

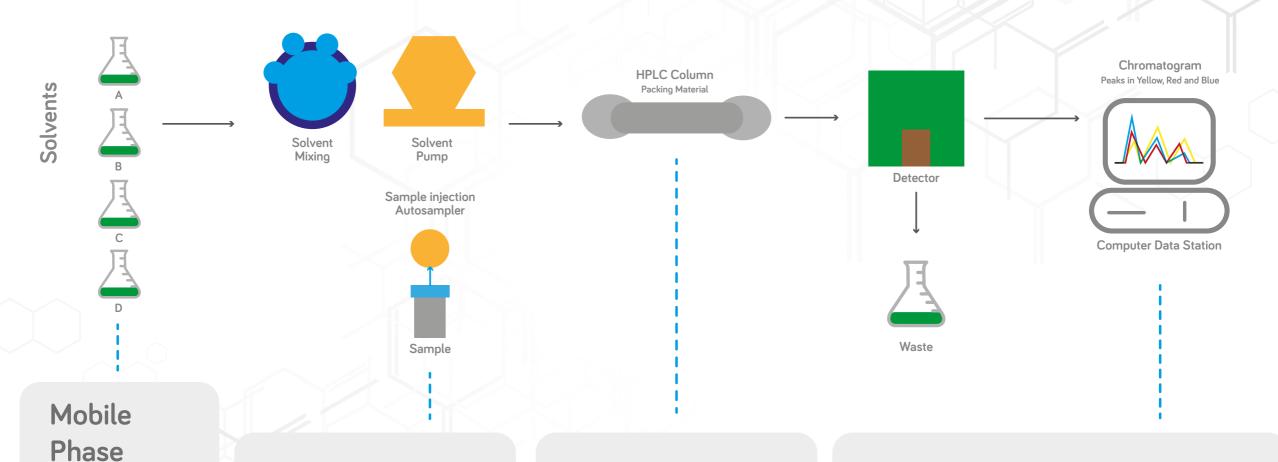
SAMPLE PREPARATION

METHOD DEVELOPMENT INSTRUMENTS AND SOFTWARE

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Many factors in HPLC and LC-MS workflows can impact analysis. Considering the effects of the whole workflow affords improved control of analysis robustness and accuracy. Here, we systematically discuss the factors central to developing methods both efficiently and reproducibly.

Sample Preparation



Column Selection



Preparation

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Instruments and Software



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Mobile Phase Preparation

Mobile phase preparation is vital to the performance of HPLC and LC-MS workflows

There are several factors that can harm analysis, such as contamination from the HPLC system or use of low-quality consumables and reagents; these can cause ghost peaks and introduce impurities, ruining your HPLC or UHPLC analyses.

Ghost peaks are problematic because these non-identified peaks can lie close to, or even overlap with, peaks of interest. Uncertainty in data quality and reliability is of course the result.

Particulates introduced during the incorrect preparation of mobile phases can lead to poor peak shape, cause the appearance of additional peaks, and can also block the HPLC system, causing costly delays.

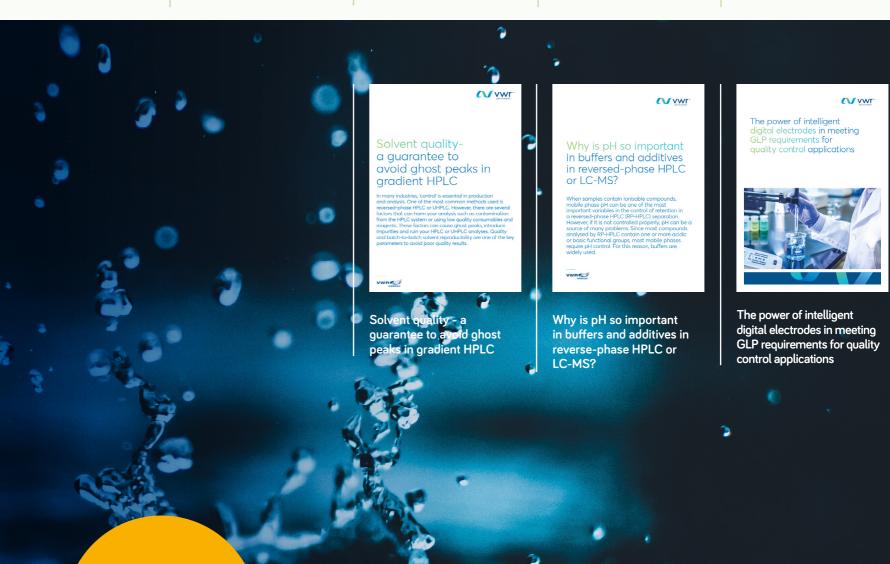
Improperly prepared buffers or buffers of the wrong concentration can also cause problems. Incorrect preparation can affect retention times, and increase the level of baseline noise in your sensitive HPLC or UHPLC runs.

Controlling all of these parameters is an important but simple way to improve performance. Here, we discuss solvent quality, buffer preparation and mobile phase filtration.

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Sample Prep with Avantor SPE

For as long as scientists have been analyzing compounds, there has been a need for sample preparation to extract and purify sample components.

Today's technology for sample preparation, solid phase extraction (SPE), is grounded in the principles of chromatography and offers increased speed, reduced hazardous solvent use and exposure, and improved reproducibility of the separation when compared to other wet chemistry methods, such as liquid/liquid extraction. Our scientists have been using and perfecting this method in our research and development labs since the 1970s.

The purpose of sample preparation is twofold:

- Sample clean up necessary to eliminate impurities and/or isolate the component of interest from the matrix. This helps to increase the lifetime of the analytical column and prevents contamination of equipment, thereby protecting expensive instrumentation.
- Sample concentration in order to reach the detection limits of the analytical equipment. Sample preparation selectively concentrates the components of interest prior to analysis.



Solid phase extraction products



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Accelerating UHPLC/HPLC Method Development and Maximizing Chromatographic Selectivity with Novel Stationary Phase Chemistries

Accurate and reproducible HPLC analyses with excellent turnaround time and throughput are increasingly important in modern analytical laboratories. For those laboratories that must develop, validate and use methods over a significant period of time, or those that must transfer methods to other laboratories around the world, it is a practical business advantage to be able to develop robust methods more quickly. For both situations, it can be beneficial to have a diverse group of column chemistries, from which you can choose and use to explore chromatographic selectivity.



Accelerating UHPLC/HPLC method development and maximizing chromatographic selectivity with novel stationary phase chemistries



The power of stationary phase selectivity



Webinar

A UHPLC/HPLC method
development strategy with
complementary stationary phases
to maximise selectivity and
resolution





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ACE UHPLC and HPLC Columns

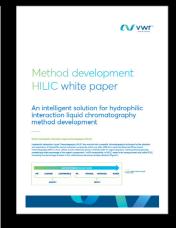
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An Intelligent Solution for Hydrophilic Interaction Liquid Chromatography Method Development

Efficient method development procedures require a logical exploration of key chromatographic parameters, leading to a robust method using a suitable combination of column and mobile phase. Rationally designed method development procedures assess key parameters that affect chromatographic selectivity (e.g. stationary phase, pH etc.) and enable analysts to make informed decisions whilst reducing the risk of developing sub-standard, non-robust methods. By following a step-by-step process, method development can be streamlined thereby and laboratory productivity increased. The approach outlined here is based on a logical assessment of the most powerful parameters affecting HILIC selectivity.



Method development

HILIC white paper



A simple step-by-step protocol for HILIC method development



Webinar

How to improve method development strategies in HILIC conditions?



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ACE UHPLC and HPLC Columns

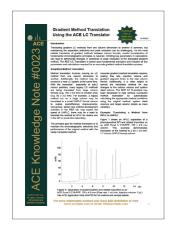
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Gradient Method Translation Using the ACE LC Translator

Translating gradient LC methods from one column dimension to another is common, but maintaining the separation selectivity and peak resolution can be challenging. For the most reliable translation of gradient methods between column formats, careful consideration of fundamental chromatographic principles is required. Omitting key parameters in calculations can lead to detrimental changes in selectivity or peak resolution of the translated gradient method. The ACE LC Translator is based upon fundamental principles and includes all key parameters and calculations required for an accurate gradient method translation process.

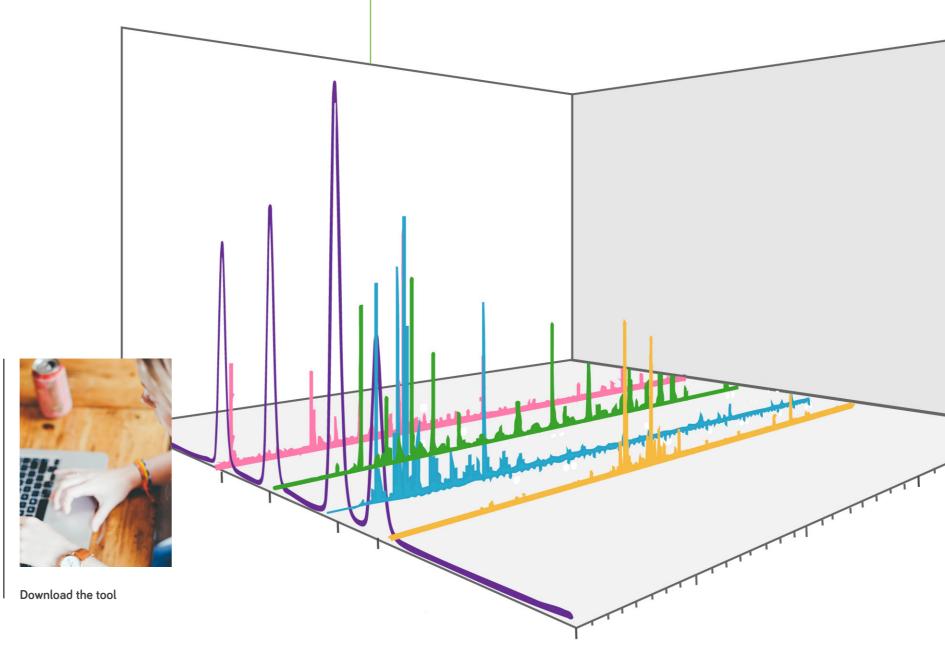


Using the ACE LC Translator



Webinar

Practical UHPLC: selectivity and rapid method development, method translations and instrument transfers









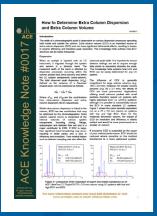




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Tips and Tricks

There are many calculations that need to be done in HPLC depending on your analysis needs. We discuss some of the common considerations and give you a tool to help you calculate them to make your HPLC/UHPLC more efficient.



How to determine extra column dispersion and extra column volume



How to determine column volume and porosity



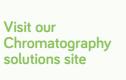
How to determine system dwell volume: theory and practice



Download the tool









ACE UHPLC and HPLC Columns



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Reversed-phase method development can be a lengthy process that consumes valuable resources in an analytical laboratory. The process followed and success obtained can vary depending upon experience, resources and time available. Often, a trial-and-error (or one factor at a time) approach is adopted, where parameters are adjusted and decisions made according to the analytical results obtained for each iterative step. This approach can produce acceptable separations but may fail to identify the most suitable method; in other words, the most robust or cost-effective methods. A structured approach to method development is helpful for many reasons: it can lead to the development of improved and more robust methods, generate useful retention knowledge for analytes, and has the potential to provide significant savings regarding both development time and costs. A popular approach is to use screening protocols to systematically explore individual chromatographic parameters (such as column stationary phase, eluent composition, pH, and so on) and their effects on retention/separation. Once screening is complete, the most promising combination of conditions can be further optimized, if needed, to produce the final method. This approach is useful, informed and highly recommended.

selectivities



ACE UHPLC and HPLC Columns











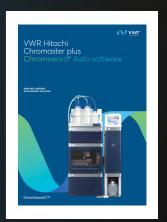
The Chromsword Auto® software package is capable of developing and optimizing HPLC methods automatically in just a few hours. Methods already in use can also be optimized, thus reducing analysis time considerably. Plan your experiment in eight simple steps, set up the mobile phases as required, place your sample in the autosampler and click "start". Chromsword Auto® then works to predict the method conditions by fully controlling the HPLC system connected. The acquired data are analyzed by the software and optimal conditions determined.

The rapid optimization mode can be used to provide an overview of the performance of the column/solvent system selected, or to rapidly screen columns and eluents considered for use. The fine optimization mode also collects and evaluates further ancillary data and produces up to ten sets of alternative optimal HPLC conditions.





Automated Method Development in Accordance with QbD Principles



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