

OMCL Network of the Council of Europe QUALITY MANAGEMENT DOCUMENT

PA/PH/OMCL (11) 04 CORR

QUALIFICATION OF EQUIPMENT ANNEX 1: QUALIFICATION OF HPLC EQUIPMENT

Full document title and reference	Qualification of Equipment Annex 1: Qualification of HPLC equipment PA/PH/OMCL (11) 04 CORR
Document type	Guideline
Legislative basis	The present document was also accepted by EA as recommendation document to be used in the context of Quality Management System audits of OMCLs
Date of first adoption	May 2005
Date of original entry into force	June 2005
Date of entry into force of revised document	17 December 2015
Previous titles/other references	This document replaces document PA/PH/OMCL (07) 17 DEF
Custodian Organisation	The present document was elaborated by the OMCL Network/EDQM of the Council of Europe
Concerned Network	GEON

**ANNEX 1 OF THE OMCL NETWORK GUIDELINE
“QUALIFICATION OF EQUIPMENT”**

QUALIFICATION OF HPLC EQUIPMENT

Introduction

The present document is the first Annex of the core document “Qualification of Equipment”, and it should be used in combination with it when planning, performing and documenting the HPLC equipment qualification process.

The core document contains the general introduction and the Level I and II of qualification, common to all type of instruments, and the present annex contains HPLC instrument-related recommendations on parameters to be checked and the corresponding typical acceptance limits, as well as practical examples on the methodology that can be used to carry out these checks.

When qualifying HPLC equipment, it should be noted that it is acceptable to check at Level III and IV several of the mentioned parameters at the same time in a combined test procedure (e.g. “overall” system performance test giving information on peak area precision, retention time precision, gradient reproducibility, etc).

TABLE III

Level III. Periodic and motivated instrument checks

Examples of requirements for HPLC instruments and detectors

Instrument module	Parameter to be checked	Typical tolerance limits
Solvent delivery system	<ul style="list-style-type: none"> • Flow rate • Proportioning accuracy and precision (gradient test) • Proportioning ripple 	<ul style="list-style-type: none"> • $\pm 5 \%$ • ± 2 • $\leq 0,2 \%$
Injector	<ul style="list-style-type: none"> • Volume precision • Carry-over 	<ul style="list-style-type: none"> • $RSD \leq 1.0 \%$ • see Annex I
Autosampler	<ul style="list-style-type: none"> • Thermostating accuracy and precision 	<ul style="list-style-type: none"> • $\pm 3 \text{ }^\circ\text{C}$
Oven or cooling device	<ul style="list-style-type: none"> • Thermostating accuracy 	<ul style="list-style-type: none"> • $\pm 2 \text{ }^\circ\text{C}$
UV/DAD detector	<ul style="list-style-type: none"> • Linearity • Wavelength accuracy 	<ul style="list-style-type: none"> • $r^2 \geq 0.999$ • $\pm 2 \text{ nm}$
Fluorescence detector	<ul style="list-style-type: none"> • Wavelength accuracy excitation • Wavelength accuracy emission • Sensitivity 	<ul style="list-style-type: none"> • $\pm 3 \text{ nm}$ • $\pm 3 \text{ nm}$ • see Annex I
Electrochemical detector	<ul style="list-style-type: none"> • Accuracy of the signal • Stability of the signal 	<ul style="list-style-type: none"> • see Annex I • see Annex I
RID detector	<ul style="list-style-type: none"> • Signal/Noise ratio • Drift over time 	<ul style="list-style-type: none"> • see Annex I • $\pm 0.1 \text{ mV/min}$
CD detector	<ul style="list-style-type: none"> • Signal/Noise ratio • Sensitivity • Drift over time • Linearity 	<ul style="list-style-type: none"> • see Annex I • see Annex I • see Annex I • $r > 0.999$

TABLE IV

Level IV. In-use instrument checks

Examples of requirements for HPLC instruments with UV or DAD detectors

Parameter to be checked	Typical tolerance limits
<ul style="list-style-type: none"> System suitability check for the method 	<p>According to Ph. Eur. or MAH dossier or validated in-house method</p>
<ul style="list-style-type: none"> Peak area precision (applicable to the main peaks in the test solution) 	<p>RSD \leq 1.5 % (unless otherwise prescribed in the system suitability of the method, e.g. specific requirements from Ph. Eur. 2.2.46, API monographs or MA dossiers)</p>
<ul style="list-style-type: none"> Retention time precision 	<p>RSD \pm 5 %</p>
<ul style="list-style-type: none"> Carry-over (by comparing consecutive standard (of the substance being quantified) and blank injections) 	<p>\leq 0.2 %</p>
<ul style="list-style-type: none"> Signal/Noise ratio (to be applied for related substances test) 	<p>According to Ph. Eur.</p>

Level III. Periodic and motivated instrument checks

This Annex contains practical examples of tests and their associated tolerance limits for several parameters related to the performance of the different modules of a HPLC. These examples can be considered by the OMCLs as possible approaches to perform the Level III of the equipment qualification process: “Periodic and motivated instrument checks”.

HPLC SOLVENT DELIVERY SYSTEM

The following tests are proposed for the periodic and motivated check of the HPLC solvent delivery system: flow rate and gradient test.

FLOW RATE

Materials:

Volumetric flask of 5 or 10 ml
Calibrated chronometer

Settings:

Mobile phase: degassed water
No column (open end)*
Flow rate: adjusted between 0.5 and 3.0 ml/min
If high-pressure mixing systems are installed, this test has to be done on each solvent channel.

* For certain equipment, e.g. in the case of low flow rates, the check would be performed by using a column or a backpressure regulator.

Method:

Set the flow rate at an appropriate level and measure the time needed to fill the volumetric flask up to the mark. Record the time needed.

$$f = V/t \qquad f = \frac{V * 60}{t}$$

f measured flow rate [ml/min]
 t elapsed time to fill up to mark [s]
 V volume of the volumetric flask [ml]

$$D = 100 * \frac{f - F}{F}$$

D deviation [%]
 F adjusted flow rate [ml/min]
 f measured flow rate [ml/min]

Limits: ± 5%

GRADIENT COMPOSITION AND RIPPLE

Settings:

Stainless steel capillary e.g. 2000 x 0.12 mm installed instead of a column

Detection: UV-Detector adjusted to 265 nm

Mobile phase A: degassed water

Mobile phase B: degassed water containing 0.5% acetone

Flow rate: 1.0 ml/min

Method:

The test is carried out in the following way by using a gradient program depending on the number of solvent channels and the configuration of the system:

A-B

A-B and A-C

A-C, A-B and B-D

time [min]	% mobile phase A (water)	% mobile phase B (water-acetone mixture)
0.0	100	0
0.1	90	10
10	90	10
10.1	50	50
20	50	50
20.1	10	90
30	10	90
30.1	0	100
40	0	100
40.1	100	0

Start the test by pumping water for at least 10 min to equilibrate the system.

The zero % value at the start of the test is the baseline. All steps are measured at the beginning of the horizontal part of the line either by software or manually on the paper print using a liner. The height of the 100% water/acetone mixture is used as the 100% value in the following calculation.

$$\%H = 100 * \frac{h}{H}$$

%H..... calculated composition

h..... height of the measured line

H..... height of the 100% water/acetone mixture line (mobile phase B)

$$d = \%H - G$$

d..... deviation

G..... gradient composition adjusted [% acetone/water solution mixture = mobile phase B]

Limits:

Absolute deviation: ± 2 of the adjusted value

The ripple of the gradient composition is the percentage of noise of the 50% line from the gradient program.

$$\%R = 100 * \frac{N}{h_{50}}$$

$\%R$ ripple

h_{50} height of the 50% line

N height of the noise line, measured during 1 minute in the linear region

Limits: $\leq 0.2 \%$

HPLC INJECTOR

Volume precision and carry-over are the tests proposed for the periodic and motivated check of the HPLC injector.

VOLUME PRECISION AND CARRY-OVER

Solutions:

Solvent A: methanol : water R, 60 : 40.

Reference solution (a): dissolve 15.0 mg methyl-, ethyl-, and propylparabene in 100.0 ml of solvent A.

Reference solution (b): Dilute 1.0 ml of reference solution (a) to 10.0 ml of solvent A.

Reference solution (c): Dilute 1.0 ml of reference solution (b) to 100.0 ml of solvent A.

Settings:

Column: Lichrospher 100 RP8, 5 μm , 125 x 4 mm, without precolumn

Mobile phase: methanol : water = 60 : 40

Flow rate: 1.0 ml/min

Detection: 254 nm

Injection volume: 20 μl

Method:

Injection scheme:

- 6x reference solution (b)
- 1x reference solution (a)
- 1x solvent A (blank injection 1)
- 1x reference solution (b)
- 1x solvent A (blank injection 2)
- 1x reference solution (c)

Limits:

Repeatability of peak areas: The relative standard deviation of the peak areas of all peaks in the chromatogram obtained with the reference solution (b) should be $\leq 1.0 \%$.

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Carry-over: The percentage of the peak area corresponding to propylparabene in the blank injection 1 does not exceed 0.5% of 10 times the peak area of the propylparabene peak in the chromatogram obtained with the reference solution (b) injected after the blank injection.

The percentage of the peak area corresponding to propylparabene in the reference solution (c) is 0.9 – 1.1% of the peak area of the propylparabene peak in the chromatogram obtained with the reference solution (b) injected after the blank injection.

HPLC AUTOSAMPLER

Thermostating accuracy and precision can be tested in the frame of the periodic and motivated check of the HPLC Autosampler.

THERMOSTATING ACCURACY

Materials:

Calibrated temperature probe.

Method:

Select a temperature along the operational or required temperature range of the equipment. Wait until the system is equilibrated.

By means of the calibrated probe, measure the actual temperature in the autosampler and compare it to the selected temperature.

Repeat the same procedure at different pre-selected points covering the temperature range.

Limits:

The actual temperature may not differ more than $\pm 3^{\circ}\text{C}$ with respect to the selected temperature.

THERMOSTATING PRECISION

Materials:

Calibrated temperature probe.

Method:

Select a temperature along the operational or required temperature range of the equipment. Wait until the system is equilibrated.

By means of the calibrated probe, make “n” measurements over a pre-established period of time. Compare the mean of the “n” measurements to the selected temperature.

Limits:

The actual temperature may not differ more than $\pm 3^{\circ}\text{C}$ with respect to the selected temperature.

HPLC OVEN/COOLING DEVICE

Thermostating accuracy is the parameter tested in this example of periodic and motivated check of the HPLC oven/cooling device.

THERMOSTATING ACCURACY

Materials:

Calibrated thermometer.

Method:

Set the column oven temperature to 40 °C, wait about 30 minutes to equilibrate the system, put a calibrated thermometer into the oven and read the temperature after 10 minutes.

Limits: 38 - 42°C.

HPLC UV/DAD DETECTOR

The periodic and motivated check of the HPLC UV/DAD detector can be performed by testing the linearity and the wavelength accuracy.

LINEARITY

Solutions:

- Std. 1: 0.5 µg caffeine/1 ml methanol HPLC Grade
- Std. 2: 1.0 µg caffeine/1 ml methanol HPLC Grade
- Std. 3: 5.0 µg caffeine/1 ml methanol HPLC Grade
- Std. 4: 25.0 µg caffeine/1 ml methanol HPLC Grade
- Std. 5: 50.0 µg caffeine/1 ml methanol HPLC Grade
- Std. 6: methanol HPLC Grade (blank)

- Std. 5: weigh 9.0 to 11.0 mg caffeine and fill up to 200.0 ml with methanol HPLC
- Std. 4: dilute 50.0 ml of Std. 5 to 100.0 ml with methanol
- Std. 3: dilute 10.0 ml of Std. 5 to 100.0 ml with methanol
- Std. 2: dilute 20.0 ml of Std. 3 to 100.0 ml with methanol
- Std. 1: dilute 10.0 ml of Std. 3 to 100.0 ml with methanol

Settings:

- Column: RP-18 5 µm 30-50 x 2,1-4.6 mm or capillary 2000 mm x 0.12 mm ID
- Mobile phase: methanol HPLC Grade
- Oven temperature: 40 °C
- Flow rate: 1.0 ml/min (adjusted by using 100% methanol)
- Detection: 273 nm
- Injection volume: 20 µl

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Method:

Injection scheme:

2 x blank
1 x Std. 1
1 x Std. 2
1 x Std. 3
1 x Std. 4
1 x Std. 5

Limits: $r^2 \geq 0.999$

Remark: As this test employs different test solutions to be injected, it covers also the check of correct positioning vials in the autosampler.

WAVELENGTH ACCURACY

If there are built-in test procedures for the determination and adjustment of wavelength accuracy, follow the instructions of the instrument manual.

In all other cases use the procedure described below.

Solutions:

DAD: caffeine Std. 5 from the linearity testing

UV/VIS: 1.0 µg/ml anthracene solution in water

Settings:

Mobile phase: 15% acetonitrile in water

Column: RP18, 5 µm 30-50 x 2,1-4.6 mm or capillary 2.0 m x 0.12 mm ID

Oven temperature: 40 °C

Flow rate: 1.0 ml/min (adjusted by using 15% acetonitrile in water)

Detection: scan from 230 nm to 290 nm (DAD)

Injection volume: 20 µl

Method:

DAD: Inject 20 µl of the caffeine solution and record the spectrum. The maximum is at 272 nm and the minimum at 244 nm.

UV/VIS: fill the cell with the anthracene solution and change the wavelength from 248 to 254 nm in 1 nm steps; record the maximum of absorption. The theoretical value is 251 nm.

Limits: ± 2 nm

HPLC FLUORESCENCE DETECTOR

The following three parameters are proposed for the performance of the periodic and motivated check of the HPLC fluorescent detector:

WAVELENGTH ACCURACY EXCITATION

Method:

Rinse and fill the measuring cell with de-ionized water
Adjust the excitation wavelength to 350 nm.
Measure the emission and subtract 397 nm (theoretical value).

Limits: ± 3 nm

WAVELENGTH ACCURACY EMISSION

Method:

Rinse and fill the measuring cell with de-ionized water
Adjust the emission wavelength to 397 nm.
Measure the excitation and subtract 350 nm (theoretical value).

Limits: ± 3 nm

SENSITIVITY

Solutions:

Quinine HCl.2H₂O solution conc. 0.015 μ g/ml (=15 ppb)

The quinine solution is prepared with the following mobile phase: dissolve 6.8 g of potassium dihydrogen phosphate R and 3.0 g of hexylamine R in 700 ml of water R, adjust to pH 2.8 with dilute phosphoric acid R, add 90 ml of acetonitrile R and dilute to 1000.0 ml with water R.

Settings:

The chromatographic conditions are set according to Ph. Eur. "Quinine HCl" (01/2005:0018), test "Other cinchona alkaloids", with modified flow rate and acetonitrile concentration.

Mobile phase: as above

Column: RP18, 5 μ m, 250 x 4.6 mm

Flow rate: 1,2 ml/min

Excitation wavelength: 350 nm

Emission wavelength: 397 nm

Flow-cell volume: 8 μ l (for this example, a Waters 2475 MultiFluorescentiedetector was used.

Flow-cell volume may vary depending on the instrument manufacturer)

Method:

Inject 10 μ l of the quinine solution and measure the peak height.

Inject 10 μ l of the blank and measure the peak height of the noise.

Divide the peak height of the quinine solution by 3 times the peak height of the noise.

Divide the concentration of the quinine solution by the previously obtained factor.

Limits: ≤ 0.5 ppb

HPLC ELECTROCHEMICAL DETECTOR

Accuracy and stability of the signal are the proposed parameters to be tested during the periodic and motivated check of the HPLC electrochemical detector.

ACCURACY AND STABILITY OF THE SIGNAL

Settings:

Cell potential of a dummy cell: 800 mV

Rise time filter: 0.1 s

Range: 0.1 nA

Temperature: 30 °C

Method:

Accuracy: Measure the electric current and subtract 2.67 nA (theoretical value)

Stability: Measure the noise over a period of 5 minutes

Limits:

Accuracy (cell current): ± 0.05 nA

Stability of the signal (Noise): max. 2 pA or 20 mV

HPLC RID DETECTOR

Signal to Noise ratio and drift over time are the parameters proposed for the periodic and motivated check of the HPLC RID (refractive index) detector.

SIGNAL TO NOISE RATIO

Solutions:

Standard solution: D-fructose concentrate solution at 4.0 mg/ml (dilute 200.0 mg fructose + 20 ml water + 25.0 ml acetonitrile up to 50.0 ml with water for HPLC)

Settings:

Column: Spherisorb NH₂ (250 x 4.6 mm 5 μ m) or equivalent

Oven temperature: 38°C

Flow rate: 1.0 ml/min

Injection volume: 20 μ l

Mobile phase: 0.253g sodium dihydrogen phosphate R in 220 ml + 780 ml acetonitrile

Method:

After equilibration, inject three times a blank solution of mobile phase over a run time where the system is stable. Measure the baseline noise over an appropriate period.

The baseline noise is accepted if the mean height of the three replicates is < 1000 μ V.

To calculate the signal to noise ratio, inject three times a solution of fructose at 0.4 mg/ml and calculate the mean of the three replicates.

Limits: S/N > 10

DRIFT OVER A DEFINED PERIOD OF TIME

Method:

Calculate the slope of the amplitude of random variations in the detector's signal over 1 minute.

Limits: ± 0.1 mV/min

Alternatively, the requirement may be expressed in $\Delta RI/\text{min}$ or in % of full scale of the selected range.

HPLC CD DETECTOR

The following tests are proposed to perform the periodic and motivated check of the HPLC CD (circular dichroism) detector.

LINEARITY AND SIGNAL TO NOISE RATIO

Solutions:

Reference solution (a): dissolve 25.0 mg D(-) pantolactone in 50.0 ml water

Reference solution (b): dilute 2.0 ml of reference solution (a) to 10.0 ml with water

Reference solution (c): dilute 4.0 ml of reference solution (a) to 10.0 ml with water

Reference solution (d): dilute 6.0 ml of reference solution (a) to 10.0 ml with water

Reference solution (e): dilute 8.0 ml of reference solution (a) to 10.0 ml with water

Reference solution (f): dilute 0.5 ml of reference solution (b) to 25.0 ml with water

Settings:

Column: C18, 150 x 4 mm, 5 μm

Mobile phase: acetonitrile : water = 10 : 90

Flow: 1.0 ml/min

Detection: 225 nm

Injection volume: 20 μl

Method:

Check the linearity of de CD- and UV-signal of D(-)pantolactone reference solution a,b,c,d,e.

Measure the noise of the CD-signal of reference solution (f) between 0 – 10 min.

- Calculate the absolute concentration (μg) in the cell
- Calculate the signal-to-noise ratio (S/N) for 0.01 μg in the cell
- Calculate the sensitivity with the calculated S/N and the specified S/N= 2
($0.01 \times 2 / S/N_{\text{calculated}}$)

Limits:

Linearity: The linearity of the calibration line obtained with reference solution (a,b,c,d,e) should be $r > 0.999$.

Sensitivity: The sensitivity at S/N= 2 should be better than 0.01 μg .

S/N ratio: The limit for S/N is > 1.0 and the sensitivity should be 0.020 μg at maximum.

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DRIFT OVER A DEFINED PERIOD OF TIME

Settings:

Column: C18, 150 x 4 mm, 5 μ m
Mobile phase: acetonitrile : water = 10 : 90
Flow: 1.0 ml/min
Detection: 290 nm
Injection volume: 20 μ l

Method:

Inject water and stop the flow after 5 minutes. Measure the CD-signal for 1 hour.
Measure with the cursor the drift of the baseline between 5 and 65 min.

Limits:

Not more than 0.1 mdeg/h

SPECTRA COMPARISON

Solutions:

Reference solution (a): dissolve 5.0 mg dexamethasone in 10.0 ml 40% acetonitrile

Settings:

Column: C18, 150 x 4 mm, 5 μ m
Mobile phase: acetonitrile : water = 40 : 60
Flow: 1.0 ml/min
Detection: 230 nm
Injection volume: 20 μ l

Method:

Compare the maxima/minima obtained at the Installation of the detector (see table).

CD max	CD min	UV max
222 nm	224 nm	236 nm
230 nm	252 nm	
284 nm		

Limits:

The maxima and minima may not differ more than ± 4 nm.