Alliance iS HPLC System: A New Era of Intuitive Simplicity

alliance™ iS

Waters™

Introduction

This application notebook details specific use cases of the Waters™ Alliance™ iS HPLC System, such as common pharmaceutical QC applications, the analysis of soft drink additives, replicating HPLC methods for water-soluble vitamins, and method migration from legacy HPLC systems.

The system demonstrates excellent performance under challenging conditions, enables reliable and quick method transfer, and has been used to achieve both scaling to modern column dimensions and method modernization for USP monograph separations. Lastly, it has improved carryover performance, addressing a common problem in HPLC systems.

To learn more about how the Alliance iS HPLC System will be your new lab ally, visit waters.com/AllianceiS.

CONTENTS

HPLC Analysis of Soft Drinks Using the Alliance iS HPLC System 4

Replicating an HPLC Method for Water-Soluble Vitamins on the Alliance iS HPLC System 5

Hydrophilic Interaction Liquid Chromatography (HILIC) Method Migration: From Legacy HPLC Systems to the Alliance iS HPLC System 6

Hydrophilic Interaction Liquid Chromatography (HILIC) Method Migration: Troubleshooting Peak Splitting of Cetirizine 7

Analysis of Azithromycin on the Alliance iS HPLC System: System Performance under Challenging Method Conditions 8

Analysis of Atorvastatin as per USP Monograph Utilizing the Alliance iS HPLC System 9

Reliable Analysis of Aspirin and Related Substances in Drug Substance and Tablet Formulation Using an Alliance iS HPLC System 10

Successful Method Migration of the USP Quetiapine Fumarate Impurities Method to an Alliance iS HPLC System 11

Simultaneous Determination of Naphazoline Hydrochloride and Pheniramine Maleate with their Related Compounds 12

Achieving Method Modernization with the New Liquid Chromatographic Gradient Allowances Provided by USP General Chapter <621> Chromatography and the Alliance iS HPLC System 13

Improved Chlorhexidine Carryover Performance Using the Alliance iS HPLC System 14

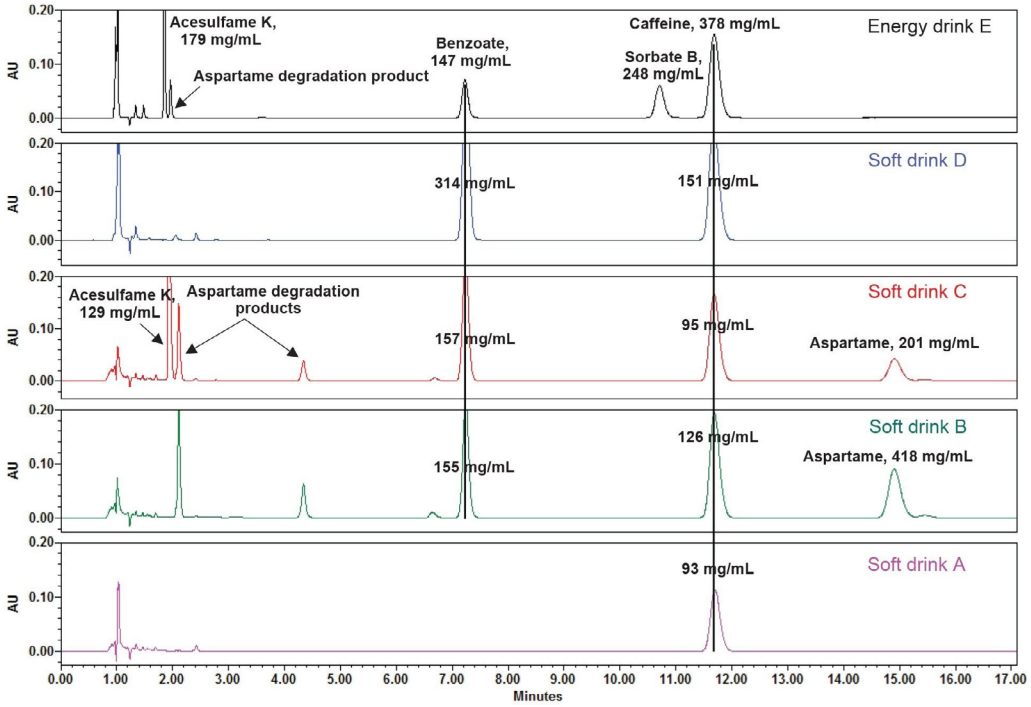
Method Migration of the USP Ibuprofen Assay and Organic Impurities Method to an Alliance iS HPLC System 15

HPLC Analysis of Soft Drinks Using the Alliance iS HPLC System

In this study an Alliance iS HPLC System was employed for the analysis of additives in soft drinks. The method successfully resolved all additives and aspartame degradation products with a critical pair resolution of 2.3. Furthermore, the method showed excellent linearity for the six additives across a broad range of injection volumes (0.2–20 µL) with a remarkable %RSD exceeding 0.9999 in six replicate injections. The instrument's precision in delivering injection volumes was beneficial for accurately quantifying caffeine aspartame and benzoate in diverse soft drink samples.

Benefits

- Using an Alliance iS HPLC System for the separation and quantification for six soft drink additives in a 17-minute isocratic method
- The Alliance iS HPLC System can accommodate higher pressures, allowing the use of longer columns, which in turn provides better separations
- The method fully resolved all additives from aspartame degradation products
- The ability to precisely and accurately inject small volumes of concentrated samples, which minimizes sample dilution

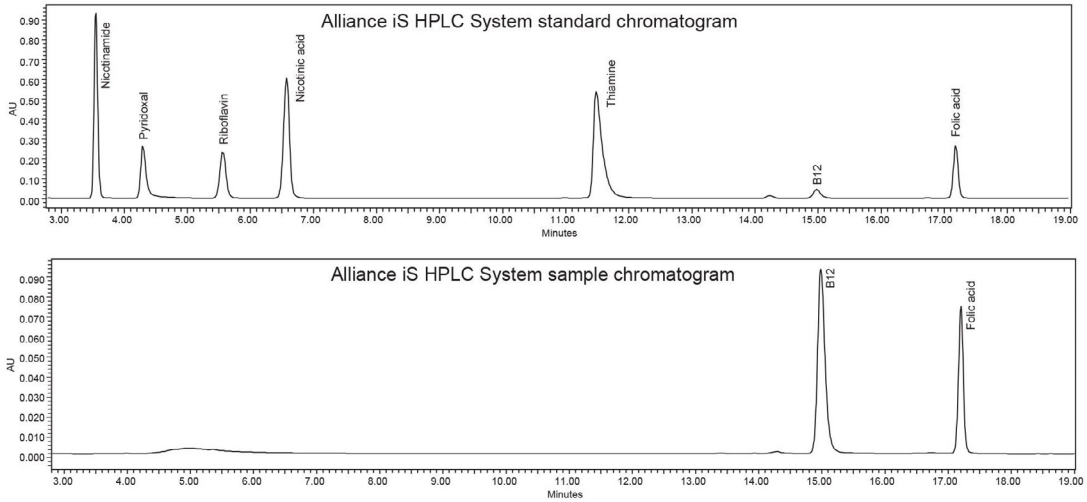


Representative separation of five different beverages using the previously outlined method, with the only deviation from prior conditions being the injection volume. All samples were injected with a 5-µL volume, except for the energy drink, which utilized a 2-µL injection volume.

Read the Application Note

Replicating an HPLC Method for Water-Soluble Vitamins on the Alliance iS HPLC System

With the increasing popularity of vitamin supplements, there is a need to ensure that these products meet the content descriptions. High-performance liquid chromatography (HPLC) is an essential analytical tool to ensure products meet label claims. However, the wide range of chemical characteristics makes analysis of vitamins with a single mode of chromatography challenging. In this work, we will document the ability to move methods for water-soluble vitamins under gradient hydrophilic interaction liquid chromatography (HILIC) conditions across HPLC systems. Here, we will demonstrate the ability to achieve the same quantitative results on legacy and newer HPLC systems. The legacy Alliance e2695 System and the Alliance iS HPLC System were chosen for method migration.



Chromatograms for the Water-Soluble Vitamin Standard (top) and Vitamin Supplement Sample (bottom) obtained on the Alliance iS HPLC System.

Benefits

- Quantitation of water-soluble vitamins in multivitamin supplements using the Alliance iS HPLC System
- Easy method transfer from legacy HPLC systems to the Alliance iS HPLC System

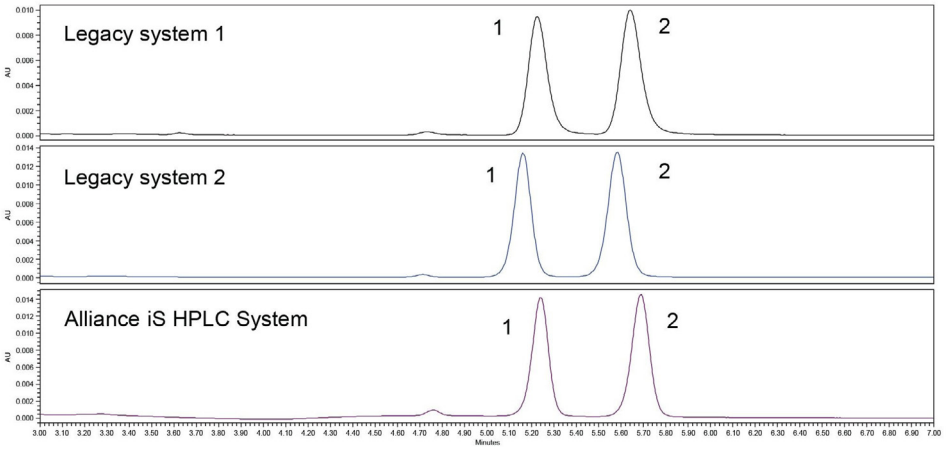
Read the Application Note

Hydrophilic Interaction Liquid Chromatography (HILIC) Method Migration: From Legacy HPLC Systems to the Alliance iS HPLC System

This application note helps illustrate a successful hydrophilic interaction liquid chromatography (HILIC) method migration from legacy HPLC systems found across industries to the Alliance iS HPLC System. Due to differences in HPLC instrument designs, HILIC method migration could be challenging since results could potentially be impacted. A well-designed system like Alliance iS HPLC System, helps overcome those challenges to allow for seamless migration. For this study, the United States Pharmacopeia (USP) monograph for cetirizine hydrochloride assay and organic impurities were analyzed on two legacy HPLC systems and migrated to the Alliance iS HPLC System. All three systems met suitability requirements and demonstrated comparable results with improved signal-to-noise observed on the Alliance iS HPLC System.

Benefits

- Seamless HILIC method migration from standard HPLC systems to Alliance iS HPLC System
- Improved signal-to-noise (s/n)

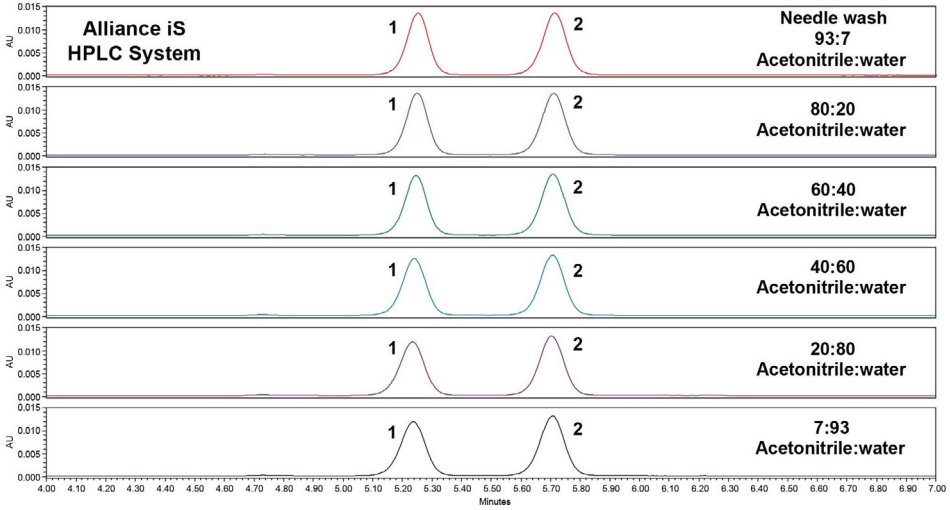


Representative chromatograms of the Organic Impurities System Suitability Solution on all three HPLC systems. Peak 1 = cetirizine related compounds A. Peak 2 = cetirizine hydrochloride.

Read the Application Note

Hydrophilic Interaction Liquid Chromatography (HILIC) Method Migration: Troubleshooting Peak Splitting of Cetirizine

Lack of familiarity with hydrophilic interaction liquid chromatography (HILIC) methods, which are known for their unique and complex separation mechanisms, can pose challenges for system set ups given the differences in strong and weak solvents as compared to reversed-phase LC. This challenge may be more prevalent when setting up a pharmacopeial analysis since only key method parameters are included in the monograph. The Alliance iS HPLC System enables the use of a very strong HILIC wash solvent by ensuring there is no interaction between the sample flow path and the wash solvent. For this study, the system suitability solution sample from the cetirizine hydrochloride organic impurities USP monograph was used to assess the impact of the needle wash and needle wash design for the Alliance iS HPLC System and a comparable HPLC system (referred to as Vendor X HPLC) on HILIC separations.



Cetirizine HCl system suitability solution chromatograms obtained using various needle wash compositions on the Alliance iS HPLC System. Peak 1: Cetirizine RC A. Peak 2: Cetirizine HCl.

Benefits

- Improved needle washing mechanism on the Alliance iS HPLC System
- HILIC method migrations

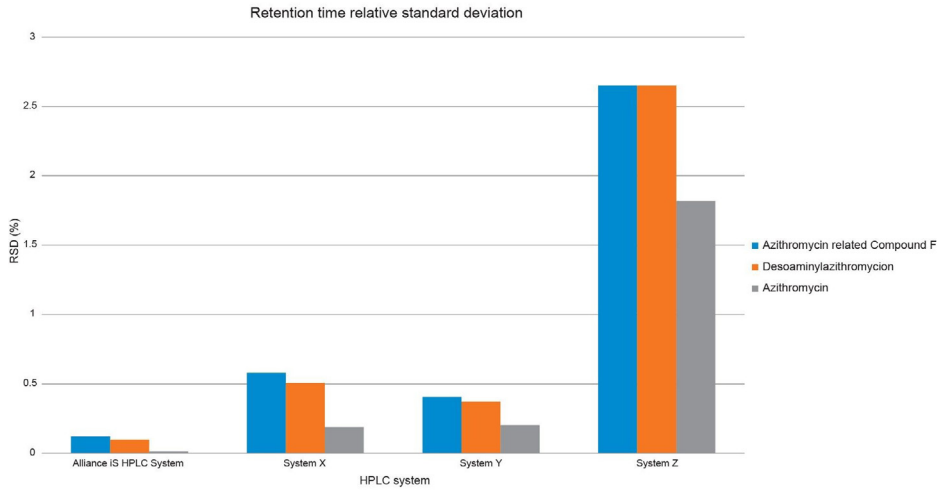
Read the Application Note

Analysis of Azithromycin on the Alliance iS HPLC System: System Performance under Challenging Method Conditions

Many existing reversed-phase monographs contain a combination of conditions that can impact long term system performance. For example, traditional HPLC gradient methods may use both buffered and organic mobile phases at proportions that the salt may be insoluble, potentially leading to precipitation of the buffer during the analysis. While these methods have been validated and can be performed on several different HPLC systems, these conditions can lead to system-to-system variability while still meeting the relevant suitability requirements listed by the USP. The USP monograph for azithromycin organic impurities is one such example. This application note will discuss the challenges of this method and compare the results obtained across several different HPLC systems.

Benefits

- Alliance iS HPLC System meets system suitability criteria for USP monograph of Azithromycin
- Superior retention time precision for high salt mobile phases under HPLC conditions
- Stable performance over 30 hours under high salt mobile phase conditions



Comparison of retention time %RSD values generated for key compounds azithromycin related compound F, desosaminylazithromycin, and azithromycin.

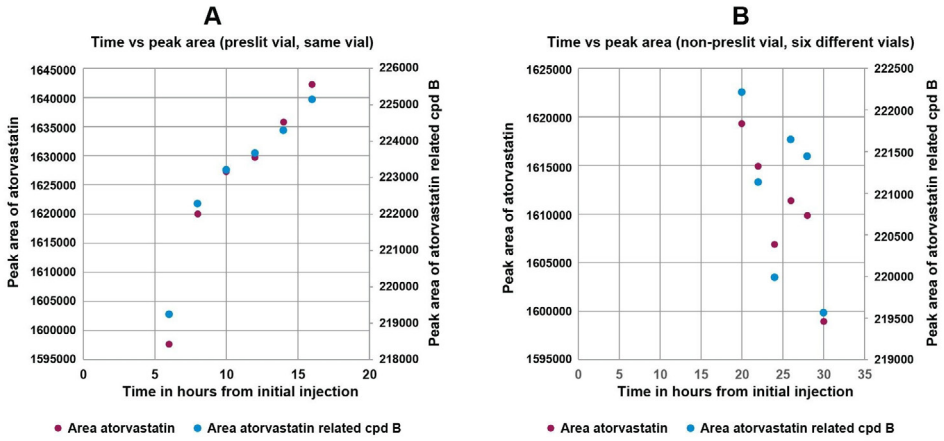
Read the Application Note

Analysis of Atorvastatin as per USP Monograph Utilizing the Alliance iS HPLC System

The USP monograph of atorvastatin requires adherence to strict criteria for the assay analysis of atorvastatin in terms of peak area/retention time precision, peak tailing, and resolution. Specifically, the monograph requires that the relative standard deviation (RSD) for multiple injections (between three and six) to be less than 0.6% for peak areas and retention time of atorvastatin. In this study, several strategies were implemented to meet the stipulated USP monograph criteria. These measures included using low evaporation caps for mobile phase bottles, utilizing non-preslit septa for vial caps, and maintaining samples at a controlled temperature of 10 °C in the autosampler. These precautions are in conjunction with the Alliance iS HPLC System and resulted in very consistent peak areas and retention times for both atorvastatin and its related compound.

Benefits

- The utilization of Alliance iS HPLC System allowed for highly consistent peak area and retention time of atorvastatin
- Excellent control of flow rate, sample temperature, and column temperature
- Low evaporation ACQUITY™ APC™ Reservoir Cap mitigates the risk of evaporation when volatile solvents are used in the mobile phase

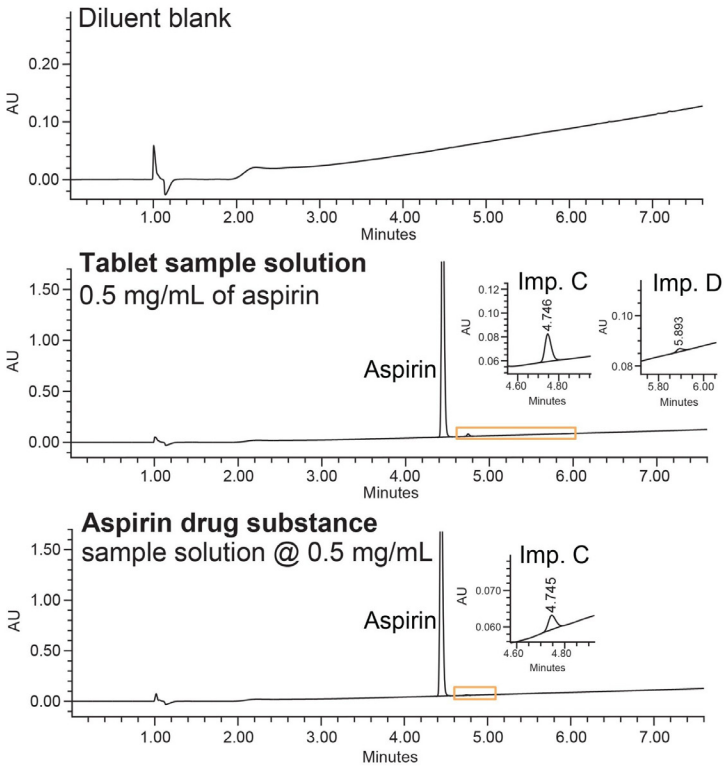


Peak area data for atorvastatin and atorvastatin related compound B over a 30-hour period. Graph A depicts six injections obtained from a single vial, capped with a preslit septa, while Graph B illustrates data from six injections using six different vials, each capped with non-preslit septa.

Read the Application Note

Reliable Analysis of Aspirin and Related Substances in Drug Substance and Tablet Formulation Using an Alliance iS HPLC System

Robust and reliable analytical test methods are essential to ensure the quality and safety of pharmaceutical drug products. This work describes a single high-performance liquid chromatography (HPLC) method for the analysis of aspirin active pharmaceutical ingredient (API) and six associated related substances. The analysis is performed on the Alliance iS HPLC System with the Waters XSelect™ HSS T3 Column. The system suitability, linearity, accuracy, intraday, and inter-day method performance are assessed, generating excellent results while meeting the USP requirements (USP43-NF38, Aspirin Tablets). This work also demonstrates applicability of the method for a reliable and accurate determination of aspirin assay and related substances content in drug substance and tablet formulation.



Sample solutions for analysis of related substances. UV at 237 nm.

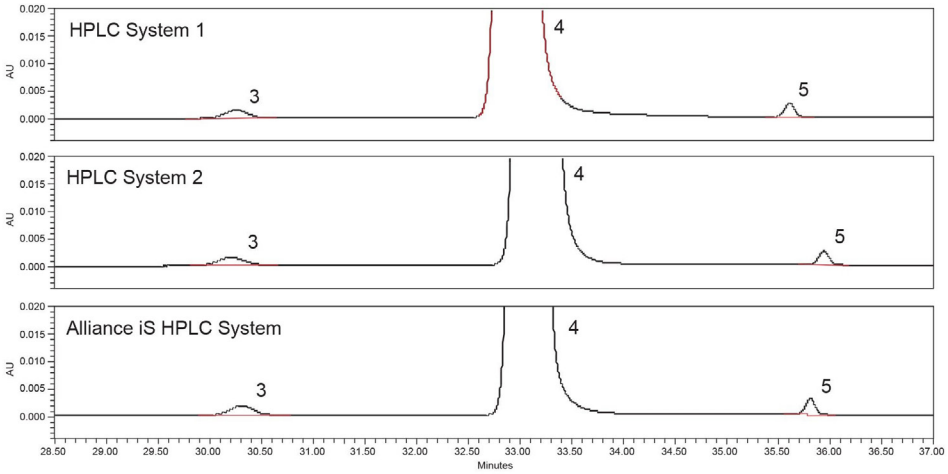
Benefits

- Reliable and quick method (within 7.6 mins) for the simultaneous determination of aspirin assay and related compounds (impurities) in the drug substance and tablet formulation
- Precise, repeatable, linear, accurate, and excellent intraday, and inter-day method performance demonstrated on the Alliance iS HPLC System

Read the Application Note

Successful Method Migration of the USP Quetiapine Fumarate Impurities Method to an Alliance iS HPLC System

In regulated laboratories, method migration is an important aspect to move an established method from one High-Performance Liquid Chromatography (HPLC) system to the same or different HPLC system. Successful method migration to a new HPLC system can be challenging due to differences in the instrumentation that may affect the chromatographic results. In this application note we will look at how the Alliance iS HPLC System has many important elements that will help to achieve a successful method migration. The USP method for quetiapine fumarate impurities will be analyzed on two legacy HPLC systems and then analyzed on the Alliance iS HPLC System to compare and evaluate the method using an unknown sample of the drug substance and the system suitability requirements found in the USP method.



The Sample Solution chromatographic results on HPLC System 1 (top), HPLC System 2 (middle), and the Alliance iS HPLC System (bottom). All quantitative results were within 0.01% of each other on all systems. Peak identification - Peak 3: quetiapine desethoxy, Peak 4: quetiapine, and Peak 5: unknown impurity.

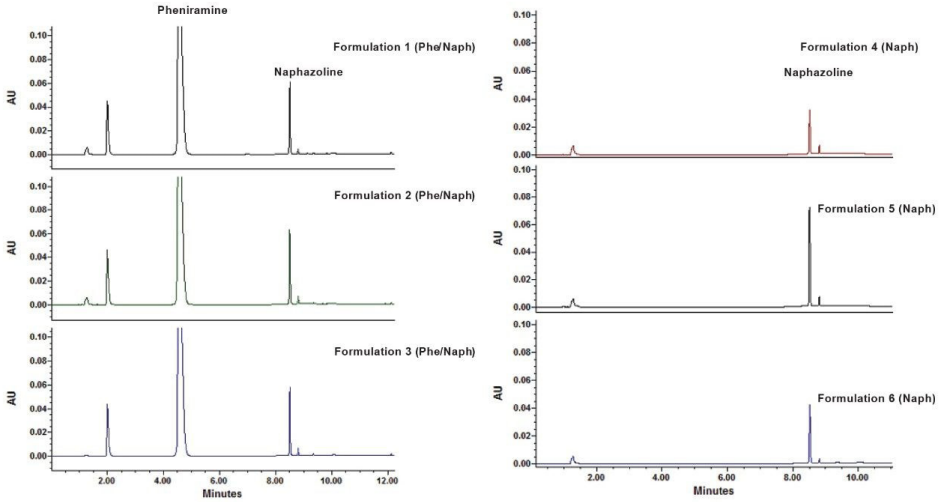
Benefits

- Straightforward and successful method migration of a USP impurity monograph to the Alliance iS HPLC System
- Reproducible quantitative results of the quetiapine fumarate drug substance
- Increased injection precision achieved on the Alliance iS HPLC System

Read the Application Note

Simultaneous Determination of Naphazoline Hydrochloride and Pheniramine Maleate with their Related Compounds

In this study, a liquid chromatographic method was developed for simultaneous determination of two active ingredients - naphazoline hydrochloride and pheniramine maleate ophthalmic, along with their related compounds. The method achieved complete separation of analytes within 20 minutes, in a single run at a temperature of 40 °C and a flow rate of 2.0 mL min−1, using a Waters XSelect CSH C_{18} Column. A comprehensive evaluation of system suitability, range, accuracy (recovery), and intraday and inter-day precision was performed as a part of this study. The method was also found to be linear in the range of 80 to 120% with respect to the API concentration in a working concentration, displaying a correlation coefficient (R2) greater than 0.997. As a practical application, the method was successfully used for the routine analysis of commercially available ophthalmic and nasal solutions, without significant interference from the excipients.



Representative separation of commercially available nasal solutions that contain pheniramine (PHE) and naphazoline APIs (formulations 1, 2, and 3), as well as solutions that contain naphazoline API only (formulations 4, 5, and 6).

Benefits

- A single LC method was developed to combine three USP monographs for naphazoline HCl and pheniramine maleate ophthalmic and nasal solutions
- Alliance iS HPLC System enabled rapid and reliable separation of multiple APIs along with their related compounds in a single HPLC method

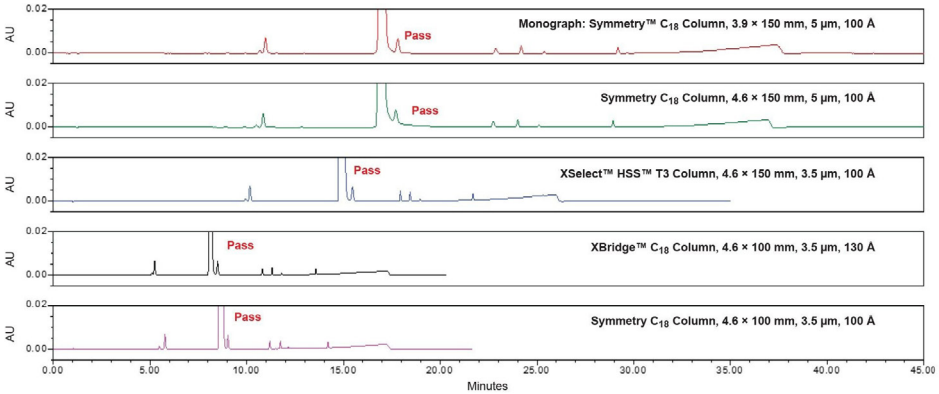
Read the Application Note

Achieving Method Modernization with the New Liquid Chromatographic Gradient Allowances Provided by USP General Chapter <621> Chromatography and the Alliance iS HPLC System

The extent to which the various parameters of a chromatographic test may be adjusted without fundamentally modifying the pharmacopeial analytical procedures is defined in U.S. Pharmacopeia (USP) General Chapter <621> Chromatography. In this application brief, we combine the gradient method adjustments described in this chapter with the Alliance iS HPLC System to achieve both column dimension and system modernization for the USP monograph separation of antiviral drug, abacavir sulfate.

Benefits

- When paired with the Alliance iS HPLC System, USP General Chapter <621> Chromatography gradient method allowances generate quality data that meet regulatory requirements
- The Alliance iS HPLC System's extended chromatographic backpressure limits provide high-efficiency separations using an array of modern column dimensions resulting in run time, injection volume, and solvent savings



Overlay of monograph and adjusted column chromatograms.

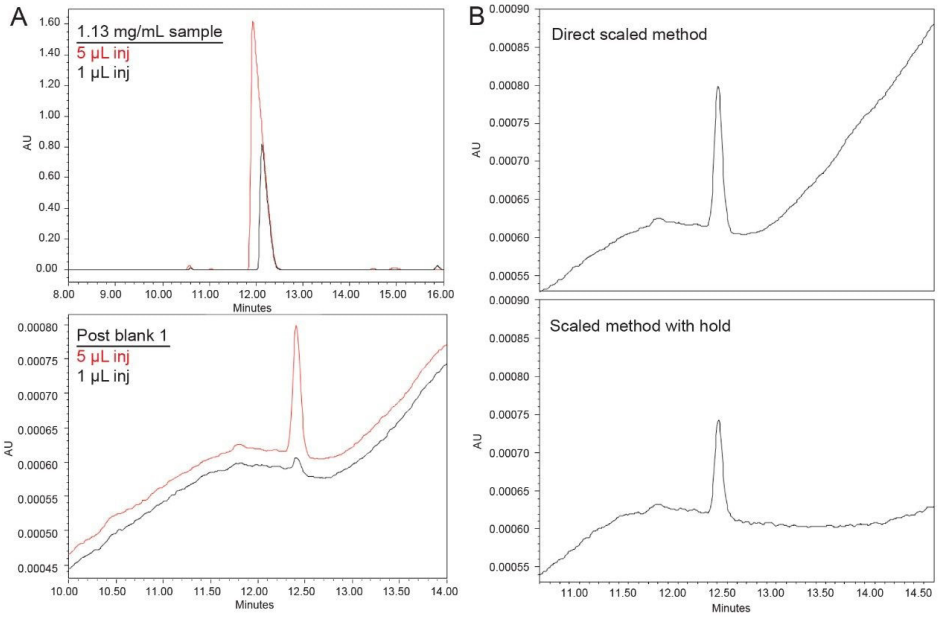
Read the Application Note

Improved Chlorhexidine Carryover Performance Using the Alliance iS HPLC System

Carryover is an all-too-common problem for many users of High Performance Liquid Chromatography (HPLC) systems. There are multiple forms of carryover, including volumetric, or carryover as a result of void volumes in the flow path, and adsorptive carryover, where sample "sticks" or adsorbs to surfaces of the flow path. If a method suffers from carryover, it is important to know which form of carryover it is so that you can most effectively eliminate the source of the carryover. In many cases, methods may display both volumetric and adsorptive carryover. In this application note, carryover of chlorhexidine will be evaluated on a variety of HPLC systems across various vendors. In addition, mitigation strategies, namely implementation of needle wash and/or extending washing will be explored as well.

Benefits

- Improved carryover performance using the Alliance iS HPLC System
- The Alliance iS HPLC System uses easy to use tool-free fittings to eliminate volumetric carryover
- Improved wash function to significantly reduce adsorptive carryover

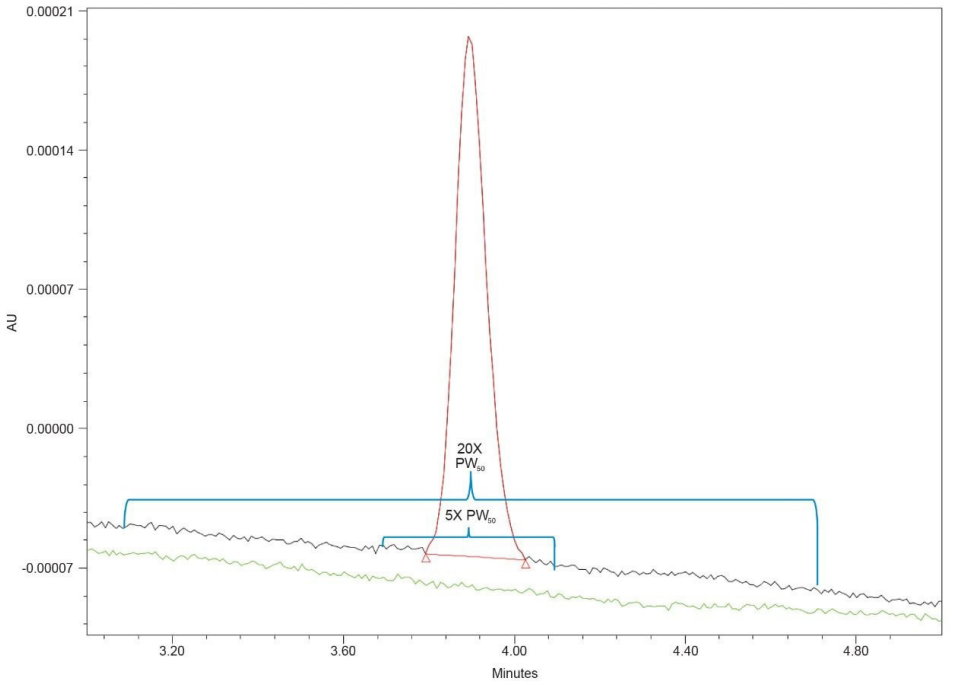


A - challenge sample (top) and post blank 1 (bottom) demonstrating measured carryover vs injection volume. B - extended gradient step to provide flat baseline in region of chlorhexidine peak elution.

Read the Application Note

Method Migration of the USP Ibuprofen Assay and Organic Impurities Method to an Alliance iS HPLC System

Method migration, or moving a method from one high-performance liquid chromatography (HPLC) system to another, is routine practice in many regulated laboratories, where there may be a variety of HPLC systems from different vendors and different models in a single lab. However, moving a method across systems can be challenging. The Alliance iS HPLC System aims to make method migration more straightforward with improved usability features that limit analyst error and improve system reliability while maintaining chromatographic and quantitative performance. In this study a United States Pharmacopeia (USP) monograph method for the analysis of Ibuprofen and related impurities is replicated on two legacy HPLC systems and subsequently migrated to the Alliance iS HPLC System. Results are analyzed and compared, with all three systems demonstrating comparable results while meeting the USP system suitability requirements.



Overlaid chromatograms of the sensitivity solution and a blank injection on the Alliance iS HPLC System. PW_{50} = peak width at half height.

Benefits

- Ability to migrate USP monographs to the Alliance iS HPLC System, meeting system suitability requirements
- Consistent and reliable quantitative results
- Decreased user error

Read the Application Note

waters.com/AllianceiS

For your local sales office, please visit waters.com/contact

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
waters.com

Waters™