HPLC and UHPLC

Method Modernization and Method Development

e-Book

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Introduction

The demand for labs to develop faster, more sensitive, and robust liquid chromatography methods capable of meeting stringent regulatory criteria is a growing challenge faced by many industries. This guide is an all-in-one technical resource with solutions to help you increase throughput by (i) optimizing existing methods and (ii) developing new methods efficiently.

Method modernization

In current analytical laboratories, vast numbers of methods are often used to analyze hundreds of samples. The problem is most labs are limited by technology and bench space, so finding ways to increase your throughput and accommodate greater demand is essential. Adopting UHPLC instruments is one solution modern labs use to scale productivity.

Method development

HPLC method development is a labor-intensive process that requires you to optimize a broad range of separation parameters such as temperature and the gradient table. Developing LC methods that meet ICH requirements and allow sufficient sample throughput is demanding, even for your experienced scientists.

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 skilled chromatographers.

Thermo Fisher Scientific offers comprehensive HPLC and UHPLC method development solutions to simplify your journey. Flexible method scouting hardware plus a suite of software tools deliver rapid, automated method development and validation testing according to the quality by design (QbD) approach.

Both the ChromSword and S-Matrix™ Fusion™ software platforms feature a complete analytical quality by design (AQbD) solution to ease your method development, validation, and transfer that includes full closed-loop experiment automation support for Thermo Scientific™ Chromeleon™ CDS (Chromatography Data System) and Thermo Scientific™ Vanquish™ LC systems.

Contents—Method modernization

HOW TO GAIN PRODUCTIVITY BY MODERINZING YOUR METHODS

Modern analytical technologies can help your lab efficiently scale productivity, with confidence. The following application notes show you how adopting new methods leads to better performance, higher sample throughput, and more sensitive detection.

CASE STUDY 002242

Maximizing laboratory productivity: How modernizing an instrument park leads to significant reduction in system suitability failures

Sebastien Morin, Jon Bardsley, *Thermo Fisher Scientific*

Keywords

HPLC, high performance liquid chromatography, UHPLC, ICH 014, Vanquish Flex UHPLC, cGMP, COMO, system suitability failures

Introduction

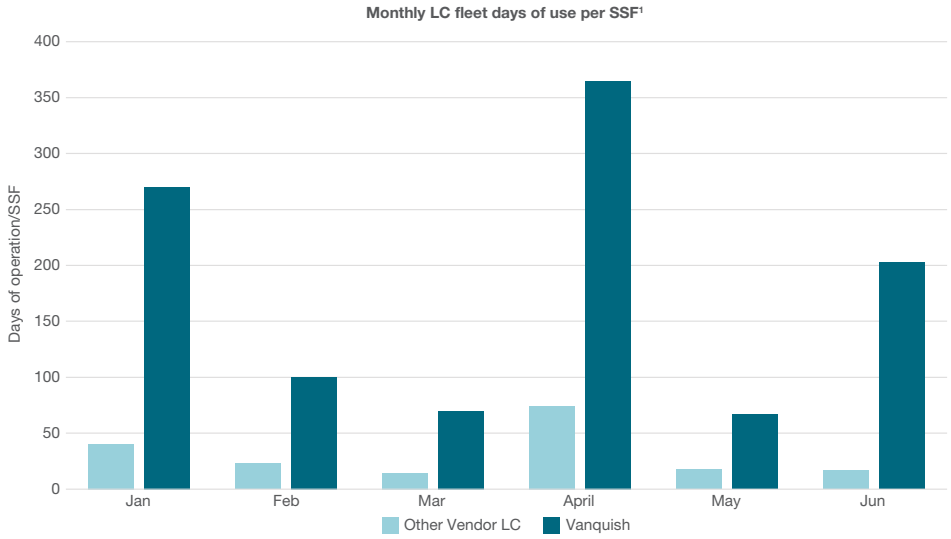
To meet the growing demands for a variety of projects Thermo Fisher Scientific Pharma Services Group (PSG, also known as Patheon) recently embarked on a technology refresh program to replace aging analytical equipment with a more modern liquid chromatography platform.

The new platform must be compatible with their existing IT infrastructure (Waters™ Empower™ 3 Chromatography Data System), suitable for the analysis of both chemical and biologic molecules, and compatible with legacy HPLC methods as well as modern UHPLC assays. Meeting all requirements, the Thermo Scientific™ Vanquish™ UHPLC platform was adopted across the PSG network.

The transition to this platform presented some hesitancy for analysts familiar with other technologies as well as clients who had developed their methods on other vendor LC systems. However, greater flexibility, ease-of-use, enhanced robustness, and serviceability leading to improved day-to-day operations, significantly outweighed these challenges

Goal

To demonstrate the capabilities of the Thermo Scientific™ Vanquish™ platform to reduce system suitability failures.



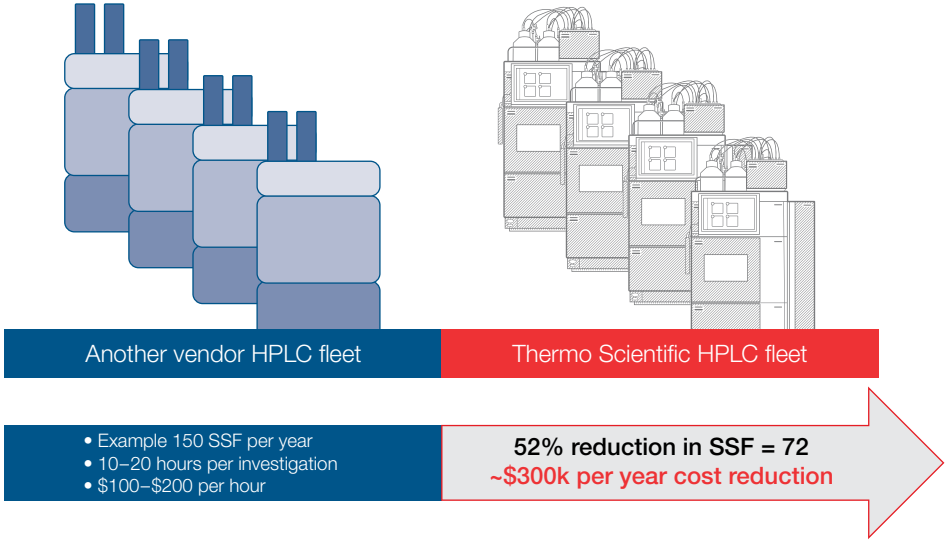
Daily usage of Vanquish system and combined other vendor LC systems broken down by month, per SSF, highlighting the Vanquish system runs for much longer without incident

Vanquish UHPLC systems significantly reduce instrument-related system suitability failures

The Patheon Toronto facility has modernized its UHPLC instrument park by converting 48% of the instrument fleet primarily Thermo Scientific™ Vanquish™ Flex UHPLC systems. Replacing aging liquid chromatography systems has led to obvious benefits like reducing instrument downtime and providing state of the art technology to build newer/faster methods. It also led to a significant reduction in system suitability failures (SSF), as the Vanquish footprint was increased from 18% to 47% of the LC fleet, system suitability failures were decreased by 34% overall.

When comparing SSF on Vanquish UHPLC systems vs. other LC systems we see that, on average, Vanquish UHPLCs account for ~24% of LC instrument-related SSF. It is important to stress that even though the Toronto LC fleet comprises 48% Vanquish systems, these systems represent on average 63% of the total hourly LC usage monthly. Therefore, even though the Vanquish systems are used much more than other LCs, they have significantly fewer instrument-related failures. Vanquish LC instrument uptime always exceeds that of competitors systems averaging at 6 times more usage.

At the current Vanquish system footprint, we can estimate a 52% reduction of instrument-related system suitability failures, which can be directly tied to significant annual savings associated with unproductive work. For example, if a laboratory had ~150 instrument-related system failures and replaced their aging instrumentation to meet the same productivity gain as in our case study, they would now have ~72 SSF/year. By industry standards, an investigation can take up to 20 hours, and each hour of investigation could represent $200 of cost/loss of revenue, this would lead to $300K annual savings.



Example of how an HPLC fleet with 150 SSF per year would have significant cost savings by replacing with Vanquish systems

Conclusion

- Modernizing an instrument park can be seen as a significant investment. However, rather than just replacing with like-for-like new systems, choosing to replace with the Vanquish UHPLC platforms can have significant advantages, especially for improving daily operations by reducing system suitability failures. This far outweighs the drawbacks associated with fleet replacement.
- Moreover, with the ICH Q14 guidelines, it is expected that cGMP laboratories have proper analytical procedure lifecycle strategy in place.
- Modernizing instrumentation is a straightforward and futureproof way to meet current and future regulatory guidance and can lead to significant cost savings.

View the full case study

APPLICATION NOTE 72593

Simultaneous high-performance and ultra-high-performance liquid chromatographic analysis of acetaminophen impurities using a single instrument

Maria Grübner, Carsten Paul, *Thermo Fisher Scientific*

Keywords

Vanquish Flex Duo UHPLC system, Vanquish Flex Dual Pump, UHPLC, Vanquish Flex Dual Split Sampler, acetaminophen

Introduction

The Vanquish Flex Duo system, allows for optimization of each flow path to specific requirements, e.g. regarding extra column or gradient delay volumes, giving the opportunity to have one HPLC and one UHPLC instrument in the same stack.

Such a setup can be utilized for parallel implementation of completely independent HPLC and UHPLC methods but also for speed-up of legacy HPLC methods at the same workstation. This application demonstrates the latter case.

- Thermo Scientific™ Vanquish™ Flex Duo UHPLC System
- Thermo Scientific™ Vanquish™ Variable Wavelength Detectors H
- Thermo Scientific™ Hypersil™ GOLD C8 columns

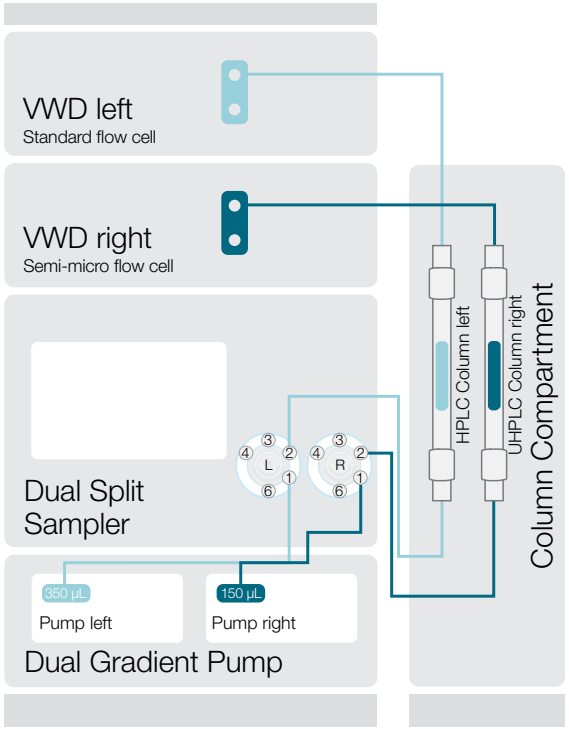
Application benefits

- Vanquish Duo LC technology provides two independent LC channels with the footprint of only one instrument.
- Established HPLC methods and their UHPLC counterparts can be implemented in parallel on the same instrument.

Goal

To demonstrate the capabilities of the Thermo Scientific™ Vanquish™ Flex Duo UHPLC system to run independent HPLC and UHPLC methods simultaneously using one instrument.

Fluidic setup of Vanquish Flex Duo system with one HPLC (light blue) and one UHPLC (dark blue) flow path.



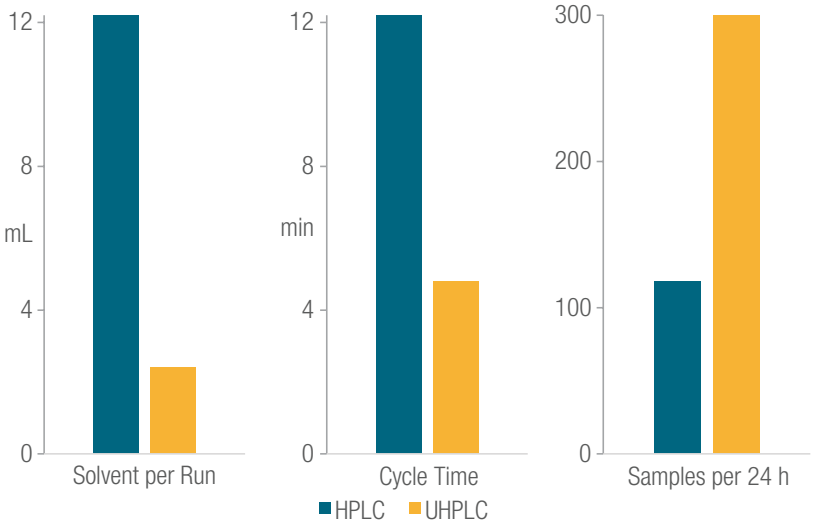
Results

The method parameters of the UHPLC channel of this experiment were derived from the original HPLC method by the Chromeleon CDS UHPLC speed-up tool with a boost factor of 1.52 for a flow rate of 0.5 mL/min and additional flush time to ensure sufficient equilibration. The average resolutions (RS) easily meet the USP requirements. The absolute and relative standard deviations (SD and %RSD) of retention times are comparable for both methods.

Regarding peak area precision and signal-to-noise values (S/N), the UHPLC method was inferior to the HPLC method. Thus, a simple improvement of the UHPLC method by increasing the injection volume from 0.17 µL to 0.5 µL is recommended to improve S/N and yield area %RSDs in a similar range as the HPLC method. However, all three methods resulted in well integrable peaks with S/N values all greater than 50.

Considerable benefits of the UHPLC method are substantial savings in sample volume, solvent consumption, and cycle time (tC), with additional optimization capabilities if the gradient delay volume and thus equilibration time were further reduced, for example by configuring the Vanquish Duo UHPLC system with a high-pressure mixing pump (HPG) for the UHPLC path. Another option to increase throughput and save costs and time is the elimination of the first isocratic step from the gradient table, as the column experiences a sufficiently long isocratic step due to gradient delay.

Compared to the HPLC analysis, the optimized UHPLC method (without isocratic step, injection volume 0.5 µL) resulted in 50% sample, 80% solvent, and 60% time savings and a 2.5-fold throughput improvement. One hundred samples could be analyzed during an 8 h working day by UHPLC instead of more than 20 h. Assuming costs of $25 per liter of solvent plus 10% for disposal, switching to UHPLC implies cost savings of around $27 per 100 samples or $5400 per year (with an estimation of 20,000 samples per year).



Comparison of the HPLC method and the most-optimized UHPLC method (without an isocratic step and injection volume of 0.5 µL) regarding throughput, solvent, and time expenses.

Conclusion

- The Vanquish Flex Duo system provides the opportunity to have one HPLC and one UHPLC channel in a single system stack, both working independently from each other.
- Speed-up of legacy HPLC methods to fast UHPLC methods can be easily conducted at the same workstation. Both channels can also be used independently for separate analyses.
- In the current study, a 2.5-fold throughput increase and savings of up to 80% mobile phase and 60% cycle time were achieved by speeding up a HPLC method to UHPLC conditions.

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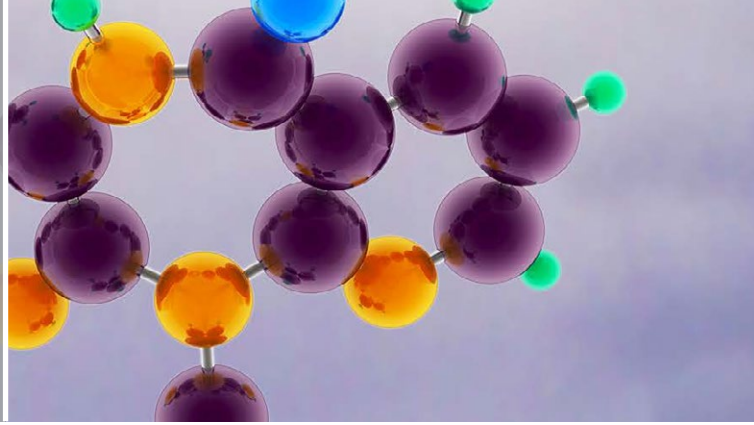
APPLICATION NOTE 72603

Simultaneous quantification of nevirapine and low-level impurities

Maria Grübner, Frank Steiner, Holger Franz, *Thermo Fisher Scientific*



Keywords

Thermo Scientific Vanquish DAD FG, nevirapine, impurity profiling, linear range

Introduction

Analytical monitoring of substances and impurities is a crucial requirement in drug development and production. The challenge for the instrumentation is the absorption difference of high-concentrated API and low-level impurities that need to be reported down to a content of 0.03% of the API.

A previously in a Thermo Fisher Scientific application brief developed UHPLC method was used in the current study to demonstrate the capabilities of the new Thermo Scientific Vanquish DAD FG to quantify both the API nevirapine and its impurities A, B, and C in a single run.

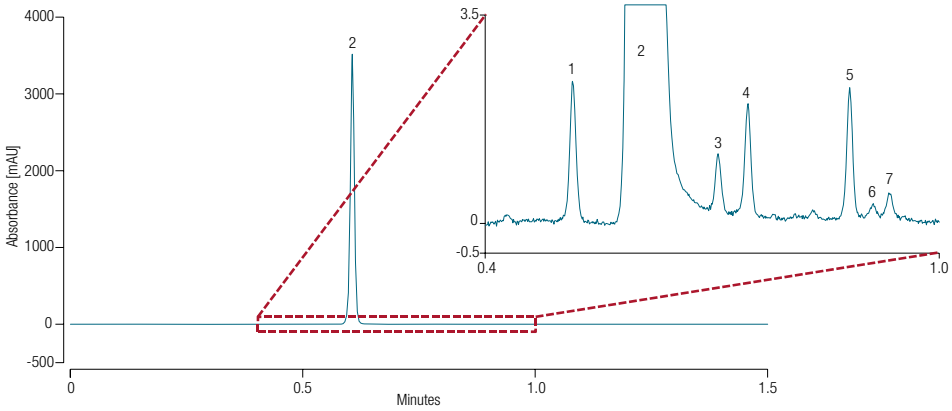
- Thermo Scientific™ Vanquish™ Horizon HPLC System
- Thermo Scientific™ Vanquish™ Diode Array Detector FG
- Thermo Scientific™ Syncronis™ C18 column

Application benefits

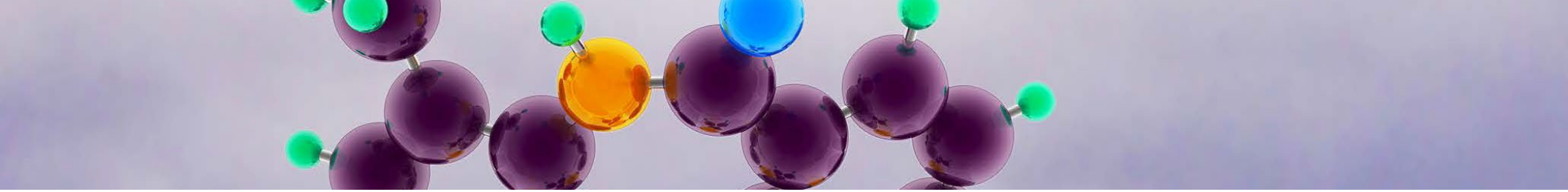
- The new Thermo Scientific ™ Vanquish ™ DAD FG provides an industry-leading linear range up to 3700 mAU.
- The detector linearity in combination with low baseline noise allows for the simultaneous quantification of nevirapine and its impurities within a single run.

Goal

To demonstrate the wide dynamic range of the new Thermo Scientific Vanquish DAD FG and how it facilitates the quantification of compounds of very different concentrations



Impurity profile of nevirapine sample (injection volume 0.9 µL) with baseline zoom.



Results

In order to discover the linear detection range provided by the new Vanquish DAD FG, the dependence of detector response on the nevirapine amount was recorded over a concentration range from 0.1 µg/mL to 1200 µg/mL. For a linear calibration with permitted offset and no weighting of data points, the regression line of peak heights exhibited a correlation coefficient of 99.965% and a relative standard deviation of less than 3% for standards from 0.1 µg/mL to 850 µg/mL. This corresponds to peak heights of 0.5 mAU to 3700 mAU. Each successive calibration point was successfully predicted to within an error of less than 5% from the respective preceding data.

Depending on the drug development stage, impurities may not be fully characterized and therefore are unavailable as reference material. For nevirapine profiling we applied this procedure by quantifying API and impurities based on the nevirapine calibration curve. We calculated a calibration curve based on peak area with permitted offset and weighting of calibration points by 1/amount. Over the same concentration range as before (0.1–850 µg/mL), a correlation coefficient of 99.984% was obtained and deviations of expected and measured response were less than 5%.

In the pure nevirapine standard (850 µg/mL), all six impurities were detectable but exhibited relative areas of 0.006% to 0.025%. This was far below the threshold of 0.05% and peaks were not quantifiable due to signal to-noise ratios (S/N) of less than 10 except for Unknown 1 and Unknown 3.

Conclusion

- The new Vanquish DAD FG combines a very wide linear range with the best noise performance, enabling for the simultaneous quantification of APIs and impurities within a single run.
- Excellent quantitative results were obtained for the API nevirapine and its impurities with an optimized UHPLC method with deviations from expected amounts of less than 2% for the API and 6–21% for impurities under the approximated assumption of equivalent responses. Impurity quantification was possible down to 0.012% relative area if the linear detection range is fully exploited.

View the full application note

CUSTOMER APPLICATION NOTE 001903

Acarbose impurity analysis: method migration from UV detection to universal charged aerosol detection

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Keywords

Acarbose, glucosidase inhibitor, impurity analysis, method transfer, Vanquish Charged Aerosol Detector H, HILIC, Amide-HILIC column, graphite column

Introduction

In this application note, we examine the impurity analysis of acarbose (according to the Ph. Eur.) and present two alternative methods for the impurity analysis by means of charged aerosol detection. The suitability of these two methods for analysis of acarbose is also discussed.

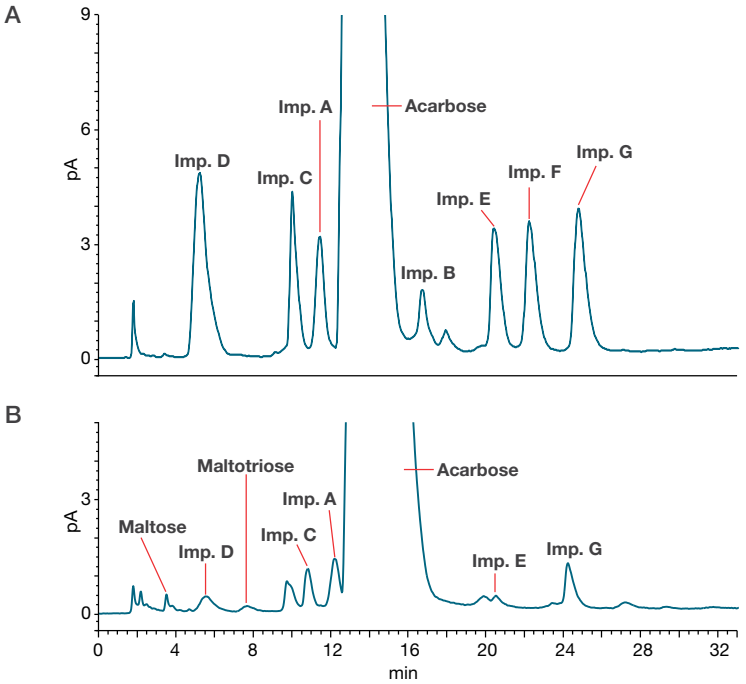
- Thermo Scientific™ Vanquish™ Flex HPLC System
- Thermo Scientific™ Vanquish™ Charged Aerosol Detector H
- Thermo Scientific™ Hypersil™ APS-2 column
- Thermo Scientific™ Accucore™ 150 Amide HILIC column
- Thermo Scientific™ Hypercarb™ column

Application benefits

Impurity analysis of acarbose using a Thermo Scientific™ Vanquish™ Flex UHPLC system with a Thermo Scientific™ Vanquish™ Charged Aerosol Detector (CAD) as an alternative to pharmacopoeial UV detection to extend the range of impurities to include those that cannot be determined due to a lack of a strong chromophore.

Goal

This application note examines the suitability of a HPLC-CAD system for impurity analysis of acarbose as an alternative/supplement to the UV detection used in the European Pharmacopoeia (Ph. Eur.) monograph 2089.



(A) Chromatogram obtained with acarbose reference solution (1) and the Hypercarb method; (B) Chromatogram obtained with acarbose test solution and the Hypercarb method. Hypercarb graphite column: (150 × 4.6 mm, 3 µm.), flow rate 1 mL/min, gradient elution, and CAD detection.

Results

The current Ph. Eur method for the related substances test of acarbose uses an aminopropyl-silyl (APS) column with phosphate buffer. Instability of these columns applied with this monograph has been observed, resulting in the motivation to look for alternative stationary phases run with volatile mobile phases compatible with charged aerosol detection to establish a more stable and sensitive method.

Running the APS column by switching the non-volatile mobile phase from phosphate buffer to the volatile mobile phase 10 mM aqueous ammonium acetate was not successful, as the CAD signal showed a relative high background current of 75 pA. The decreased sensitivity due to increased noise level made it necessary to investigate other stationary phase options.

Most of the analytes show epimerization, which can lead to peak splitting or distortion. The epimerization speed can be increased by a higher column temperature or a higher pH of the mobile phase. However, higher pH values of the mobile phase may cause silica-based stationary phases to degrade.

A compromise of acceptable peak shape and stability of the stationary phase was found in the Accucore 150 Amide HILIC column. All compounds were separated. Although the validation results were in an acceptable range, for improvement of sensitivity further stationary phases were evaluated.

Hypercarb columns, unlike those based on bonded silica, can be used at high temperatures and high acidic or alkalinic conditions. Good separation of the components was achieved by the method described.

Furthermore, the CAD has the potential to determine additional impurities in the acarbose batches that are not detectable with a UV detector used in the compendial monograph.

Conclusion

- Impurity profiling of acarbose is generally challenging.
- Successful method migration from UV detection to the Vanquish CAD system was demonstrated. Both CAD methods met the requirements of the Ph. Eur. “related substances” test for the impurity analysis of acarbose.
- Both the Accucore 150 Amide HILIC column and the Hypercarb column are suitable for the impurity profiling of acarbose, while being more stable than the APS column used in the monograph method and operating under MS compatible conditions.
- Detection of additional impurities without a chromophore at low level is enabled by CAD.
- The Vanquish UHPLC system with the Vanquish Charged Aerosol Detector is versatile. For example, the ability to adjust evaporation temperature enables improved performance.
- Usage of LC-MS grade chemicals is recommended.

View the full application note

Contents—Method development

SAVE TIME AND MONEY WITH AUTOMATED METHOD DEVELOPMENT

Traditional method development shouldn't be a time or resource-intensive process for your lab. Here, we demonstrate how you can use automated method development tools to save on time and labor costs.

- **Accelerating method development on HPLC and UHPLC utilizing ChromSwordAuto Chromeleon Connect**
- **Automated method scouting and mass detection for a stability-indicating method utilizing ChromSword Chromeleon Connect**
- **Simultaneous reversed-phase and anion-exchange method scouting with a dual flow path system for mRNA impurity determination**
- **Automated HPLC method development and robustness tests for drug products utilizing ChromSwordAuto Chromeleon Connect**
- **Automated development of a fast, robust UHPLC method for an API and its related impurities utilizing ChromSwordAuto Chromeleon Connect**
- **Analytical Quality-by-Design (AQbD) approach to develop a fast and robust UHPLC method utilizing S-Matrix™ Fusion™ QbD Software**

PRODUCT SPOTLIGHT 73997

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

Alec Valenta, Carsten Paul, Peter Zipfell, *Thermo Fisher Scientific*

Keywords

HPLC, High Performance Liquid Chromatography, UHPLC, Method Development Systems, Vanquish Core, Vanquish Flex, Vanquish Horizon, ISQ EC, ISQ EM, charged aerosol detection (CAD), Chromeleon CDS, ChromSwordAuto, mass spectrometry (MS)

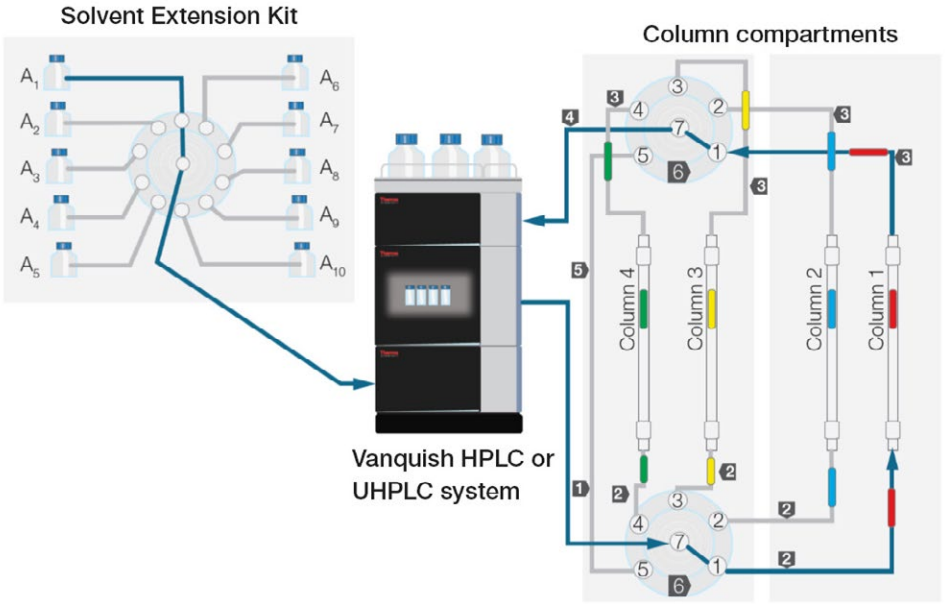
Introduction

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.

Automated method scouting hardware

The Thermo Scientific Vanquish HPLC and UHPLC Method Development Systems offer multiple solutions for a wide range of applications. The Thermo Scientific™ Viper™ Method Scouting Kit includes all fluidic connections and capillaries required to scout 4 column chemistries. The solvent extension kit includes an external selection valve for automated scouting of up to 10 solvents per channel.



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.

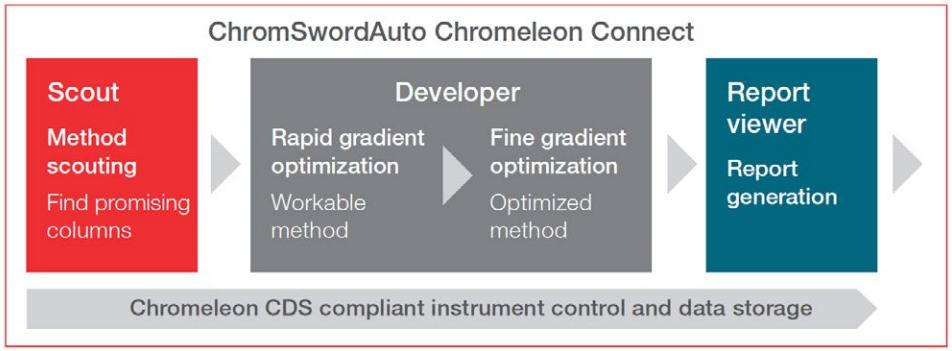
Results

The Vanquish Method Development System hardware including two column compartments, solvent extension kit, Viper method scouting kit, and two 7 port-6 position valves can be used for automated scouting of up to 6 columns and 13 mobile phases.

Automated method development software

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.

- 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.



Method development workflow using ChromSwordAuto Chromeleon Connect

Automated robustness testing and method validation software

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.

The Chromeleon 7 Extension Pack ICH Templates deliver a complete set of eWorkflow procedures that have been created in accordance with the ICH Q2 guidelines and include a series of method validation tests to ensure characteristics, such as accuracy, linearity, precision, etc. are considered.

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.
- 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.
- 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.

View the full application note

APPLICATION NOTE 000460

Development of a stability-indicating method for esomeprazole and related degradation products by automated method scouting and mass detection

Soo Hyun Park, Sylvia Grosse, Mauro De Pra, Michaela Scherz, Frank Steiner,
Thermo Fisher Scientific

Keywords

ChromSword Chromeleon Connect, Vanquish Flex UHPLC, ISQ EM, ISQ EC, esomeprazole, forced degradation, impurity profiling, degradation product, peak tracking, HPLC, LC-MS, method development

Introduction

In this application note, initial scouting experiments for the development of a stability-indicating method for esomeprazole are reported. An automated method scouting workflow is presented, which is easy to perform and delivers faster method development.

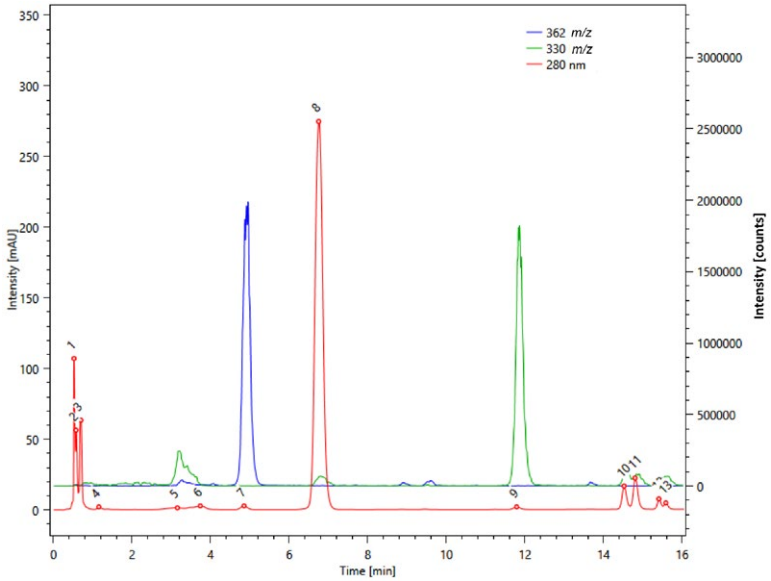
- Thermo Scientific™ Vanquish™ Quarternary Flex UHPLC System
- Thermo Scientific™ ISQ™ EM mass spectrometer
- Thermo Scientific™ ChromSword Chromeleon Connect

Application benefits

- Thermo Scientific™ Vanquish™ Method Development UHPLC system in combination with ChromSword Chromeleon Connect, enables automated and unattended method development, reducing method development time significantly.
- ChromSword Chromeleon Connect accelerates the screening of column and mobile phases for the determination of esomeprazole and related degradation products.
- Coupling a Thermo Scientific™ ISQ™ EM single quadrupole mass detector to a Vanquish UHPLC Method Development system increases peak tracking capability in method development by the combined use of mass and UV spectra.

Goal

Demonstrate a streamlined method scouting workflow for the analysis of esomeprazole and related degradation products.



UV chromatogram (red) and extracted ion chromatograms (XICs, blue and green) of esomeprazole and related degradation products, confirming impurity-3 (green, *m/z* 330.4) and impurity-4 (blue, *m/z* 362.4). The separation was obtained on the Hypersil GOLD C8 column with ammonium bicarbonate buffer at pH 8.0 and acetonitrile. The wavelength of 280 nm was used for UV detection.

Results

The Vanquish Method Development system, combined with ChromSword Chromeleon Connect, streamlines the entire method development process, providing automation of all method development tasks such as method scouting, method optimization, and method robustness testing. This application note focuses on step 1, the method scouting step. Three columns and three mobile phases were screened to develop a stability-indicating method for esomeprazole and its degradation products.

Step 1: Method scouting
- ChromSword Chromeleon Connect software module: Scout
- Screen for a promising combination of column, solvent, and mobile buffer pH

Step 2: Data analysis
- ChromSword Chromeleon Connect software module: ReportViewer
- Process chromatograms, UV and mass spectra, generate reports

Step 3: Method optimization
- ChromSword Chromeleon Connect software module: Developer
- Fine tune method parameters such as gradient, flow rate, and temperature

Step 4: Method robustness testing
- ChromSword Chromeleon Connect software module: AutoRobust
- Verify the method is robust when changing separation parameters (such as pH, flow rate, and temperature)

General workflow for automated method development using ChromSword Chromeleon Connect

The column screening was performed by evaluating parameters related to the column selectivity and peak shape, namely total number of resolved peaks, resolution, peak asymmetry, and peak width. An Accucore C18 column yielded the greatest number of peaks resolved with Rs >1.5 and better peak shapes, and thus was selected as the most promising column.

Six scouting runs using three mobile phase buffers with acidic, neutral, and alkaline pH on the Accucore C18 column were evaluated for buffer screening. The use of ammonium bicarbonate buffer at pH 8 produced more peaks and better resolution, resulting in selection of this mobile phase buffer as the best.

As a result, the Accucore C18 column and ammonium bicarbonate buffer of pH 8.0 were selected as a starting condition for further method optimization. The method optimization can be performed using the Developer module of ChromSword Chromeleon Connect.

Conclusion

- The Scout module of ChromSword Chromeleon Connect, combined with the Vanquish Flex UHPLC system and ISQ EM mass spectrometer, enabled unattended method screening for column and mobile phases.
- A proposed workflow resulted in rapid screening of the most suitable column and mobile phase buffer within 10 hours.
- By a systematic approach using method development software, the Accucore C18 column was rapidly selected as the most suitable column for separating esomeprazole and related degradation products.
- The combined use of UV and mass spectra facilitated accurate, straightforward, and rapid peak tracking during method scouting.

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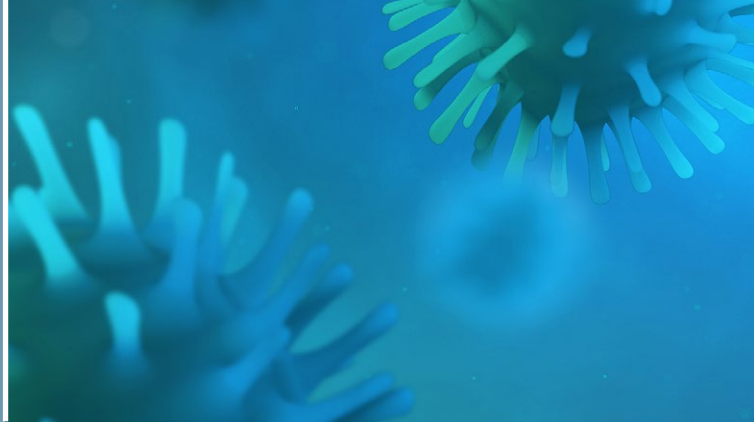
APPLICATION NOTE 000471

Simultaneous reversed-phase and anion-exchange method scouting with a dual system for mRNA impurity determination

Denis Andrei Ispan, Mauro De Pra, Katherine Lovejoy, *Thermo Fisher Scientific*



Keywords

Vanquish Duo UHPLC, Dual-LC, mRNA, ion-exchange chromatography, ion-pairing reversed-phase chromatography, method development, solvent scouting, mRNA purification

Introduction

In this work, a large number of mobile phase conditions are tested with minimal preparation work. The most promising methods for each chromatography type are discussed.

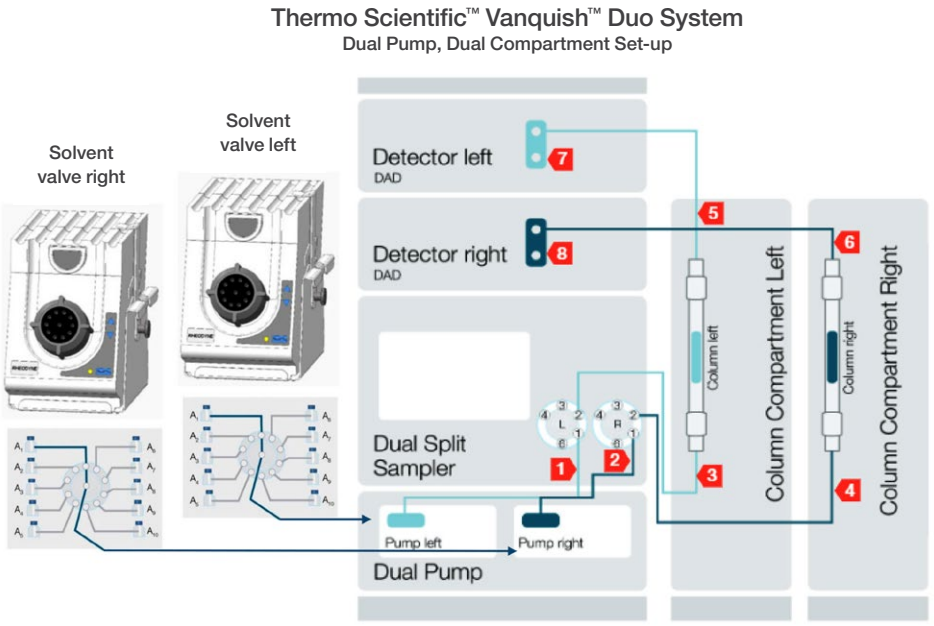
- Thermo Scientific™ Vanquish™ Flex Duo UHPLC System
- Thermo Scientific™ Diode Array Detectors H
- Thermo Scientific™ Extension Kits for Automated Method Scouting, Vanquish Systems

Application benefits

- Leverage two different separation chemistries in the same instrument at the same time: ion-exchange (IEX) and ion-pairing reversed-phase (IP-RP)
- Scout up to 10 solvents for each column type to find most suitable eluent condition
- Accelerate method development
- Maximize sample knowledge
- Perform mRNA profiling and detection of post-in vitro transcription (IVT) purification impurities
- Use corrosive mobile phases, such as NaCl 1 M, with the fully biocompatible flow path of the Thermo Scientific™ Vanquish™ Duo UHPLC system

Goal

Determine the most suitable conditions for the detection of post-transcriptional impurities in mRNA with a time-effective scouting approach.



Flow scheme overview: Thermo Scientific Vanquish Duo for Dual LC with Solvent Extension Kits for automated method scouting. Dual pump and dual column compartment set-up.

Results

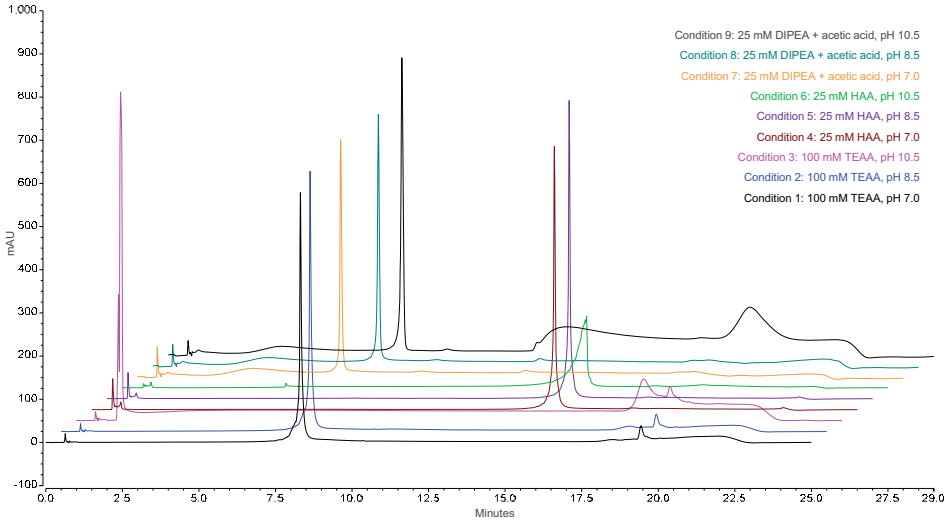
Ion pair chromatography and ion exchange chromatography (IEX) method have been compared.

Initial scouting was performed using the purified sample. All three ion pairing agents at high pH (10.5) give unacceptable chromatograms, showing either poor shape of the peak assigned to mRNA, very low retention of the main peak, or high background signal. The remaining conditions yield mRNA as the main peak with acceptable peak shape and some impurities detected at low retention times.

The method assessment was continued with the non-purified sample. HAA is the only tested IP agent with acceptable retention and separation of most impurities; therefore, this method was deemed the most suitable for impurity profiling.

As for IEX, Tris buffer with perchlorate in MeCN at 80 °C was the only condition capable of eluting the RNA and which gave reasonable peak shape. The mobile phases based on a combination of perchlorate and acetonitrile were the most promising with MeCN increased to 20% resulted in better mRNA peak elution and baseline signal.

A rough estimation of the purity from the chromatograms based on the IP-RP and IEX selected methods was calculated. The selected methods provided a comparable estimation of the purity of the mRNA sample. some low-level impurity peaks remaining in the purified sample and the assumed impurity after the mRNA are better detected with IEX.



IP-RP purified mRNA scouting conditions overlay at 50 °C. 1 µL injection. mRNA is the most intense peak.

Conclusion

- Two methods were developed for the assessment of purification efficiency of mRNA and for purity profiling of purified and non-purified mRNA.
- The DNAPac RP and DNAPac PA 200RS columns deliver high selectivity and efficiency for the separation of mRNA impurities.
- The Vanquish Duo system extended with the Method Scouting Kit is a valuable solution that enables simultaneous scouting of columns with different chemistries, thereby, greatly reducing the time investment for complex method development tasks.
- Suitable method conditions for the detection of mRNA and impurities were found to be 25 mM HAA for IP-RP and 40 mM Tris/0.8 M perchlorate/20% MeCN for IEX.

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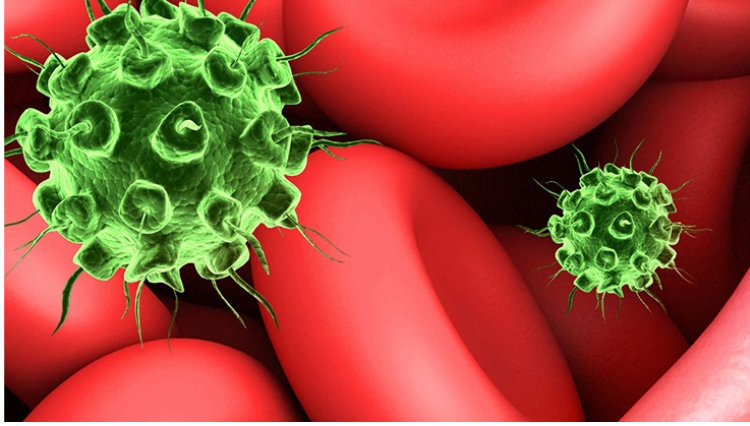
APPLICATION NOTE 001193

Automated HPLC method development and robustness tests for abacavir, lamivudine, dolutegravir, and their related compounds in Triumeq drug product

Xuepu Li, Min Du, *Thermo Fisher Scientific*



Keywords

Automated method development, ChromSwordAuto Chromeleon Connect, Vanquish Core HPLC, abacavir, lamivudine, dolutegravir, Triumeq, aQBD

Introduction

In this application note, ChromSwordAuto Chromeleon Connect and a Thermo Scientific Vanquish Core HPLC system were used for the automated method development.

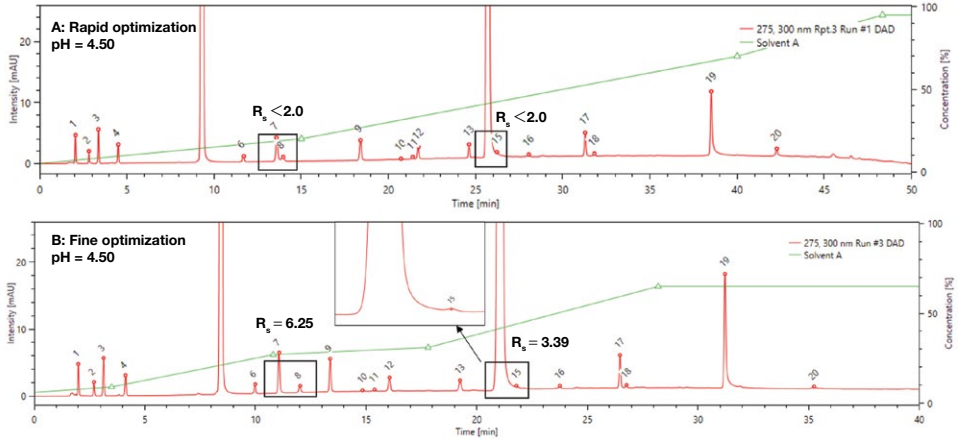
- Thermo Scientific™ Vanquish™ Quaternary Core HPLC System
- Thermo Scientific™ Vanquish™ Diode Array Detector CG
- Thermo Scientific™ ChromSwordAuto Chromeleon Connect

Application benefits

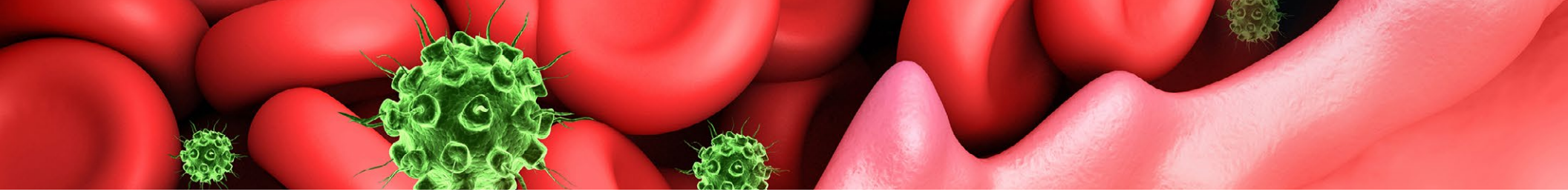
- The seamless integration between ChromSword and Thermo Scientific™ Chromeleon™ Chromatography Data System fully automates the method development and robustness testing process.
- HPLC method development is accelerated significantly by using ChromSwordAuto Chromeleon Connect and Thermo Scientific™ Vanquish™ switching valves.
- The statistical design of experiments (DoE) and the design space in the robustness test are in accordance with analytical quality by design (aQbD) principles.

Goal

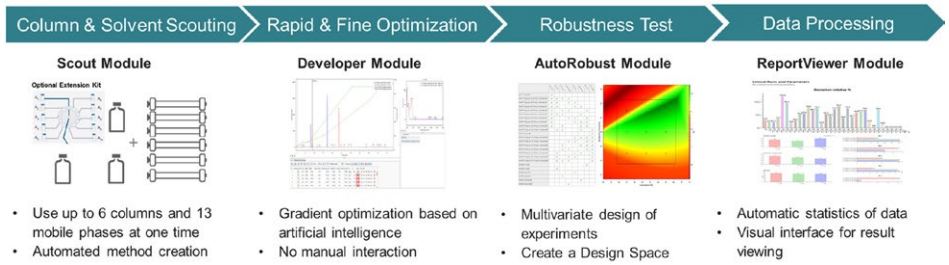
Demonstrate an automated method development and robustness test workflow for abacavir, lamivudine, dolutegravir, and their related compounds in the drug Triumeq by using ChromSwordAuto Chromeleon Connect and a Thermo Scientific™ Vanquish™ Core HPLC



The best chromatogram selected from rapid and fine optimization results on an Acclaim 120 C18 column, with column temperature 30 °C, injection volume 2 µL. After fine optimization, the resolution between peak 7 and 8, peak 14 and 15 was improved and the run time reduced. The green line represents the gradient.



Results



Workflow overview and benefits of automated method development and robustness testing based on ChromSwordAuto Chromeleon Connect and Thermo Scientific Vanquish automated method scouting kit

Method scouting study: The main task in this phase was to find the most powerful parameters that influence the separation. According to how influential these factors are in affecting selectivity, the key three parameters are column stationary phase, pH of aqueous phase, and organic modifier. The Scout module of ChromSwordAuto Chromeleon Connect was used for this phase. In this experiment, we selected five different columns for the method screening, and the five columns were screening at one time.

Method optimization study: In this phase, other parameters were adjusted to get a better separation and peak shape, such as gradient breakpoint time, ratio of the organic phase, column temperature, and injection volume. The Developer module of ChromSwordAuto Chromeleon Connect was used for this phase. It shows that after fine optimization, all the compounds can be separated well

Robustness test: After the method development, the robustness was studied to demonstrate the method is reliable even under some variations in the lab. The AutoRobust module of ChromSwordAuto Chromeleon Connect was used for this phase. Here, we used the full factorial design to study the method robustness.

Data processing: The ReportViewer module of ChromSwordAuto Chromeleon Connect was used for all data processing, including peak integration, data analysis and statistics, design space analysis, report creation, and export.

As this automated workflow does not require any manual intervention, the instrument can run fully unattended and continuously even during night time and over the weekend, which supports increased efficiency in an analytical lab significantly.

Conclusion

- In this study, we have demonstrated an automated workflow for HPLC method development and a robustness test for abacavir, lamivudine, dolutegravir, and their related compounds in the drug Triumeq.
- The workflow reduced both the development time and manual operation, enabling the analytical laboratory to be more efficient and cost-effective in HPLC method development.
- The final method provides an adequate separation for all analytes with a USP resolution ≥ 2.0, and USP peak asymmetry within 0.9 to 2.1.
- The full factorial robustness test provided a robust region for the method.

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APPLICATION NOTE 000754

Automated UHPLC method development for mebendazole and related impurities, from method scouting to robustness testing

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Thermo Fisher Scientific

Keywords

ChromSword Chromeleon Connect, Vanquish Flex, mebendazole, impurity analysis, UHPLC, automated method development, robustness test

Introduction

In this application note, we present an automated method development workflow utilizing a Vanquish Flex UHPLC system combined with ChromSword Chromeleon Connect.

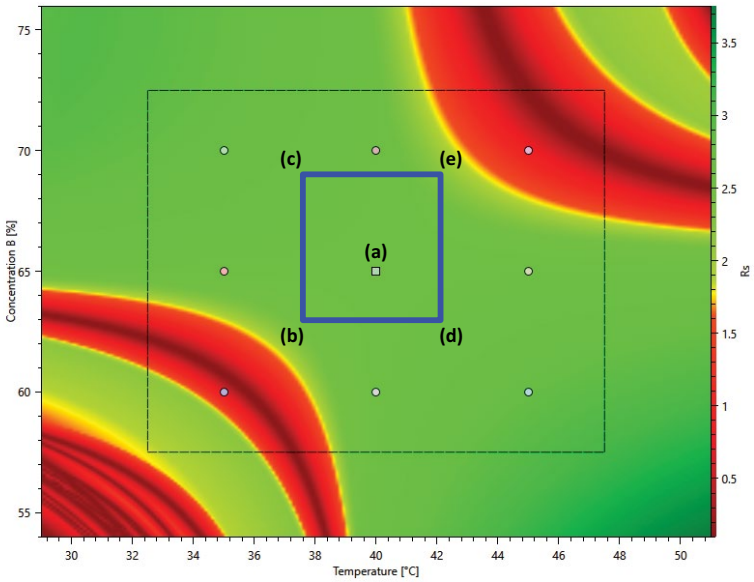
- Thermo Scientific™ Vanquish™ Quarternary Flex HPLC System
- Thermo Scientific™ Vanquish™ Diode Array Detector FG
- Thermo Scientific™ ChromSwordAuto Chromeleon Connect

Application benefits

- The Thermo Scientific™ Vanquish™ UHPLC Method Development system in combination with ChromSword Chromeleon Connect enables automated and unattended method development.
- The proposed method development workflow for the analysis of mebendazole and related impurities considerably reduces method development time and cost.
- The complete workflow includes method scouting, method optimization, and method robustness testing.
- The AutoRobust module of ChromSword Chromeleon Connect provides the robust region to afford assurance of quality of the final method via the design space between method parameters.

Goal

To develop a fast, robust UHPLC method for mebendazole and related impurities using an automated method development workflow.



A 2D resolution map illustrating the effect of temperature and concentration of organic solvent B (%), with the design space (or robust region) indicated by a blue box. (a) Final method, and (b) to (e)—four corners of the blue box. Eight circles and a square (i.e., the final method) in the 2D space represent the experimental measurements. The region of the blue box was determined by identifying common robust green regions, obtained at pH 4.6, pH 4.7, and pH 4.8.

Results

Step 1: Method scouting

- ChromSword Chromeleon Connect software module: Scout
- Screen for a promising combination of column, solvent and mobile phase pH

Method scouting was done by evaluating performance criteria related to column selectivity and peak shape, namely total number of peaks, minimum peak resolution, peak asymmetry, and peak width.

Step 2: Rapid and fine optimization

- ChromSword Chromeleon Connect software module: Developer
- Optimize the gradient profile with the column, mobile phase buffer, and organic solvent chosen in method scouting

The rapid optimization algorithm automatically performs three or four runs to find a good separation for target analytes. Based on the first run, rapid optimization is generally achieved in the second or subsequent runs.

For fine optimization, a total of 30 runs consisting of 18 isocratic and 22 gradient methods were performed to maximize resolution of mebendazole and related impurities.

Step 3: Method robustness test

- ChromSword Chromeleon Connect software module: AutoRobust
- Create design space (or robust region) by multivariate study

The software supports design of experiments (DoE) through one of three different design principles: one-by one (or one parameter at a time), Plackett-Burman, and full factorial design.

Conclusion

- The Vanquish Method Development System, consisting of a combination of ChromSword Chromeleon Connect and the Vanquish Flex UHPLC system, enabled rapid, unattended development of a fast and robust method for the analysis of mebendazole and related impurities.
- The proposed workflow substantially reduced both development time and user intervention.
- By a systematic approach using method development software, the Thermo Scientific Hypersil GOLD column was rapidly selected as the most promising column for separating mebendazole and related impurities.

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APPLICATION NOTE 001631

Development of a robust LC method for metolazone and related impurities using analytical quality by design best practices

Sylvia Grosse, Soo Hyun Park, Richard Verseput, ***Thermo Fisher Scientific;***
Frank Steiner, ***S-Matrix Corporation, Eureka, CA, USA***

Keywords

Quality by design, AQbD, system suitability, SST, Vanquish Flex, UHPLC, robustness, MODR, verification

Introduction

In this application note the analytical quality by design (AQbD approach is described for the development of a fast and robust UHPLC method for metolazone and its related impurities. A QbD software combined with a Vanquish Flex UHPLC system enables automated method scouting, optimization, and robustness evaluation as well as enhanced data visualization and reporting.

- Thermo Scientific™ Vanquish™ Quarternary Flex HPLC System
- Thermo Scientific™ Vanquish™ Diode Array Detector FG
- Thermo Scientific™ Extension Kit for Automated Method Scouting, Vanquish Systems
- S-Matrix™ Fusion™ QbD Software

Application benefits

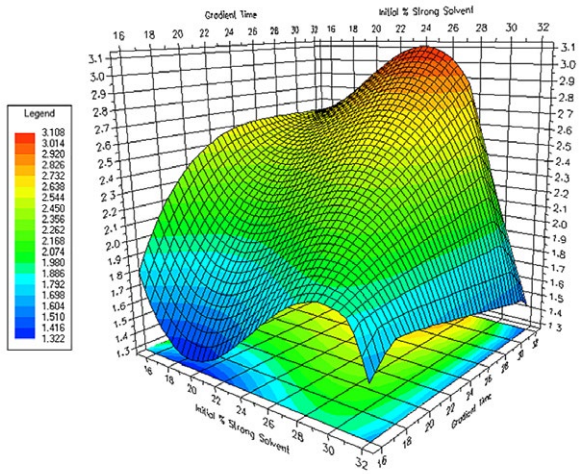
- Thermo Scientific™ Vanquish™ UHPLC Method Development System in combination with QbD software enables automated and unattended method development.
- The workflow presented herein significantly reduces the number of experiments, method development time, and related costs.

Analytical QbD benefits

- Statistically valid study protocols
- Independently verifiable, data-driven decisions
- Quantitative characterization of the effects of all study parameters on critical performance characteristics, i.e., mean (average) performance and robustness

Goal

Demonstrate the benefits of the Analytical Quality-by-Design (AQbD) approach by developing a fast and robust UHPLC method for metolazone and related impurities.



Resolution map – 2D contour and 3D overlay for the critical peak pair (here always impurities A and B). The color represents the resolution.

Results

The AQbD approach involves the following general workflow steps.

1. ATP (Analytical Target Profile)
CMA (Critical Method Attribute)

2. Fishbone Diagram
CMP (Critical Method Parameter)
Process (method) understanding/
Risk assessment

3. Method Screening / 4. Method Optimization
DoE (Design of Experiments)

5. *In-silico* Robustness / 6. MODR Validation
Knowledge space

AQbD workflow steps

In Phase I - Screening - the most appropriate stationary phase, aqueous and organic solvents, pH range, and initial gradient conditions were determined by examining the main effectors of separation.

Using a multi-factor DoE-based screening experiment enabled quantitative characterization of all interactions between column type, strong solvent type, and gradient time. The screening study showed that these interaction effects have the greatest influence on separation. Therefore, characterizing these effects enabled identification of the column type and organic solvent combination to use for the optimization study along with the workable regions of both pH and tG.

The Robustness Simulator within QbD is used to automatically model the variation in method performance for each included performance characteristic resulting from simultaneous variations in the method parameters entered by the user in the setup dialog.

The target method will also have excellent robustness performance in terms of the resolution of the critical pairs and the % RSD of the API at the target setpoint conditions of pump flow rate, oven temperature, and pH.

The optimized method resulted in a faster total run time than the method described in the EP Monograph method, which is now 22 minutes versus 48 minutes for the EP method. This represents a 54% reduction in overall run time relative to the current EP method. In addition, the resolutions between impurities E & C and impurities A & B are significantly improved.

Conclusion

- Thermo Scientific Vanquish UHPLC Method Development system in combination with Chromeleon CDS and QbD software enabled rapid, successful modernization of the current EP monograph method for metolazone and related impurities.
- The seamless connectivity between the Chromeleon CDS and QbD, which included full QbD experiment automation support, enabled quick execution of a best practices AQbD approach to method development.
- The project yielded a fast and robust final method for separation of metolazone and five related known impurities which met all the project's method performance goals.

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