

# Speeding Up Pharmaceutical UHPLC Method Development with an Integrated, Ultrafast Automated Method Scouting Solution

Marco Karsten,<sup>1</sup> Wim Decrop,<sup>1</sup> Remco Swart,<sup>1</sup> Wulff Niedner,<sup>2</sup> Frank Steiner,<sup>2</sup> Fraser McLeod,<sup>2</sup> Xiaodong Liu,<sup>3</sup>  
<sup>1</sup>Dionex Corporation, Amsterdam, The Netherlands, <sup>2</sup>Dionex Corporation, Germering, Germany,  
<sup>3</sup>Dionex Corporation, Sunnyvale, CA USA

## UHPLC METHOD SCOUTING

HPLC method development is still considered to be one of the crucial bottlenecks that impede productivity in analytical laboratories. Due to the variety of available columns, the proper selection of the stationary phase usually represents the greatest challenge. With the progress in UHPLC which enables generic gradient separations within 5 min or less, ultrafast method development techniques become possible. While conventional LC rarely supported more than one generic gradient run per hour, UHPLC can do a multitude of chromatographic runs in the same amount of time. This allows changing the experimental approach from thoroughly designing a small number of initial experiments towards a more comprehensive screening along any possible combination of column/eluent/temperature. Thanks to the very short run times of UHPLC, this can be accomplished within the same time frame as the conventional approach. However, this screening provides a significantly broader set of results, more information on the influence of the parameters within the design space, and thus more confidence in the robustness of the new method.

In this poster, we present an automated ultrafast method scouting solution. The separation of a mixture of diuretics has been screened with two different UHPLC columns, acetonitrile, and methanol as organic modifiers and five different buffers.

**Table 1. LC Conditions for Automated Column and Eluent Scouting**

|                                  |  |
|----------------------------------|--|
| <b>Columns</b>                   | Acclaim C18, 2.2 $\mu$ m,<br>120 Å, 100 mm $\times$ 2.1 mm (068982)<br>Acclaim PolarAdvantage II (PA2), 2.2 $\mu$ m,<br>120 Å, 100 mm $\times$ 2.1 mm (068990)                                 |
| <b>Aqueous Mobile Phases (A)</b> | 50 mM ammonium acetate pH 2.5 with TFA<br>50 mM ammonium acetate pH 3.6 with FA<br>50 mM ammonium acetate pH 4.8 with FA<br>50 mM ammonium acetate pH 6.5<br>50 mM ammonium bicarbonate pH 8.0 |
| <b>Organic Modifiers (B)</b>     | Acetonitrile<br>Methanol   |
| <b>Sample Set</b>                | Amiloride<br>Ethacrynic acid<br>Furosemide<br>Triamterene<br>Bumetanide<br>Chlortalidone   |

## INSTRUMENT AND METHOD SETUP

In this study, we have employed a set of UHPLC columns and have screened them for the analysis of pharmaceutical samples on an integrated HPLC system designed for ultrafast automated method scouting. The system is comprised of:

- A pump with dual binary gradient capabilities for pressures up to 800 bar extended with an additional 10-position, 11-port solvent selection valve.
- A split loop autosampler compatible with very short cycle times.
- A powerful diode array detector with data collection rates up to 100 Hz providing compound identification based on UV/VIS spectra.
- Two high-pressure 6-position, 7-port column selection valves integrated in the thermostatted column compartment for maximum scouting flexibility.
- Intelligent software for easy parameter permutation, fully automated system control and automated identification of the optimum chromatograms from the large data set.

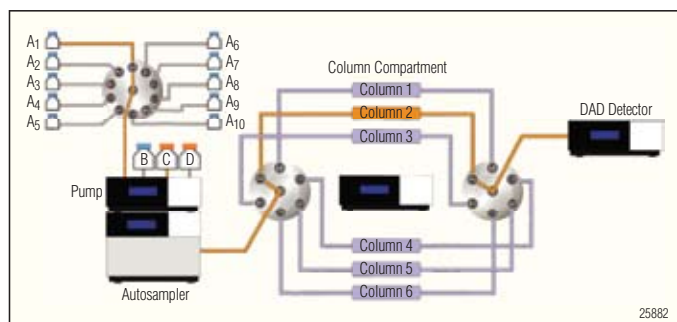


Figure 1. System configuration for automated method scouting with optional 10-position solvent selection valve.

The following method parameters were used during the method scouting study:

- Flow rate 0.55 mL/min, unless stated otherwise
- Gradient 15%–90% B in 2 min, 2.5 min equilibration, unless stated otherwise
- UV detection at 254 nm, 3-D field from 210 nm to 360 nm
- Injection volume 2  $\mu$ L, 0.03 mg/mL each compound (15  $\mu$ L on conventional column)
- Column compartment at 25  $^{\circ}$ C

## SOFTWARE

The Chromeleon<sup>®</sup> Chromatography Data System software allows fully automated control of the complete system as well as manual control. The work flow for automated method scouting involves the steps shown in Figure 2.

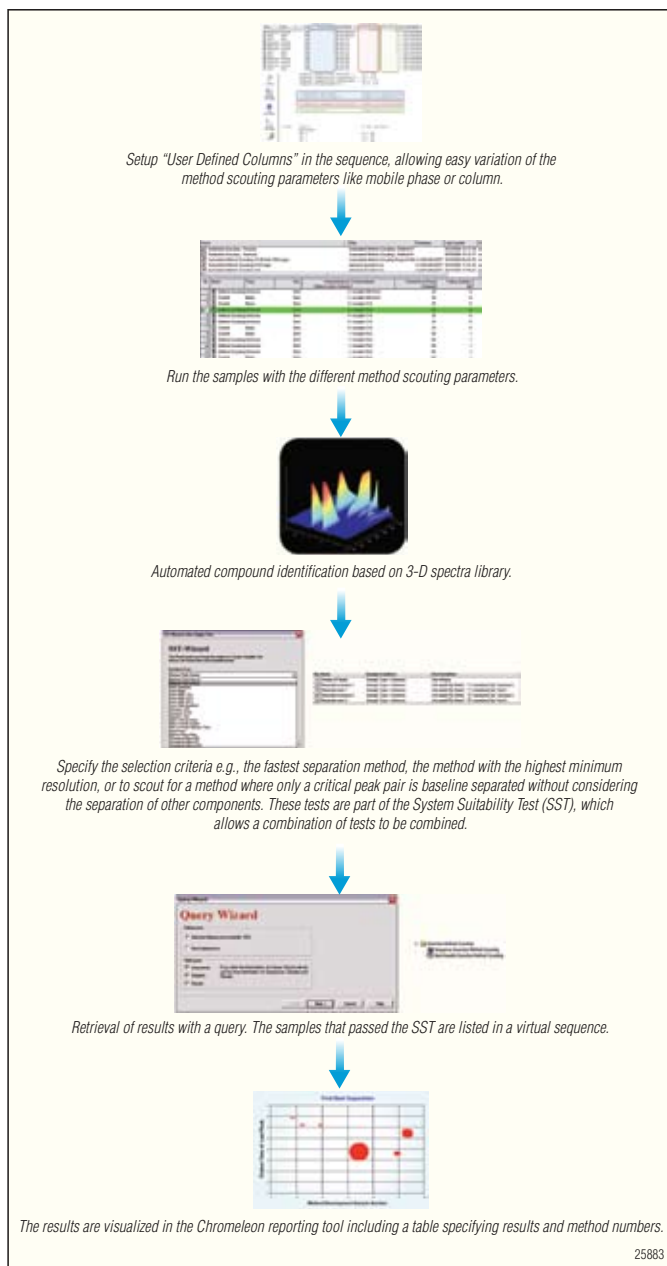


Figure 2. Method scouting workflow.

## METHOD TRANSFER

Columns with small particle sizes can be employed at higher mobile phase velocities while maintaining resolution compared to conventional columns, thereby reducing analysis time. The original LC method on a  $4.6 \times 150$  mm Acclaim® C18 column packed with  $3 \mu\text{m}$  particles was transferred to a  $2.1 \times 100$  mm column packed with Acclaim C18 2.2  $\mu\text{m}$  particles. To adjust the LC method parameters from the original column to the new UHPLC column, the gradient volume concept was followed using the formulas below.

$$t_{G2} = t_{G1} \cdot \frac{F_1}{F_2} \cdot \frac{V_{C2}}{V_{C1}}$$

- $t_{G1}$  Original gradient segment duration
- $t_{G2}$  New gradient segment duration
- $F_1$  Original flow rate
- $F_2$  New flow rate
- $V_{C1}$  Original column volume
- $V_{C2}$  New column volume

$$F_2 = F_1 \cdot \frac{(d_2)^2}{(d_1)^2} \cdot \frac{d_{p1}}{d_{p2}}$$

- $F_1$  Original flow rate
- $F_2$  New flow rate
- $d_1$  Original column diameter
- $d_2$  New column diameter
- $d_{p1}$  Original particle size
- $d_{p2}$  New particle size

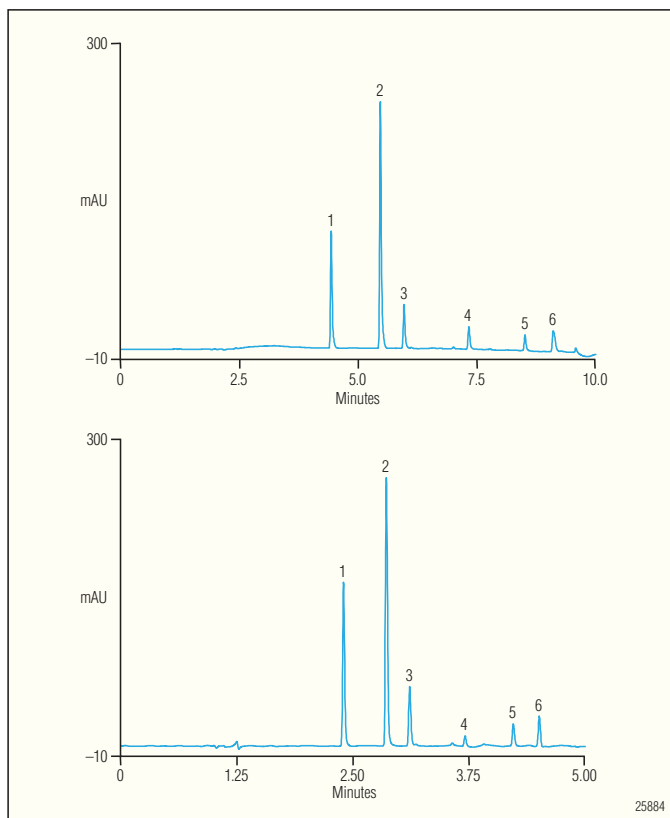


Figure 3. Method transfer from a conventional column ( $3 \mu\text{m}$ ,  $4.6 \times 150$  mm, flow rate  $1.0$  mL/min, gradient  $8$  min) and system to an RSLC column ( $2.2 \mu\text{m}$ ,  $2.1 \times 100$  mm, flow rate  $0.275$  mL/min, gradient  $4$  min) and system.

**Table 2. Results from the Method Transfer from a Conventional Column and System to an RSLC Column and System**

| Peak # | Component       | Resolution         |                    | Peak Width Half Height (s) |                    |
|--------|-----------------|--------------------|--------------------|----------------------------|--------------------|
|        |                 | 4.6 mm I.D. 150 mm | 2.1 mm I.D. 100 mm | 4.6 mm I.D. 150 mm         | 2.1 mm I.D. 100 mm |
| 1      | Amiloride       | 17.4               | 12.0               | 2.06                       | 1.36               |
| 2      | Ethacrynic acid | 7.9                | 5.9                | 2.16                       | 1.41               |
| 3      | Furosemide      | 20.6               | 14.3               | 2.27                       | 1.47               |
| 4      | Triamterene     | 17.2               | 12.0               | 2.41                       | 1.50               |
| 5      | Bumetanide      | 6.6                | 5.9                | 2.46                       | 1.57               |
| 6      | Chlortalidone   | –                  | –                  | 3.97                       | 1.85               |

Besides the advantages of a reduction of the analysis time and an increase in sensitivity, the use of UHPLC columns with a smaller I.D. and smaller particles also reduces the solvent consumption. In Table 3, example data is listed for the method scouting study whereby the speed was further increased by doubling the linear flow rate and adjusting the gradient time.

| Table 3. Comparison for Solvent Consumption and Analysis Time between Conventional and UHPLC Method Scouting |                   |                                       |   |
|--|-------------------|---------------------------------------|---|
|  | Conventional HPLC | UHPLC with the Same Number of Methods | UHPLC with More Method Variation in the Same Time |
| Runtime for one condition min  | 12                | 4.5                                   | 4.5   |
| Number of runs per conditions (blank, repro)   | 4                 | 4                                     | 4   |
| Number of conditions   | 40                | 40                                    | 106   |
| Total number of runs   | 160               | 160                                   | 424   |
| Total time (h)   | 32                | 12                                    | 32  |
| Solvent consumption L (flow rate, mL/min)  | 1.9 (1.0)         | 0.4 (0.55)                            | 1.1 (0.55)  |

The time for a method scouting study is reduced from 32 h to 12 h, a total reduction of 20 h or 62.5%. In addition, the solvent consumption was reduced from 1.9 L to 0.4 L, a reduction of solvent of 1.5 L or 78.9%.

Changing the experimental approach from a small number of initial experiments towards a more comprehensive screening with more possible combinations of column / eluent / temperature in the same time frame is also possible with UHPLC. This provides a significantly broader set of results, more information on the influence of the parameters within the design space, and thus more confidence in the robustness of the new method. The number of method parameters can be increased from 40 different conditions to 106 different conditions in the same time frame with UHPLC method scouting. This is a productivity increase of 265%.

## SELECTIVITY TUNING WITH pH

Figure 4 shows the effect of pH on the selectivity for an Acclaim C18 column. Depending on the pKa of the sample analyte and pH of the mobile phase the retention of the analyte can be tuned to optimize the separation.

Ethacrynic acid (component 2, pKa = 3.5) contains a carboxylic acid group and will be negatively charged at high pH. Changing the pH from below the pKa to above the pKa (from pH 2.5 to pH 3.6) influences the retention time of ethacrynic acid, while a further increase in pH from pH 3.6 to pH 4.8 shows no difference in retention time for ethacrynic acid.

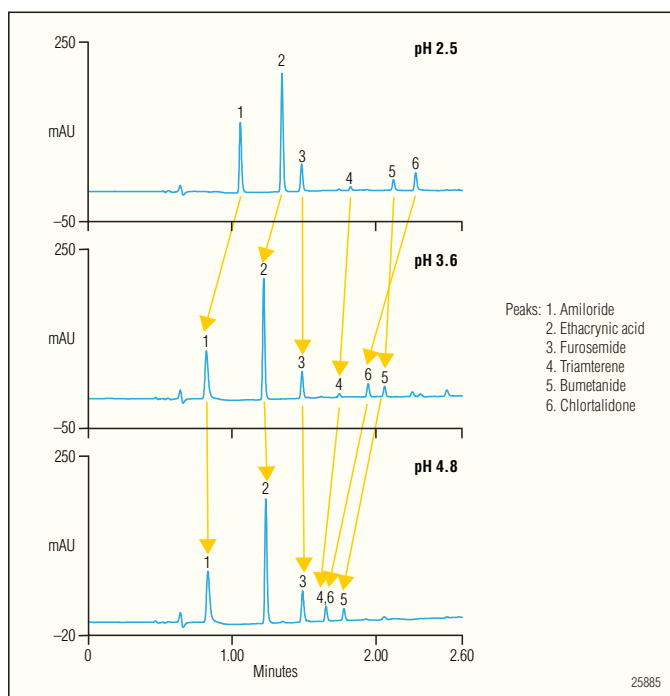


Figure 4. Influence of pH on diuretics separation on Acclaim C18 with acetonitrile as organic modifier.

## INFLUENCE OF ORGANIC MODIFIER

The most common organic modifiers methanol and acetonitrile can be used to influence the selectivity. With methanol, a protic solvent, the retention times of most components will increase as it is a weaker elution solvent in equivalent concentration compared to acetonitrile, an aprotic solvent. The influence of the weaker elution solvent methanol is demonstrated in Figure 5, whereby all diuretic compounds are eluting later compared to the separation with acetonitrile as organic modifier. In addition, a shift in elution order can be observed between ethacrynic acid and furosemide.

## INFLUENCE OF STATIONARY PHASE

The Acclaim PA2 stationary phase features an amide-embedded chemistry with excellent hydrolytic stability from pH 1.5 up to pH 10 and provides selectivity complementary to conventional Acclaim C18 stationary phases. The influence of the stationary phases Acclaim C18 and Acclaim PA2 on the selectivity of the diuretics compounds was investigated as well. A comparison of the separations on these stationary phases can be seen in Figure 6.

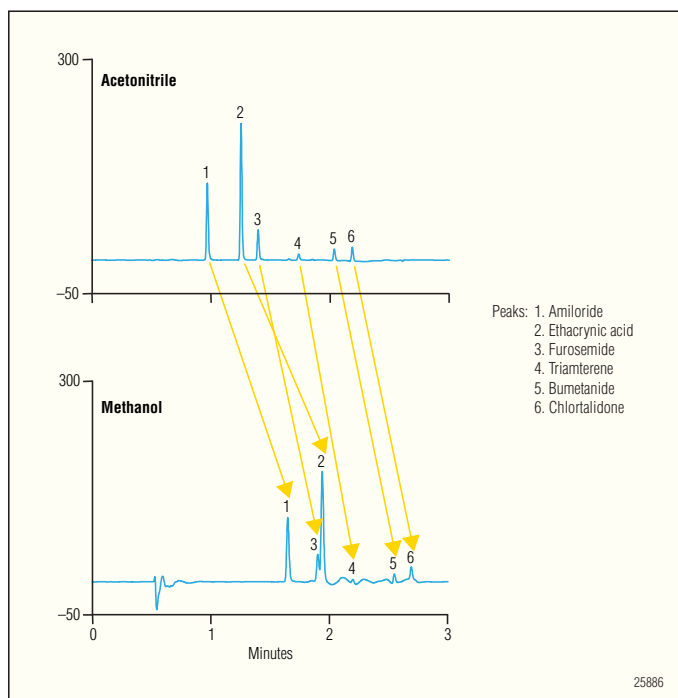


Figure 5. Influence of the organic modifier on Acclaim C18 with pH 2.5.

## METHOD SELECTION

The method selection in this example is based on the fastest separation whereby all six components are separated. The run time and resolution map for the samples that meet the SST criteria are shown in Figure 6. The shorter the elution time of the last peak, the lower on the Y-axis the bubble is. The diameter of the bubble corresponds to the resolution. The separation on the C18 column, employing acetonitrile as modifier and a acetate buffer pH 2.5 yielded the best method.

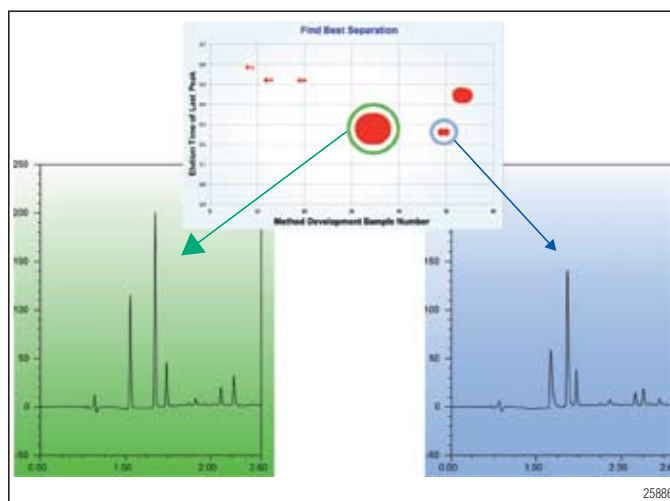


Figure 6. Reporting of the best separations based on SST criteria with the fastest conditions and the conditions for the highest minimum resolution. Left: Acclaim C18 with acetonitrile as organic modifier and 50 mM ammonium acetate pH 2.5. Right: Acclaim PA2 with acetonitrile as organic modifier and 50 mM ammonium acetate pH 2.5.

## CONCLUSIONS

- The UltiMate® 3000 RSLC method scouting system, Acclaim RSLC columns, and Chromeleon Chromatography Data System software offer a flexible, automated, and integrated solution for UHPLC method development.
- UHPLC technology not only offers a reduction in method development time of more than 60%, it also can help to reduce solvent consumption (acetonitrile) by almost 80% compared to conventional method scouting. Alternative to the time saving, the number of investigated method parameters can be increased up to 265%.
- The best result for the separation of diuretics is obtained on the Acclaim C18 RSLC column with acetonitrile as organic modifier and 50 mM ammonium acetate pH 2.5. It combines high resolution with short analysis time.

Acclaim, Chromeleon, and UltiMate are registered trademarks of Dionex Corporation.

Passion. Power. Productivity.



### Dionex Corporation

1228 Titan Way  
P.O. Box 3603  
Sunnyvale, CA  
94088-3603  
(408) 737-0700

### North America

U.S./Canada (847) 295-7500

### South America

Brazil (55) 11 3731 5140

### Europe

Austria (43) 1 616 51 25 Benelux (31) 20 683 9768; (32) 3 353 4294  
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0  
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380  
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

### Asia Pacific

Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735  
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190  
Taiwan (886) 2 8751 6655

[www.dionex.com](http://www.dionex.com)



LPN 2289-01 06/09  
©2009 Dionex Corporation