HPLC Calibration and Operational Qualification

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Objectives

- Concepts and Principles in HPLC Qualification and Calibration
  - Overview of equipment qualification
  - Intro to HPLC, a dominant analytical tool for research and quality control
  - HPLC equipment qualification vs. calibration
  - DQ, IQ, OQ and PQ
- A Procedure for HPLC Calibration and Operational Qualification
  - Outsourcing vs. internal execution
  - Describe a generic HPLC calibration procedure (OQ)
  - Setting acceptance criteria for pump, detector, autosampler and column oven
  - Learn how to calibrate an HPLC system within one day using internal personnel
Instrument Qualification

<table>
<thead>
<tr>
<th>Pre-Installation</th>
<th>Upon Installation at User Site</th>
<th>Post-Installation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DQ</strong></td>
<td><strong>IQ</strong></td>
<td><strong>OQ</strong></td>
</tr>
<tr>
<td>Defining user requirements and setting functional and performance specification</td>
<td>Verification procedure and documentation of the installation</td>
<td>Equipment testing to verify system is operating according to predefined functional and performance specifications</td>
</tr>
<tr>
<td><strong>PQ</strong></td>
<td><strong>DQ</strong></td>
<td><strong>OQ</strong></td>
</tr>
<tr>
<td>Verification that system is consistently operating as intended for the selected application, (system suitability testing)</td>
<td>Calibration and Maintenance</td>
<td>Perform periodic performance verification (OQ) and preventive maintenance</td>
</tr>
</tbody>
</table>

**Timeline**

IQ includes
Verification of installation of each module

• A procedure designed to establish that the instrument is installed and configured according to the requirements established by the instrument manufacturer.
• Instrument functional description
  – Specific instrument ID (i.e. serial number), location
  – Software / firmware revision
  – Verification of certificates and manuals
• Installation/configuration procedures
  – Site compatibility
  – Electrical, fluidic, communication connections
• User approval of IQ completion
  – With signature and date

OQ typically includes
Function qualification of each module

• A procedure designed to establish that the system is functioning according to manufacturer specifications throughout its intended operating range.
• Functional qualification procedure
  – Power-up initialization and diagnostic for each LC component
• Acceptance criteria
• User’s approval of OQ completion
  – With signature and date
What is PQ?

- A procedure designed to establish that the overall system is performing as intended with actual use.
  - If electronic data is stored via a computer network, network access should be verified as part of the performance qualification.
- PQ protocols are developed by the user, though generic procedures using test mixtures might be adequate
- Typically, PQ involves performing system suitability test.
  - Check precision, peak resolution, tailing factor, etc.

Traditional column liquid chromatography and HPLC Systems

- Agilent Series 1200
- JASCO
- Waters Alliance (6000 psi)
- Waters Acquity – UPLC (15,000 psi)

Modern HPLC for Practicing Scientists, Wiley, 2006, Chap. 4.
Advantages and limitations

**Advantages**
- Amenable to diverse analyte/sample types
- Precise and highly reproducible quantitative analysis
- LC/MS
- Flexible customizable automated operation
- High separation power with sensitivity detection

**Perceived Limitations**
- Lack of an ideal universal detector
- Less separation efficiency than capillary GC
- Relatively difficult for novices
- Still arduous for regulatory testing

Potency Assay of a Drug Product

- **Column**: Symmetry C18 (100 x 3.0 mm i.d., 3.5 µm)
- **Mobile phase**: 20% ACN in 0.2% phosphate buffer pH 2.5 with 8.6% of sodium dodecyl sulfate
- **Flow rate**: 1.2 mL/min at 60 °C
- **Sample**: 20 µL injection of capsule extract

Impurity Testing of a Drug Product

- **Full Scale**
- **Expanded Scale**

*Modern HPLC for Practicing Scientists, Wiley, 2006, Chap. 6.*
SEC comparison of UHPLC and HPLC

Column: TOSOH 22 Minutes
Flow rate: 0.5 mL/min
Mobile phase: 200 mM potassium phosphate, 250 mM potassium chloride pH 6.2

Column: Waters BEH200 5 Minutes

RP-HPLC for Protein Separation

Samples were incubated with varied concentrations of CuSO₄ in centrifuge tubes

Column: Pursuit 3 Diphenyl (4.6 x 150 mm, 3.5 µm); Temperature: 75 °C; 1mL/min; injection: 20 µL (20 µg); Solvent A: 0.1% TFA in Water; Solvent B: 0.1% TFA in CAN; gradient from 36%B to 45%B in 15 minutes.
Ion Exchange Chromatography for Protein Separation

Isolation and characterization of therapeutic antibody charge variants using cation exchange displacement chromatography; Taylor Zhang, Justin Bourne and Tony Cano, *J. Chromatogr. A*, 1218 (2011) 5079–5086

Column: Dionex ProPac WCX-10 analytical column (4 mm × 250 mm).

The chromatography was performed on an Agilent 1100 HPLC system at 34 °C with the mobile phase flow rate at 0.8 ml/min.

Separation was obtained in 20 mM MES buffer and 1 mM EDTA at pH 6.0 with a gradient of sodium chloride from 70 mM to 145 mM in 60 min.

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Calibration: Outsourced vs. internal

• Outsourcing of HPLC calibration is typical: performed by service of instrumental vendors
• Calibration can also be implemented by internal analysts
  – Time and cost saving, and can promote better understanding of instrumentation function.
  – Relatively easy with a 1-day template and procedure for UV detector, pump, autosampler and column oven.

Detector Calibration

• use a calibrant with a well defined uv spectrum
• perform the test under static conditions
• check two wavelengths

Spectrum of Anthracene solution:
at 1μg/mL using 996 PDA
Calibration of UV/Vis detector (2487)

near 340 nm

\[ \lambda_{\max} \] is 339 nm

Detector Wavelength Calibration Template

START-UP DIAGNOSTICS
Was the system started without any problems?  
\[ \square \] YES \[ \square \] NO

DETECTOR CALIBRATION (Non PDA or PDA)
Manufacturer:  
PFRC #:  

Wavelength maximum near 340 nm:  
Specification: 337 - 343 nm

Millennium Result ID:  
\[ \square \] PASS \[ \square \] FAIL

Wavelength maximum near 251 nm:  
Specification: 248 - 254 nm

Millennium Result ID:  
\[ \square \] PASS \[ \square \] FAIL
LC Pump:  
Compositional accuracy of A/C & B/D

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Flow (mL/min.)</th>
<th>A water (%)</th>
<th>B water (%)</th>
<th>C 0.1% acetone (%)</th>
<th>D 0.1% acetone (%)</th>
<th>Curve</th>
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<td>100</td>
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</tr>
</tbody>
</table>

- New Specification of +/- 1% absolute.
- Run at 2 mL/min with an extended initial and final segment.
- Higher flow yields better data for all 4 lines in 60 min.

Typical Step Gradient Profile  
using the new solvent program
# Pump Compositional Accuracy Template

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A water (%)</th>
<th>B water (%)</th>
<th>C 0.1% acetone (%)</th>
<th>D 0.1% acetone (%)</th>
<th>Height (mm or peak height unit)</th>
<th>% Ratio</th>
<th>Specification (%)</th>
<th>Pass/Fail</th>
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<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Millennium Result ID: ___________ □PASS □FAIL

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## Pump Flow Accuracy Template

**Pump Calibration**

Pump Manufacturer: ___________ PFRC #: ___________

Column type: Waters Symmetry C18, 3.9 x 150 mm

Stopwatch Manufacturer: ___________ Serial #: ___________

Calibration Due: ___________

Set flow rate to 1.0 mL/min:

Time to collect 10 mL of mobile phase: ________ min ______ Sec.

_________ min.

Measured flow rate: ________ mL/min

Specification: Within 0.95 and 1.05 mL/min

Flow accuracy □PASS □FAIL
Autosampler Precision

- Ten 10-μL injections using ethyl paraben solution
- Specification remains at ±0.5% RSD
- Use a modern 5-μm high-purity silica column

Column: Waters Symmetry (5 μm, 150 x 3.9 mm)
Mobile Phase: MeOH/water (65/35)
Flow Rate: 1.0 mL/min at rm. Temp.
Detection: UV at 258 nm
Sample: 10 μL @ 25 μg/mL

Plate (n): 3150
Pressure (psi) 2900
Retention (min) 2.53
k' 1.1
Tailing factor 1.4

Autosampler Linearity and Carryover

- Perform four injection levels at 5, 10, 40 and 80 μL
  - Reason for using an upper limit of 80 μL due to default sampling loop for 2690 is 100-μL

- Linearity specification
  R>0.999
- Carryover specification remain at <1%
HPLC calibrate 7/2/2014

Method Linearity

Autosampler Sampling Accuracy using Waters OQ procedure

- Inject six 50-μL injections from a tared vial of water and weighing the difference. Run time ~ 30 min.
- Spec. are 50±2μL

Sample Data

<table>
<thead>
<tr>
<th></th>
<th>Run 1</th>
<th>Run 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of filled vial in g: W1</td>
<td>2.98872 g</td>
<td>2.93152 g</td>
</tr>
<tr>
<td>Weight of vial after six injections in g: W2</td>
<td>2.68380 g</td>
<td>2.63300 g</td>
</tr>
<tr>
<td>Average injection volume (μL) = (W1-W2)/6*1000</td>
<td>50.89 μL</td>
<td>49.75 μL</td>
</tr>
<tr>
<td>Specification</td>
<td>50 μL ± 2 μL</td>
<td>50 μL ± 2 μL</td>
</tr>
</tbody>
</table>
1-day calibration feasible

- **Detector**
  - Wavelength calibration using a single anthracene solution at 1 \(\mu g/mL\), Check \(\lambda_{max}\) at 251 ± 3 nm and 340 ± 3 nm.

- **Pump**
  - Compositional accuracy test run at 2 mL/min can test all 4 lines in 60 min. ± 1% absolute.
  - Flow accuracy of 1.00 ± 0.05 mL/min

- **Autosampler**
  - Precision (± 0.5%) and linearity (R>0.999) - modified using Symmetry C18 columns and injection volumes up to 80 \(\mu L\).
  - Waters gravimetric OQ procedure for testing autosampler sampling accuracy. 50 ± 2 \(\mu L\).

- **Others**: Column oven (35 ± 2 °C)
References

4. W. Maxwell and J. Sweeney, Applying the validation timeline to HPLC system validation. LC/GC, 12(9), 1994, 678-82.

Extra resources
Three Domains of an Analytical System

- Calibration
- Method Validation
- System Suitability

Method Validation Overview

Specificity
- Linearity
- Accuracy
- Limit of Detection
- Limit of Quantitation
- Precision
- Range
- Robustness
- System Suitability

### Validation Parameters

- **Accuracy**
  - agreement between true value/accepted value and the value found

- **Precision**
  - agreement (degree of scatter) between a series of measurements, Repeatability (same test), Intermediate Precision (different tests)

- **Specificity**
  - unequivocally determine analyte in the presence of components which may be expected to be present. (e.g. impurities, degradants, matrix)

- **Sensitivity**
  - Detection Limit/Quantitation Limit
    - lowest amount detected but not necessarily quantitatively determined/quantitatively determined (suitable precision and accuracy)

- **Linearity**
  - test results are directly proportional to the concentration

- **Range**
  - Interval with suitable level of precision, accuracy and linearity

- **Robustness**
  - capacity to remain unaffected by small, but deliberate variations in method parameters

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L. Wigman, CACO QC workshop, Nov 2012.