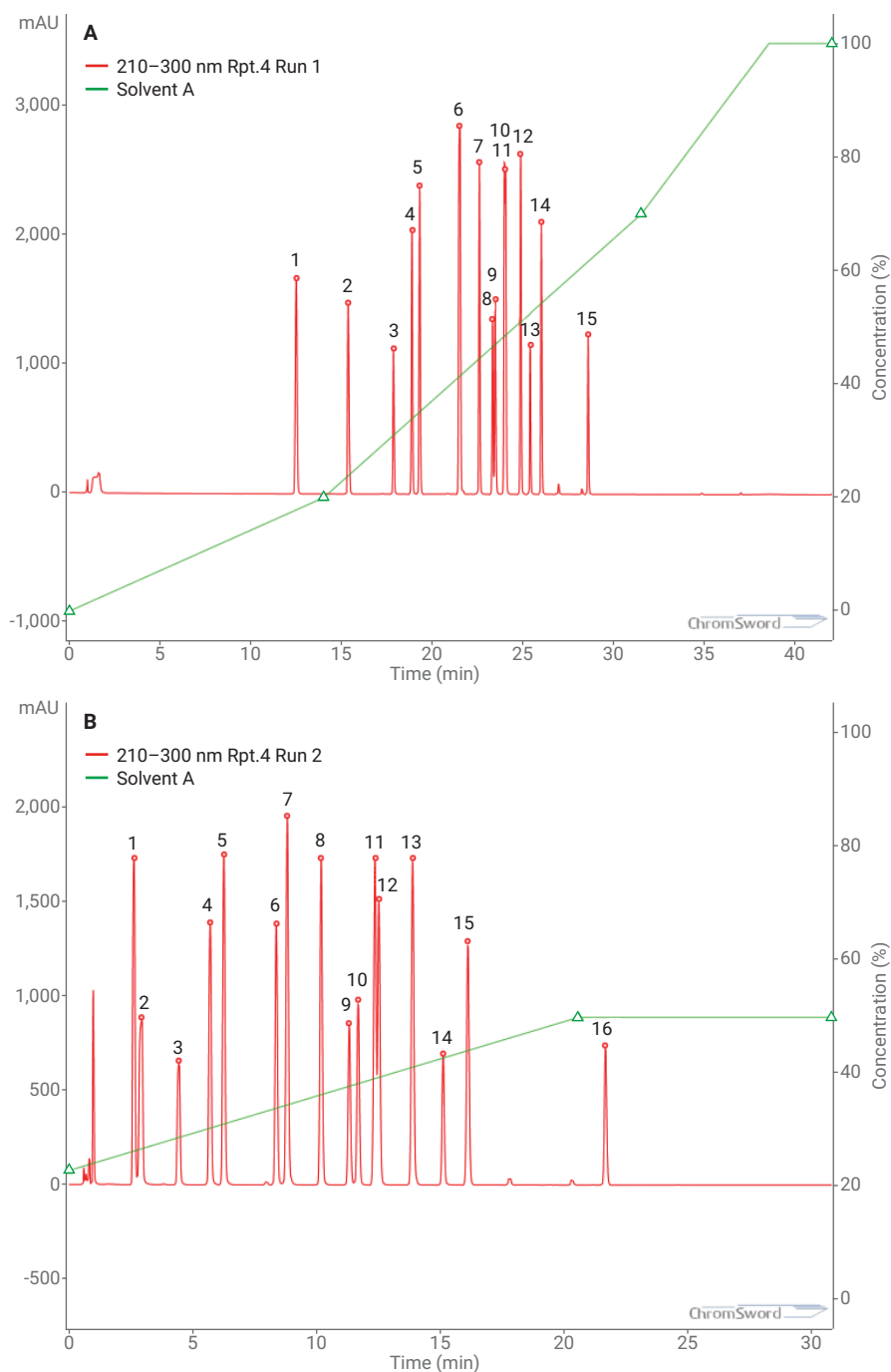
The following method development strategy was applied for this application:

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

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

The best results obtained in the rapid optimization 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 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 adaptation of the gradient, which was applied in the third experiment.



**Figure 1.** Rapid optimization of the separation of 16 pesticides. A) Initial gradient for the separation of the complex test sample. B) First optimization, with an increased content of organic solvent in the starting conditions and a shallower gradient.

In this case, the peak pairs 9/10 and 11/12 were clearly separated with sufficient resolution (Figure 2).

Following this optimization, all compounds were separated with maximized resolution in less than 22 minutes. In the Fine Optimization mode, the software could perform detailed sample profiling, peak tracking, and optimization.

### Robustness study

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

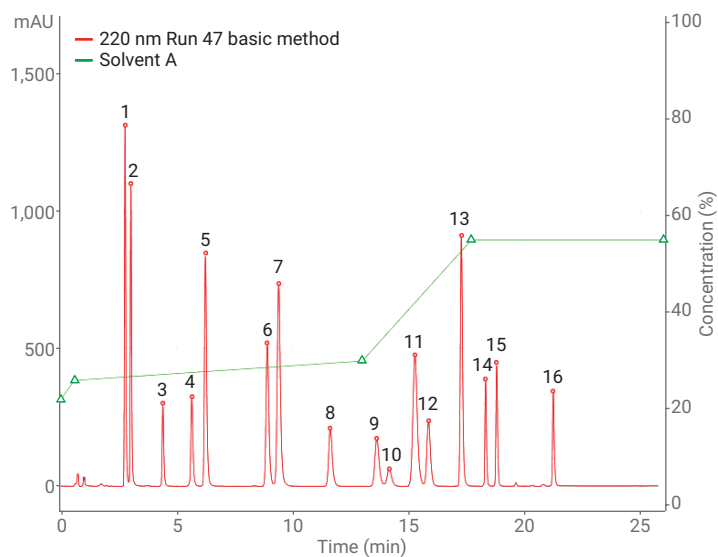
For robustness studies, AutoRobust supports different designs of experiment (DoEs):

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

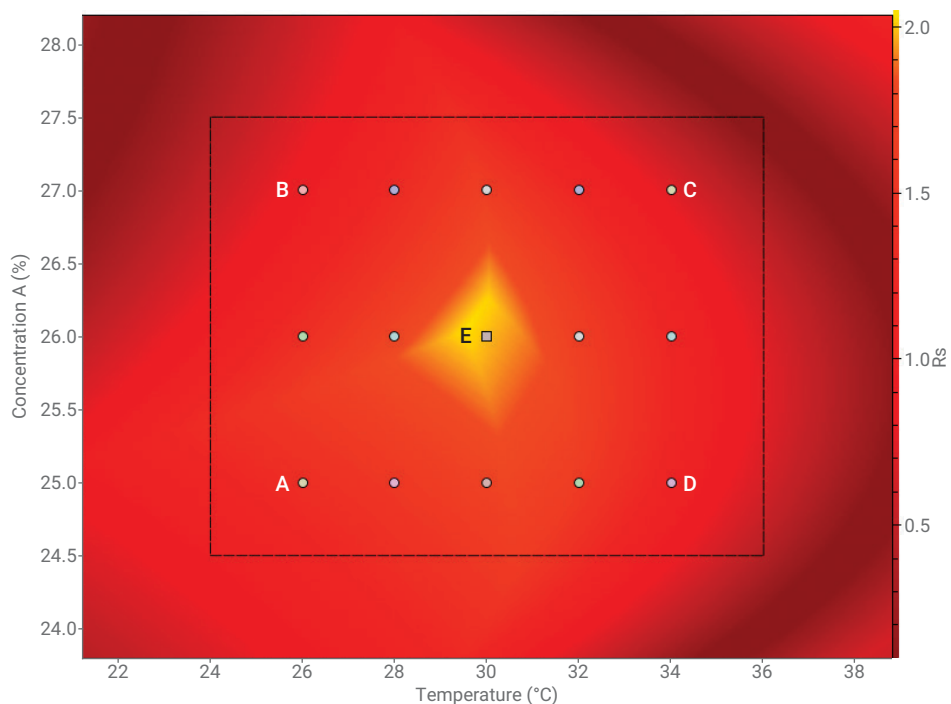
We applied the full factorial design, which tested all possible combinations of flow rate, temperature, and concentration of solvent A (%). The applied gradients were exactly parallel, with 1 % distance between them.

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

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 in the gradient (Figure 3). The real experiments appearing in the 2D space are indicated by circles. The center point, which is the basic method, is marked by a square.



**Figure 2.** Final, optimized gradient and separation, which was achieved in Rapid Optimization mode.



**Figure 3.** Two-dimensional resolution map for the effect of concentration of solvent A and column temperature on compound resolution. The optimum space is shown in yellow. The measured points marked A to E are shown in Figure 4. All available chromatograms and resolutions between these points are calculated.

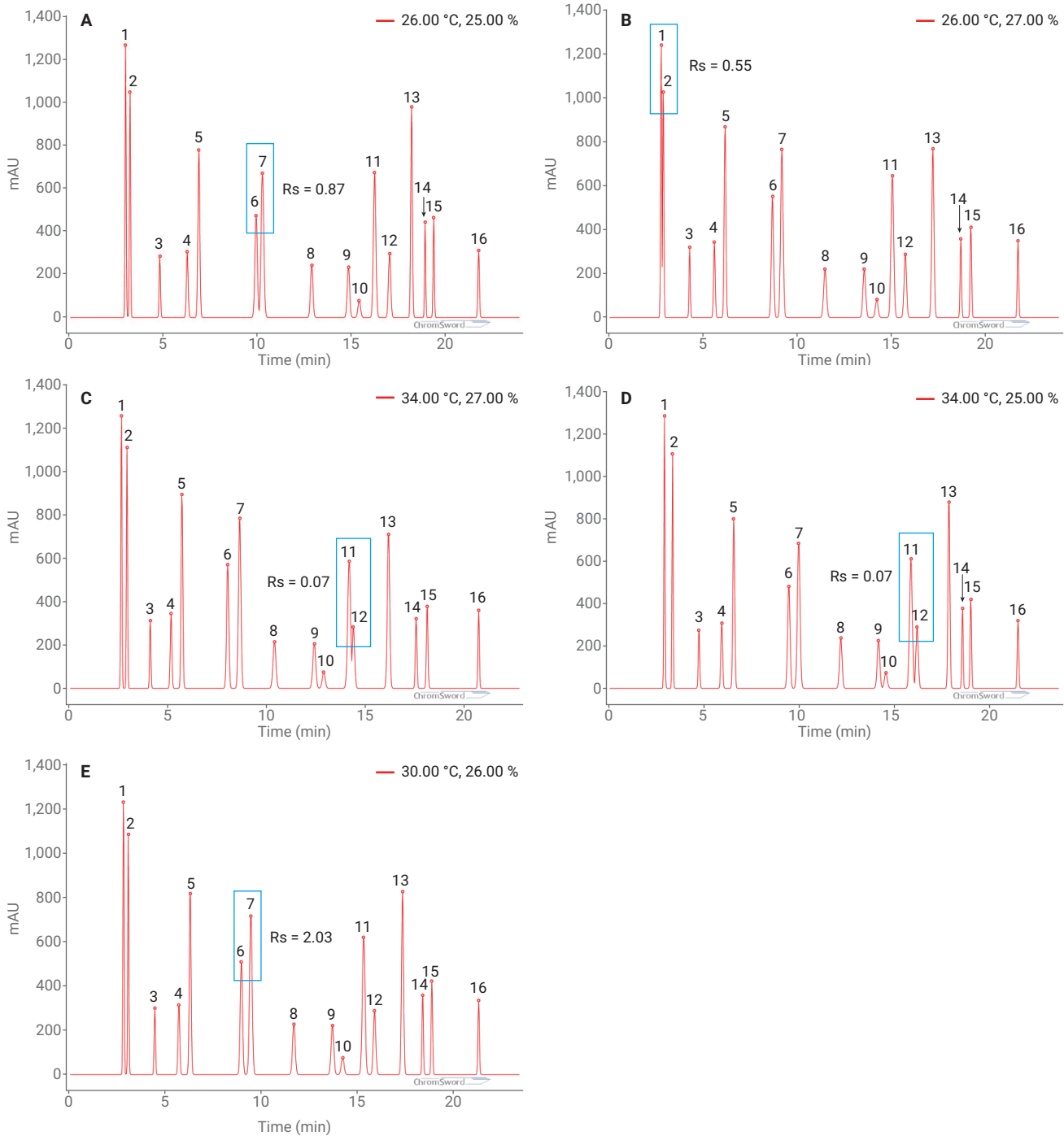
The flow rate, which is the third dimension, was 0.3 mL/min for the basic method. Additional runs under these concentration and temperature conditions were performed at 0.2 and

0.4 mL/min. The yellow area is the optimum resolution range, where measured resolution is above 2.0 for all peak pairs.

Figure 4 shows the data points, which are indicated A to E. The chromatogram shown for point A has its lowest resolution of  $R_s = 0.78$  for the critical pair of peaks 6 and 7.

By moving to point B, the situation changes, and the lowest resolution ( $R_s = 0.55$ ) 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 4). All available chromatograms in this 2D space are calculated from those obtained



**Figure 4.** Measured chromatograms A to D as indicated in the two-dimensional resolution space shown in Figure 3. The minimum resolution under the given separation conditions are marked and indicated. Chromatogram E shows the chromatogram obtained under optimum conditions with no resolution of critical pairs below 2.03.

experimentally. By moving through the third dimension, the applied flow rate, the optimized condition could be identified at 0.28 mL/min. Together with a temperature of 29.5 °C and an initial organic solvent concentration of 25.9 %, the critical peak pair resolution remained above 2.03 (Figure 4, chromatogram E). For flow rates below 0.23 mL/min and above 3.3 mL/min, the space for this optimum resolution disappeared.

## Conclusion

Automated method development with the 1290 Infinity II LC and ChromSwordAuto software is an effective means to find optimal conditions for the separation of complex mixtures in a short time. The results, shown in this Technical Overview have also been published in *Chromatography Today*, Nov/Dec **2018**.

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