

## System Operation Qualification (OQ) Analytical

AZURA® Systems with UV Detector used in reversed phase mode

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## Operation Qualification (OQ) for analytical systems

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### System data

|                          |   |             |                                |  |                         |  |
|--------------------------|---|-------------|--------------------------------|--|-------------------------|--|
| <b>Pump type</b>         | AZURA® P 6.1L HPG <sup>1</sup>              |             | AZURA® P 6.1L LPG <sup>2</sup> |  | AZURA® P 6.1L isocratic |  |
|                          | Other:                                      |             |                                |  |                         |  |
|                          | Serial no./firmware version:                |             |                                |  |                         |  |
| <b>Pump head</b>         | A   | Serial no.: |                                |  | Volume: ml              |  |
|                          | B   | Serial no.: |                                |  | Volume: ml              |  |
| <b>Mixing chamber</b>    | AZURA® Mixer 50 µl                          |             | AZURA® Mixer 100 µl            |  | AZURA® Mixer 200 µl     |  |
|                          | Dynamic Mixer                               |             | Other:                         |  |                         |  |
| <b>Column thermostat</b> | AZURA® CT 2.1                               |             | None                           |  | Other:                  |  |
|                          | Serial no./firmware version:                |             |                                |  |                         |  |
| <b>Detector</b>          | AZURA® DAD 6.1L                             |             | AZURA® DAD 2.1L                |  | AZURA® MWD 2.1L         |  |
|                          | AZURA® UVD 2.1L                             |             | AZURA® UVD 2.1S                |  | Other:                  |  |
|                          | Serial no./firmware version:                |             |                                |  |                         |  |
| <b>Flow cell type</b>    | KNAUER LightGuide                           |             | KNAUER PressureProof           |  | Other:                  |  |
| <b>Cell path length</b>  | 10 mm                                       |             | 50 mm                          |  | Other:                  |  |
| <b>Cell volume</b>       | 2 µl  |             | 6 µl                           |  | 10 µl                   |  |
|                          | Other:                                      |             |                                |  |                         |  |
|                          | Serial no.:                                 |             |                                |  |                         |  |
| <b>Accessories</b>       | Fiber optic cables and fiber optics adapter |             |                                |  |                         |  |
|                          | Serial number adapter:                      |             |                                |  |                         |  |
| <b>Software</b>          | OpenLAB®EZChrom                             |             | ClarityChrom®                  |  | PurityChrom®            |  |
|                          | Other:                                      |             |                                |  |                         |  |
|                          | Software version:                           |             |                                |  |                         |  |
|                          | Driver version:                             |             |                                |  |                         |  |

<sup>1)</sup> HPG = high pressure gradient, <sup>2)</sup> LPG = low pressure gradient

### Sample Injection:

|                    |                                      |  |                  |  |        |  |
|--------------------|--------------------------------------|--|------------------|--|--------|--|
| <b>Autosampler</b> | AS 3950                              |  | AZURA® AS 6.1L   |  | Other: |  |
|                    | Serial no./firmware version:         |  |                  |  |        |  |
| <b>Valves</b>      | Manual injection valve               |  | Valve with drive |  |        |  |
|                    | Serial no. (valve):                  |  |                  |  |        |  |
|                    | Serial no./firmware version (drive): |  |                  |  |        |  |
| <b>Loop</b>        | 10 µl                                |  | 20 µl            |  | 100 µl |  |
|                    | Other:                               |  |                  |  |        |  |

### Reason for OQ

|                      |          |  |   |  |                |  |
|----------------------|----------|--|---|--|----------------|--|
| <b>Reason for OQ</b> | First OQ |  | Periodic OQ                                     |  | No periodic OQ |  |
| <b>Date of OQ</b>    |          |  | <b>Date of next recommended OQ<sup>3)</sup></b> |  |                |  |

<sup>3)</sup> Refer to the section „Test intervals“.

## Operation Qualification (OQ) for analytical systems

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### Customer and manufacturer data

|                        | Customer | Manufacturer                         |
|------------------------|----------|--------------------------------------|
| Company                |          | KNAUER Wissenschaftliche Geräte GmbH |
| Customer number        |          |                                      |
| Contact person         |          |                                      |
| Address                |          | Hegauer Weg 38                       |
| Postal Code/City       |          | 14163 Berlin                         |
| Phone                  |          | +49 30 80 97 27 111                  |
| E-Mail                 |          | support@knauer.net                   |
| Device set-up location |          | -                                    |

### Materials and software used



**Note:** If you implement the OQ in a different manner or with other OQ methods, please modify the OQ in accordance with these instructions. Perform the different OQ and document it in writing.



**Note:** The eluent must be degassed in order to avoid malfunctioning caused by air bubbles. Please ensure that the solvents are completely equilibrated with regard to temperature and gases in the laboratory atmosphere.



**Note:** The software OQ needs to be done separately. You may use the [OQ for ClarityChrom 8.x](#).

| Material/Solvent/Item                 |                                | Additional information     | Comment |
|---------------------------------------|--------------------------------|----------------------------|---------|
| <b>Eluent A</b>                       | Water (HPLC quality)           | Manufacturer:<br>_____     |         |
| <b>Eluent B</b>                       | Eluent A with tracer           | Manufacturer:<br>_____     |         |
| <b>Tracer</b>                         | Caffeine CAS 58-08-2           | Manufacturer:<br>_____     |         |
| <b>Flow cell</b>                      | Path length 3 mm               | Tracer: 0.05 g/L caffeine  |         |
|                                       | Path length 10 mm              | Tracer: 0.015 g/L caffeine |         |
|                                       | Path length 50 mm              | Tracer: 0.003 g/L caffeine |         |
|                                       | Other path length <sup>4</sup> |                            |         |
| <b>Liquid for piston backflushing</b> | E.g. ethanol/water 10/90 (v/v) | Manufacturer:<br>_____     |         |
| <b>Autosampler wash solution</b>      | Water                          | Manufacturer:<br>_____     |         |

<sup>4)</sup> For flow cells with different path length the concentration of the tracer has to be modified.

## Operation Qualification (OQ) for analytical systems

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| Material/Solvent/Item        |   | Additional information |  | Comment |
|------------------------------|---|------------------------|--|---------|
| Restriction capillary        | Order no. G2071<br>p~9 MPa at flow rate of 1 mL/min<br>ID = 0.18 mm<br>L = about 15 m |                        |  |         |
|                              | Alternatively used:<br>_____  |                        |  |         |
| Chromatography software      | ClarityChrom®   |                        |  |         |
| Separate degasser (optional) | Model:<br>_____   |                        |  |         |
|                              | Serial number:<br>_____   |                        |  |         |
| Mixing chamber               | Model:<br>_____   |                        |  |         |
|                              | Serial number:<br>_____   |                        |  |         |

### Test sample sets

| Cell path length | Test sample set |
|------------------|-----------------|
| 10 mm            | Set 1           |
| 50 mm            | Set 2           |

### Concentration test sample

| Set | Sample 1 [µg/mL] | Sample 2 [µg/mL] | Sample 3 [µg/mL] | Sample 4 [µg/mL] | Sample 5 [µg/mL] | Sample 6 [µg/mL] | Sample 7 [µg/mL] |
|-----|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 1   | 10               | 60               | 140              | 220              | 300              | 2000             | Blank            |
| 2   | 5                | 10               | 30               | 60               | 100              | 2000             | Blank            |

## Operation Qualification (OQ) for analytical systems

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### Confirmation - Result table

| Test                  | Data                                | Specification              | Result      |        |              |  |
|-----------------------|-------------------------------------|----------------------------|-------------|--------|--------------|--|
|                       |                                     |                            | Passed      | Failed | Not relevant |  |
| <b>1. Pump</b>        | Gradient accuracy                   | ≤ 1 %                      |             |        |              |  |
|                       | Precision gradient                  | ≤ 0.4 %                    |             |        |              |  |
|                       | Ripple                              | ≤ 0.4 %                    |             |        |              |  |
|                       | Flow precision                      | ≤ 0.008 min                |             |        |              |  |
| <b>2. Autosampler</b> | Precision of injection volume       | ≤ 0.4 %                    |             |        |              |  |
|                       | Carry-over                          | ≤ 0.01 %                   |             |        |              |  |
|                       | Linearity of injection volume $R^2$ | ≥ 0.9995                   |             |        |              |  |
| <b>3. Detector</b>    | Cell path length 10 mm              |                            |             |        |              |  |
|                       | Baseline noise                      | AZURA® DAD 6.1L            | ≤ 0.03 mAU  |        |              |  |
|                       |                                     | AZURA® DAD 2.1L / MWD 2.1L | ≤ 0.045 mAU |        |              |  |
|                       |                                     | AZURA® UVD 2.1L / UVD 2.1S | ≤ 0.06 mAU  |        |              |  |
|                       | Cell path length 50 mm              |                            |             |        |              |  |
|                       | All detectors                       | ≤ 0.06 mAU                 |             |        |              |  |
|                       | Drift                               | Cell path length 10 mm     |             |        |              |  |
|                       |                                     | AZURA® DAD 6.1L            | ≤ 1 mAU/h   |        |              |  |
|                       |                                     | AZURA® DAD 2.1L / MWD 2.1L | ≤ 1 mAU/h   |        |              |  |
|                       |                                     | AZURA® UVD 2.1L / UVD 2.1S | ≤ 1 mAU/h   |        |              |  |
|                       |                                     | Cell path length 50 mm     |             |        |              |  |
|                       |                                     | AZURA® DAD 6.1L            | ≤ 2.5 mAU/h |        |              |  |
|                       |                                     | AZURA® DAD 2.1L / MWD 2.1L | ≤ 2.5 mAU/h |        |              |  |
|                       |                                     | Linearity $R^2$            | ≥ 0.9995    |        |              |  |

### Results: Accuracy $X_G$ of gradient formation

| Specification: ≤ 1 % |                   |         |              |                    |
|----------------------|-------------------|---------|--------------|--------------------|
| Repetition           | Level of eluent B | H [mAU] | Mixing ratio | Accuracy $X_G$ [%] |
| <b>1</b>             | 0 %               |         |              |                    |
|                      | 10 %              |         |              |                    |
|                      | 50 %              |         |              |                    |
|                      | 90 %              |         |              |                    |
|                      | 100 %             |         |              |                    |
| <b>2</b>             | 0 %               |         |              |                    |
|                      | 10 %              |         |              |                    |
|                      | 50 %              |         |              |                    |
|                      | 90 %              |         |              |                    |
|                      | 100 %             |         |              |                    |

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|   |      |  |  |  |
|---|------|--|--|--|
| 3 | 0%   |  |  |  |
|   | 10%  |  |  |  |
|   | 50%  |  |  |  |
|   | 90%  |  |  |  |
|   | 100% |  |  |  |

### Results: Gradient precision

| Specification: ≤ 0.4 % |     |     |     |      |
|------------------------|-----|-----|-----|------|
| Repetition             | 10% | 50% | 90% | 100% |
| 1                      |     |     |     |      |
| 2                      |     |     |     |      |
| 3                      |     |     |     |      |
| Mean [mAU]             |     |     |     |      |
| StdDev [mAU]           |     |     |     |      |
| Precision:             |     |     |     |      |
| RSD [%]                |     |     |     |      |

### Results: Ripple

 **Note:** Depending on the length of your capillary the time interval must be adapted to a meaningful range on the gradient steps. The time interval should have a least a range of 1.5 min.

| Specification: ≤ 0.4 % |                |                |                |       |            |
|------------------------|----------------|----------------|----------------|-------|------------|
| Repetition             | Runtime 18 min | Runtime 35 min | Runtime 70 min | Noise | Ripple [%] |
| Time interval [min]    |                |                |                |       |            |
| 1                      | 3.5-5.0        | 9-10.5         | 15-16.5        |       |            |
|                        | 6.5-8.0        | 14-15.5        | 25-26.5        |       |            |
|                        | 9.5-11.0       | 19-20.5        | 35-36.5        |       |            |
| 2                      | 3.5-5.0        | 9-10.5         | 15-16.5        |       |            |
|                        | 6.5-8.0        | 14-15.5        | 25-26.5        |       |            |
|                        | 9.5-11.0       | 19-20.5        | 35-36.5        |       |            |
| 3                      | 3.5-5.0        | 9-10.5         | 15-16.5        |       |            |
|                        | 6.5-8.0        | 14-15.5        | 25-26.5        |       |            |
|                        | 9.5-11.0       | 19-20.5        | 35-36.5        |       |            |

## Operation Qualification (OQ) for analytical systems

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### Results: Flow precision Y and precision of injection volume

| Specification: $\leq 0.008$ min. and 0.4 % |          |              |              |
|--|----------|--------------|--------------|
| Repetition                                 | RT [min] | Area [mAU*s] | Height [mAU] |
| 1  |          |              |              |
| 2  |          |              |              |
| 3  |          |              |              |
| 4  |          |              |              |
| 5  |          |              |              |
| 6  |          |              |              |
| 7  |          |              |              |
| 8  |          |              |              |
| 9  |          |              |              |
| 10   |          |              |              |
| <b>Average:</b>                            |          |              |              |
| <b>StdDev:</b>                             |          |              |              |
| <b>RSD [%]:</b>                            |          |              |              |

### Results: Carry over

| Specification: 0.01 % |              |
|-----------------------|--------------|
|                       | Area [mAU*s] |
| Blank 1               |              |
| 10 µg/mL              |              |
| 2000 µg/mL            |              |
| Blank 2               |              |
| <b>Carry over [%]</b> |              |



## Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

### Results: Linearity of injection volume $R^2$

| Specification: $\geq 0.9995$     |          |              |              |
|----------------------------------|----------|--------------|--------------|
| Injection Vol. [ $\mu\text{L}$ ] | RT [min] | Area [mAU*s] | Height [mAU] |
| 1                                |          |              |              |
| 2                                |          |              |              |
| 3                                |          |              |              |
| 4                                |          |              |              |
| 5                                |          |              |              |

### Results: Linearity of the detector $R^2$

| Specification: $\geq 0.9995$              |          |              |              |
|---|----------|--------------|--------------|
| Concentration [ $\mu\text{L}/\text{mL}$ ] | RT [min] | Area [mAU*s] | Height [mAU] |
|   |          |              |              |
|   |          |              |              |
|   |          |              |              |
|   |          |              |              |

### Results: Baseline drift

|               |                        |                              |                  |
|---------------|------------------------|------------------------------|------------------|
| Specification | 10 mm cell path length | AZURA® DAD 6.1L              | $\leq 1$ mAU/h   |
|               |                        | AZURA® DAD 2.1L/<br>MWD 2.1L | $\leq 1$ mAU/h   |
|               |                        | AZURA® UVD 2.1L/ UVD 2.1S    | $\leq 1$ mAU/h   |
|               | 50 mm cell path length | AZURA® DAD 6.1L              | $\leq 2.5$ mAU/h |
|               |                        | AZURA® DAD 2.1L/<br>MWD 2.1L | $\leq 2.5$ mAU/h |
|               | Drift [mAU/h]          | 1-20 min                     |                  |

### Results: Detector noise

|               |                        |                              |                  |
|---------------|------------------------|------------------------------|------------------|
| Specification | 10 mm cell path length | AZURA® DAD 6.1L              | $\leq 0.03$ mAU  |
|               |                        | AZURA® DAD 2.1L/<br>MWD 2.1L | $\leq 0.045$ mAU |
|               |                        | AZURA® UVD 2.1L/<br>UVD 2.1S | $\leq 0.06$ mAU  |
|               | 50 mm cell path length | All detectors                | $\leq 0.06$ mAU  |
| Noise [mAU]   | 1-20 min               |                              |                  |

## Evaluation

Export the data to an [Excel file](#) (see: [www.knauer.net](http://www.knauer.net) → Partner area → Qualification (PQ/IQ/OQ/PV) → Operation Qualification → Tab: Systems → OQ Calculator: System OQ Report).

## Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

### Comments/recommendations

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### Change list

| No. | Type of change | Additional information | Date/signature |
|-----|----------------|------------------------|----------------|
| 1   |                |                        |                |
| 2   |                |                        |                |
| 3   |                |                        |                |

### Certificate

A KNAUER employee or an employee authorized by KNAUER has checked the device and performed all tests described in the OQ.

The OQ form must be signed by a person who is authorized by the customer's head of laboratory. The scope of the OQ meets the customer's requirements.

The results of the OQ, any changes made as well as the OQ process have been documented in this form in writing. Both parties confirm that the OQ has been performed to the customer's satisfaction by signing with date and signature.

**Customer**

\_\_\_\_\_  
Name (capital letters)

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

**KNAUER agent**

\_\_\_\_\_  
Name (capital letters)

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

## Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

### Description

#### Definition of the System Operation Qualification

The qualification document „System Operation Qualification (OQ)“ is part of the quality management system at the company KNAUER.

#### OQ

The Operation Qualification includes extensive functionality tests of pump, autosampler and detector. A KNAUER employee or an employee authorized by KNAUER performs these tests after installation, after changes on the devices and after specified time intervals.

The OQ is a standardized KNAUER document and includes the following:

- Documentation on device specifications
- All necessary method parameters to perform the OQ
- Device functionality checks

#### Goals

The system runs reliably within the documented specifications. The OQ is a summary of the results with comments and evaluations.

#### Target group

The test can be carried out either by the technical support of KNAUER, from a provider authorized by KNAUER or by the customer.

#### About this document

The information in this document is subject to change without prior notice. The original version of this document is the English version. This document contains confidential information and may not be reproduced without written consent of KNAUER.

Depending on the customer's quality assurance system, the signed document must either be filed in the device folder or scanned and stored in an electronic archive.

#### Test intervals

To make sure that the device operates within the specified range, you should test the device using the OQ at the following intervals:

| Average use   | OQ test        |
|---|----------------|
| 1 to 5 days/week  | Every 6 months |
| More than 5 days/week or 24 hours/day                   | Every 3 months |
| Operation with buffer solutions or other salt solutions | Every 3 months |

## Operation Qualification (OQ) for analytical systems

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### Settings

#### Settings UV detector

| Material | $\lambda$ [nm] |
|----------|----------------|
| Caffeine | 272            |

### Function and performance tests

Enter all results in the „Confirmation - Result table“.

### Preparations

**Note:** The HPLC should not be stored under 100% water for more than 24 hours, including the restriction capillary and washing solution in the autosampler. After performing the OQ, flush the system, capillaries and autosampler with organic solvent.

Sometimes a pre-washing with isopropanol is helpful to increase precision of the signals.

#### Detector

The detector is prior to the OQ in operation at least for 1 hour and is ready for use.

#### Pump

**Note:** Only use fresh eluents.

Prepare and deaerate the pump. Set the flow rate to 1 mL/min and follow this flow pattern:

| Eluent        | Time   |
|---------------|--------|
| 100% Eluent A | 10 min |
| 100% Eluent B | 10 min |
| 50% A / 50% B | 10 min |

| Optional:                 |        |
|---------------------------|--------|
| 100% Channel C (Eluent A) | 10 min |
| 100% Channel D (Eluent B) | 10 min |

| Eluent | Time  |
|--------|-------|
| 100% A | 2 min |

#### Autosampler

Ensure that the autosampler wash solution is water. Ensure the dispenser syringe is free from air bubbles.

Place the samples in the correct positions in the sample tray.

#### Software

Check that all adjustments in the methods are correct, especially the installed sample loop, the sample tray in the autosampler and the syringe volume. Also make sure that the pressure trace is recorded. Load the sequence and check if the methods and vial positions are correct.

## Operation Qualification (OQ) for analytical systems

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### System preparation

| Cell path length: |                   | 10 mm                              | 50 mm                              |
|-------------------|-------------------|------------------------------------|------------------------------------|
| Sample position   | Substance         | Concentration [ $\mu\text{g/mL}$ ] | Concentration [ $\mu\text{g/mL}$ ] |
| 1                 | Caffeine in water | 60                                 | 10                                 |
| 2                 | Caffeine in water | 60                                 | 10                                 |
| 3                 | Water (blank)     | -                                  | -                                  |
| 4                 | Caffeine in water | 10                                 | 5                                  |
| 5                 | Caffeine in water | 2000                               | 2000                               |
| 6                 | Caffeine in water | 140                                | 30                                 |
| 7                 | Caffeine in water | 220                                | 60                                 |
| 8                 | Caffeine in water | 300                                | 100                                |

### Duration

The entire test takes appr. 3.5-7 hours.

### Order of the methods in the sequence



**Note:** For all methods an autozero at start is mandatory. It is also recommended to record the pressure trace (auxiliary signal) for all methods.

| Method | Repetition | Vial position | Sample volume [ $\mu\text{L}$ ] | Concentration of standard [ $\mu\text{g/mL}$ ] |           | Duration [min]                                     | Parameter                                       |
|--------|------------|---------------|---------------------------------|--|-----------|--|---|
|        |            |               |                                 | 10 mm  | 50 mm     |  |   |
| M1     | 1          | -             | -                               | -  | -         | 20   | Noise Drift                                     |
| M2     | 11         | 1             | 5                               | 60   | 10        | 44 (11x 4 min)                                     | Flow precision<br>Precision of injection volume |
| M3     | 1          | 2             | 1                               | 60   | 10        | 4  | Linearity of injection volume                   |
| M3     | 1          | 2             | 2                               | 60   | 10        | 4  |   |
| M3     | 1          | 2             | 3                               | 60   | 10        | 4  |   |
| M3     | 1          | 2             | 4                               | 60   | 10        | 4  |   |
| M3     | 1          | 2             | 5                               | 60   | 10        | 4  |   |
| M4     | 1          | 4             | 5                               | 10   | 5         | 4  | Linearity of detector                           |
| M4     | 1          | 2             | 5                               | 60   | 10        | 4  |   |
| M4     | 1          | 6             | 5                               | 140  | 30        | 4  |   |
| M4     | 1          | 7             | 5                               | 220  | 60        | 4  |   |
| M4     | 1          | 8             | 5                               | 300  | 100       | 4  |   |
| M5     | 1          | 3             | 5                               | 0 (Blank)                                      | 0 (Blank) | 4  | Carry over                                      |
| M5     | 1          | 4             | 5                               | 10   | -         | 4  |   |
| M5     | 1          | 1             | 5                               | -  | 10        | 4  |   |
| M5     | 1          | 5             | 5                               | 2000   | 2000      | 4  |   |
| M5     | 1          | 3             | 5                               | 0 (Blank)                                      | 0 (Blank) | 4  |   |
| M6     | 4          | -             | -                               | -  | -         | 72 (4x18 min)<br>140 (4x 35min)<br>280 (4x 70 min) | Gradient (precision and accuracy)               |

## Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

### Method 1

#### Settings: Baseline noise and drift



**Drift:** Use the baseline-chromatogram to determine the drift from minute 1 to 20.

**Noise:** Use the baseline-chromatogram to determine the ASTM-noise from minute 1 to 20 (set the value to 0.5 min).

| Device      | Parameter     | Setting    |
|-------------|---------------|------------|
| Pump        | Flowrate      | 1 mL/min   |
|             | Eluent        | 100% water |
| Autosampler | Injection     | None       |
| Detector    | Wavelength    | 254 nm     |
|             | Data rate     | 1 Hz       |
|             | Time constant | 1 sec      |
|             | Duration      | 20 min     |

### Method 2

#### Settings: Flow precision

##### Precision of injection volume



**Note:** The flow precision test should be performed 11 times. The first run should not be used for calculation.

| Device      | Parameter          | Setting                |
|-------------|--------------------|------------------------|
| Pump        | Flowrate           | 1 mL/min               |
|             | Eluent             | 100% water             |
| Autosampler | Injections         | 11                     |
|             | Injection volume   | 5 µL                   |
|             | Vial position      | 1                      |
|             | Injection method   | Partial loopfill       |
|             | Syringe speed      | normal                 |
|             | Flush volume       | 30 µL                  |
|             | Air segment        | No                     |
|             | Headspace pressure | No                     |
|             | Needle wash        | Between injections, 2x |
| Detector    | Wavelength         | 272 nm                 |
|             | Data rate          | 10 Hz                  |
|             | Time constant      | 0.1 sec                |
|             | Duration per run   | 3 min                  |

## Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

### Calculation: Flow precision

| Value          | Calculation   | Legend   |
|----------------|---|--|
| Flow precision | $Y_F = \sqrt{\frac{\sum (t_x - \bar{t}_x)^2}{n_x}}$ | <p><math>Y_F</math> = Flow precision<br/> <math>t_x</math> = Retention time of the peak<br/> <math>\bar{t}_x</math> = Mean of retention times<br/> <math>n_x</math> = Quantity of measurement (10)</p> |

### Calculation: Precision of injection volume

| Value                         | Calculation   | Legend  |
|-------------------------------|---|---|
| Precision of injection volume | $Y_V = \sqrt{\frac{\sum (A_x - \bar{A}_x)^2}{n_x}} \cdot \frac{100}{\bar{A}_x}$ | <p><math>Y_V</math> = Precision of injection volume<br/> <math>A_x</math> = Peak area<br/> <math>\bar{A}_x</math> = Mean of peak areas<br/> <math>n_x</math> = Quantity of measurement (10)</p> |

## Method 3

### Settings: Linearity of injection volume

| Device             | Parameter          | Setting                |
|--------------------|--------------------|------------------------|
| <b>Pump</b>        | Flowrate           | 1 mL/min               |
|                    | Eluent             | 100 % water            |
| <b>Autosampler</b> | Injections         | 5                      |
|                    | Injection volume   | 1, 2, 3, 4, 5 µL       |
|                    | Vial position      | 2                      |
|                    | Injection method   | Partial loopfill       |
|                    | Syringe speed      | normal                 |
|                    | Flush volume       | 30 µL                  |
|                    | Air segment        | No                     |
|                    | Headspace pressure | No                     |
| <b>Detector</b>    | Needle wash        | Between injections, 2x |
|                    | Wavelength         | 272 nm                 |
|                    | Data rate          | 10 Hz                  |
|                    | Time constant      | 0.1 sec                |
|                    | Duration per run   | 3 min                  |

### Calculation: Linearity of injection volume

| Value                        | Calculation  | Legend  |
|------------------------------|--|---|
| Coefficient of determination | $R^2 = \frac{\sum [(x_i - \bar{x}) \cdot (y_i - \bar{y})]}{\sqrt{\sum (x_i - \bar{x}) \cdot (y_i - \bar{y})^2}}$ | <p><math>R^2</math> = Coefficient of determination<br/> <math>x_i</math> = Injection volume of measurement i [µL]<br/> <math>y_i</math> = Peak area of measurement i [mAU·s]<br/> <math>\bar{x}</math> = Mean of injection volumes [µL]<br/> <math>\bar{y}</math> = Mean of standard concentrations [mAU·s]</p> |

## Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

### Method 4

#### Settings: Linearity of the detector

| Device      | Parameter          | Setting                |
|-------------|--------------------|------------------------|
| Pump        | Flowrate           | 1 mL/min               |
|             | Eluent             | 100 % water            |
| Autosampler | Injections         | 5                      |
|             | Injection volume   | 5 µL                   |
|             | Vial position      | 4, 2, 6, 7, 8          |
|             | Injection method   | Partial loopfill       |
|             | Syringe speed      | normal                 |
|             | Flush volume       | 30 µL                  |
|             | Air segment        | No                     |
|             | Headspace pressure | No                     |
| Detector    | Needle wash        | Between injections, 2x |
|             | Wavelength         | 272 nm                 |
|             | Data rate          | 10 Hz                  |
|             | Time constant      | 0.1 sec                |
|             | Duration per run   | 3 min                  |

#### Calculation: Linearity of the detector

| Value                        | Calculation  | Legend  |
|------------------------------|--|---|
| Coefficient of determination | $R^2 = \frac{\sum[(x_i - \bar{x}) \cdot (y_i - \bar{y})]}{\sqrt{\sum(x_i - \bar{x}) \cdot (y_i - \bar{y})^2}}$ | <p><math>R^2</math> = Coefficient of determination</p> <p><math>x_i</math> = Concentration of the standard of measurement i [mAU·s]</p> <p><math>y_i</math> = Peak area of measurement [µL]</p> <p><math>\bar{x}</math> = Mean of standard concentrations [mAU·s]</p> <p><math>\bar{y}</math> = Mean of peak areas [µL]</p> |

### Method 5

#### Settings: Carry over

| Device | Parameter | Setting     |
|--------|-----------|-------------|
| Pump   | Flowrate  | 1 mL/min    |
|        | Eluent    | 100 % water |



## Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

| Device      | Parameter          | Setting                |
|-------------|--------------------|------------------------|
| Autosampler | Injections         | 4                      |
|             | Injection volume   | 5 µL                   |
|             | Vial position      | 3, 4, 5, 3             |
|             | Injection method   | Partial loopfill       |
|             | Syringe speed      | normal                 |
|             | Flush volume       | 30 µL                  |
|             | Air segment        | No                     |
|             | Headspace pressure | No                     |
|             | Needle wash        | Between injections, 2x |
| Detector    | Wavelength         | 272 nm                 |
|             | Data rate          | 10 Hz                  |
|             | Time constant      | 0.1 sec                |
|             | Duration per run   | 3 min                  |

### Calculation: Linearity of the detector



| Value         | Calculation   | Legend   |
|---------------|---|--|
| Carry over CO | $CO = \frac{A_{Blank2} - A_{Blank1}}{A_{10} \cdot \frac{C_{2000}}{C_{10}}} \cdot 100$ | CO = Carry over<br>A <sub>Blank1</sub> = Peak area of first water injection<br>A <sub>Blank2</sub> = Peak area of second water injection<br>A <sub>10</sub> = Peak area of reference solution 10 µg/mL<br>C <sub>2000</sub> = Caffeine concentration of the reference solution 2000 µg/mL<br>C <sub>10</sub> = Caffeine concentration of the reference solution 10 µg/mL |

## Method 6

### Settings: Gradient



The gradient test should be performed four times.  
The results from the first run should not be used for calculation.

| Device  | Parameter        | Setting                  |
|---|------------------|--------------------------|
| Pump  | Flowrate         | 1 mL/min                 |
|   | Eluent A         | 100% water               |
|   | Eluent B         | Eluent A with tracer     |
|  <b>Note:</b> For flow cells with other path length, the concentration of the tracer must be modified. |                  |                          |
|  <b>Note:</b> For LPG quaternary pump a second set should be performed using Channel C/D.              |                  |                          |
| Autosampler   | Injections       | -                        |
| Detector  | Wavelength       | 272 nm                   |
|   | Data rate        | 10 Hz                    |
|   | Time constant    | 0.1 sec                  |
|   | Duration per run | 18 min   35 min   70 min |

## Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

### Gradient program

- When using a mixing chamber of  $\leq 100 \mu\text{L}$ , a runtime of 18 min is used.
- When using a mixing chamber of  $> 100 \mu\text{L}$ , a runtime of 35 min is used.
- When operating with a pulsation damper, a runtime of 70 min is used.

Depending on the runtime and use of a pulsations damper, one of the following step gradients must be programmed.

| Program without pulsation damper:<br>Runtime 18 min |               |               |
|---|---------------|---------------|
| Time [min]  | Channel A [%] | Channel B [%] |
| 0.00  | 100           | 0             |
| 2.00  | 100           | 0             |
| 2.06  | 90            | 10            |
| 5.00  | 90            | 10            |
| 5.06  | 50            | 50            |
| 8.00  | 50            | 50            |
| 8.06  | 10            | 90            |
| 11.00   | 10            | 90            |
| 11.06   | 0             | 100           |
| 14.00   | 0             | 100           |
| 14.06   | 100           | 0             |
| 18.00   | 100           | 0             |

| Program without pulsation damper:<br>Runtime 35 min |               |               |
|---|---------------|---------------|
| Time [min]  | Channel A [%] | Channel B [%] |
| 0.00  | 100           | 0             |
| 5.00  | 100           | 0             |
| 5.06  | 90            | 10            |
| 10.00   | 90            | 10            |
| 10.06   | 50            | 50            |
| 15.00   | 50            | 50            |
| 15.06   | 10            | 90            |
| 20.00   | 10            | 90            |
| 20.06   | 0             | 100           |
| 25.00   | 0             | 100           |
| 25.06   | 100           | 0             |
| 35.00   | 100           | 0             |

Example: Step gradient with duration and eluent percentages for channel A/B. Also applies to A1/B1, A2/B2, C/D, A/C.

| Program with pulsation damper:<br>Runtime 70 min |               |               |
|--|---------------|---------------|
| Time [min]                                       | Channel A [%] | Channel B [%] |
| 0.00   | 100           | 0             |
| 10.00  | 100           | 0             |
| 10.06  | 90            | 10            |
| 20.00  | 90            | 10            |
| 20.06  | 50            | 50            |
| 30.00  | 50            | 50            |
| 30.06  | 10            | 90            |
| 40.00  | 10            | 90            |
| 40.06  | 0             | 100           |
| 50.00  | 0             | 100           |
| 50.06  | 100           | 0             |

## Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

|       |     |   |
|-------|-----|---|
| 70.00 | 100 | 0 |
|-------|-----|---|

### Calculation: Accuracy of gradient formation

| Value              | Calculation                           | Legend   |
|--------------------|---------------------------------------|--|
| Mixing ratio $C_x$ | $C_x = \frac{H_x}{H_{100}} \cdot 100$ | $C_x$ = Mixing ratio [%]<br>$H_x$ = Measured value at x level of eluent with tracer [mAU]<br>$H_{100}$ = Measured value at 100 % level of eluent with tracer [mAU] |
| Accuracy $X_G$     | $X_G = G - C_x$                       | $X_G$ = Accuracy of gradient formation<br>$G$ = mixing ratio [%]<br>$C_x$ = Mixing ratio [%]   |

### Calculation: Precision of gradient formation

Example for 50 % A / 50 % B:

|                                       |   |   |
|---------------------------------------|---|---|
| $Y_G$ precision of gradient formation | $Y_G = \sqrt{\frac{\sum(x_{50} - \bar{x})^2}{n_x}} \cdot \frac{100}{\bar{x}}$ | $Y_G$ = precision of gradient formation<br>$x_{50}$ = signal height at 50 % B<br>$\bar{x}$ = mean of the step heights [mAU]<br>$n_x$ = number of runs |
|---------------------------------------|---|---|

### Calculation: Ripple

| Value    | Calculation                       | Legend   |
|----------|-----------------------------------|--|
| R Ripple | $R = 100 \cdot \frac{N}{h_{100}}$ | $R$ = Ripple<br>$N$ = Noise, measured for 1.5 min [mAU]<br>$h_{100}$ = signal at 100 % B [mAU] |

### Calculation

1. Calculate the resulting step gradient with the chromatography software (see Fig.1 for an example).
2. Note the measured values at levels 10%, 50%, 90% and 100% of eluent with tracer.
3. Calculate the mixing ratio  $C_x$ .
4. Calculate the accuracy  $X_G$ .

### Operation Qualification (OQ) for analytical systems

AZURA® Systems with UV detector used in reversed phase mode

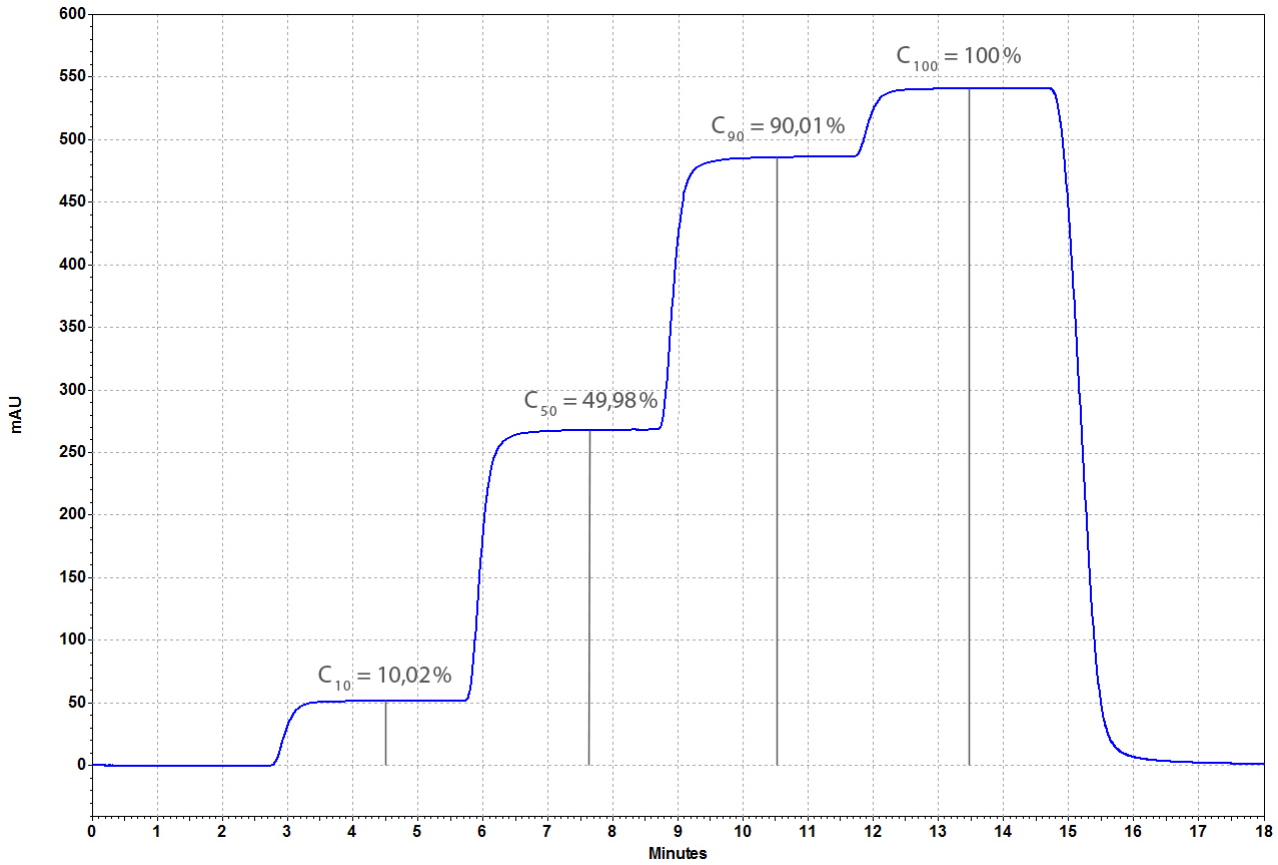


Fig.1 Step gradient with calculated values ( $C_x$ )