GAIN GREATER CONFIDENCE

AGILENT SOLUTIONS FOR QUALITY-BY-DESIGN IMPLEMENTATION IN PHARMACEUTICAL DEVELOPMENT

Primer

The Measure of Confidence

Agilent Technologies

CONTENTS

INTRODUCTION: AN OVERVIEW OF QUALITY BY DESIGN

Quality by design (QbD) is a systematic approach to drug development that begins with predefined objectives and uses science and risk management to gain better product and process understanding and, ultimately, process control.[1]

Applying QbD principles at every stage of development and manufacturing helps enhance process robustness and prevent failures at late stages.

In essence, QbD helps ensure consistent drug quality by moving beyond traditional quality-control methods to instead test intermediates and end-products against a risk-based control strategy for well-understood products and processes.[1]

QbD places extra emphasis on quality controls upstream through detailed scientific investigation into eliminating variability.[3] Implementation of QbD also warrants a closer look at impurities and degradation products.

The QbD concept was introduced by the International Conference on Harmonization (ICH) document Q8, supported by Q9, Q10, and Q11. Both the U.S. Food and Drug Administration (FDA) and the European Medicine Agency (EMA) are supporting the pharmaceutical industry in the implementation of QbD principles. A three-year pilot program was announced in March 2011 by the FDA in partnership with the EMA to ensure consistent implementation of ICH Q8-11. As part of this pilot program, two sets of Q&A documents were published in 2013, clarifying certain QbD elements.[2] Among other things, the agencies expect companies to provide a list of critical quality attributes (CQAs) and acceptance limits for each CQA for drug substance, finished product, and excipients, plus a rationale for designating these properties as CQAs.

Traditional approach	Quality by Design approach
Product specifications: primary means of control based on batch data available	**Product specifications:** based on desired product performance and overall quality-control strategy
Quality control: mainly by testing of intermediates and end products	**Quality control:** risk management-based control strategy for well-understood products and processes
Life cycle management: Reactive	**Life cycle management:** based on continuous improvement.

Comparing the traditional approach to drug development and manufacturing with the QbD approach.[1–3]

Pharmaceutical laboratories have started applying QbD concepts, viewing analytical methods as processes.[3] These labs are using risk assessments and statistically designed experiments to define analytical target profiles (ATPs) and method operational design ranges (MODRs) for analytical methods.

Beyond just keeping the focus on process validation, full exploration of method variables is critical to develop robust analytical methods that are applicable throughout the life of the product. It is widely anticipated that QbD in analytical development will significantly reduce the effort and cost related to post-approval variation.

QbD approaches for analytical methods could logically involve understanding how analytical results are affected by variation in input parameters; evaluating multivariate interactions originating from the instrument, laboratory, sample, method parameters, analyst, etc.; and incorporating prior knowledge of analytical techniques and methods.

Agilent strives to provide the tools and technologies you need to be successful, and earn your trust as a partner in implementing solutions. This document provides information about the tools Agilent offers to support QbD implementation in drug development. Agilent offers tools that support science- and risk-management-based approaches. Our tools are designed to ensure consistency in product quality through detailed investigation of variability. With the help of our tools, you can set the design space for robust analytical methods, eliminate variability in method transfer, and evaluate CQAs.

Agilent Solutions Support QbD Implementation

Eliminating variability in analytical method development

Solution: Automated LC method development solution with multivariate analysis for design space evaluation

Products: 1200 Infinity Series Method Development Solution + QbD software (ChromSword, ACD labs/AutoChrom, S-Matrix/FusionAE)

Solution: Instrument compliance for robust analytical method development in a quality by design environment

Products: Agilent Analytical Instrumentation Qualification (AIQ) services

Eliminating variability in analytical method transfer

Solution: Method transfer of any legacy or latest UHPLC methods to deliver the same chromatographic results without the need to modify the original method or the instrument hardware

Products: 1290 Infinity LC + ISET

Solution: Automated/dynamic online mixing of solvents to minimize the instrument specific contribution and operator contribution to the variance in method transfer

Products: 1290 Infinity Quaternary Pump

Quality risk assessment and evaluation of critical quality attributes (CQAs) for drug substances and drug products

Solution: Dissolution testing for evaluation of CQAs

Products: Dissolution apparatus, online UV

Solution: Increased precision in identification and quantification of impurities for quality risk assessment

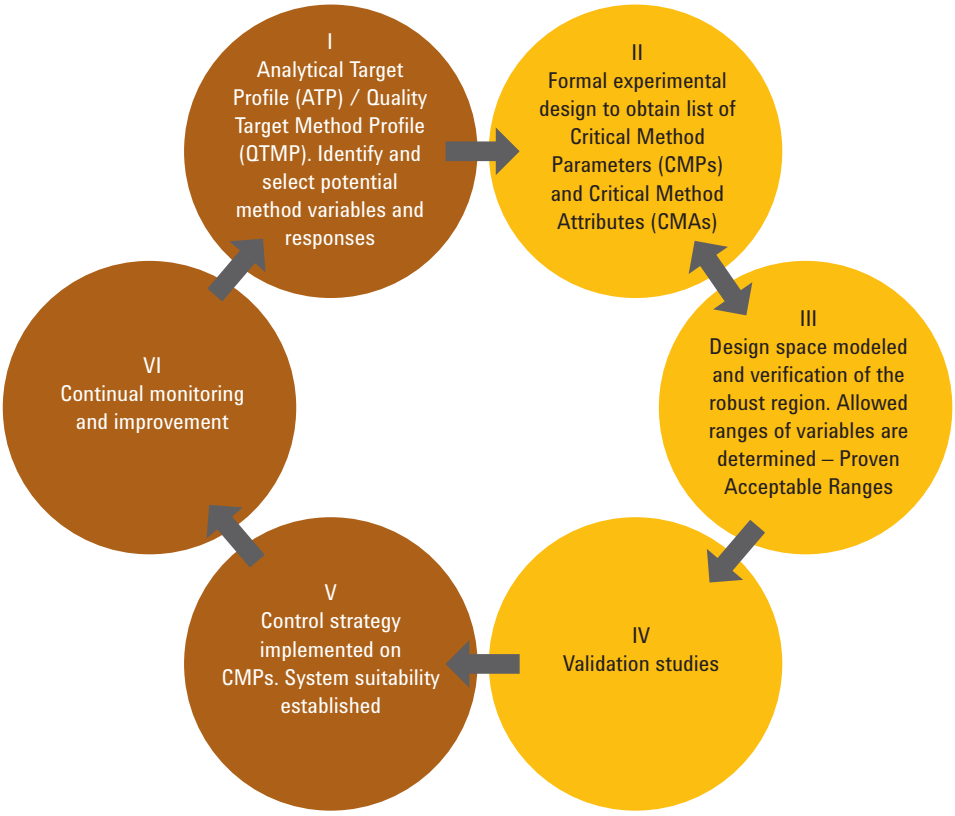
Products: 1290 Infinity Series HDR DAD, 1290 Infinity 2-D LC, 7900 ICP-MS, 700 Series ICP-OES

NOTE: The solutions and experimental data provided as part of this brochure are for illustrative purposes and, depending on the nature of each project, the approach and degree of experimentation for a particular product or process may vary. The concepts and ideas expressed do not reflect official company policies. We recommend that all readers consult official regulatory websites and other reliable sources for the latest information.

ELIMINATING VARIABILITY IN ANALYTICAL METHOD DEVELOPMENT

QbD requires the application of science- and risk-management-based methodology to analytical method development, which involves assessing risk, defining a design space, establishing a control strategy, and continuously improving method robustness. QbD-based analytical method development helps to identify and minimize sources of variability that may lead to poor method performance. It also ensures that the method meets its intended performance requirements throughout the product and method life cycle. Quality is built into the development of the method itself, resulting in improved separations. The systematic examination of variables prior to experimentation also allows you to focus your experiments on the optimal range for each variable.[3–5]

The following represents a typical workflow for QbD-based analytical method development designed to create a reliable method through the life cycle of its use:



Typical workflow for QbD based analytical method development. Steps II, III, and IV represent software-assisted sections.

The first step in developing QbD-based analytical methods is to define the goals and select variables before starting any experiments. The analytical target profile (ATP) states the intended purpose – what the method is designed to measure and the desirable responses are.[3]

In this definition phase, you need to thoroughly examine potential variables, particularly variables and their ranges that may impact the performance of the method. Next, conduct a risk assessment of critical variables and their range of attributes/responses. Then, using science-based statistical design of experiments, select those parameters which have the greatest influence on selectivity and method performance. With the help of statistical software, you can model the experimentally measured responses to determine the design space. The allowed deviations of the variables – the proven acceptable ranges – form the robust region where deliberate variations in the method parameters do not change the critical method attributes. Studying the multivariate interaction of variables and process parameters will enhance your understanding of variability in method performance, which further provides assurance of quality and prevents method failure downstream. If the modeling experiments do not lead to desired results, you can adjust the method variables and perform further experiments to validate the developed method.

The next step is to evaluate your control strategy to determine if the method serves its intended goals. System suitability should be implemented to ensure that the desired method attributes are delivered. One of the key components of QbD-based analytical method development is continual improvement. If the process requires changes after monitoring the method performance, implementation of continual improvement helps to redefine the ATP.

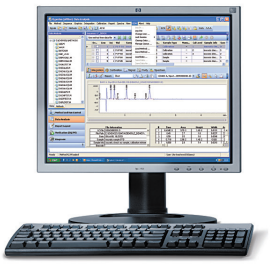
Analytical QbD terminology	Examples
Analytical Target Profile (ATP)	Accurate quantitation of API without interferences from degradants
Quality Target Method Profile (QTMP)	pKa, Log P, solubility
Critical Method Parameters (CMP)	Flow rate, temperature, pH
Critical Method Attributes (CMA)	Resolution, peak tailing, peak capacity
Control strategy	pH ± 0.1; Wavelength ± 2nm

Automated Method Development Solution with Multivariate Analysis for Evaluation of the Design Space

Agilent supports the QbD approach to analytical method development by providing instrumentation and software tools that automate the process and facilitate the multivariate experiments required to implement QbD principles. We offer a wide range of column selectivities. You can use these to explore design options under various conditions. More than 1,000 unique separation conditions can be realized. With the Agilent Method Scouting Wizard, a simple-to-use but highly effective tool, you can define a sequence and all methods to screen a multidimensional matrix of columns, solvents, gradients, and temperatures. The Agilent 1200 Infinity Series Method Development Solution can be combined with sophisticated QbD method development software such as ChromSword, ACD labs/AutoChrom, and S-Matrix/Fusion AE.[6]

Agilent 1200 Infinity Series Method Development Solution

- Up to eight columns and 26 solvents
- Over 1000 unique separation conditions
- Four to six temperature zones
- ID columns from 2.1 to 4.6 mm and up to 300 mm length
- Different configurations/detectors
- Highly scalable from a 1260 Infinity LC-based version up to a full-blown 1290 Infinity LC-based solution, and older 1100 or 1200 Series LCs can be upgraded



OpenLAB CDS ChemStation Edition combined with sophisticated method development tools

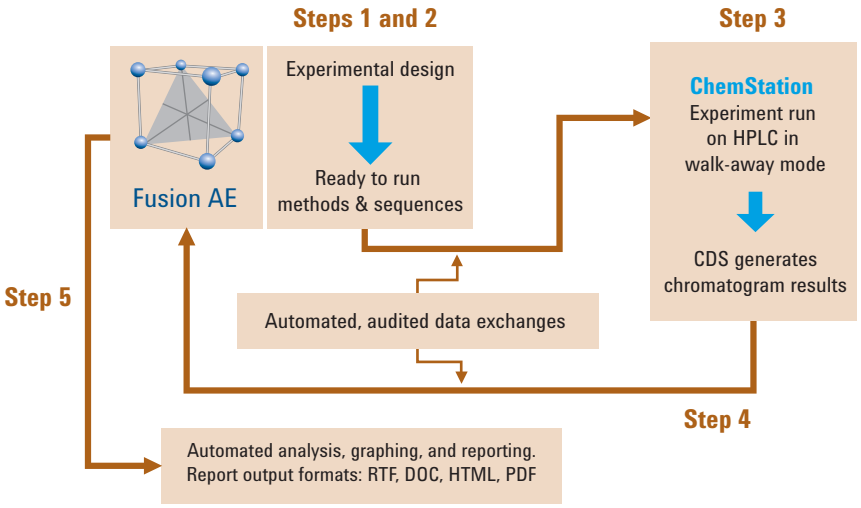
- Agilent Method Scouting Wizard
  - Guides you through setup of sample sequence; takes flushing and column equilibration into consideration automatically
  - Screens samples against a multidimensional matrix of columns, solvents, gradients, and temperatures
- Can be combined with QbD software such as ChromSword, ACD labs/Autochrom and SMatrix/Fusion AE
  - Application of QbD principles to develop robust chromatographic methods
  - Characterization of a method design space
  - Rapid results browsing and automatic report generation

A robust LC columns portfolio

- A wide range of selectivities can be used to optimize resolution, including phases used for pH and temperature extremes
- Varying dimensions and pressure capabilities help optimize your productivity
- Multiple lot availability enables thorough evaluation

Applying QbD Principles to Linagliptin Stability-Indicating Method Development

In the workflow depicted below for Fusion AE automated method development software, QbD principles are applied to stability-indicating method development for linagliptin on the Agilent 1200 Infinity Series Method Development System.[7] Fusion AE software runs the instrument unaided, sets up the design of experiments, tracks components, and models the responses. A statistical design-of-experiments (DoE) concept was applied to screen the mobile phase and column chemistry and for method optimization experiments. Multivariate analysis of critical method parameters (CMPs) such as mobile phase composition, pH, and column temperature was used to determine the final design space.



Fusion AE software: Overnight execution in walk-away mode.

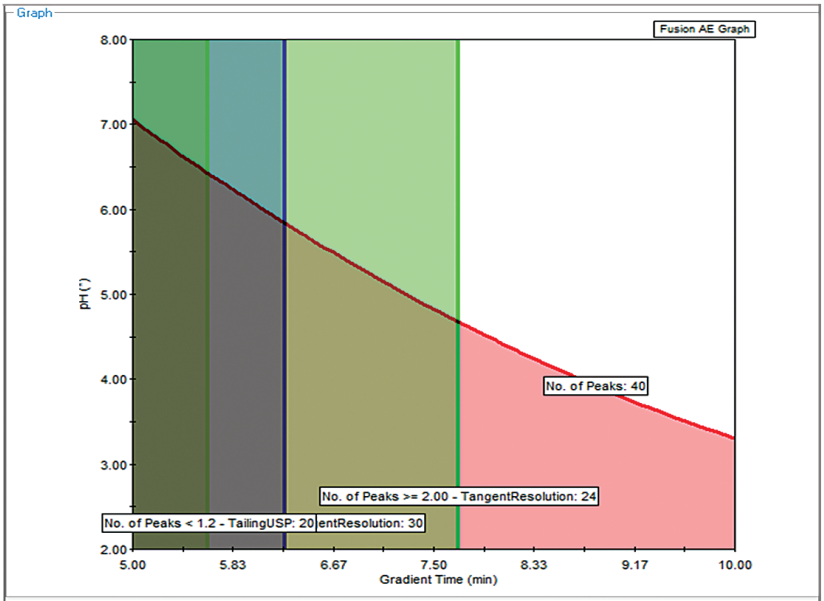
After the quality target method profile (QTMP) is set, the next stage involves initial chemistry screening based on prior knowledge and early risk assessment with mobile phase type, pH, column chemistry, and run time as the variables. The chosen elution mechanism was a gradient based on the QTMP. You can use a statistical design of experiments utilizing full factorial design or other default designs.

Column, Solvent, and Gradient Conditions Used in Screening Experiments

Columns	
Agilent ZORBAX RRHD StableBond C18, 3.0 X 50 mm, 1.8 µm (p/n: 857700-302)	
Agilent ZORBAX RRHD Bonus-RP 2.1 x 50 mm, 1.8 µm (p/n: 857768-901)	
Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 x 50 mm, 1.8 µm (p/n: 959757-306)	
Agilent ZORBAX RRHD StableBond Phenyl 3.0 X 50 mm, 1.8 µm (p/n: 857700-312)	
Agilent PLRP-S 4.0 x 50 mm, 3.0 µm (p/n: PL1512-1300)	
Agilent ZORBAX RRHD Extend-C18, 3.0 x 50 mm, 1.8 µm (p/n: 757700-302)	
Solvents	
Mobile phase A1	pH 2.0, 10 mM TFA in water
Mobile phase A2	pH 5.0, 10 mM ammonium acetate and 5 mM acetic acid in water
Mobile phase A3	pH 6.4, 10 mM ammonium acetate in water
Mobile phase A4	pH 8.0, 10 mM ammonium hydrogencarbonate in water
Mobile phase A5	pH 11.0, 10 mM ammonia in water*
Mobile phase B1	Acetonitrile
Mobile phase B2	Methanol
Gradient	
Initial hold	0.6 min, 5 % B
Gradient time	**Condition 1, 5 min – 5 % B to 95 % B** **Condition 2, 10 min – 5 % B to 95 % B**
Hold	1 minute at 95 % B
Re-equilibrate	3 minutes at 5 % B
Experimental details (constants)	
Pump flow	0.6 mL/min
Injection volume	1 µL
Oven temperature:	40 °C
Wavelength	292 nm ± 4 nm (ref 400 ± 20nm)

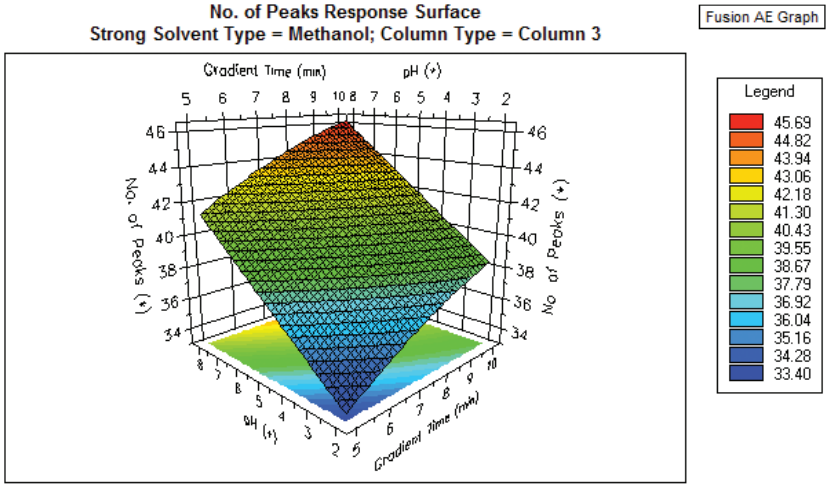
For more information, see Agilent publication 5991-3834EN.

After peak integrations, the data is exported to Fusion AE software, which is used to model the data. The critical method attributes, including number of peaks, resolution and peaks having tailing less than 1.2, are optimized and the software models the contour plot for various columns. The contour plot of the modeled data for Zorbax Eclipse Plus C8 (below) shows the unshaded region as the acceptable region where all the critical attributes are met. The surface plot (further down) shows the region where gradient time greater than 8 minutes and pH greater than 5.5 leads to the maximum number of integrated peaks. In setting up the models in Fusion AE, minimizing the resolution of the largest peak (API) can also provide optimal screening conditions, which show peaks that are eluting near the main peak. This method, in general, was found to be sensitive to pH and gradient time/slope within a range thereby indicating a stringent control on these method parameters.



Response Settings

	Name	Goal	Lower Bound	Upper Bound	Pointer Predictions	Contour Label	Color
☑	No. of Peaks	Maximize	40		42.93	☑	Red
☑	No. of Peaks >= 1.50 - TangentResolution	Maximize	30		33.09	☑	Blue
☑	No. of Peaks >= 2.00 - TangentResolution	Maximize	24		25.19	☑	Green
☑	Max Peak #1 - TangentResolution	Maximize	2.000000000		3.987	☑	Orange
☐	Max Peak #2 - TangentResolution	---	---	---	---	---	---
☐	No. of Peaks >= 1.0 - TangentResolution	---	---	---	---	---	---
☑	No. of Peaks < 1.2 - TailingUSP	Maximize	20		23.81	☑	Lime



The two-dimensional plot [a] of pH as the function of gradient time. The surface plot [b] shows the maximum number of peaks based on gradient time and pH of the mobile phase. For more information, see Agilent publication 5991-3834EN.

Six columns were compared, including Agilent ZORBAX Rapid Resolution High Definition (RRHD) Eclipse Plus C18, ZORBAX RRHD Bonus-RP, and ZORBAX RRHD Phenyl Hexyl. The ZORBAX RRHD Eclipse Plus C8 provided the optimal resolution for the next stage of experiments. The best overall answer from the screening experiments, as shown below, suggests Eclipse Plus C8, pH 7, and gradient time of 10 minutes to be best among the screening experiments. In the next stage, these parameters can be varied further in small increments.

Best overall answer	
Strong solvent	Methanol
Gradient time	10 minutes
pH	7
Column type	Agilent ZORBAX RRHD Eclipse Plus C8 3 × 50 mm, 1.8 µm

For more information, see Agilent publication 5991-3834EN.

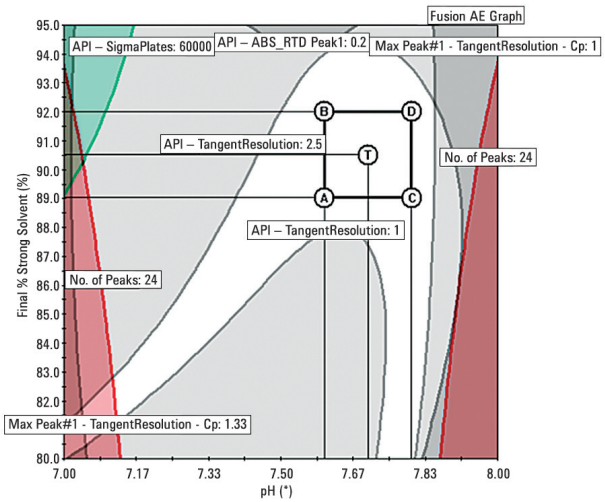
The next stage involves mean performance optimization with starting/ending gradient percentage/slope, run time, a narrower pH range, and temperature as variables. The column chemistry and mobile phase pH are kept constant for determining optimal conditions. The results of mean performance optimization are shown in the table below.

Best overall answer from the optimization study	
Gradient time	15 minutes
Final % strong solvent	90.5 %
pH	7.7
Oven temperature	45 °C

For more information, see Agilent publication 5991-3834EN.

In the final stage, the design space is determined for the critical method attributes (CMAs) in terms of critical method parameters (CMPs). The design space is a region in which changes to method parameters will not significantly affect the results. Operating within the design space leads to a more robust method, as small deviations from the method will not significantly impact the analysis. The design space incorporates both the mean method performance and method robustness (method performance variation). The unshaded region in the graph on the next page defines the design space for the critical responses in terms of the critical method parameters studied, since the design space now incorporates both mean method performance and method robustness.

Final Percentage of Organic Mobile Phase Versus pH of Aqueous Mobile Phase



The unshaded region shows the design space (at 45 °C), and the square marks the region with maximum robustness. For more information, see Agilent publication 5991-3834EN.

With greater understanding of the method (variability from pH, percentage of strong solvent, oven temperature), you can define robust operating ranges (control limits). Deviations of the variables within the design space represent the allowed changes where you can still expect to meet the method performance of resolution and peak tailing criteria. The table below summarizes the findings based on the desired method attributes. Experiments can be performed to validate the robustness of the developed method.

Critical Method Parameters (CMPs)	Proven Acceptable Range (PARs)	Critical Method Attributes (CMAs)
Column: Agilent ZORBAX RRHD Eclipse Plus C8 3.0 x 50 mm, 1.8 µm	—	No. of peaks (> 40) API resolution (> 1.5) Peak purity (≥ 98 %) Peak tailing (< 1.5)
Strong solvent: Methanol	—	
% Strong solvent: 90.5 %	± 1.5 %	
Aqueous solvent pH:7.7	± 0.1	
Gradient range: 5 % to 90.5 %	—	
Oven temperature: 45 °C	—	
Gradient time: 15 min	—	
Flow rate: 0.6 mL/min	—	
Wavelength: 292 nm	—	

For more information, see Agilent publication 5991-3834EN.

QbD method development helps to better understand the variables and define robustness parameters and resolution limits. As part of this QbD implementation, several column chemistries were evaluated in addition to the mobile phase and other method parameters. (After conducting validation experiments, you should implement a control strategy. Over time, you can achieve continuous improvement by expanding the robust region to include more variables.) The automated method development described here not only takes less time compared to manual method development but also brings the advantage of low failure rates during method validation and transfer.

Agilent Method Development Kits and Method Validation Kits Help Streamline Your Process

Agilent offers kits that contain a range of column chemistries and selectivities, giving you the tools you need to save time and perfect your separation. See the list of method development kits for UHPLC methods below. You can find a complete list of kits at **www.agilent.com/chem/methoddevkits**.

Agilent Method Development Kits

Part Number	Kit Contents
5190-6152	ZORBAX RRHD pH Method Development Kit (SB-C18, Eclipse Plus C18, Extend-C18), 2.1 x 50 mm
5190-6153	ZORBAX RRHD Eclipse Plus Method Development Kit (Eclipse Plus C18, Eclipse Plus C8, Eclipse Plus Phenyl-Hexyl), 2.1 x 50 mm
5190-6154	ZORBAX RRHD Aqueous Method Development Kit (SB-Aq, Bonus RP, Eclipse Plus Phenyl-Hexyl), 2.1 x 50 mm
5190-6155	Poroshell 120 Selectivity Method Development Kit (EC-C18, Phenyl-Hexyl, Bonus RP), 2.1 x 50 mm
5190-6156	Poroshell 120 Selectivity Method Development Kit (EC-C18, Phenyl-Hexyl, Bonus RP), 4.6 x 50 mm
5190-6157	Poroshell 120 Aqueous Method Development Kit (SB-Aq, Phenyl-Hexyl, Bonus RP), 2.1 x 50 mm
5190-6158	Poroshell 120 Aqueous Method Development Kit (SB-Aq, Phenyl-Hexyl, Bonus RP), 4.6 x 50 mm
5190-6159	Poroshell 120 L1, L7, and L10 USP Method Development Kit (EC-C18, EC-C8, EC-CN), 4.6 x 100 mm
5190-6160	Poroshell 120 L1, L7, and L10 USP Method Development Kit (EC-C18, EC-C8, EC-CN), 3.0 x 100 mm

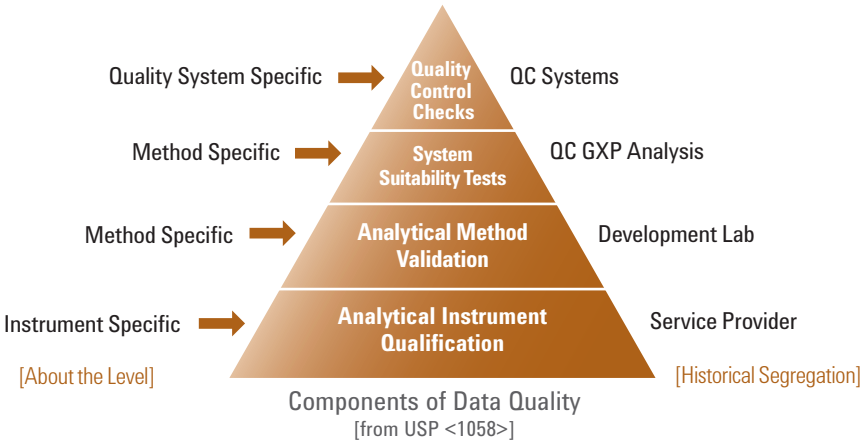
Once you have developed your method, use Agilent Method Validation Kits, prepackaged with three different lots of the same phase column for your method validation. You can find more information about these kits at **www.agilent.com/chem/methodvalidationkits.**

Instrument Compliance for Robust Analytical Method Development in a QbD Environment

The components of the data quality triangle defined in United States Pharmacopeial Convention (USP) general chapter <1058> (Analytical Instrument Qualification), document the relationship between:

- Knowing the instrument is working correctly (instrument qualification)
- Knowing the analytical method is suitable for its intended use
- Knowing the system (instrument, method analyst) is working on the day (system suitability criteria).

This is represented below with an example of the segregation of roles and hierarchical thinking that might typically be applied:

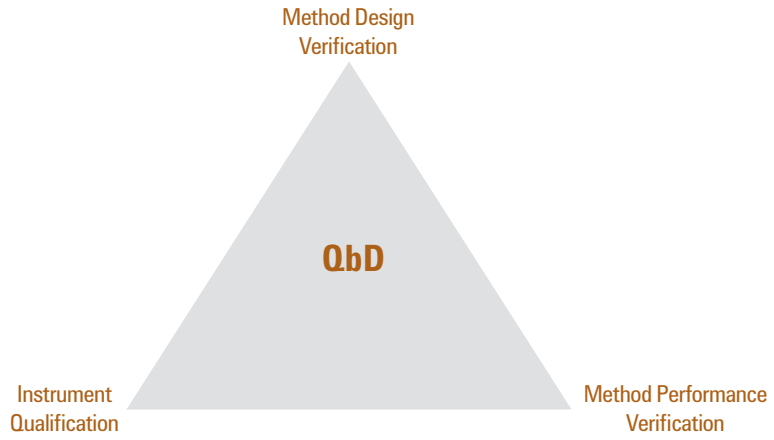


Components of Data Quality
[from USP <1058>]

The principles of the data quality triangle universally apply to all laboratories because they are based on good science. However, when considered in this way, the segregation of the work and linear hierarchical thinking is clearly visible.

In a QbD environment, special emphasis is placed on product and process understanding as well as controlling variability. The top three levels of the triangle can be considered components of a continuum, rather than a hierarchical model. This means analytical methods will be designed to be suitable for their intended use, and the variability and performance of the analytical method will be better understood. However, within this QbD framework, there is still a fundamental requirement that you be able to independently test/verify and monitor the performance of the analytical instrument, as well as the analytical method validation/design space and the QbD-based system suitability. For HPLC, for example, the performance of the instrument is defined in the equipment qualification plan. The principles of system suitability that should be achieved (injection precision, for example) are defined in the Pharmacopeia general chapters such as USP <621>. While broad principles of chapters such as USP <621> will continue to be applied, we anticipate a move toward the acceptance criteria for point-of-use holistic testing (system suitability criteria, for example) being defined in the analytical method. In the QbD environment, some of the parameters for analytical method performance may no longer be fixed. Though variable, they will be specifically designed to achieve the desired quality measurement or CQA.

With QbD principles being applied to analytical methods with increasing regularity, we expect the hierarchy presented in USP <1058> for instrument qualification to evolve. In USP <1058>, Analytical Instrument Qualification is presented as the foundation layer of the triangle. The principles for this are unchanged. If the analytical instrument is not installed correctly, or if the environment is not suitable for the instrument, or the instrument is not operating correctly, then the results may not be valid. Because of the integrated thinking that drives QbD, the levels of the data-quality triangle may be considered in a different way in order to represent the true equilibrium between instrument qualification, the design space, and QbD-based system suitability.



The application of QbD principles to analytical method development and validation will probably result in a move away from the fixed method validation criteria defined in ICH Q2, toward criteria designed for specific requirements. Just as fixed method validation requirements may become a thing of the past, fixed instrument qualification criteria, such as those typically followed in paper protocols, may become obsolete. Instead, you will most likely need a configurable electronic qualification. The flexibility needs to be fully controlled to satisfy regulatory requirements, while the move from paper-based protocols toward electronic qualification (and including the option of electronic approval, rather than ink signature) also satisfies the data-integrity concerns of regulatory agencies such as the FDA.

Agilent Automated Compliance Engine (ACE) software is designed to satisfy the most stringent regulatory requirements while providing controlled flexibility to satisfy future requirements. Qualified software and analytical instrumentation will always remain a key to the reliability of the analytical data generated in a laboratory. Monitoring and maintaining calibration under a robust pharmaceutical quality system (ICH Q10) is essential.

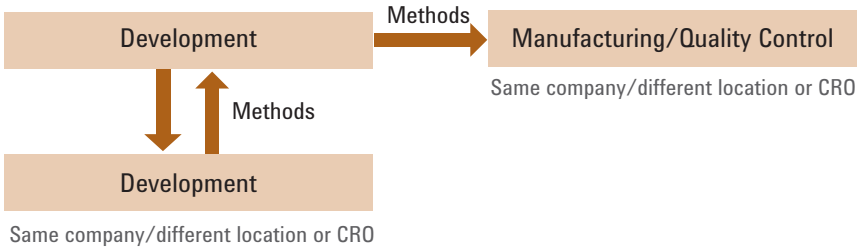
Agilent provides a comprehensive portfolio of qualification services for your analytical lab.[9] With additional coverage for multivendor systems, Agilent has been independently ranked as a market leader for over a decade. All services are designed to fulfill Good Manufacturing Practice/Good Laboratory Practice requirements and meet the pharmaceutical industry's evolving qualification needs. Agilent provides installation qualification and operational qualification that can be adapted to your specifications, according to our contractual agreement. The controlled flexibility and designed-in quality of Agilent's qualification services cover a spectrum of systems and techniques with a harmonized, cost-effective approach based on scientific risk assessment and oriented to your business requirements.

- Successful delivery of more than 150,000 qualification services worldwide for confidence in the methodology and results.
- Proven automated protocols, calibrated tools, and certified engineers to quickly and reliably deliver your qualification report and certificate for a cost-effective solution.
- Easy review and approval for all qualification services, with complete documentation of regulatory compliance for your instrument.

Agilent has been rated number one in compliance by independent survey five consecutive times since 1995.[9]

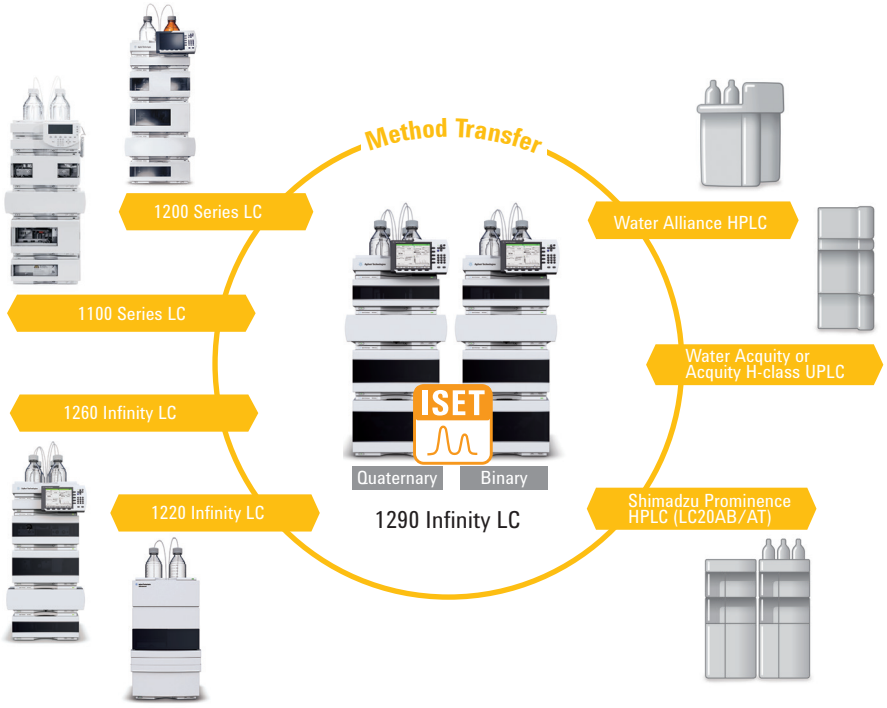
ELIMINATING VARIABILITY IN ANALYTICAL METHOD TRANSFER

Transferring analytical methods between research and development departments, contract research organizations (CROs), and manufacturing is essential in the life cycle of a new pharmaceutical product. Several hundred FDA observations and a proposal for a new chapter in USP <1224>, "Transfer of Analytical Procedures," emphasize the importance of this topic. Differences in design between LC instrumentation (such as power range, delay volume, mixing behavior, temperature control, extra column volume, and detector cell design), methods, and in the level of analyst experience in redeveloping methods affect your ability to transfer a method from one system to another. QbD principles advocate eliminating variability in the method transfer process.



Agilent's Intelligent System Emulation Technology

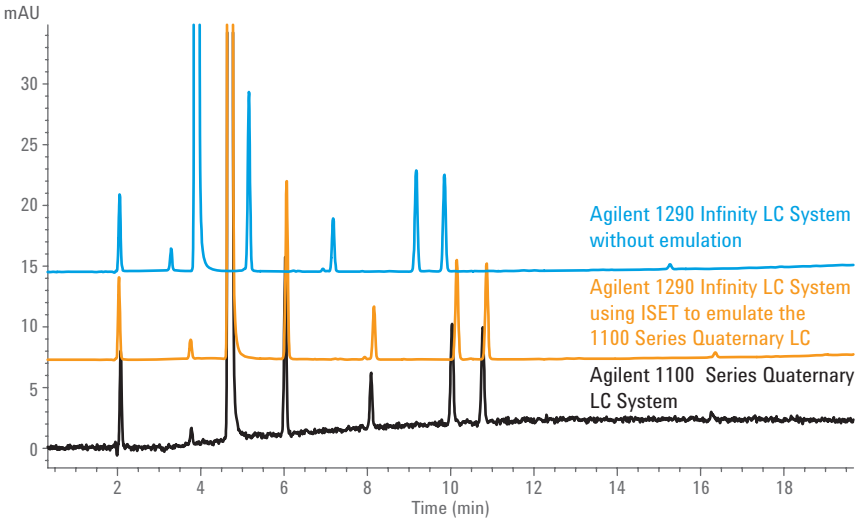
Agilent's Intelligent System Emulation Technology (ISET) can execute any legacy HPLC or latest UHPLC method and deliver the same chromatographic results, without the need to change the original method or modify the instrument hardware.[10] This new technology delivers best-in-class performance, a direct result of Agilent's revolutionary emulation algorithm.



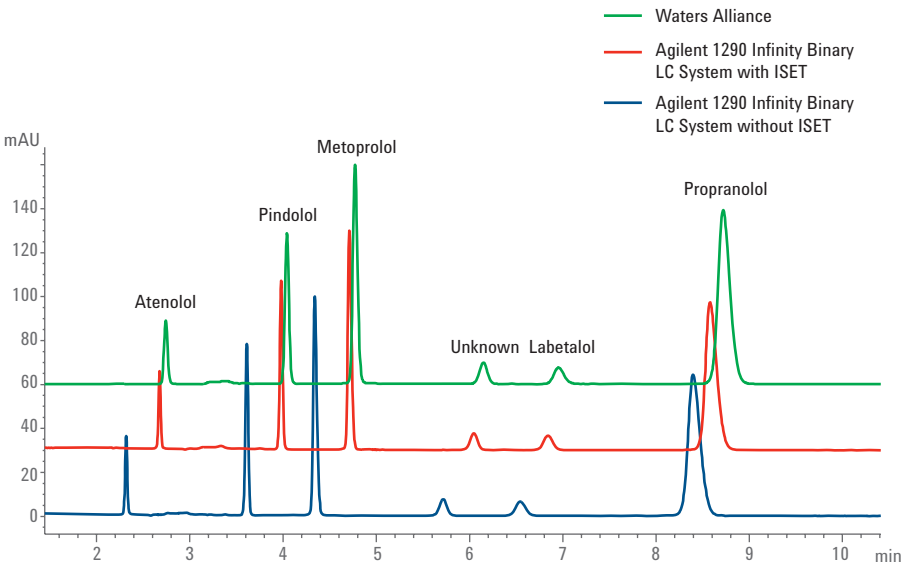
With exact knowledge of the behavior of a selected LC instrument, ISET creates an emulation function so that the Agilent 1290 Infinity LC delivers the same gradient conditions as the selected LC – no shifts in retention times, and no changes in resolution.

- Emulates other (U)HPLC instruments by a simple mouse click
- Runs existing (U)HPLC methods without modifying method or system
- Delivers same retention times and peak resolution for infinitely better method transfer

Application examples as shown below demonstrate that ISET can help you address the challenge of variability in method transfer.[10]



Overlay of chromatograms at 270 nm obtained for paracetamol and its impurities on the Agilent 1290 Infinity LC System (blue), the Agilent 1290 Infinity LC System with ISET (orange), and on the Agilent 1100 Series Quaternary LC System (black). For more information, see Agilent publication 5990-9715EN.



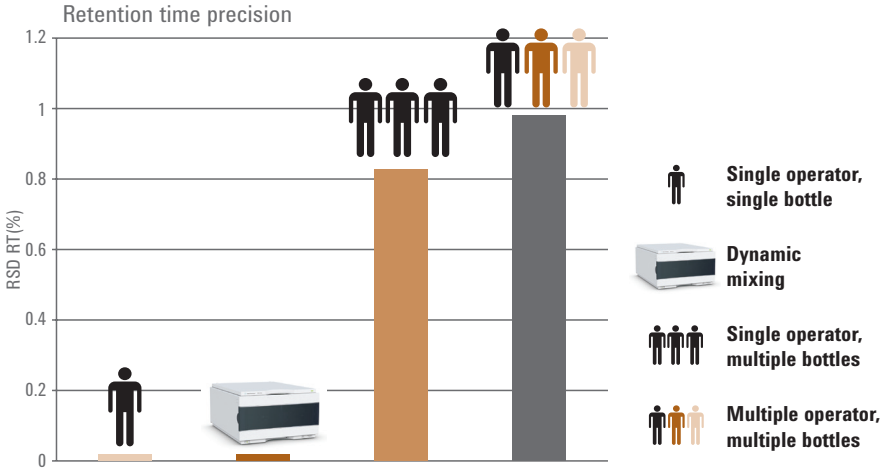
Overlay of chromatograms of b-blockers obtained on the Alliance and the Agilent 1290 Infinity Binary LC System with and without ISET. For more information, see Agilent publication 5991-1603EN.

Automated/Dynamic Online Mixing with Agilent 1290 Infinity Quaternary Pump

Agilent 1290 Infinity Quaternary Pump.

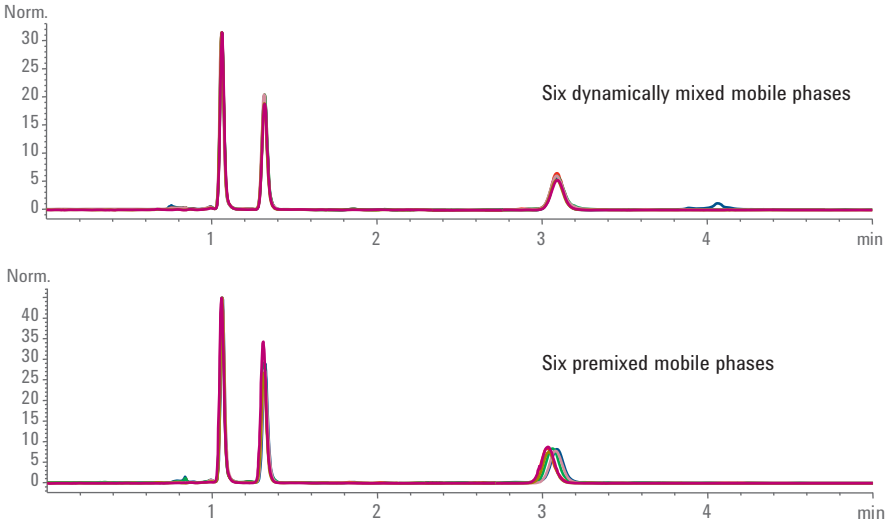
In the pharmaceutical industry, quality control analyses of drug preparations are frequently performed using isocratic UHPLC conditions. To ensure the highest precision for the retention times, premixed phases are often preferred. To ensure that the premixed phases always have the same compositions, the mixing procedures have to be consistently reproducible. Slight composition changes could occur between preparations and between different users. For compounds that react to even small composition changes, non-reproducible mixing will result in lower precision of retention times.

The Agilent 1290 Infinity Quaternary Pump offers mixing performance that can help you meet your intended method performance in the receiving laboratory.[11] The pump provides the highest precision for retention times using dynamically mixed mobile phases over prolonged periods. The retention time precision is comparable to results obtained from different charges of premixed mobile phases. By replacing the manual solvent mixing with automated/dynamic online mixing by pump, you can minimize not only the instrument-specific contribution to the variance, but also the operator contribution.

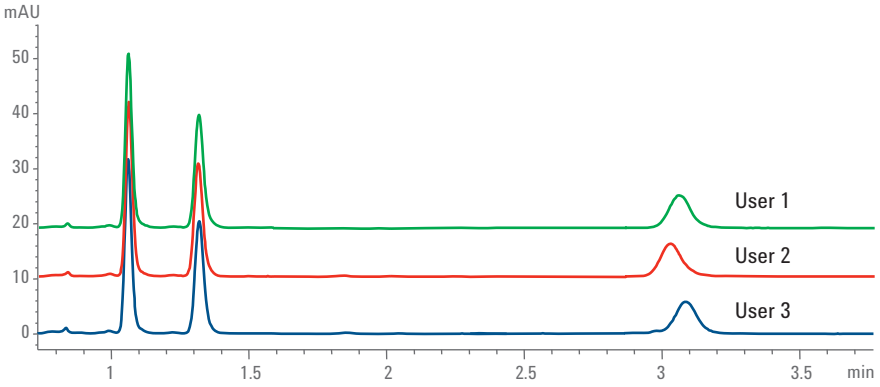


Variability in flow accuracy and precision for dynamically mixed versus pre-mixed solvents. For illustration only. For more information, see Agilent publication 5991-0098EN.

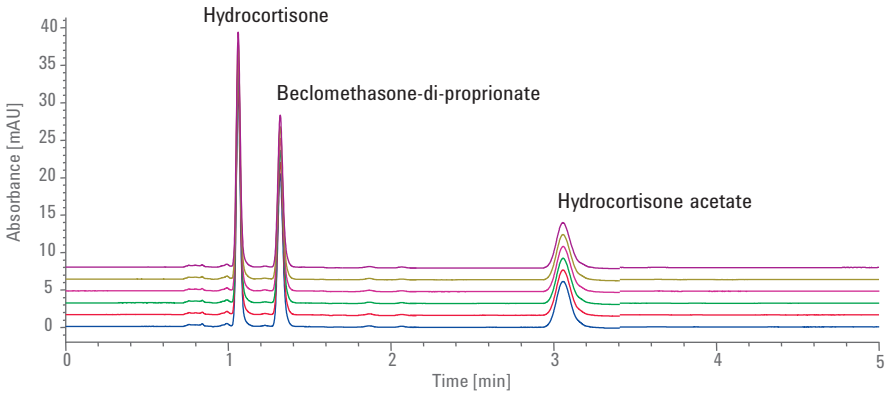
The Agilent 1290 Infinity Quaternary Pump can eliminate the need to premix the mobile phase to achieve better day-to-day reproducibility as shown in the analysis of three glucocorticoids below.



Comparison of chromatograms from six sequences using dynamically mixed and six premixed mobile phases.



Comparison of chromatograms obtained by premixed phases prepared by three different users.



Analysis of glucocorticoids using dynamically mixed mobile phases.

For more information, see Agilent publication 5991-0098EN.

QUALITY RISK ASSESSMENT AND EVALUATION OF CRITICAL QUALITY ATTRIBUTES FOR DRUG SUBSTANCES AND DRUG PRODUCTS

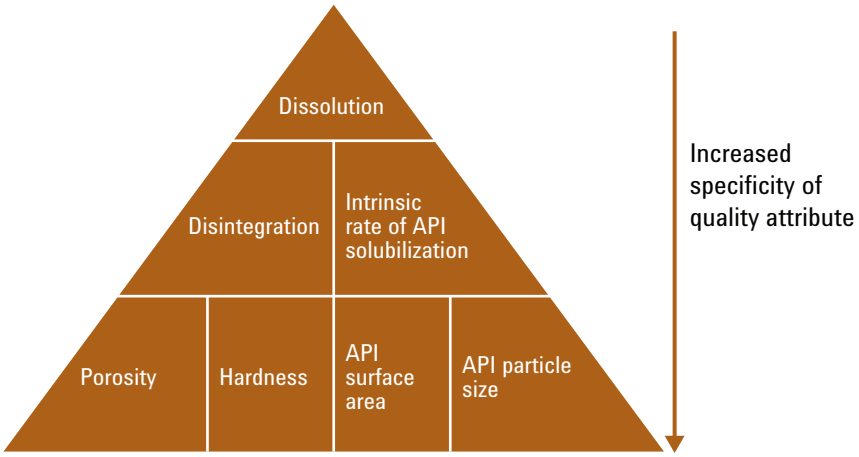
A critical quality attribute(CQA) is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.[1] As stated in ICH Q11, drug substance CQAs typically include those properties or characterstics that affect identity, purity, biological activity, and stability. For example, the dissolution capability of solid oral dose forms is recognized as a CQA. *In vitro* dissolution is used throughout the product development cycle and is vital in demonstrating how critical process parameters (CPPs) like blending time, granulation, excipients, or tablet hardness influence the dissolution rate. Dissolution testing can also be used to define CQAs such as the particle size of an active pharmaceutical ingredient (API). Here dissolution rates may be correlated with particle size and adjusted to provide controlled release of the API.

Impurities are an important class of potential drug substance CQAs (as stated in ICH, Q11) due to their potential impact on drug safety.[4] Organic impurities (including potential genotoxic impurities), inorganic impurities, and residual solvents are the three major types of impurities commonly evaluated as per regulatory guidelines. Agilent's detailed primer, Pharmaceutical Impurity Analysis Solutions, covers a broad range of solutions to analyze all three.[13]

Dissolution Testing Solutions for QbD Implementation

The dissolution test has evolved to become a definitive tool used to characterize the biopharmaceutical performance of solid oral dosage forms. The traditional objectives of dissolution testing are to assure consistent batch-to-batch quality, optimize formulations, monitor drug stability over time, and establish bioequivalence between future and generic formulations. However, it plays a more critical role in the contemporary world of pharmaceutical product manufacturing because it employs QbD principles, not only throughout the product development stages, but also through the dissolution analytical method development stages. By establishing scientifically sound dissolution methods and specifications, we are better able to identify and monitor critical quality attributes and product performance characteristics. This is consistent with the FDA's guidance to apply knowledge of the API, excipients, and manufacturing processes (plus a better mechanistic understanding of the drug product under development) to yield product quality through consistent and controlled manufacturing.

Dissolution becomes an even more critical tool for understanding product performance through QbD and for measuring the impact of changes in input parameters or processes. It is equally important to apply a mechanistic approach to drug design along with the intelligent design of the dissolution method based on the design space.



Dissolution testing in QbD implementation.[8]

Traditional dissolution methods were designed to optimize drug formulations in terms of biopharmaceutical performance. Such methods had known limitations and have occasionally yielded methods with inherent variability. The use of dissolution methods that are not scientifically appropriate for unique formulations may result in methods that are either poorly discriminating or overly discriminating. That can lead to inappropriate interpretation of product performance and the specification setting. Cascading issues may arise from the unreliable method, which will jeopardize the development process by obscuring critical manufacturing attributes, yielding poor in vitro/in vivo correlation, and ultimately causing significant and costly delays in submission, regulatory review, and approval.

Applying QbD principles to the development of dissolution methods (principles that are currently recommended for the design, development, and manufacture of dosage forms) will lead to a more consistent and precise measurement of biopharmaceutical performance. If we take a closer look at applying QbD principles to dissolution method development through a knowledge-based approach, we are able to:

- Understand dissolution method variability and its sources
- Build precise methods based on knowledge of API, and excipients
- Identify critical attributes of the dissolution method in terms of biorelevance
- Identify critical attributes of manufacturing processes related to product performance
- Set meaningful product release specifications
- Provide assurance of rugged and reliable methods with an analytical design space
- Allow for regulatory flexibility for potential analytical method changes
- Provide continuous improvement to methodology
- Contribute to a knowledge library for development of future products

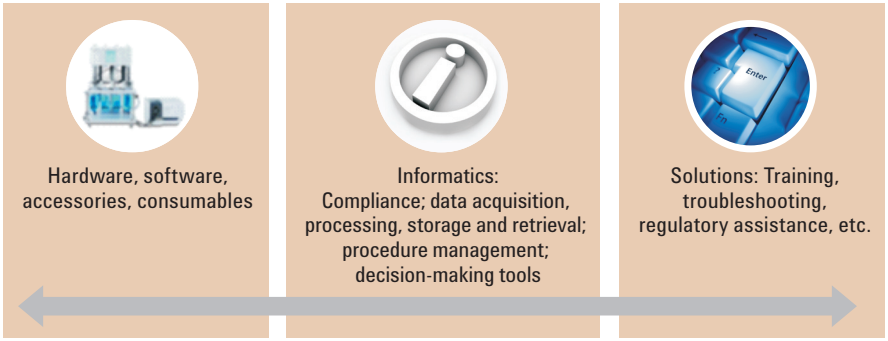
The Agilent 708-DS Dissolution Apparatus.

Agilent offers complete dissolution testing solutions that can help you successfully implement QbD principles in pharmaceutical development.[12] The cornerstone of scientifically sound method development rests with DoEs conducted on a dissolution apparatus that leads the industry in its innovative design, eliminating variability and conforming to the strictest mechanical qualification specifications and tolerances. The Agilent 708-DS System and 709-DS Waterless System provide state-of-the-art dissolution platforms that afford you the flexibility to test traditional and novel dosage forms via:

- Traditional paddle and basket apparatus
- Intrinsic dissolution – critical for determination of the dissolution rate constant
- Manual or automated dissolution sampling
- Sampling into capped HPLC vials
- Online analysis for unattended operation
- Fiber-optic analysis for numerous profile data points and microparticle analysis

Reliance on Agilent's dissolution apparatus and QbD principles to develop meaningful dissolution methods will lower the overall cost of bringing dosage forms to market by reducing variability and unforeseen reformulation issues, and by increasing method robustness, enhancing product understanding, and identifying critical attributes of the manufacturing process. This approach will ultimately lead to continuous improvement of the product and the product development cycle.

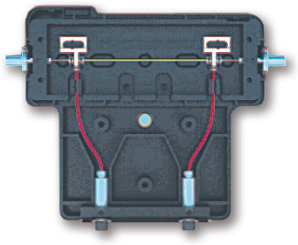
Agilent Dissolution Testing Portfolio



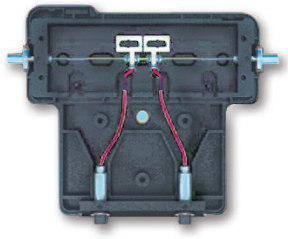
Improving Precision in Impurity Analysis for Quality Risk Assessment

Analysis of trace-level organic impurities in the presence of large amounts of drug substances with Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector (HDR-DAD) Solution

Identifying and quantifying trace-level impurities in the presence of large amounts of drug substances is particularly challenging. For example, analyzing trace impurities can be very complex when testing fixed dose combination drugs where the percentage of the active ingredients can vary depending on the desired physiological effect. If high-dose and low-dose ingredients are combined, the analysis using conventional HPLC and UHPLC diode array detectors may need at least two injections to ensure all compounds are quantified within the linear range of the detector with reliable integration and quantification of trace compounds.



Detector 1 with a 60 mm path length flow cell



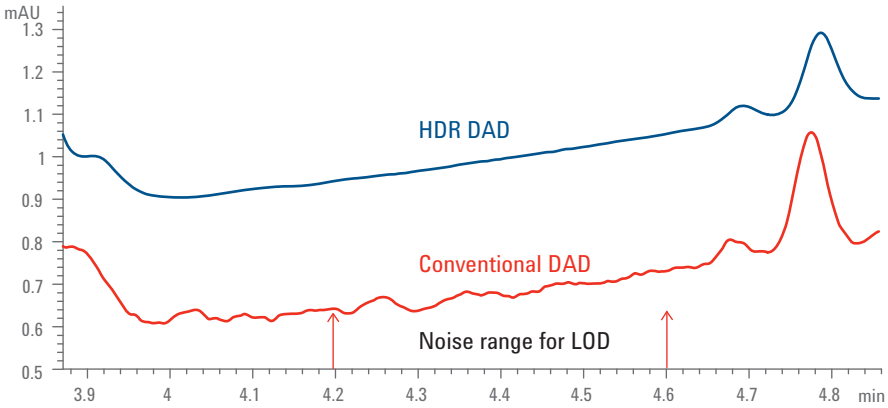
Detector 2 with a 3.7 mm path length flow cell

Agilent 1200 Infinity Series HDR DAD Solution with two clustered detectors. For more information, see Agilent publication 5991-1409EN.

The power of the Agilent 1290 Infinity HDR DAD Solution was demonstrated in the analysis of combination drug sample 1:80 paracetamol and chlorphenamine wherein significantly improved precision and lower detection limits were achieved compared to the conventional DAD.[14] The limit of detection for chlorphenamine was 10 times better.

	LOD with S/N=3 for conventional DAD	LOD with S/N=3 for HDR DAD
Chlorphenamine	~1 ng	~0.1 ng

Limit of detection chlorphenamine for the conventional DAD and HDR DAD signals. For more information, see Agilent publication 5991-0115EN.



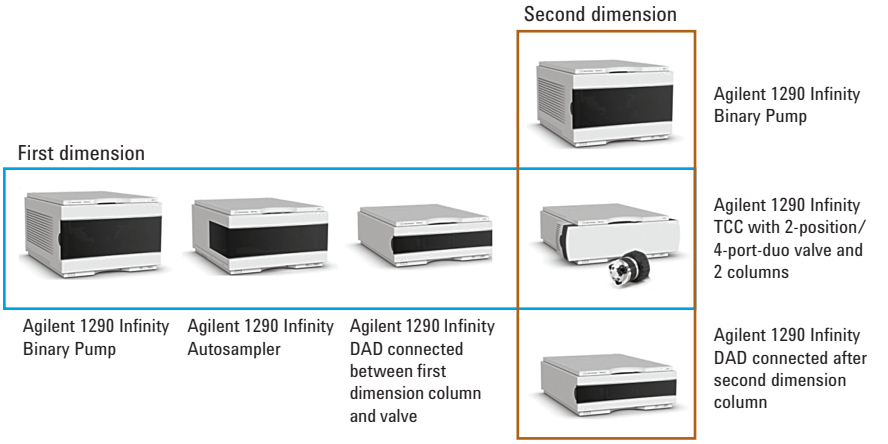
Analysis of combination drug sample 1:80 paracetamol and chlorphenamine, along with vitamin C, caffeine and further small unknown impurities. Significantly improved precision and lower detection limits were achieved with the Agilent 1290 Infinity HDR-DAD Solution compared to the conventional DAD. The limit of detection for chlorphenamine was 10 times better. For the high-dosed ingredient paracetamol, the area precision was 30 times better for the HDR DAD signal. For more information, see Agilent publication 5991-0115EN.

Analysis of trace-level organic impurities co-eluting with main compounds using Agilent 1290 Infinity 2D-LC Solution

Because most impurities are structurally similar to each other and the main compound, their separation is often very challenging as the impurities can elute with each other or with the main compound. In the worst case, separation may be impossible with the chosen stationary phase-eluent combinations.

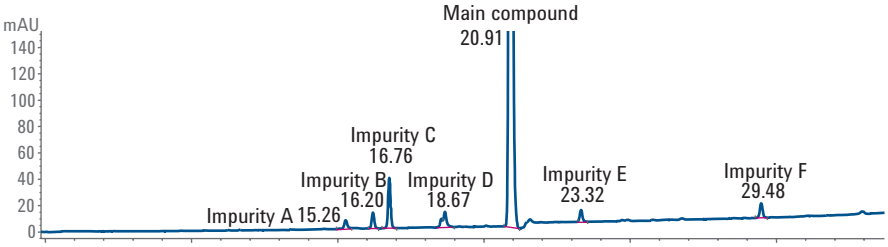
The Agilent 1290 Infinity 2D-LC System offers a solution to this challenge, giving you the ability to perform not only comprehensive 2D-LC (LC×LC) but also heart-cutting 2D-LC (LC-LC).[15] In a heart-cutting experiment, a defined amount of the eluent from the first-dimension column is eluted into a loop capillary and then transferred by a switching valve to the second-dimension column. On this second column, it is possible to separate the co-eluting compounds from the first-dimension column because stationary phase-eluent combinations with different selectivities and longer, adapted gradients can be used.

Instrument Set Up for Heart Cutting 2D-LC Applications

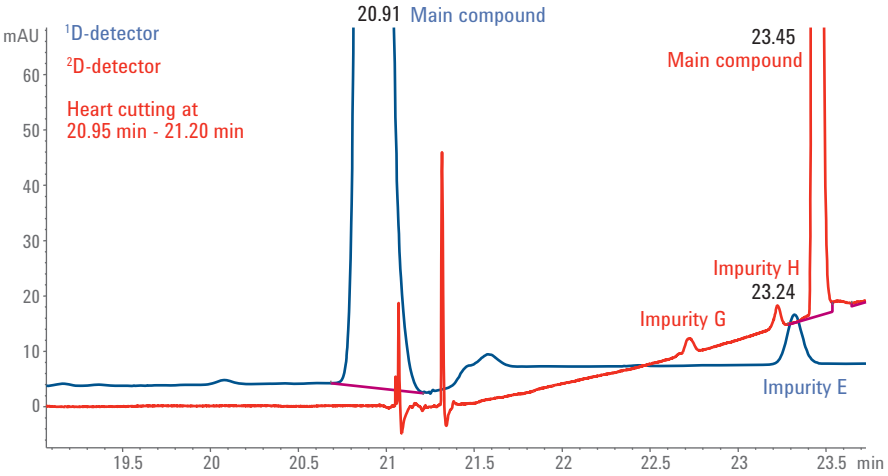


In this configuration, the first and second dimension pumps are identical. Typically, the second dimension pump must be a 1290 Infinity Pump to deliver fast gradients to the second dimension column. The first dimension pump is flexible and could also be a 1260/1290 Infinity Quaternary Pump or a 1260 Infinity Binary Pump. For more information, see Agilent publication 5991-0834EN.

The data below shows the Agilent 1290 Infinity 2D-LC Solution's proficiency with heart-cutting applications. The minor impurity is resolved by heart cutting from a co-eluting main compound. The minor impurity, which is isolated on a second-column dimension after heart cutting, is verified by additional spiking and statistical evaluation of the separation performance.



Main compound and detected impurities A to F. For more information, see Agilent publication 5991-0834EN.



Main chromatogram (blue) and chromatogram of heart cut (red) from the main peak between 20.75–21.00. The main compound elutes at 23.26 minutes from the second-dimension column. For more information, see Agilent publication 5991-0834EN.

Analysis of elemental impurities using ICP-MS and ICP-OES

In 2013, ICH published the "Guideline for Elemental Impurities Q3D" with information on acceptable safety limits of potentially toxic elemental impurities, or permitted daily exposure levels.[16] The guidance describes four toxicity classes as in the table below.

	Included Elemental Impurities	Include in Risk Assessment?
Class 1	As, Pb, Cd, Hg	Yes
Class 2A	V, Mo, Se, and Co	Yes
Class 2B	Ag, Au, Tl, Pd, Pt, Ir, Os, Rh, and Ru	Yes, only if intentionally added
Class 3	Sb, Ba, Li, Cr, Cu, Sn, Ni	Dependent upon route of administration – see Class 3 description
Class 4	B, Fe, Zn, K, Ca, Na, Mn, Mg, W, Al	No

Elemental impurity classification as per ICH Q3D (step 2B version).[16]

The draft guidance recommends a four-step process in regard to elemental impurities: identify, analyze, evaluate, and control. The control component requires the manufacturer to obtain data from suppliers, and to test the finished product to account for impurities that may arise during manufacturing or packaging. As stated in the draft guidance, "the determination of elemental impurities should be conducted using appropriate procedures suitable for their intended purposes. Unless otherwise justified, the test should be specific for each elemental impurity identified for control during the risk assessment. Pharmacopeial procedures or suitable validated alternative procedures for determining levels of elemental impurities should be used."

The current method used for monitoring inorganic contaminants in pharmaceutical samples is a 100-year-old colorimetric test, defined in USP General Chapter <231>. Besides the obvious potential bias associated with a subjective visual comparison, USP<231> is a limit test based on the sum of the 10 elements, and therefore does not give individual concentrations for each individual element. USP<231> is expected to be replaced with new General Chapters USP<232> (Limits) and <233> (Procedures). The new methods will address the limitations of the current method, in particular with respect to the list of analytes, sample preparation, retention of volatile analytes, and the use of closed-vessel sample digestion and modern instrumental techniques to recover and accurately determine the concentrations of individual analytes.

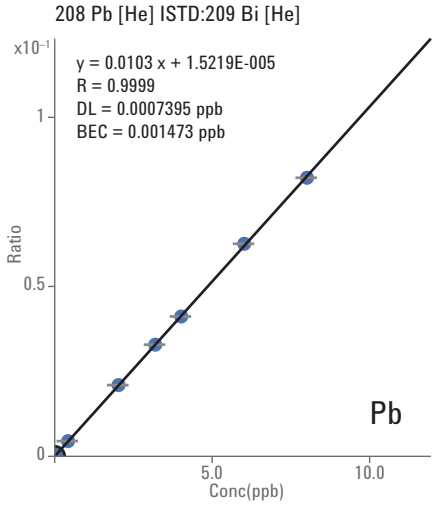
USP<232> covers a wider range of analytes, including catalysts, and the maximum permitted levels are defined according to toxicity, rather than method capability. The new heavy metals test (USP<233>) requires that an instrument-based method be used to determine the elemental impurities, and that the reference methods be based on either inductively coupled plasma-mass spectrometry (ICP-MS) or inductively coupled plasma-optical emission spectrometry (ICP-OES), where both use closed-vessel microwave digestion. This eliminates the specificity issue that characterized the previous colorimetric method, removes the major limitation of analyte loss during the sample ashing step, and significantly lowers the limit of detection.

Agilent offers industry-leading systems for both ICP-MS and ICP-OES to meet the demands of the new, stringent regulations.[16–17] With both methods, sample analysis can be accomplished in three ways: directly (unsolvated), following sample preparation by solubilization in an aqueous or organic solvent, or after acid digestion using a closed-vessel microwave system.

Agilent 7900 ICP-MS

Agilent 700 Series ICP-OES

Agilent Solutions for Elemental Impurities Analysis	
7900 ICP-MS	• Powerful and sensitive system for research and routine, high throughput and high matrix applications • Provides reliable (interference-free) analysis of all 16 regulated elements at and below the regulated levels in the new USP <233> method, even when large sample dilutions are required • The 7900 also provides a unique screening capability, in combination with helium (He) cell mode, which uniquely removes the polyatomic interferences from all analytes, regardless of the sample matrix. It also provides a simple, easily interpreted spectrum and a comprehensive elemental composition from a single rapid scan
700 Series ICP-OES	• Covers the basic requirements of USP<232> that do not necessitate the lowest detection limits • Addresses the needs of all users, including those with budget restrictions and those seeking unrivaled OES performance • Offers superb stability, speed, and flexibility • Provides parts per billion (ppb) detection for most regulated elements • All models provide extended dynamic range, robust plasma, and one-view, one-step measurement of major, minor, and trace elements
1200 Infinity Series LC + 7900 ICP-MS	• Provides speciation of certain regulated elements (As and Hg) • Allows the same instrument to address research applications
OpenLAB ECM, OpenLAB Data Store or Agilent SDA	• Provides full support of all requirements mandated by 21 CFR Part 11 in a closed system, including IQ/QQ services • SDA provides a single PC compliance solution for the 700 Series ICP-OES and 7900 ICP-MS
ICP-MS and ICP-OES supplies	• Ensure optimal and reliable instrument performance with cones, nebulizers, tubes, and torches from Agilent



Calibration curve for Pb in He mode, demonstrating limits of detection of 1 ng/L or below, and good sensitivity and linearity. See Agilent publication 5990-9365EN for data on all the regulated elements.

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(2) (a) EMA-FDA pilot program for parallel assessment of quality-by-design applications: lessons learned and Q&A resulting from the first parallel assessment, 20 August 2013; (b) Questions and Answers on Design Space Verification; EMA-FDA pilot program for parallel assessment of quality-by-design applications, 24 October 2013.

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(5) Borman, Phil, et. al., "The Development Phase of an LC Method Using QbD Principles," Separation Science, 2012.

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(7) Quality-by-Design Approach to Stability Indicating Method Development for Linagliptin Drug Product, Agilent publication 5991-3834EN.

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(12) Dissolution Testing and Analysis: Dissolution Systems Source Book, Agilent publication 5991-3085EN.

(13) (a) PHARMACEUTICAL IMPURITY ANALYSIS SOLUTIONS, Agilent publication 5991-0090EN; (b) Genotoxic Impurities in Pharmaceutical Products: Regulations and Analysis, Agilent publication 5991-1876EN; (c) Pharmaceutical Applications Compendium PURITY AND IMPURITY ANALYSIS, Agilent publication 5991-2796EN.

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(15) (a) Detection of Impurities by Heart Cutting Using the Agilent 1290 Infinity 2D-LC Solution, Agilent publication 5991-0834EN; (b) Heart-Cut 2D-LC/MS Approach for Pharmaceutical Impurity Identification Using an Agilent 6540 Q-TOF LC/MS System, Agilent publication 5991-1873EN.

(16) GUIDELINE FOR ELEMENTAL IMPURITIES QbD: Current Step 2b version dated 26 July 2013.

(17) (a) Pharmaceutical Analysis by ICP-MS: New USP Test for Elemental Impurities to Provide Better Indication of Potentially Toxic Contaminants, Agilent publication 5990-5427EN; (b) Validating the Agilent 7700x ICP-MS for the determination of elemental impurities in pharmaceutical ingredients according to draft USP general chapters <232>/<233>, Agilent publication 5990-9365EN.

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