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TECHNICAL NOTE

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A UHPLC method development system for efficient scouting of chromatographic elution parameters

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Key words

Column switching, eluent screening, column screening, solvent selection valve, optimization, method development

Goal

Show the straightforward method development capabilities of the Thermo Scientific™ Vanquish™ UHPLC platform in combination with Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) software

Introduction

The speed of analysis in HPLC has dramatically improved over the last decade due to the development of columns with sub-2 µm particles and the respective UHPLC instrumentation. (U)HPLC method development is, however, still a bottleneck in laboratory workflows and can take from weeks to months, especially if extensive column and eluent scouting is required to complete the development. This often limits the laboratory productivity and increases operational costs. A high degree of automation in column and eluent scouting is required to fully exploit the speed potential of UHPLC.

In reversed-phase HPLC, several parameters are subject to optimization, such as mobile phase pH, column chemistry, and separation temperature, in addition to the gradient profile. The pH value, for instance, is of high importance when analyzing ionizable compounds. In general, the eluent pH should be adjusted according to the pKa value of the compound, which can be a challenge for mixtures of acidic and basic compounds and requires a screening of various pH values. For polar compounds the selectivity might vary between different C18 chemistries, depending on endcapping and additional polar selectivities, and it can be helpful to investigate the separation on a range of C18 columns. Finally, the separation temperature can change the selectivity dramatically and is worth exploring during method development workflows. In order to avoid thermal

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mismatch between column temperature and incoming eluent, best chromatographic practice should include the use of an eluent pre-heater.[1] If all these parameters are used for the method development, a large number of chromatographic runs is required. An instrument and software package enabling an automated sequence and instrument method setup facilitates the task of method development significantly.

In this technical note we present an Automated Method Scouting solution for all Thermo Scientific Vanquish UHPLC systems. This solution combines the leading Vanquish technology with the intelligence of Chromeleon CDS software. It provides quaternary or binary (two out of six solvents) solvent blending and column scouting capabilities of up to six columns using 6-position 7-port switching valves. Other features include advanced thermostatting scouting options, and extensive solvent screening possibilities using a low-pressure solvent selection valve of a dedicated extension kit. With this extension kit, for example, up to 12 aqueous buffers can be screened in an automated manner (Figure 1). These large data sets are automatically evaluated by Chromeleon CDS, and methods providing the best results according to pre-defined criteria, for example best resolution between critical peak pairs, are reported.

This development approach was used for the separation of two isomeric forms of budesonide, a steroid used in the long-term treatment of asthma. Four different columns, spanning a broad selectivity range, were scouted with six different aqueous buffers from pH 3 to pH 8. The method results were evaluated by the best resolution between the critical peak pair.

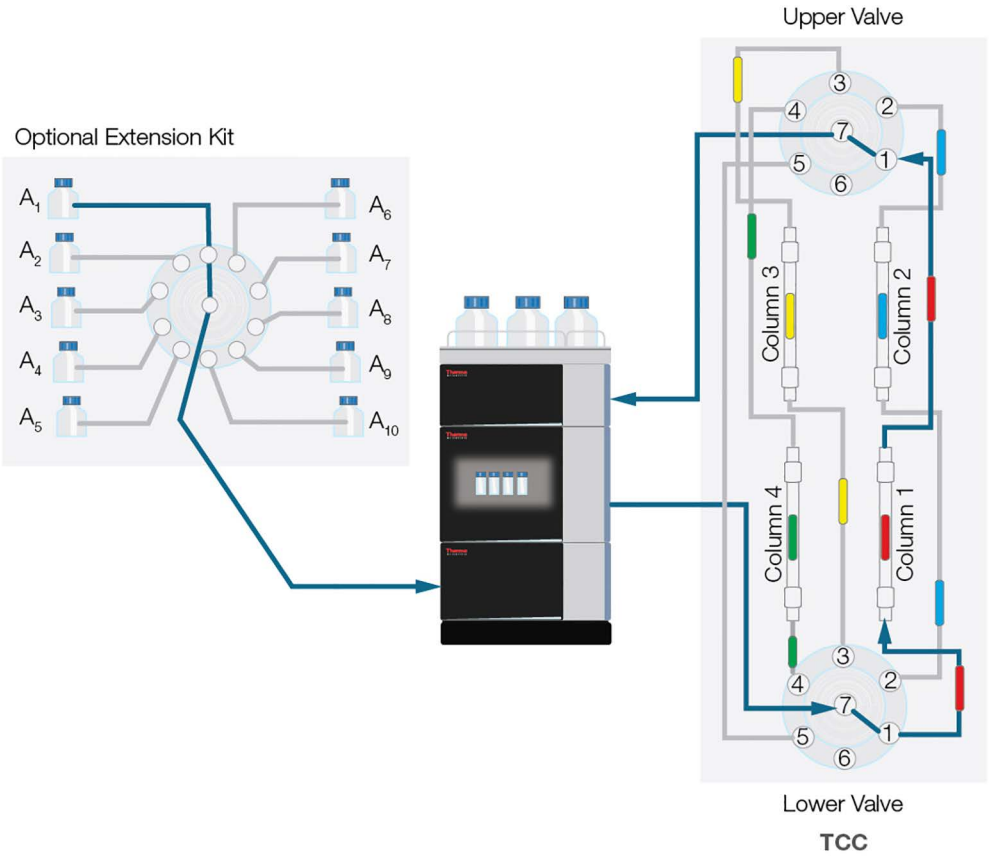

Figure 1. Vanquish flow scheme.

Experimental Conditions	
Columns	Thermo Scientific™ Hypersil GOLD™ VANQUISH™ (2.1 × 100 mm, 1.9 µm), P/N 25002-102130-V Thermo Scientific™ Hypersil GOLD™ VANQUISH™ aQ (2.1 × 100 mm, 1.9 µm), P/N 25302-102130-V Thermo Scientific™ Accucore™ Vanquish™ C18+, (2.1 × 100 mm, 1.9 µm), P/N 27101-102130 Thermo Scientific™ Hypersil GOLD™ C4 (2.1 × 100 mm, 1.9 µm), P/N 25502-102130
Mobile Phase	A1: 20 mM Ammonium formate in water, pH 3, (P/N Ammonium formate A114-50) A2: 20 mM Ammonium formate in water, pH 4 A3: 20 mM Ammonium acetate in water, pH 5, (P/N Ammonium acetate A115-50) A4: 20 mM Ammonium acetate in water, pH 5.6 A5: 20 mM Sodium phosphate in water, pH 7, (P/N NaH_2PO_4 BP329-500, P/N Na_2HPO_4 BP332-500) A6: 20 mM Sodium phosphate in water, pH 8 B: Acetonitrile (v/v), P/N TS-51101
Gradient	0–6.5 min: 5–80% B, 6.5–7.5 min: 80% B 7.5–7.6 min: 80–5% B 7.6–10.5 min: 5% B
Flow Rate	0.5 mL/min
Temperature	30 °C Still Air
Injection Volume	1 µL
Detection	254 nm
Data Collection Rate	20 Hz
Response Time	0.2 s

Experimental

Equipment

Thermo Scientific™ Vanquish™ Horizon UHPLC system consisting of:

- System Base (P/N VH-S01-A)
- Binary Pump H (P/N VH-A10-A)
- Split Sampler HT (P/N VH-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Diode Array Detector HL (P/N VH-D10-A)
- Flow Cell, 10 mm Thermo Scientific™ LightPipe™ (P/N 6083.0100)
- Viper Automated Method Scouting Kit, Vanquish Systems (P/N 6036.2807)
- Column Switching Valve 6-pos 7-port 150 MPa, (P/N 6036.1570)
- Extension Kit for Automated Method Scouting, Vanquish Systems (P/N 6036.0100)

Chromeleon 7.2 CDS software was used.

Sample preparation

Commercially available budesonide powder (Sigma-Aldrich®) was dissolved in acetonitrile (P/N A955-212) to a concentration of 1 mg/mL. This stock solution was diluted 1:1 with water to give a working sample of a concentration of 0.5 mg/mL.

Method scouting workflow

eWorkflow download and sequence setup

For a fast and efficient sequence setup and experimental design, an eWorkflow can be downloaded from the Thermo Scientific™ AppsLab Library of Analytical Applications (name: *Method Scouting on Vanquish Horizon*).[2] After launching the eWorkflow, a sequence with instrument methods for scouting and column switching will appear. This sequence also includes Custom Variables. Custom Variables are associated with a sequence and can be used to set parameters for instrument methods in an elegant way. In this eWorkflow, the active column position, aqueous buffer, flow rate, column temperature, and solvent selector can be set with customer variables (Figure 2). All important parameters of a method development process can be changed with one universal instrument method, making the sequence setup very easy. Details on the use of Custom Variables in Method Scouting experiments were also published in a previous Technical Note.[3]

#	UV_VIS_1	Name	Type	Level	*PercentageASele	*PercentageBSele	*ColumnSele	*ColumnNam	*SSV_Pos	*BufferName	*FlowRate	*ColumnOvenTem
1	None	Column Switching	Blank		%A1	%B1	1	<enter nam..	1	<enter name of...	0.600	30
2	None	Equilibration	Unknown		%A1	%B1			1	<enter name of...	0.600	30
3	None	Blank injection	Unknown		%A1	%B1			1	<enter name of...	0.600	30
4	None	Standard, injectio..	Calibrat..		%A1	%B1			1	<enter name of...	0.600	30
5	None	Standard, injectio..	Calibrat..		%A1	%B1			1	<enter name of...	0.600	30
6	None	Equilibration	Blank		%A1	%B1			2	<enter name of...	0.600	30
7	None	Blank injection	Unknown		%A1	%B1			2	<enter name of...	0.600	30
8	None	Standard, injection...	Calibrat..		%A1	%B1			2	<enter name of...	0.600	30
9	None	Standard, injection...	Calibrat..		%A1	%B1			2	<enter name of...	0.600	30
10	None	Equilibration	Blank		%A1	%B1			3	<enter name of...	0.600	30
11	None	Blank injection	Unknown		%A1	%B1			3	<enter name of...	0.600	30
12	None	Standard, injection...	Calibrat..		%A1	%B1			3	<enter name of...	0.600	30
13	None	Standard, injection...	Calibrat..		%A1	%B1			3	<enter name of...	0.600	30
14	None	Equilibration	Blank		%A1	%B1			4	<enter name of...	0.600	30
15	None	Blank injection	Unknown		%A1	%B1			4	<enter name of...	0.600	30
16	None	Standard, injectio..	Calibrat..		%A1	%B1			4	<enter name of...	0.600	30
17	None	Standard, injectio..	Calibrat..		%A1	%B1			4	<enter name of...	0.600	30

Figure 2. Sequence setup as created by the Automated Method Scouting for Vanquish Horizon eWorkflow. The red boxes highlight the used Custom Variable (please see Reference 3 for a detailed explanation).

For the development of a separation of two epimeric forms of budesonide, four different reversed-phase columns were utilized, one of which was the Accucore Vanquish C18+ column, employing 1.5 µm solid core particles. In addition, the effect of six aqueous buffers ranging from pH=3 to pH=8 was investigated. The separation temperature was kept at 30 °C including passive pre-heaters for all four columns. In general, up to three different organic solvents can be used in the standard configuration of the Vanquish Horizon system, which allows additional screening of different organic eluent types like methanol or solvents blended with modifiers. However, in this example, only acetonitrile was used as organic eluent.

Efficient data evaluation

Chromeleon 7 CDS features the *Intelligent Run Control* to check which injections pass certain criteria. Common test cases are the number of detected peaks, minimal resolution between critical peak pairs, or the peak asymmetry. The most promising conditions are easily found with the powerful Query tool of Chromeleon CDS, which is also part of the downloadable eWorkflow. This tool condenses the most promising injections into a virtual sequence allowing comfortable access to the raw data of the best results. Details on the use of the Intelligent Run Control and the Query functionality were published previously[3] and will not be discussed in more detail here. Figure 3 gives the retention time of all detected critical peak pairs, while the resolution is represented by the bubble size, with larger bubbles meaning better resolution. In total, 24 different chromatographic conditions were tested in less than 20 hours and needed no manual interaction. The data processing was facilitated by Chromeleon 7.2 CDS software, which shortened the time effort for the data analysis to less than 1 hour.

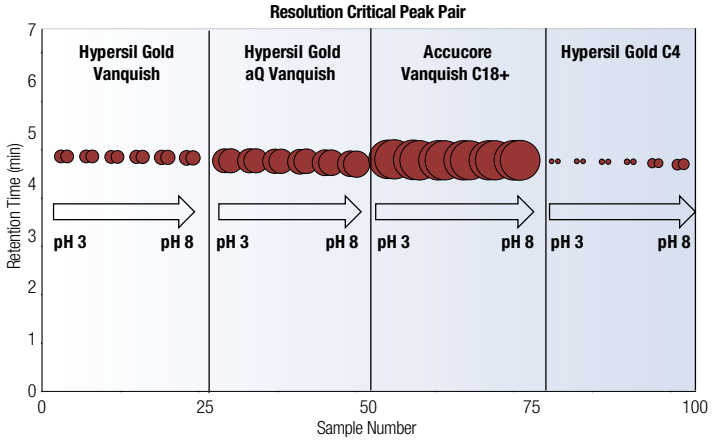

Figure 3. Retention time and resolution of the method scouting of budesonide epimers using the Vanquish Horizon system. The resolution correlates with the bubble size. Two injections were performed for each of the six buffers, resulting in 12 injections on each column. The bubble chart is part of the default report template within the eWorkflow.

As Figure 3 illustrates, the retention time is very similar among all columns and is not affected by the pH of the eluent. The Accucore Vanquish C18+ column with the 1.5 µm solid core particles clearly gave the best resolution and was the only column that delivered a resolution of more than 1.5 as required by the USP method[4] (Figure 4). The Accucore Vanquish C18+ column not only offers an improved separation but also improves the signal-to-noise ratio by 20 percent compared to the second-best resolution method due to the decreased peak width achieved with that column.

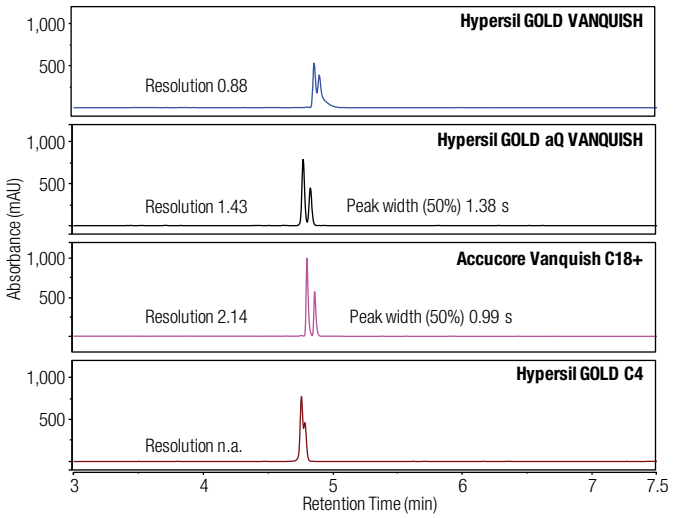

Figure 4. Comparison of the separation of budesonide epimers with four different columns during the method development.

Clearly this method could be further optimized especially regarding the mobile phase gradient and separation temperature to increase the speed of the analysis. This intuitive and fast method development approach would recommend performing the subsequent optimization with the Accucore Vanquish C18+ column. This optimization was done for the separation temperature. For an improved separation of the two epimers, the effect of a sub-ambient separation temperature was investigated using the passive pre-heating capability of the Vanquish UHPLC system.

Figure 5 illustrates the effect of the separation temperature on the resolution of the two epimers of budesonide. While the resolution is 2.04 and lower for a separation temperature of 40 °C and above, the resolution can be increased when working at sub-ambient temperatures. In this case the difference in resolution between 10 °C and 20 °C is marginal. Still, the highest resolution of 2.2 was obtained at a temperature of 10 °C. With the Vanquish systems these temperature settings can be scouted while simultaneously using a passive eluent pre-heater for all four columns in order to avoid any thermal mismatch. This can be very helpful for separations of labile substances or enantiomers.

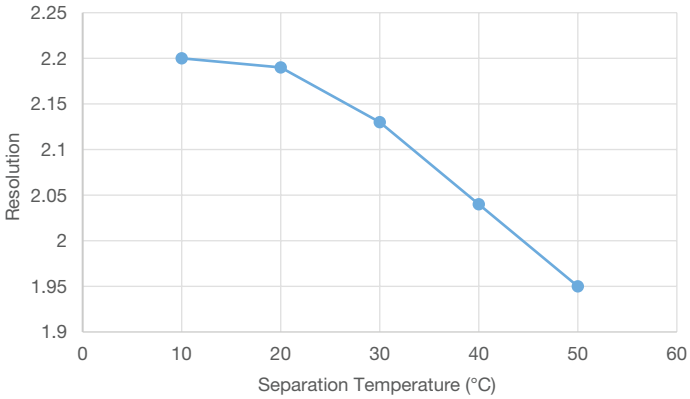

Figure 5. Effect of separation temperature on resolution for the separation of budesonide epimers.

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Conclusion

The Automated Method Scouting solution for the Vanquish UHPLC platform, in combination with Chromeleon 7.2 CDS, enables chromatographers to develop (U)HPLC methods easily and efficiently because of the automation. The instrument enables the screening of up to six columns and an extensive range of solvents. Chromeleon CDS facilitates this workflow by automated sequence setup using pre-defined eWorkflows and Custom Variables, post-processing tools, and graphical user interfaces for method evaluation. This way the complete method development process can be accelerated significantly.

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