Accelerating method development with Thermo Scientific Vanquish HPLC and UHPLC Method Development Systems

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Introduction

The demand for faster, more sensitive, and more robust liquid chromatography methods capable of meeting stringent regulatory criteria is a major challenge faced by many industries. Moreover, it is often crucial that all compounds and impurities within a sample are separated and detected reliably to ensure product quality and safety. Traditional chromatographic method development is a labor-intensive process that requires the operator to optimize a broad range of separation parameters such as temperature and the gradient table. During the process, decisions must be made regarding the complex interactions between these separation parameters. As such, successful method development can take days, weeks, or months for even skilled chromatographers. Thermo Fisher Scientific offers comprehensive HPLC and UHPLC method development systems including flexible method scouting hardware as well as a suite of software tools that deliver rapid, automated method development and validation testing according to the quality by design (QbD) approach (Figure 1).

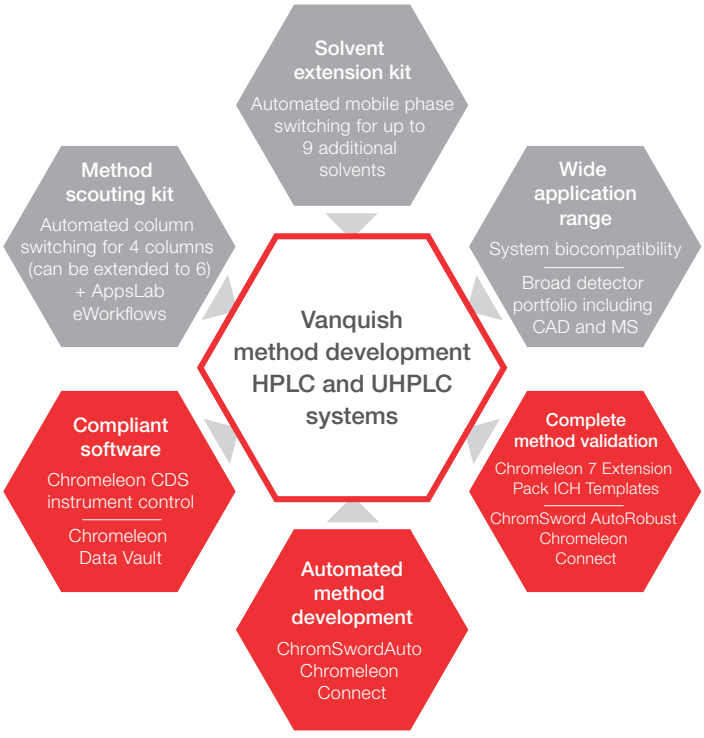

Figure 1. Hardware (gray) and software (red) tools for automated method development using Thermo Scientific™ Vanquish™ HPLC and UHPLC systems.

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Automated method scouting hardware

The Thermo Scientific Vanquish HPLC and UHPLC Method Development Systems offer multiple solutions for a wide range of applications with the Thermo Scientific™ Vanquish™ Core HPLC, Vanquish Flex UHPLC, and Vanquish Horizon UHPLC systems paired with diode array detection (DAD). For situations where even higher confidence in component confirmation or peak purity is required, Thermo Scientific™ ISQ™ EM and EC single quadrupole mass spectrometers can be used to ensure accurate MS-based peak tracking between scouting runs and reduce the risk of overlooking co-eluting peaks. Charged aerosol detection (CAD) ensures that you never miss a peak because all non- or semi-volatile compounds in a sample, even those without a chromophore, can be detected (Figure 2). This is particularly useful for challenging analyses including forced degradation studies.

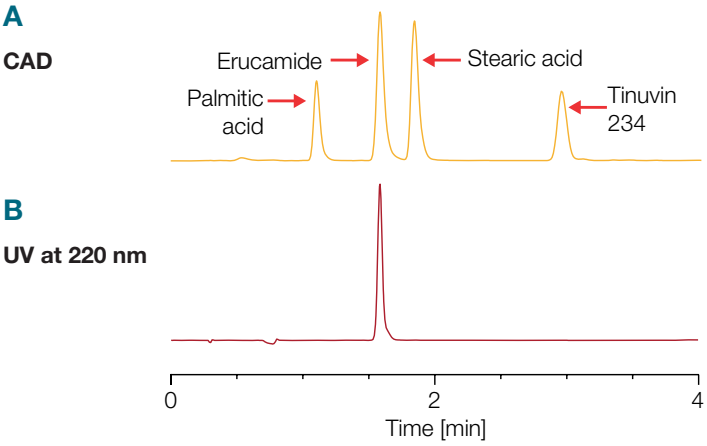


Figure 2. Comparison of compounds detected using either CAD (A) or UV detection at 220 nm (B). Only erucamide absorbs strongly with UV detection while all four analytes are detectable using CAD.

Two hardware kits are available for the Vanquish HPLC and UHPLC Method Development Systems. The Thermo Scientific™ Viper™ Method Scouting Kit includes all fluidic connections and capillaries required to scout 4 column chemistries, which can be used in conjunction with either one or two column compartments (depending on column length and required number of temperature zones) and one additional bypass line for efficient system rinsing. The solvent extension kit includes an external selection valve for automated scouting of up to 10 solvents per channel.

The Vanquish Column Compartment can host up to four 100 mm or two 250 mm columns in a single temperature zone. Additional column compartments provide multiple temperature zones and the ability to screen up to six columns. The Method Development System comes with the option of a Thermo Scientific™ Accucore™ column selectivity kit providing three different column chemistries for column scouting experiments. Figure 3 shows a Vanquish Method Development System with two column compartments and all kits installed.

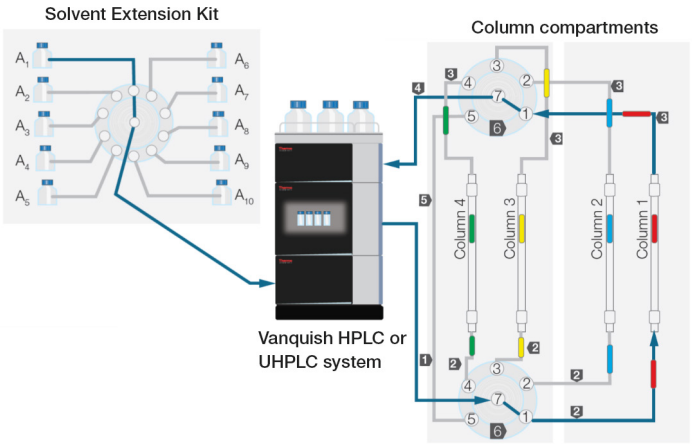


Figure 3. Vanquish Method Development System hardware including two column compartments, solvent extension kit, Viper method scouting kit, and two 7 port-6 position valves for automated scouting of up to 6 columns and 13 mobile phases. For 4 columns ≤10 cm, only one column compartment required.

In addition to the Method Scouting Kit, the Thermo Scientific™ AppsLab Library of Analytical Applications, a searchable online repository of thousands of applications with detailed method information and prepopulated eWorkflow™ procedures.[1] These procedures can be downloaded and directly executed in Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), eliminating unnecessary method development steps when a suitable method exists in the AppsLab library. In addition, a dedicated method scouting eWorkflow procedure can be found in AppsLab.

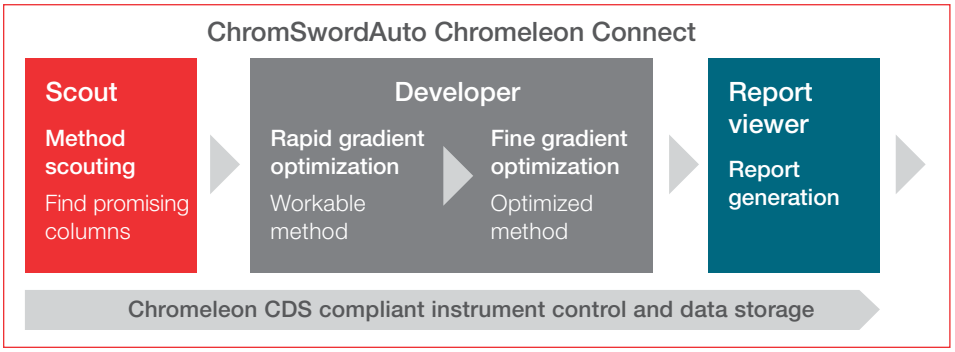


Figure 4. Method development workflow using ChromSwordAuto Chromeleon Connect

Automated method development software

The Vanquish HPLC and UHPLC Method Development Systems utilize ChromSwordAuto Chromeleon Connect software for fast and automated method development, safe data storage and compliant method validation. The ChromSwordAuto Chromeleon Connect software modules, which include Scout, Developer, and ReportViewer, have been integrated with Chromeleon CDS and generated data is safely stored in any Chromeleon Data Vault for regulatory compliance (Figure 4). From generating method scouting plans to fine optimization, ChromSwordAuto Chromeleon Connect enables users of any skill level to efficiently develop reliable (U)HPLC methods with minimal time and effort.

Previous methods and knowledge of analyte retention characteristics are unnecessary for column/mobile phase scouting and rapid gradient generation, leading to a workable method in as few as 3–5 runs. Quality by Design is supported through extensive documentation during all stages of method development.

- Scout generates column scouting sequences for screening mobile phases and column chemistries to streamline initial column selection.
- Developer employs artificial intelligence to provide real-time, automated decision making between injections, enabling rapid, fully unattended method development.
- ReportViewer facilitates simple navigation and comparison of chromatograms and spectra, including MS peak tracking.

For further method improvement, Developer can also perform fine gradient optimization to generate the best possible separation conditions through sample and impurity profiling. It not only ensures that resolution requirements are met, but also that separations are optimized for speed. In ReportViewer advanced plots can be generated to simultaneously analyze chromatographic parameters across multiple chromatograms to guide method development and enable users to quickly identify

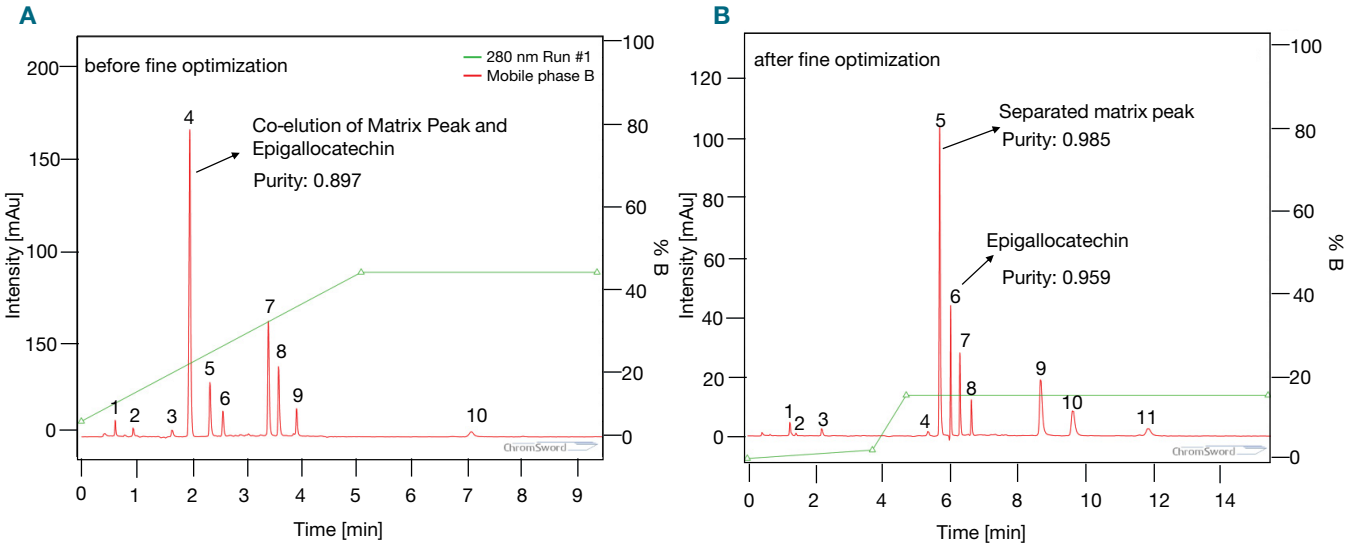


Figure 5. Comparison of chromatograms using ChromSwordAuto Chromeleon Connect Developer after rapid optimization (A) and after sample profiling (B) for separation of catechins and matrix components in green tea. Fine optimization yielded a baseline separation of all 11 peaks including caffeine and epigallocatechin, which co-eluted (peak 4) in method A.

key parameters without searching through individual chromatograms. Figure 5 shows chromatograms for the separation of catechins in green tea.[2] The first chromatogram was collected after Developer rapid gradient optimization (A), while the second was collected after sample profiling (B). Peak purity analysis using UV spectral data revealed that peak 4 in chromatogram A represented the co-elution of epigallocatechin (ECG) and a major matrix peak. After sample profiling, the matrix component (identified as caffeine) was successfully separated from EGC and all peaks were completely resolved. The entire method development process required 50.5 h of instrument time (24 h/day) and 24 h of analyst time (8 h/day).

Automated robustness testing and method validation software

Method robustness is crucial for generating reproducible results, especially when transferring methods to other instruments or labs. According to the Food and Drug Administration (FDA) Guidance for Industry, method robustness should be evaluated during early stages of method development because of its importance for further method validation.

ChromSword AutoRobust Chromeleon Connect automatically evaluates method robustness and system stability using a straightforward experimental design employing a single or multivariate approach, expediting method validation and future method transfer processes. A design space describing the impact of method parameters on resolution of target compounds can easily be developed using AutoRobust (Figure 6). All data is stored in the Chromeleon Data Vault for security and easy exchange between groups within an organization. A final robust method can be further validated in accordance with corresponding guidelines.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) sets out Q2 (R1) guidelines for demonstrating the validity of analytical methods for their intended uses. The Chromeleon 7 Extension Pack ICH Templates deliver a complete set of eWorkflow procedures that have been created in accordance with these guidelines and include a series of method validation tests to ensure characteristics, such as accuracy, linearity, precision, etc. are considered. These templates can be easily customized with minimal user effort to meet any method validation criteria.[3]

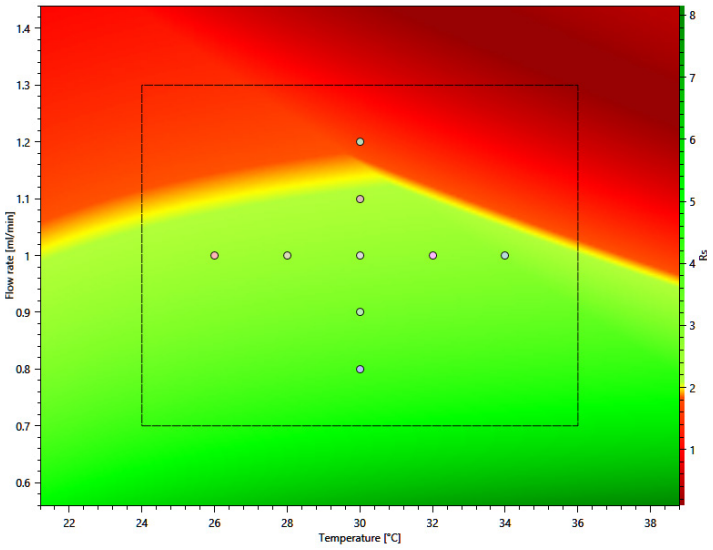
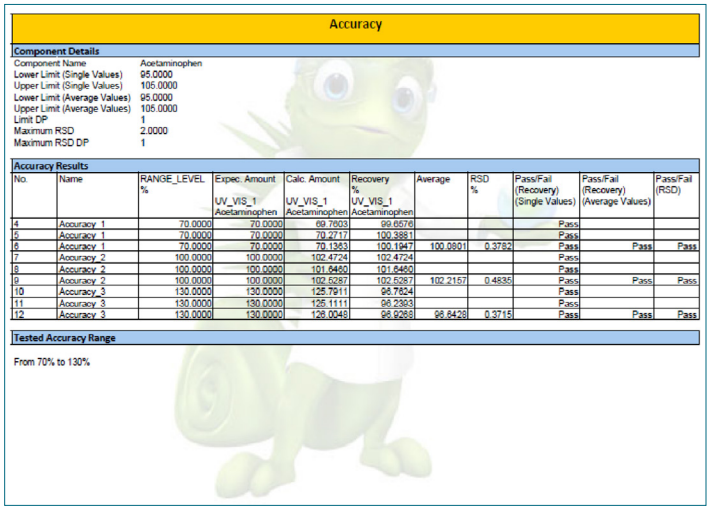


Figure 6. ReportViewer plot evaluating the effect of flow rate and temperature on the resolution of a critical pair.

All method validation results are consolidated in Chromeleon CDS for final reporting (Figure 7), providing an automated and streamlined approach to method validation while ensuring full compliance throughout the validation process.



Accuracy

Component Details

Component Name	Acetaminophen
Lower Limit (Single Values)	95.0000
Upper Limit (Single Values)	105.0000
Lower Limit (Average Values)	95.0000
Upper Limit (Average Values)	105.0000
Limit DP	1
Maximum RSD	2.0000
Maximum RSD DP	1

Accuracy Results

No.	Name	RANGE_LEVEL %	Expec. Amount UV_VIS_1 Acetaminophen	Calc. Amount UV_VIS_1 Acetaminophen	Recovery % UV_VIS_1 Acetaminophen	Average	RSD %	Pass/Fail (Recovery) (Single Values)	Pass/Fail (Recovery) (Average Values)	Pass/Fail (RSD)
4	Accuracy_1	70.0000	70.0000	69.7603	99.6576			Pass		
5	Accuracy_1	70.0000	70.0000	70.2717	100.3881			Pass		
6	Accuracy_1	70.0000	70.0000	70.1353	100.1947	100.0801	0.3782	Pass	Pass	Pass
7	Accuracy_2	100.0000	100.0000	102.4724	102.4724			Pass		
8	Accuracy_2	100.0000	100.0000	101.5460	101.5460			Pass		
9	Accuracy_2	100.0000	100.0000	102.5287	102.5287	102.2157	0.4835	Pass	Pass	Pass
10	Accuracy_3	130.0000	130.0000	125.7911	96.7624			Pass		
11	Accuracy_3	130.0000	130.0000	125.1111	96.2393			Pass		
12	Accuracy_3	130.0000	130.0000	126.0048	96.9268	96.6428	0.3715	Pass	Pass	Pass

Tested Accuracy Range

From 70% to 130%

Figure 7. ICH method accuracy report generated in Chromeleon CDS. Accuracy values as well as pass/fail criteria are included in report.

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Table 1. Method development approaches.

Requirement	Manual	Method development hardware kits	Vanquish Method Development System + ChromSwordAuto Chromeleon Connect
Analyst knowledge	Expert	Expert	Novice
Manual instrument operation	High	Medium	Low
Instrument utilization	Low	Medium	High
Total development time	Weeks–months	Weeks–months	Days
Assisted method validation	No	No	Yes

Conclusion

The Vanquish HPLC and UHPLC Method Development Systems are ideal for fast, unattended method development with a full suite of hardware and software tools (summarized in Table 1). Method scouting and solvent extension kits extend the capacity to scout multiple columns and mobile phases without manual solvent purging and fluidic changes. A wide range of applications can be performed using conventional detection techniques, such as DAD, with more accurate peak tracking made possible by MS support and universal detection provided by CAD. Using the AppsLab Library can save time and resource by presenting a strong starting point for method development with downloadable and executable eWorkflow procedures.

The integration of Chromeleon CDS with ChromSwordAuto means methods can be developed and evaluated quickly, while full compliance coverage is delivered throughout the validation process. In addition, the Chromeleon Data Vault ensures secure, centralized data storage and the Chromeleon Extension Pack ICH Templates with the ChromSword AutoRobust Chromeleon Connect delivers fast, compliant method validation according the QbD approach.

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