

PQ

for DECADE Elite, Lite, I, II
and Intro

171.0023P, Edition 13, 2018



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CHAPTER 1

Introduction

This document describes the Qualification procedure as advised by the manufacturer. It is a result from our interpretation of many regulations and laboratory practices. In addition, feedback from users and representatives helped us to finalize this procedure.

In this document, all qualification checks have to be approved, or should be marked "n.a." if not applicable. Any deviation observed must be documented in the 'non-conformance' record. All relevant documents regarding this operational qualification must be filed together in one location.

As regulations and customer requirements may change, manufacturer reserves the right to introduce changes without prior notice. For details on functionality, operation and theory refer to the instrument user manuals.

Supported configurations

The PQ procedure in this document is applicable to an HPLC-ECD system with an Antec electrochemical detector (DECADE Elite™, DECADE Lite, DECADE II™, DECADE™ or INTRO™) and Antec flow cells with **glassy carbon** (GC) or **gold** (Au) working electrodes. Flow cells with working electrodes other than Au or GC are not supported with PQ procedures.

CHAPTER 2

PQ test

Principle

For testing the performance of the HPLC/ECD system, a test substance is injected onto a Flow Injection Analysis (FIA) tubing assembly, and repeatability, linearity & peak height is measured.

Different detection modes for GC and Au working electrodes

In case of the use of a flow cell with glassy carbon electrode, the detection method is set to direct current (DC) mode, which will apply one working potential. In case of testing with a gold working electrode, Pulsed Amperometric Detection (PAD) mode is used.

Test parameters

Important parameters to characterize and check the performance of all relevant parts of a detection system are:

- Repeatability
- Linearity
- Detector response
- Background current (only with GC electrode)
- Signal-to-noise ratio (only with GC electrode)

Not only the detector and flow cell performance but also those of pump and autosampler are taken into account with these parameters. In case of testing the performance with a gold flow cell, the background current, noise and S/N measurement are not relevant, as in PAD mode the signal is digitally processed.

As the detector response is affected by electrode size and injection volume, different values are specified for different combinations of hardware.

For Clarity software, pre-configured PQ method files are available on our website for download.



Fig. 1. PQ tubing kit installed in detector oven and connected to flow cell.

Required parts and consumables

To perform the PQ, a (reusable) PQ FIA tubing kit and PQ consumables kit are necessary (Table 1). Two different PQ consumables kits are available: one specific for testing glassy carbon electrodes (pn. 250.3048), and another specific for gold working electrodes (pn. 250.3046).

Table 1. Required parts (reordering info of Antec)

| Part | Part no | Qty |
|--|-----------------|-----|
| PQ FIA tubing set | 250.1052 | 1* |
| Tubing assembly for Flow Injection Analysis | | |
| PQ consumables, ECD in DC mode | 250.3048 | |
| Concentrated buffer for PQ | 250.1064 | 1 |
| MOPEG 4.0 µmol/L, 2 mL for PQ | 250.1062 | 4 |
| Document: PQ for D2 Elite Lite and ROXY | 171.0023P | 1 |
| Document: OQ for D2 Elite Lite and ROXY | 171.0023O | 1 |
| PQ consumables, ECD on Au at pH 13 | 250.3046 | |
| Glucose for PQ (dry powder; for 3 mL stock solution of 20 mM) | 250.1067 | 1 |
| Document: OQ for D2 Elite Lite and ROXY | 171.0023O | 1 |
| Document: PQ for D2 Elite Lite and ROXY | 171.0023P | 1 |

* For testing in parallel set-up with DCC detector, 2 sets are necessary.

Additional consumables and chemicals that are necessary to perform the PQ procedure are:

- Autosampler vials
- 1 L ultra-pure water (resistivity > 18 MΩ·cm, low TOC)
- For test in DC mode on GC: 50 mL methanol, HPLC-grade or better
- For test in PAD mode on Au: 50% sodium hydroxide solution, HPLC-grade (commercially available)

Preparations

For a successful performance test it is important that the HPLC-EC system has been optimized and is in top condition.

OQ

1. Perform the Operation Qualification procedure (pn. 171.0023O) before performing the PQ test. Results of the OQ are to be summarized in the PQ results table on page 12.

Flush the system

2. Prepare a bottle with degassed water and use it to flush the system up to the injector.
3. Connect the red-striped end of the PQ FIA tubing directly to the valve. If needed use the 1/32"OD tubing adapter.
4. Flush the FIA tubing for 10 min with water at 1 mL/min.
5. Prepare the mobile phase according to Table 2.
6. Connect the mobile phase and run about 30 mL through the system.

Install the flow cell and let the signal stabilize

7. Clean the flow cell (see user manual) and assemble with a 50 μm spacer.
8. Connect the flow cell to the orange striped end of the FIA tubing set using the correct connectors, and ensure that the flow cell is filled air-free with mobile phase.
9. Set the detector conditions as given in Table 2 and turn on the cell.



Do not turn the cell on when mobile phase has not yet completely filled the cell. Turning on a cell that still has air inside can damage the cell!

10. Wait for the system to stabilize for at least 30 min before starting the PQ HPLC tests.

Prepare the sample

11. For testing flow cells with glassy carbon electrode only: the MOPEG solution is a ready to use standard and only needs to be diluted in case the linearity check is done with diluted standard instead of injection volume variation.
12. For testing flow cells with gold electrode only: Glucose needs to be dissolved and diluted:
 - Add 3 mL of mobile phase to the bottle with Glucose and shake to dissolve thus making a 20 mM stock solution.
 - Dilute the 20 mM stock solution 1000x with MP. For example, dilute in 3 consecutive steps: mark 3 empty vials clearly as "1", "2", "3" and add 900 μL mobile phase to each of the 3 vials. Add 100 μL stock solution in vial 1, close and shake well. With a new clean pipet tip take 100 μL solution from vial 1 and add to vial 2, close and shake. With a new clean pipet tip take 100 μL solution from vial 2 and add to vial 3. Vial 3 now contains 1 mL of 20 $\mu\text{mol/L}$ Glucose standard ready for a PQ test injection.

For gold cells the PQ standard solution is 20 $\mu\text{mol/L}$ Glucose in mobile phase (MP). Dissolve and dilute the glucose powder prior to use.

HPLC-ECD test conditions for PQ

Table 2. HPLC-EC conditions for PQ.

| General settings | |
|--|---|
| PQ hardware | PQ FIA tubing set (250.1052) |
| Flow rate (signal) | 1 mL/min (up to 1.5 mL/min is allowed) |
| Temperature | 35 °C (flow cell and FIA tubing set inside the detector oven) |
| Integrator | Data acquisition frequency: < 5 Hz |
| Flow cell spacing | 50 μ m spacer ,or AST setting '2' for SenCells |
| Settings for testing with <u>glassy carbon</u> working electrodes | |
| Mobile phase | 1 bottle concentrated PQ buffer (pn. 250.1064), 50 mL MeOH (HPLC grade or better) and water (18 MOhm.cm, low TOC) mixed to a final total volume of 1000 mL. PQ buffer already contains chloride required in case of using an ISAAC. |
| Sample | MOPEG 4 μ mol/L (pn. 250.1062) |
| E cell | +610 mV (vs HyREF); +800 mV (vs sb); +650 mV (vs ISAAC) |
| Filter | 0.1 Hz |
| Range (for signal) | 200 nA/V for 2 mm, 3 mm and Flexcell GC electrodes 10 nA/V for 0.7 mm GC electrodes |
| Range (for noise) | 1 nA/V |
| Flow rate (for noise) | 0 mL/min or set to an optimum flow rate |
| Settings for testing with <u>gold</u> working electrodes | |
| Mobile phase | 100 mM NaOH in water (HPLC grade, low TOC, and >18 MOhm.cm) |
| Sample | 20 μ mol/L Glucose in mobile phase |
| E (PAD mode) same for HyREF & Ag/AgCl sb REF | For <u>DECADE Elite</u> : E1, E2, E3, E4 = 100, -2000, 600, -100 mV (E5 0 mV); t1, t2, t3, t4, ts = 400, 20, 10, 70, 200 ms (t5: 0 ms) For <u>DECADE II</u> : E1, E2, E3 = 100, -2000, -100 mV t1, t2, t3, ts = 400, 20, 80, 200 ms Icell is about 0.5 - 2 μ A |
| Filter | off |
| Range | 1 μ A/V |
| Specific settings for DECADE I and INTRO (GC electrodes only) | |
| Noise filter | Signal: 0.1 s; Noise: 1 s |
| Noise analysis | DECADE: pA range; INTRO: att x100 = off |

PQ procedure

Test injection

Run a test chromatogram with the test solution. Check the test-chromatogram peak time, and optimize the automated integration parameters if necessary.

In case of using a 20 or 100 μL sample loop on the injector: adjust the detector range if the test peak height is larger than 50% of the full scale range. The analyses for linearity require a larger injection volume and the signal may run off scale if the range setting is too low.

Linearity test

A 5-point calibration curve has to be set up for linearity evaluation. Data can be generated by varying the concentration or injection volume:

- Varying concentrations: dilute the test solution by hand thus generating equidistant concentrations, for example: 0.8, 1.6, 2.4, 3.2 and 4.0 $\mu\text{mole/L}$ MOPEG (for gold cells: 4, 8, 12, 16, 20 $\mu\text{mole/L}$ Glucose).
- Varying injection volumes: inject 5 different volumes of the test solution. For example: 4, 5, 6, 7, 8 μL (20 μL loop) or 20, 25, 30, 35, and 40 μL (100 μL loop).

The latter is the most preferred method because it eliminates manual dilution errors.

Repeatability test

Repeatability is evaluated based on 8 subsequent analysis of the test solution.

Noise measurement and background current (for GC electrodes only)

Check the cell current (**I cell**) at the detector (e.g. read from the display) and write it in the Results Summary table (page 10).

Record a 15 min baseline trace using the setting specified in Table 2. When pump is suspected to contribute to the noise level, the pump may be switched off or set to another optimum flow rate.

Processing the PQ results

The PQ procedure results in linearity data, 8 repeatability chromatograms, and a baseline trace (for GC electrodes only).

1. Select the 8 'repeatability' chromatograms and create a report containing retention time, peak heights and peak areas. Calculate the average values and the relative standard deviation as percentage of the average (%RSD).
2. Process the linearity data and calculate the correlation coefficient between peak area and concentration or injection volume.

Write the results down in the PQ results table on page 11

Contact your supplier if the final result of the HPLC PQ procedure is 'failed'. Note also that a 'failed' PQ is not necessarily related to the detector. For example, repeatability failures are often an HPLC problem (autosampler).

Additional parameters for GC electrodes:

3. Open the baseline trace and evaluate the noise as described in the OQ document (171.0023O), or use any other software that is capable of calculating the baseline peak-to-peak noise level (ASTM).
4. Calculate the S/N ratio (=peak height/noise level) after obtaining the noise value. The average peak height from the repro-evaluation is used as "signal".

Write the results down in the PQ results table on page 11.



To expand the life time of a PQ tubing set flush it with water before storage.
Before flushing the system, SWITCH OFF AND REMOVE THE CELL!

Recalculation to the correct unit - signal in mV or nA

In case the detector is connected to an AD convertor, this will usually generate a signal with the units in mV. Use the detector range setting to convert to current.

$$\text{Range (nA/V)} * \text{Signal (mV)} = \text{Signal (pA)}$$

For example, at the 2 nA/V range, a signal of 750 mV is actually a current of:

$$2 \text{ nA/V} * 750 \text{ mV} = 1500 \text{ pA (1.5 nA)}$$



Use the proper units nA or pA by multiplying mV with range if necessary!

CHAPTER 3

Specifications

PQ test on **gold** electrodesPeak height specification of 20 μM glucose test solution

| Injection volume | Au working electrode | | | |
|------------------|----------------------|----------|---------|----------|
| | 0.7 mm* | 2 mm* | 3 mm* | Flexcell |
| 5 μL | > 1.3 nA | > 6.3 nA | > 13 nA | > 25 nA |
| 20 μL | > 5 nA | > 25 nA | > 50 nA | > 100 nA |

*AU WE: 0.7 mm is μVT03 , 3mm is VT03, 2mm is SenCellPQ test on **glassy carbon** electrodesPeak height specification of 4 μM MOPEG test solution

| Injection volume | GC working electrode | | | |
|------------------|----------------------|----------|--------|----------|
| | 0.7 mm* | 2 mm* | 3 mm* | Flexcell |
| 2 μL | > 0.1 nA | > 0.5 nA | > 1 nA | > 1 nA |
| 5 μL | > 0.2 nA | > 1 nA | > 2 nA | > 2.5 nA |
| 10 μL | > 0.4 nA | > 2 nA | > 4 nA | > 5 nA |
| 20 μL | > 0.8 nA | > 4 nA | > 8 nA | > 10 nA |

*GC WE: 0.7mm is μVT03 , 3mm (2.7mm) is VT03 sn 4xxxx 2mm is SenCell or VT03 sn 2xxxx.SN-ratio specification for 4 μM MOPEG test solution

| Injection volume | GC working electrode | | | |
|------------------|----------------------|--------|--------|----------|
| | 0.7 mm | 2 mm | 3 mm | Flexcell |
| 2 μL | > 500 | > 500 | > 500 | > 250 |
| 5 μL | > 1000 | > 1000 | > 1000 | > 500 |
| 10 μL | > 2000 | > 2000 | > 2000 | > 1000 |
| 20 μL | > 4000 | > 4000 | > 4000 | > 2000 |

Noise and background current specification

| | GC working electrode | | | |
|-----------|----------------------|--------|---------|----------|
| | 0.7 mm | 2 mm | 3 mm | Flexcell |
| I cell | < 3 nA | < 8 nA | < 12 nA | < 20 nA |
| noise p-p | < 2 pA | < 6 pA | < 8 pA | < 14 pA |

Dummy cell test noise

The noise spec for a dummy cell test with a ROXY: noise < 4.0 pA. For all other devices: noise < 2.0 pA.

CHAPTER 4

OQ-PQ results summary

Injected volume of sample for response and repeatability tests: μL

Electronic test results and HPLC test results

| | Specified ^{*1} | Measured | Result ^{*2} |
|---|-------------------------|-----------|----------------------|
| <u>ELECTRONIC TESTS</u>^{*3} | | | |
| Dummy cell test | | | |
| Current (I-cell) | 2.67 \pm 0.05 nA | nA | |
| Noise p-p | < pA | pA | |
| Analog output test | | | |
| Output at 5 nA/V | 530 \pm 10 mV | mV | |
| <u>HPLC TESTS</u> | | | |
| Chromatogram | | | |
| Peak time | < 3 min | min | |
| Response | | | |
| Height | > nA | nA | |
| Repeatability | | | |
| %RSD t | < 0.5 % | % | |
| %RSD area | < 3.0 % | % | |
| Linearity | | | |
| Correlation coefficient r | > 0.997 | | |
| Tests for GC WE only^{*4} | | | |
| I-cell | < nA | nA | |
| Noise | < pA | pA | |
| S/N ratio | > | | |

*1 Specifications for some of the HPLC tests are hardware dependent: check the applicable specs on page 11 and copy the specified value into this table.

*2 Fill in 'passed' or 'failed'

*3 for 'electronic test' results, copy the measurement values from the OQ test.

*4 Tests for background current and sensitivity are applicable only in case of using a glassy carbon working electrode in the flow cell. When testing with a **gold** working electrode, fill in n.a.

Final result^{*2} _____

Verified by (customer):

Deviations (Y/N):

Comments:

CHAPTER 5

OQ-PQ results summary of optional 2nd cell

This page is for use on DCC detectors that are tested in parallel or serial configurations, otherwise fill in n.a. (not applicable)

Injected volume of sample for response and repeatability tests: μL

Electronic test results and HPLC test results on 'Cell 2'

| | Specified ^{*1} | Measured | Result ^{*2} |
|---|-------------------------|-----------|----------------------|
| <u>ELECTRONIC TESTS</u>^{*3} | | | |
| Dummy cell test | | | |
| Current (I-cell) | 2.67 ± 0.05 nA | nA | |
| Noise p-p | < pA | pA | |
| Analog output test | | | |
| Output at 5 nA/V | 530 ± 10 mV | mV | |
| <u>HPLC TESTS</u> | | | |
| Chromatogram | | | |
| Peak time | < 3 min | min | |
| Response | | | |
| Height | > nA | nA | |
| Repeatability | | | |
| %RSD t | < 0.5 % | % | |
| %RSD area | < 3.0 % | % | |
| Linearity | | | |
| Correlation coefficient r | > 0.997 | | |
| Tests for GC WE only^{*4} | | | |
| I-cell | < nA | nA | |
| Noise | < pA | pA | |
| S/N ratio | > | | |

*1 Specifications for some of the HPLC tests are hardware dependent: check the applicable specs on page 11 and copy the specified value into this table.

*2 Fill in 'passed' or 'failed'

*3 for 'electronic test' results copy the measurement values from the OQ test.

*4 Tests for background current and sensitivity are applicable only in case of using a glassy carbon working electrode in the flow cell. When testing with a **gold** working electrode, fill in n.a.

Final result^{*2} _____

Verified by (customer):

Deviations (Y/N):

Comments:

C H A P T E R 6

PQ certification

The Performance Qualification has been carried out in accordance to the PQ procedure and to the satisfaction of both parties.

All tests as described in this document have been completed, and all results are within specifications, or clearly indicated if not.

Engineer

The undersigned engineer certifies that he/she is trained and qualified to perform a PQ on Antec devices.

Name

Initials

Company

.....

Date

.....

Signature

Reviewer/customer

The undersigned reviewer/customer is authorized to sign and accepts that the above-mentioned engineer is trained and qualified to perform a PQ on Antec devices.

Reviewer/Customer

Initials

Job title

Company & Dept.

.....

Date

.....

Signature

Instrument

Antec detector and flow cell(s)

DECADE (Elite, Lite, I, II) or Intro p/n: s/n:

Detector has DCC option (Y/N)

Flow cell p/n: s/n:

Working electrode type:

Reference electrode type:

Flow cell* p/n: s/n:

Working electrode type:

Reference electrode type:

* enter the info of second flow cell only in case of a DCC detector.

(U)HPLC instrument

Pump* s/n:

Autosampler/injector s/n:
Sample loop volume*

Degasser s/n:

Acquisition software & rev. nr.

*entering two values is allowed for post column addition or parallel set-up with DCC detectors.

Verified by (customer):

Deviations (Y/N):

Comments:

Test materials

- Standard*
Lot nr Exp. date
- Mobile phase
.....

* Fill in 'glucose' or 'MOPEG'

Test devices

- Dummy cell* p/n: 250.0040 s/n:
- Volt meter or AD signal p/n: s/n:

*s/n: entering more than one s/n is allowed for DCC detectors.

Other relevant information

| Description | |
|-------------|--|
| | |
| | |
| | |
| | |
| | |

Verified by (customer):

Deviations (Y/N):

Comments:

Comments

Verified by (customer):

Deviations (Y/N):

Comments:

C H A P T E R 7

Non-conformance record

Any case of non-conformance found during the qualification procedure should be documented and signed for acceptance or corrective action taken.

| Ref. | Non-conformance and action taken or acceptance | Signature customer | Sign. executing technician |
|------|--|--------------------|----------------------------|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |

Verified by (customer):

Deviations (Y/N):

Comments: