Quality by Design (QbD) Solutions for Analytical Method Development

A systematic approach to reducing variability
Content

• Introduction
  - Traditional Development and Transfer of Methods
  - QbD Approach for Method Development

• Agilent Solutions for QbD Method Development
  - Agilent Method Development Systems
  - Intelligent System Emulation Technology (ISET)

• QbD Method Development Workflow
  - Screening
  - Optimization
  - Robustness study, Design of Experiments
  - Transfer & Verification
Introduction

- QbD (*Quality by Design*) is defined in the ICH guideline Q8(R2)

- The ICH guidelines suggest to apply *Quality by Design* principles in each step to eliminate risk or failures in drug development processes

- Analytical method development for a drug is also a process therefore quality principles from the ICH guideline should be implemented

(ICH = International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Founded in 1990 by an FDA initiative)
## Appendix

### Analytical QbD Terminology

<table>
<thead>
<tr>
<th>QbD process terminology</th>
<th>Analytical QbD terminology</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality Target Product Profile (QTPP)</td>
<td>Analytical Target Profile (ATP)</td>
<td>Accurate quantitation of API without interferences from degradants</td>
</tr>
<tr>
<td>Critical Process Parameters (CPP)</td>
<td>Quality Target Method Profile (QTMP)</td>
<td>pKa, Log P, Solubility</td>
</tr>
<tr>
<td>Critical Quality Attributes (CQA)</td>
<td>Critical Method Parameters (CMP)</td>
<td>Flow rate, Temperature, pH</td>
</tr>
<tr>
<td>Control Strategy</td>
<td>Critical Method Attributes (CMA)</td>
<td>Resolution, Peak Tailing, Peak Capacity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH ± 0.1; Wavelength ± 2 nm</td>
</tr>
</tbody>
</table>
Traditional Development and Transfer of Methods
A chromatographic challenge!

- Develop
- Validate
- Transfer
  - R & D
  - QA / QC

Fixed method specifications determined manually:
- Slope 14.5% min MeOH
- pH 7
- 35°C
- 1.0 mL/min

± 50%, ± 0.2, ± 10%, ± 10%, ± 10%, ± 0.2, ± 10°C
Variability in resolution of critical peaks from day to day

Different results on different systems

Buffer solutions prepared by different operators deliver different results

Even small changes in pH show a large effect
71 % of HPLC warning letters include reference to “stability”
QbD Approach for Method Development

• Analytical QbD begins by defining goals (Analytical Target Profile, ATP) and identifying potential method variables and responses that affect method quality.

• Statistical „Design of Experiments“ (DOE) has been applied to the selected method variables leading to process and method understanding and to create a list of critical method parameters (CMPs - flow rate, temperature etc.) and critical method attributes (CMAs - resolution, peak tailing).

• The experimentally measured responses were modeled to determine the Design Space (defined in ICH Q8 (R2)).

• A verified and validated method can be modified within the Design Space to compensate any unforeseen variables yet delivering consistent results.
Design Space: Definition

- Combination and interaction of variables that provides assurance of quality
- Working within the design space is not considered as a change
- Movement out of the design space is considered a change and would require regulatory approval
- Design space is proposed by the applicant and is subject to regulatory assessment and approval (ICH Q8)
Traditional Approach vs. QbD Approach

Traditional Approach
• Fixed Protocol

QbD Approach
• Variable Protocol

Method specifications:
- Slope 14.5% MeOH
- 20 mM buffer
- pH 7
- 45°C
- 1.0 mL/min

Working within the design space will ensure the method’s robustness
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  - High Dynamic Range Detection System (HDR)

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Agilent Solutions for QbD Method Development

Method Development System

Intelligent System Emulation Technology

In How Instruments Are Qualified

In Usage

Integrates Critical Compliance Services

Harmonized Qualification

ACE

Equipment Qualification Report

Remote Advisor

Instrument Monitoring – e.g. Injections

Number of Daily Injections
1352 different combinations of column chemistries and eluents
A nearly infinite number of separation conditions is created by including different temperature and flow rates as variable parameters
1290 Infinity II Method Development Solution

New: *Multi Column Thermostat*

1290 Infinity I System

Multi Column Thermostat (MCT)

1290 Infinity II System
### Exampl...om. | pH | %A | %B | %C | %D
---|---|---|---|---|---
1 | 2.7 | 70 | 20 | 10 | 0
 |  | 70 | 20 | 9 | 1
 |  | 70 | 20 | 8 | 2
 |  | 70 | 20 | 7 | 3
2 | 4.9 | 70 | 20 | 5 | 5
 |  | 70 | 20 | 6 | 4
3a | 5.5 | 70 | 20 | 4 | 6
 |  | 70 | 20 | 3 | 7
 |  | 70 | 20 | 2 | 8
 |  | 70 | 20 | 1 | 9
 | 7.8 | 70 | 20 | 0 | 10

**Method:**
- **Column:** Zorbax Eclipse Plus C18 4.6 x 50 mm, 1.8 µm
- **Mobile Phase:**
  - A = Water
  - B = Acetonitrile
  - C = 1M HOAc
  - D = ~1M HOAc, pH ~7.5 with AmmOH
Blend Assist with Infinity II Quat Pump

![Method Editor screenshot]

- Flow: 0.400 mL/min
- Solvents:
  - Solvent 1/HAA (15 mM)
  - Solvent 2
  - Solvent 4
- Pressure Limits
- Timetable (empty)
- Blend Assist
- Channels:
  - Channel A: Solvent 1
    - Calibration: 100.0% Water V.03
    - Name: Solvent 1
    - Stock conc.: 100.00
    - Final conc.: 15.00 mM
  - Channel B: Solvent 2
    - Calibration: 100.0% Methanol V.03
    - Name: Solvent 2
  - Channel C: Solvent 1 Additive
    - Calibration: 100.0% Water V.03
    - Name: HAA
    - Stock conc.: 100.00
    - Final conc.: 15.00 mM
  - Channel D: Solvent 4
    - Calibration: 100.0% Water V.03
    - Name: Solvent 4

Enable Blend Assist checkbox is checked.
1290 Infinity II Series Method Development Solution
New: *Column Centric View*

Assign the mounted columns to the available locations in the MCT
1290 Infinity II Series Method Development Solution
New: Column Centric View

One click column selection

Unequivocal column selection in the instrument method for higher confidence
Column Selection:  

Use to find similar columns

Step #1: Select a Column to Compare

Select a column to compare from the list below. A similarity factor, $F_3$, will be calculated for each of the other columns in the database (below).

<table>
<thead>
<tr>
<th>ID</th>
<th>$F_3$</th>
<th>Name</th>
<th>Manufacturer</th>
<th>Silica type</th>
</tr>
</thead>
<tbody>
<tr>
<td>519</td>
<td>2.80</td>
<td>HSS.C18</td>
<td>Waters</td>
<td>B</td>
</tr>
<tr>
<td>667</td>
<td>3.09</td>
<td>Cortecs C18</td>
<td>Waters</td>
<td>B</td>
</tr>
<tr>
<td>521</td>
<td>7.12</td>
<td>HSS.T3</td>
<td>Waters</td>
<td>B</td>
</tr>
<tr>
<td>303</td>
<td>7.76</td>
<td>XTerra MS C18</td>
<td>Waters</td>
<td>B</td>
</tr>
<tr>
<td>327</td>
<td>8.07</td>
<td>Atlantis T3</td>
<td>Waters</td>
<td>B</td>
</tr>
<tr>
<td>291</td>
<td>8.09</td>
<td>Atlantis dC18</td>
<td>Waters</td>
<td>B</td>
</tr>
<tr>
<td>292</td>
<td>8.98</td>
<td>DeltaPak C18 100A</td>
<td>Waters</td>
<td>B</td>
</tr>
<tr>
<td>304</td>
<td>9.02</td>
<td>XTerra MS C8</td>
<td>Waters</td>
<td>B</td>
</tr>
<tr>
<td>404</td>
<td>10.92</td>
<td>XBridge C18</td>
<td>Waters</td>
<td>B</td>
</tr>
<tr>
<td>484</td>
<td>11.25</td>
<td>XBridge C8</td>
<td>Waters</td>
<td>B</td>
</tr>
</tbody>
</table>
Extracted Ions of 10 congeners overlaid with DAD UV signal
Phenyol Hexal
Extend C18

New Bidentate C18-C18 Bonding for Extend-C18 Bonded Phase

The combination of the unique bidentate structure and double endcapping permits long life at high pH.

Agilent Technologies
Cyno column with ACN

SB-CN
Sterically Protected StableBond Bonded Phase

Bulky disobutyl (C18) or disopropyl (C8, C3, CN, Ar, Phenyl) side-chain groups are used to stabilize both long and short chain bonded phases.

R = Isopropyl, isobutyl (C18)
R₁ = C8, C18, CN, C3, etc.
Experiment 2
CN 2.1 x 50mm 1.8
Determination of Solvent Choice ACN or MeOH
<table>
<thead>
<tr>
<th>Peak #</th>
<th>Mass</th>
<th>Compound</th>
<th>Peak #</th>
<th>Mass</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>127.0390</td>
<td>5-HMF</td>
<td>6</td>
<td>153.0546</td>
<td>Vanillin</td>
</tr>
<tr>
<td>2</td>
<td>97.0284</td>
<td>Furfural</td>
<td>7</td>
<td>199.0601</td>
<td>Syringic acid</td>
</tr>
<tr>
<td>3</td>
<td>171.0288</td>
<td>Gallic acid</td>
<td>8</td>
<td>183.0652</td>
<td>Syringaldehyde</td>
</tr>
<tr>
<td>4</td>
<td>111.0441</td>
<td>5-methylfuran</td>
<td>9</td>
<td>179.0703</td>
<td>Coniferaldehyde</td>
</tr>
<tr>
<td>5</td>
<td>169.0495</td>
<td>Vanillic acid</td>
<td>10</td>
<td>209.0808</td>
<td>Sinapaldehyde</td>
</tr>
</tbody>
</table>
Intelligent System Emulation Technology (ISET)

- Seamless transfer of methods between LCs, regardless of the brand
Method Transfer Between Different LC Instruments

Method transfer from a UHPLC system with a minimized dwell volume and optimized mixing behavior to any other LC system is often challenging and affects retention time and resolution.

Example: Method Transfer from a 1290 UHPLC to a 1100 HPLC system
Method Transfer Between Different LC Instruments

Gradient differences due to different dwell volumes and different mixing behavior

Results from a tracer experiment
Method Transfer Between Different LC Instruments

Approach # 1: Isocratic holding step to synchronize

Still gradient differences due to different mixing behavior

Results from a tracer experiment
Approach # 1: Applying Isocratic Holding Steps

Results

- Results shows a low constistency
- Requires manual determination of the dwell volume/isocratic hold
  (in solvent delivery systems equipped with dampeners the dwell volume is pressure dependent and variable)
- Requires modification of the methods
  (should be avoided in validated environment, but doesn’t require revalidation USP Chapter <621>)
Method Transfer Between Different LC Instruments
Approach # 2: Adding a physical void volume

Almost consistency of both gradient curves

Results from a tracer experiment
Approach # 2: Adding a Physical Void Volume

Results

- Results shows a good consistency
- Manual determination of dwell volumes required (issues of a variable dwell volume with systems containing damperners)
- Mechanical changes are laborious and not flexible
Agilent Solution: Intelligent System Emulation Technology (ISET)

Software controlled compensation of dwell volume differences and synchronization of mixing behaviors

- Programmed gradient
- 1290 gradient
- 1200 gradient
Agilent Solution: Method Transfer by ISET

Results: 1260 Infinity Binary LC to 1290 Infinity LC

- Consistency of results
ISET Setup: Agilent Configuration
ISET Setup: Different Vendor

- **Emulation**
  - Enable ISET
  - Model: ISET 3 V1.0
  - Manufacturer: Waters

- **Model Parameter**
  - Emulated Pump: Alliance 2690, 2695 V1.0
  - Emulated Sampler: Alliance 2690, 2695 (100 µL Loop) V1.0

- Manually select ISET solvent model
  - Generic

- Enable manual fine tuning
Agilent Application Notes

• Fast screening of mobile and stationary phases with the Agilent 1290 Infinity LC and seamless method transfer to an Agilent 1200 Series LC using ISET

  Agilent Application Note 5991-0989EN

• Developing faster methods for generic drugs within USP <621> allowed limits

  Agilent Application Note 5991-0278EN

• Effective use of pharmacopeia guidelines to reduce cost of chromatographic analysis

  Agilent Application Note 5991-1053EN

• Developing faster methods for generic drugs within EP 2.2.46E allowed limits

  Agilent Application Note 5991-0394EN
30x wider linear UV range
Quantification of widely different concentration levels in one single run

Agilent 1200 Infinity High Dynamic Range (HDR-DAD) Solution
Optofluidic Waveguides: Max-Light Flow Cells

Total-internal reflection in a non-coated fused silica fiber

1260 / 1290 Infinity DAD

Deuterium Lamp

Mirror

Max-Light Cartridge Cell
10 mm or 60 mm path length

Programmable or fixed slit

1024 element diode-array

Grating
Effects of path length increase

Conventional flow cells:

10 mm pathlength
small geom. cell volume (13 µL)
high light transmission

60 mm pathlength
large geom. cell volume (78 µL)
lower light transmission

Max-Light High Sensitivity cell:

Optofluidic waveguides
(total internal reflection)

60 mm pathlength
4 µL \( \sigma_V \) dispersion volume

- Loss of resolution
- Loss of signal height
10x Higher Sensitivity

1290/1260 Infinity DAD compare to 1200 Series DAD SL

<table>
<thead>
<tr>
<th></th>
<th>1260/1290 DAD 60 mm</th>
<th>1200 DAD SL 10mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height [mAU]</td>
<td>28.876</td>
<td>4.938</td>
</tr>
<tr>
<td>Noise [mAU]</td>
<td>0.0098</td>
<td>0.0190</td>
</tr>
<tr>
<td>Signal/ noise</td>
<td>2944</td>
<td>259</td>
</tr>
<tr>
<td>Increase</td>
<td>11.4 x</td>
<td></td>
</tr>
</tbody>
</table>

Columns: 150 x 4.6mm Zorbax SB C18, 5µm
Sample: Anthracene: 835 pg/µL
Mobile phase: A: Water, B: Acetonitrile
Elution: isocratic 80 % B
Injection volume: 5 µL
Flow: 1.5 mL/min
DAD: 251/4nm, Ref= 450/80nm, 2.5Hz, slit width 4nm

1260/1290
Infinity DAD

1200 Series
DAD / DAD SL
Linearity, linear range and dynamic range

1200 Series Diode Array Detector, 10 mm flow cell

Detector Signal [AU]

Upper limit 2.0

Detector noise $7 \times 10^6$

Slope = Response Factor (Sensitivity)

HDR- DAD

Linear range →

Dynamic range →

$Linear\_range = \frac{Upper\_Limit}{(3 \times Detector\_Noise)} \approx 10^5$
30x Wider Linear Range with HDR-DAD

3.7 mm and 60 mm and Max-Light flow cell

Detector Signal [AU/cm]

Computing the signals from

60 mm path-length for the low concentration
3.7 mm path-length for the high concentration
30x Wider Linear Range with HDR-DAD

3.7 mm and 60 mm and Max-Light flow cell

30x lower Limit of Detection (LOD) for impurity analysis
- for more reliable automated peak integration
- higher area precision

Detector Signal [AU/cm]

UV range with High Dynamic Range HDR-DAD

Today 1200 DAD

10 x higher sensitivity

~3x higher upper limit

Conc [µg/mL]
Application Example
Sample & chromatographic conditions

Sample: Fixed Dose Combination drug consisting of paracetamol and chlorphenamine (80:1), other compounds vitamin C, caffeine and impurities.

Column: 4.6 x 100 mm Eclipse plus C18, 5 µm
Mobile Phases: A = Water + 0.1 % TFA, B = Acetonitrile + 0.09 % TFA
Flow rate: 1 mL/min
Gradient: at 0 min 5 % B, at 0.5 min 5 % B, at 6.1 min 40 % B, at 6.5 min 95 %, at 8 min 5 %
Stop time: 8 min
Post time: 4 min
Injection vol.: var.
UV: 254/20 nm, Ref 380/80, 10 Hz
Column temp.: 40 °C
Analysis using conventional DAD

Two injections required to quantify main compounds and impurities

Vitamin C
Paracetamol
Caffeine
Chlorphenamine

1 µL injection
To determine the first three peaks
Analysis using conventional DAD

Two injections required to quantify main compounds and impurities

Chlorphenamine

1 µL injection

Imp. 3

Imp. 4

5 µL injection
Analysis using conventional DAD

Two injections required to quantify main compounds and impurities

1 µL injection
To determine the first three peaks

5 µL injection
To determine last peak and further impurities

- Vitamin C
- Paracetamol
- Caffeine
- Chlorphenamine

Linear range
(> 2.0 AU (5 %) at 265 nm)
Analysis using HDR DAD

Comparison with conventional DAD

**Conventional DAD**
2 peaks out of linear range.

**HDR DAD**
All peaks within linear range (up to 6 AU).
Analysis using HDR DAD

Comparison with conventional DAD

Conventional DAD
Determination of impurities and chlorphenamine in second run.

HDR DAD
Determination of all peaks in one run.

Paracetamol
Vitamin C
Caffeine
Chlorphenamine

mAU

Imp. 1 Imp. 2 Imp. 3 Imp. 4

1 2 3 4 5 min
Analysis using HDR DAD

Additional Advantage: Higher sensitivity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conventional DAD LOD (S/N = 3)</th>
<th>HDR DAD LOD (S/N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chorphenamine</td>
<td>~ 1 ng on column</td>
<td>~ 0.1 ng on column</td>
</tr>
</tbody>
</table>

Noise range for LOD

Chlorphenamine

HDR DAD

Conventional DAD
Application Example – Overlay

Blue: 1290 Infinity HDR-DAD
Red: 1290 Infinity DAD 60mm cell
Green: 1100 Series DAD

HDR1 B, Sig=220,10 Ref=450,80 (SOP 132 NE...X10UL_9OCT 2012-10-10 08-54-32;HDRDAD_ANTIOX_20UL_10OCT10.D)
DAD1 B, Sig=220,10 Ref=450,80 (SOP 132 NE...X10UL_9OCT 2012-10-10 08-54-32;HDRDAD_ANTIOX_20UL_10OCT10.D)
DAD2 B, Sig=220,10 Ref=450,80 (SOP 132 NE...ANTIOXINT_10OCT2012-10-10 13-41-34;ANTIOX_G1315B_10OCT10.D)
Reliable Automated Peak Integration

Saving Time – Increasing Confidence

Area Precision

- 1100 Series DAD (no manual integration events)
- 1100 Series DAD (with manual integration events)
- 1290 Infinity HDR-DAD
- 1290 Infinity DAD 60mm cell

31.4%
### Overview 1200 Infinity HDR-DAD Solution

#### Applications

<table>
<thead>
<tr>
<th>Publication Number</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>5991-4121EN</td>
<td>Determination of Log P for Compounds of Different Polarity Using the Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System</td>
</tr>
<tr>
<td>5991-4044EN</td>
<td>Analysis of Degradation Products of Doxycycline in Solution Exposed to Light and Elevated Temperatures Using the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Impurity Analyzer System</td>
</tr>
<tr>
<td>5991-3877EN</td>
<td>Comparison of sensitivity and linearity of the Agilent 1200 Infinity Series high Dynamic Range Diode Array Detector Solution and the conventional Agilent 1290 Infinity DAD</td>
</tr>
<tr>
<td>5991-3876EN</td>
<td>Analysis of high- and low-dosed vitamins in a single run using the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Solution</td>
</tr>
<tr>
<td>5991-3875EN</td>
<td>Reliable and automatic integration of trace compounds using the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector solution</td>
</tr>
<tr>
<td>5991-3874EN</td>
<td>Single-run assay and impurity testing of fixed-dose combination drugs using the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Solution</td>
</tr>
</tbody>
</table>

For latest applications check the [application finder](#)
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QbD Method Development & Method Transfer: Workflow from UHPLC to HPLC in a nutshell

1. **QbD Method development**
   - Sub-2-micron particles

2. **Method transfer**
   - Using Agilent Method translator (Ver 2.2)
   - USP particle sizes

3. **QbD based Method Optimization**
   - as 1260 system, USP column

**QA/QC Routine System**

*Agilent Technologies*
QbD Based Method Development Workflow

Overall workflow which consists of four main steps namely

• Step # 1: Screening

Agilent Method Development Software

Screening

• Column Chemistry
• pH conditions
• Organic Solvent Selection
• Gradients, temperatures
Step # 1: Screening
Agilent Chemstation Method Scouting Wizard Software

- **Define project**
  Choose scouting combinations and base method.

- **Select columns**
  All installed columns are shown automatically.

- **Select solvents**
  Pump types and valves are automatically detected.

- **Define gradients**
  Select between different gradients and temperatures.

- **Review and select screening methods**
  Check for incompatible combinations.
Choose Columns to Be Used
Note: Column Properties Obtained From Column Table

<table>
<thead>
<tr>
<th>Use</th>
<th>Name</th>
<th>TCC #</th>
<th>Location</th>
<th>Void Vol</th>
<th>Max Temp</th>
<th>Max pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td>Zorbax SB C18 (USWEY01663)</td>
<td>1</td>
<td>upper left</td>
<td>140.000</td>
<td>75.00</td>
<td>9.5</td>
</tr>
<tr>
<td>✓</td>
<td>Zorbax Eclipse plus C18 (USWEY01056)</td>
<td>1</td>
<td>upper right</td>
<td>210.000</td>
<td>35.00</td>
<td>9.5</td>
</tr>
<tr>
<td>✓</td>
<td>Zorbax Extend C18 (USWEX10038)</td>
<td>1</td>
<td>middle left</td>
<td>780.000</td>
<td>39.12</td>
<td>9.5</td>
</tr>
<tr>
<td>✓</td>
<td>Zorbax Eclipse plus C8 (USSQF01009)</td>
<td>1</td>
<td>lower right</td>
<td>75.000</td>
<td>30.00</td>
<td>9.5</td>
</tr>
<tr>
<td>✓</td>
<td>Zorbax Eclipse plus PheHex (USFA00786)</td>
<td>1</td>
<td>middle left and right</td>
<td>2200.000</td>
<td>58.90</td>
<td>9.5</td>
</tr>
<tr>
<td>✓</td>
<td>Zorbax Bonus RP (USRJ02456)</td>
<td>1</td>
<td>lower left</td>
<td>360.000</td>
<td>50.00</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>Zorbax SB Aq (USSQG01002)</td>
<td>1</td>
<td>lower right</td>
<td>1894.000</td>
<td>50.00</td>
<td>9.5</td>
</tr>
</tbody>
</table>

6 of 7 columns selected.

Select All  Invert Selection
Choose Solvents to be Used

Note: Solvents Choices Obtained from Valve Table

Dialog box displaying solvent selection options for channel A and channel B. The image shows a method scouting wizard with choices for solvents such as Phosphate buffer 10mM pH 3.5, Phosphate buffer 10mM pH 6.2, Phosphate buffer 10mM pH 8.0, Ammonia pH 10.2, Formic Acid 0.1% pH 3.1, NH4Ac 10mM pH 2.5, NH4Ac 10mM pH 4.8, NH4Ac 10mM pH 7.0, (NH4)2CO3 10mM pH 9.1, Water, Water/ACN 80/20, Methanol, Acetoniitrile, and Ethanol.
Setup Gradient(s) Profile(s)
Gradients May be Setup with Timetable or Graphically
### Review and Select Methods

Note Methods are Flagged if Outside Limits (Temp & pH)

---

<table>
<thead>
<tr>
<th>Method Key</th>
<th>Method</th>
<th>Column</th>
<th>Solvent A</th>
<th>Solvent B</th>
<th>Gradient</th>
<th>Temp °C</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection0028.m</td>
<td>Zorbax Eclipse plus C8 (UISQF01009)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary solvent 1 increasing</td>
<td>18.7</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0027.m</td>
<td>Zorbax Eclipse plus C8 (UISQF01009)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary plateau</td>
<td>18.7</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0028.m</td>
<td>Zorbax Eclipse plus C8 (UISQF01009)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary default</td>
<td>18.7</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0029.m</td>
<td>Zorbax Eclipse plus C8 (UISQF01009)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary solvent 1 only</td>
<td>24.2</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0030.m</td>
<td>Zorbax Eclipse plus C8 (UISQF01009)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary solvent 1 increasing</td>
<td>24.2</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0031.m</td>
<td>Zorbax Eclipse plus C8 (UISQF01009)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary plateau</td>
<td>24.2</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0032.m</td>
<td>Zorbax Eclipse plus C8 (UISQF01009)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary default</td>
<td>24.2</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0033.m</td>
<td>Zorbax Bonus RP (USFRU02456)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary solvent 1 only</td>
<td>18.7</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0034.m</td>
<td>Zorbax Bonus RP (USFRU02456)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary solvent 1 increasing</td>
<td>18.7</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0035.m</td>
<td>Zorbax Bonus RP (USFRU02456)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary plateau</td>
<td>18.7</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0036.m</td>
<td>Zorbax Bonus RP (USFRU02456)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary default</td>
<td>18.7</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0037.m</td>
<td>Zorbax Bonus RP (USFRU02456)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary solvent 1 only</td>
<td>24.2</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0038.m</td>
<td>Zorbax Bonus RP (USFRU02456)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary solvent 1 increasing</td>
<td>24.2</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0039.m</td>
<td>Zorbax Bonus RP (USFRU02456)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary plateau</td>
<td>24.2</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0040.m</td>
<td>Zorbax Bonus RP (USFRU02456)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B1: Acetonitrile</td>
<td>Binary default</td>
<td>24.2</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Injection0041.m</td>
<td>Zorbax Bonus RP (USFRU02456)</td>
<td>A1:04: Ammonia pH 10.2</td>
<td>B2: Ethanol</td>
<td>Binary solvent 1 only</td>
<td>18.7</td>
<td>7.0</td>
<td></td>
</tr>
</tbody>
</table>

---

Exclusion may be overridden
Step # 1: Screening Results

Bubble size represents the number of integrated peaks and, consequently, best mobile and stationary phase combination.

For more details please see Agilent Application Note 5991-0989EN
Infinitely better reporting

Many options for sorting and filtering....

Scouting conditions sorted for highest number of peaks

<table>
<thead>
<tr>
<th>Peaks</th>
<th>#</th>
<th>Datafile</th>
<th>Column</th>
<th>Solvent1</th>
<th>Solvent2</th>
<th>Solvent3</th>
<th>Temp</th>
<th>Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>7-1</td>
<td>001-1101.D</td>
<td>SBAq</td>
<td>A1: water</td>
<td>B1: acetonitrile</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
<tr>
<td>10</td>
<td>3-1</td>
<td>001-0501.D</td>
<td>Poroshell 120EC18</td>
<td>A1: water</td>
<td>B1: acetonitrile</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
<tr>
<td>10</td>
<td>6-1</td>
<td>001-0901.D</td>
<td>SB CN</td>
<td>A1: water</td>
<td>B1: acetonitrile</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
<tr>
<td>10</td>
<td>9-1</td>
<td>001-1401.D</td>
<td>SB C8</td>
<td>A1: water</td>
<td>B1: acetonitrile</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
<tr>
<td>10</td>
<td>11-1</td>
<td>001-1701.D</td>
<td>Poroshell 120 SBC18</td>
<td>A1: water</td>
<td>B1: acetonitrile</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
<tr>
<td>10</td>
<td>19-1</td>
<td>001-2901.D</td>
<td>SB C8</td>
<td>A1: water</td>
<td>C1: Methanol</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
<tr>
<td>9</td>
<td>1-1</td>
<td>001-0201.D</td>
<td>Eclipse Plus C18</td>
<td>A1: water</td>
<td>B1: acetonitrile</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
<tr>
<td>9</td>
<td>15-1</td>
<td>001-2301.D</td>
<td>Eclipse Plus C18</td>
<td>A1: water</td>
<td>C1: Methanol</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
<tr>
<td>9</td>
<td>17-1</td>
<td>001-2601.D</td>
<td>Poroshell 120 SBC18</td>
<td>A1: water</td>
<td>C1: Methanol</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
<tr>
<td>9</td>
<td>21-1</td>
<td>001-3201.D</td>
<td>SBAq</td>
<td>A1: water</td>
<td>C1: Methanol</td>
<td>D1: 2procent TFA</td>
<td>40.0 °C</td>
<td>Gradient 1 T</td>
</tr>
</tbody>
</table>
Infinitely better reporting

And of course chromatograms – with scouting conditions!

Data-file, column, solvents, temperature, gradient-name from Method Scouting Wizard
QbD Based Method Development Workflow

Overall workflow which consists of four main steps namely

- **Step # 1: Screening**
  - Agilent Method Scouting wizard
  - Column Chemistry
  - pH conditions
  - Organic Solvent Selection
  - Gradients, temperatures

- **Step # 2: Optimization**
  - QbD Software
  - Optimize Gradient Profiles
  - pH conditions
  - Flow rates, temperatures

- **Screening**
  - Column Chemistry
  - pH conditions
  - Organic Solvent Selection
  - Gradients, temperatures

- **Optimization**
  - Optimize Gradient Profiles
  - pH conditions
  - Flow rates, temperatures
Add-on Software Options for QbD Method Development

**ChromSword**
- Minimizes effect of human factors by studying method robustness.
- Tests effects of method variables on resolution and other parameters.
- Explores the design space of your method through changing simultaneously up to 7 method variables.
- Fully documents all method development and robustness study steps to meet regulatory compliance.

**AutoChrom (ACD Labs)**
- Runs unattended method development with Agilent LC and LC-MS instruments.
- Rapidly finds methods for unknown samples to separate 100 or even more compounds.
- Starts method development without any preliminary information about a sample.
- Utilizes revolutionary peak tracking procedure to work with peaks less than 0.01% of total area.
- Provides total support for method development and robustness test workflow.

**Fusion AE (S-Matrix)**
- Experimental Design
- Automated, Audited Data Exchanges
- Automated analysis, graphing, and reporting.
- Report output formats: RTF, DOC, HTML, PDF.

Agilent Technologies
Step # 2: Optimization
Fusion AE QbD Software

Optimization of flow rate, gradient slope, pH, column temperature

<table>
<thead>
<tr>
<th>Variable Parameters</th>
<th>Study Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump Flow rate (mL/min)</td>
<td>0.550, 0.600, 0.650</td>
</tr>
<tr>
<td>Intermediate hold time (min)</td>
<td>3.00 &lt;= Intermediate Hold Time &lt;= 7.00</td>
</tr>
<tr>
<td>Final % Strong Solvent (Gradient 1)*</td>
<td>30.0 &lt;= Final % Strong Solvent &lt;= 35.0</td>
</tr>
<tr>
<td>Oven Temperature (°C)</td>
<td>33.0, 36.0, 39.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Constant Parameters</th>
<th>Constant Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Type</td>
<td>3.0X100 mm, 1.8 µm ZORBAX RRHD Eclipse Plus Phenyl-Hexyl</td>
</tr>
<tr>
<td>Wavelength</td>
<td>245 nm ± 4 nm (ref off)</td>
</tr>
<tr>
<td>Strong Solvent type</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>1µl</td>
</tr>
<tr>
<td>Equilibration Time</td>
<td>2.50 min</td>
</tr>
<tr>
<td>Initial Hold Time</td>
<td>1.00 min</td>
</tr>
<tr>
<td>Gradient 1 Time*</td>
<td>5.17 min</td>
</tr>
<tr>
<td>Gradient 2 Time*</td>
<td>9.28 min</td>
</tr>
<tr>
<td>Final Hold Time</td>
<td>2.00 min</td>
</tr>
<tr>
<td>Final Hold % Organic</td>
<td>90.0 %B</td>
</tr>
<tr>
<td>Initial % Strong Solvent</td>
<td>5.0% B</td>
</tr>
</tbody>
</table>

Gradient 1: 5%B-(30-35)%B
Gradient 2: (30-35)%B-90%B
Step # 2: Optimization
Results: peak purity and separation after optimization 99.8%
**QbD Based Method Development Workflow**

Overall workflow which consists of four main steps namely:

- **Step #1: Screening**
- **Step #2: Optimization**
- **Step #3: Robustness study**

### Screening
- Column Chemistry
- pH conditions
- Organic Solvent Selection
- Gradients, temperatures

### Optimization
- Optimize Gradient Profiles
- pH conditions
- Flow rates, temperatures

### Design of Experiments
- Multivariate study
- Robust region
- Design Space
Step # 3: Design of Experiments
Investigate multivariate relationships

Moving critical pair in a more robust region

Optimized Parameters

<table>
<thead>
<tr>
<th></th>
<th>Center Point of Robust region – Prediction</th>
<th>Center Point of Robust region – Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>API-Symmetry</td>
<td>0.71±0.04</td>
<td>0.66</td>
</tr>
<tr>
<td>API-tailing USP</td>
<td>1.4±0.07</td>
<td>1.4</td>
</tr>
<tr>
<td>API-Tangent Width</td>
<td>0.081±0.004</td>
<td>0.083</td>
</tr>
<tr>
<td>API-Resolution</td>
<td>2.3±0.3</td>
<td>2.0</td>
</tr>
</tbody>
</table>

3D Graph
Called Response Surface

### Example - Why QbD is Needed

- Step # 3: Design of Experiments
- Investigate multivariate relationships

#### Center Point of Robust region

- Prediction
- Experiment

#### Moving critical pair in a more robust region

#### 3D Graph
Step #3: Design of Experiments

Results: Design Space

<table>
<thead>
<tr>
<th>Critical Method Parameters (CMPs)</th>
<th>Proven Acceptable Range (PARs)</th>
<th>Critical Method Attributes (CMAs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column: Agilent ZORBAX RRHD Eclipse Plus C8 3.0X50 mm, 1.8 µm</td>
<td>No. of peaks (&gt;40)</td>
<td>No. of peaks (≥ 98%)</td>
</tr>
<tr>
<td>Strong solvent: Methanol</td>
<td>API resolution (&gt;1.5)</td>
<td>API resolution (&lt;1.5)</td>
</tr>
<tr>
<td>% Strong solvent: 90.5%</td>
<td>Peak purity (≥ 98%)</td>
<td>Peak purity (&lt;1.5)</td>
</tr>
<tr>
<td>Aqueous solvent pH: 7.7</td>
<td>Peak tailing (&lt;1.5)</td>
<td>Peak tailing (&gt;1.5)</td>
</tr>
<tr>
<td>Gradient range: 5% to 90.5%</td>
<td>Oven Temperature: 45°C</td>
<td></td>
</tr>
<tr>
<td>Gradient time: 15 min</td>
<td>Flow rate: 0.6 mL/min</td>
<td></td>
</tr>
<tr>
<td>Flow rate: 0.6 mL/min</td>
<td>Wavelength: 292 nm</td>
<td></td>
</tr>
</tbody>
</table>

CMAs to create a Design Space

- The Design Space is a region in which changes to method parameters will not significantly affect the results.
Overall workflow which consists of four main steps namely

- **Step #1: Screening**
- **Step #2: Optimization**
- **Step #3: Robustness study**
- **Step #4: Method Transfer & Verification**
Step # 4: Method Transfer & Verification
From UHPLC to HPLC

Method transfer
• Using Agilent Method translator (Ver 2.2)
• USP particle sizes

QbD based Method Optimization as 1260 system, USP column
• Step # 2 Optimization
• Step # 3 Robustness

QA/QC Routine System
• Verification
Robustness Study After Transfer
Modeling a HPLC design space on an emulated 1260 system

HPLC design space parameters

<table>
<thead>
<tr>
<th>Critical Method Parameters (CMPs)</th>
<th>Proven Acceptable Range (PARs)</th>
<th>Critical Method Attributes (CMAs)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column:</strong> Agilent ZORBAX Eclipse Plus C8 4.6X150 mm, 3.5 µm</td>
<td>No. of peaks (&gt;40)</td>
<td><strong>No. of peaks ≥ 15</strong></td>
</tr>
<tr>
<td><strong>Strong solvent:</strong> Methanol</td>
<td>API resolution (&gt;4)</td>
<td><strong>Tangent Resolution ≥ 1.33</strong></td>
</tr>
<tr>
<td>% Strong solvent: 87.5%</td>
<td>Peak purity (≥ 98%)</td>
<td><strong>Peak purity ≥ 98%</strong></td>
</tr>
<tr>
<td>± 1.5%</td>
<td>Peak tailing (&lt;1.3)</td>
<td><strong>Peak tailing &lt; 1.3</strong></td>
</tr>
<tr>
<td>Aqueous solvent pH: 7.7</td>
<td>Flow rate: 1.4 mL/min</td>
<td><strong>Gradient range:</strong> 5% to 87.5%</td>
</tr>
<tr>
<td>± 0.1</td>
<td>Gradient time: 45 min</td>
<td><strong>Oven Temperature:</strong> 37°C</td>
</tr>
<tr>
<td>Gradient range: 5% to 87.5%</td>
<td>Flow rate: 1.4 mL/min</td>
<td><strong>Wavelength:</strong> 292 nm</td>
</tr>
<tr>
<td>Oven Temperature: 37°C</td>
<td>Wavelength: 292 nm</td>
<td><strong>Critical Method Attributes (CMAs):</strong></td>
</tr>
<tr>
<td>Gradient time: 45 min</td>
<td>Wavelength: 292 nm</td>
<td><strong>HPLC design space with new CMA values:</strong></td>
</tr>
</tbody>
</table>
Proof Of Robustness After Transfer
Conditions applied from center point and the four corner points of the Design Space

Critical Method Attributes are within the design space

API Rs > 4 and API tailing = 1.2 for all runs

A
% B max: 86 %; pH: 7.6

B
% B max: 89 %; pH: 7.6

T
% B max: 87.5 %; pH: 7.7

D
% B max: 89 %; pH: 7.8

C
% B max: 86 %; pH: 7.8

% B max: 86 %; pH: 7.6

% B max: 89 %; pH: 7.6

% B max: 87.5 %; pH: 7.7

% B max: 89 %; pH: 7.8

% B max: 86 %; pH: 7.8
Verification Of The Final Method With The Target System

Results: 1260 Infinity data compared to 1290 Infinity data in emulation mode

**1260 target system**

API Rs = 4.1  
API tailing = 1.2

**1290 emulated as 1260**

API Rs = 4.2  
API tailing = 1.2

Critical Method Attributes are within the defined limits
Overall Summary

- QbD is a state of the art approach to remove variability of analytical methods
- ISET enables cross platform method development workflows
- Method Scouting Wizard Software enables even non expert chromatographers to develop robust methods
- The combination of ISET, Method Scouting Wizard Software and QbD software provides a unique and efficient way to develop and transfer robust methods
QbD based Method Development: Agilent Application Notes

- Quality-by-Design Approach to Stability Indicating Method Development for Linagliptin Drug Product,
  - Agilent Application Note 5991-3834EN

- Automated QbD Based Method Development and Validation of Oxidative Degraded Atorvastatin
  - Agilent Application Note 5991-4944EN

- Development of an UHPLC Method for Azithromycin Tablets Using ChromSword Auto Software
  - Agilent Application Note 5991-5428EN
Key Points
Quality by Design (QbD) Solutions for Analytical Method Development

• Agilent 1290 Infinity Series Method Development Solutions support automated method development workflows

• Method transfers from Agilent 1290 Infinity Series UHPC systems to other (U)HPLC systems are seamless by ISET (Intelligent System Emulation Technology)

• 1290 Infinity Series Method Development Solutions in combination with ISET and additional QbD software are supporting fast method development processes and method transfers to other (U)HPLC systems under QbD aspects

• Agilent Remote Advisor and ACE are excellent solutions to support method development workflows under QbD aspects for regulated environments
THANK YOU