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QUALIFICATION OF EQUIPMENT
ANNEX 1: QUALIFICATION OF LIQUID CHROMATOGRAPHY EQUIPMENT

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ANNEX 1 OF THE OMCL NETWORK GUIDELINE "QUALIFICATION OF EQUIPMENT"

QUALIFICATION OF LIQUID CHROMATOGRAPHY EQUIPMENT

Note: Mandatory requirements in this guideline and its annexes are defined using the terms «shall» or «must». The use of «should» indicates a recommendation. For these parts of the text other appropriately justified approaches are acceptable. The term «can» indicates a possibility or an example with non-binding character.

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 Liquid Chromatography (LC) equipment qualification process.

The core document "Qualification of Equipment" contains the general introduction and the Level I and II of qualification, common to all types of instruments. The present Annex 1 contains a general introduction and requirements for LC (HPLC and UHPLC) instruments.

Level III and IV qualifications must be carried out being an ISO 17025 requirement.

Requirements and (if applicable) corresponding typical acceptance limits given in bold should be applied; however other appropriately justified approaches are acceptable.
Exemplary procedures provided in Annexes have non-binding character. They can be helpful to carry out the required qualification. Nevertheless, it is left to the professional judgement and background experience of each OMCL to decide on the most relevant procedures to be undertaken in order to give evidence that their LC systems are working properly and are suitable for their intended use.

Moreover, combined test procedures can be applied to carry out Level III and IV qualifications to check several parameters simultaneously (e.g. "overall" system performance test for peak area precision, retention time precision, gradient reproducibility, etc.)

TABLE I

Level III. Periodic and motivated instrument checks

Recommendations for LC instruments and related typical acceptance limits

Instrument module	Parameter to be checked	Typical Acceptance limits
Solvent delivery system	• **Flow rate accuracy (HPLC)** • **Flow rate accuracy (UHPLC)** • **Flow rate precision (HPLC and UHPLC)** • **Gradient composition accuracy** • **Gradient ripple**	± 5.0 % ± 3.0 % RSD ≤ 0.5 % ± 2.0 % ≤ 0.2 %
Injector	• **Volume precision (HPLC and UHPLC)** • **Carry-over**	RSD ≤ 1.0 % ≤ 0.2 %
Autosampler	• **Thermostating accuracy**	± 3 °C
Oven or cooling device (column)	• **Thermostating accuracy** • **Thermostating stability**	± 2 °C ≤ 1 °C
Multi-wavelength detector	• **Linearity** • **Wavelength accuracy** • **Drift**	$r^2 \geq 0.9990$ ± 2 nm Annex I
Fluorescence detector	• **Wavelength accuracy (excitation and emission)** • **Signal/Noise ratio**	± 3 nm ≥ 400

Instrument module	Parameter to be checked	Typical Acceptance limits
Electrochemical detectors:		
-Amperometric detection	• **Drift (cell current)** • **Noise**	≤ 8 pA/h ≤ 2 pA
- Integrated Amperometric detection	• **Linearity** • **Drift** • **Noise**	$r^2 \geq 0.999$ ≤1250 pC/20 min $MW_{20 read outs} < 160$ pC
- Conductivity detection	• **Linearity** • **Drift** • **Noise**	$r^2 \geq 0.999$ ≤2 nS ≤ 20 nS/h
- Coulometric detection	Annex I	Annex I
Refractive Index detector	• **Signal/Noise ratio** • **Drift over time** • **Linearity**	≥ 10 ± 0.1 mV/min $r^2 \geq 0.9950$
Circular Dichroism detector	• **Linearity** • **Signal/Noise ratio** • **Drift over time** • **Spectra comparison**	$r^2 \geq 0.9950$ > 1.0 ≤ 0.1 mdeg/h ± 4 nm
Charged Aerosol detector	• **Baseline noise** • **Largest Random spike** • **Baseline drift** • **Repeatability** • **Signal/Noise ratio** • **Signal calibration**	≤ 0.04 pA ≤ 0.2 pA ≤ 0.04 pA/min RSD ≤ 10 % ≥ 10 $r^2 \geq 0.9990$

Instrument module	Parameter to be checked	Typical Acceptance limits
Evaporative Light Scattering detector	• **Noise** • **Baseline drift** • **Repeatability**	≤ 2 mV ≤ 2.0 mV/h RSD ≤ 3.0 %

TABLE II

Level IV. In-use instrument checks

Recommendations for LC instruments and related typical acceptance limits

Parameter to be checked	Typical acceptance limits
• **System suitability**	According to Ph. Eur. or MAH dossier or validated in-house method
• **Peak area precision (Assay, applicable to the main peak of the analyte when not saturated)**	RSD ≤ 1.5 % (min. 5 injections of test or reference sol.) (unless otherwise prescribed in the system suitability of the method, e.g. specific requirements from Ph. Eur. Chapter 2.2.46, Ph. Eur. Monographs, or MAH dossiers)
• **Peak area precision (Related substances)**	RSD ≤ 5.0 % (minimum 3 injection of the diluted solution or reference solution used for quantification) (unless otherwise prescribed in the system suitability of the method, e.g. specific requirements from Ph. Eur. Chapter 2.2.46, Ph. Eur. monographs or MAH dossiers)
• **Retention time precision (applicable to the main peak of the standard solution when not saturated)**	RSD ≤ 2.0% (min. 5 injections of test or reference sol.)
• **Carry-over (by comparing consecutive injections of a standard solution of the substance being quantified and a blank injection)**	≤ 0.2 % (Assay) Below disregard limit (Related substances)
• **Signal/Noise ratio (to be applied for related substances test only)**	According to Ph. Eur. 2.2.46. (unless otherwise prescribed in Ph. Eur. monographs or MAH dossier)

ANNEX I

Level III. Periodic and motivated instrument checks

This Annex contains practical examples of procedures and their typical acceptance limits for several parameters to perform the Level III qualification of different modules of HPLC/UHPLC instruments.

In case more than one method is described for testing one parameter, method 1 or method 2 or method 3 can be used.

For qualification procedure, whenever a column is described, it can be considered to use a dedicated column.

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 ACCURACY and PRECISION

Method 1

Materials:
Beaker (capacity of 5-10 mL) or weighing vessel
Calibrated thermometer
Calibrated chronometer
Calibrated analytical balance

Settings:
Mobile phase: degassed water for chromatography R
Capillary e.g. 2000 x 0.12 mm capillary for back pressure
Flow rate:

- HPLC system: 0.5 mL/min and 5.0 mL/min or maximum flow rate used
- UHPLC system: 0.2 mL/min and 2.0 mL/min or maximum flow rate used

If high-pressure mixing systems are installed, this test has to be done on each solvent channel.

Procedure:
Set the flow rate at a desired level (at least test one flow rate, the lower operational level in use) and leave water to flow into the beaker or weighing vessel, previously weighed (empty). After a defined time (e.g. 5.0 min) weigh the beaker again containing the delivered water. Measure the temperature of the water used. Repeat the procedure at least 3 times. Calculate the volume delivered to the beaker and the flow rate:

$V = m/\varrho$

m……weight of water delivered in the measured time [g]
ϱ…….density of water at actual temperature from table [g/mL]

V ..... volume delivered [mL]

$$f = V / t$$

f..... measured flow rate [mL/min]
t..... elapsed time [min]
V ..... volume delivered [mL]

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

D ..... deviation [%]
F ..... nominal flow rate [mL/min]
f..... measured flow rate [mL/min]

Method 2
Use calibrated flow meter for determination of flow rate

Method 3

Materials:
Use calibrated volumetric glassware e.g. flask of 5.0 or 10.0 mL class A
Calibrated chronometer

Settings:
Mobile phase: degassed water for chromatography R
Capillary e.g. 2000 x 0.12 mm capillary for back pressure
Flow rate:
For HPLC system: 0.5 mL/min and 5.0 mL/min or maximum flow rate used
For UHPLC system: 0.2 mL/min and 2.0 mL/min or maximum flow rate used

If high-pressure mixing systems are installed, this test has to be done on each solvent channel.

Procedure:

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.

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

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:
Accuracy: *HPLC: D* ≤ 5.0%
UHPLC: D ≤ 3.0%

Precision (HPLC and UHPLC): The relative standard deviation should be ≤ 0.5 %.

GRADIENT COMPOSITION ACCURACY AND RIPPLE

Settings:
Capillary e.g. 2000 x 0.12 mm for back pressure
Detection: 265 nm
Mobile phase A: water for chromatography R
Mobile phase B: water for chromatography R containing 0.5% acetone
Flow rate: 1.0 mL/min

Procedure:
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

Table III - Example for A-B system configuration:

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 (as absorption units) or manually on the paper print using a ruler. The height of the 100% water/acetone mixture is used as *H* 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..... nominal gradient composition [% water/acetone mixture = mobile phase B]

Limits:
Accuracy: ± 2.0 %

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, i.e. the difference between the minimum and the maximum of the 50 % line measured during 1 minute in the linear region

Limit: ≤ 0.2 %

INJECTOR

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

VOLUME PRECISION AND CARRY-OVER

Method 1

Solutions:
Solvent A: water for chromatography R: methanol R (40:60 V/V)
Reference solution (a): dissolve 15.0 mg methylparaben and 15.0 mg of propylparaben in solvent A and dilute to 100.0 mL with the same solvent.
Reference solution (b): Dilute 1.0 mL of reference solution (a) to 10.0 mL with solvent A.

Settings:
Column: Lichrospher 100 RP8, 5 µm, 125 x 4 mm, without pre-column or equivalent
Mobile phase: water for chromatography R: methanol R (40:60 V/V)
Flow rate: 1.0 mL/min
Detection: 254 nm
Injection volume:

- HPLC ("short pathway" flow-cell, for example 5 or 10 mm): 20 µL
- UHPLC ("short pathway" flow-cell, for example 5 or 10 mm): 10 µL
- HPLC/UPLC ("long pathway" flow-cell, for example 60 or 85 mm): 2 µL

Procedure:
Injection scheme:
– 1 x solvent A (blank injection 1)
– 6 x reference solution (b)
– 1 x reference solution (a)
– 1 x solvent A (blank injection 2)
– 1 x reference solution (b)
–
Limits:
Repeatability of peak areas: The relative standard deviation of the peak areas of methylparaben and propylparaben in the chromatograms of the six consecutive injections of reference solution (b) should be ≤ 1.0 %

Carry-over: The percentage of the peak area corresponding to propylparaben in the blank injection 2 does not exceed 2 % of the peak area of the propylparaben peak in the chromatogram obtained with reference solution (b) injected after the blank injection 2, this corresponds to 0.2 %

Method 2

Solutions:
Reference solution (a): 0.5 mg caffeine/mL water for chromatography R
Reference solution (b): 50.0 µg caffeine/mL water for chromatography R

Settings:
Column: RP-18, 5 µm, 30-150 x 2.1- 4.6 mm
Mobile phase: water for chromatography R: acetonitrile R (85:15 V/V)
Oven temperature: 40 °C
Flow rate: 1.0 mL/min (flow rate may be reduced according to column diameter)
Detection: 273 nm
Injection volume:

- HPLC ("short pathway" flow-cell, for example 5 or 10 mm): 20 µL
- UHPLC ("short pathway" flow-cell, for example 5 or 10 mm): 10 µL
- HPLC/UPLC ("long pathway" flow-cell, for example 60 or 85 mm): 2 µL

Procedure:
Injection scheme:
– 1 x Mobile phase (blank injection 1)
– 6 x Reference solution (b)
– 1 x Reference solution (a)

- 1 x Mobile phase (blank injection 2)
- 1 x Reference solution (b)

Limits:
Volume precision: The relative standard deviation of the peak areas of caffeine in the chromatograms of the six consecutive injections of reference solution (b) should be ≤ 1.0 %

Carry-over: The percentage of the peak area corresponding to caffeine in the blank injection 2 does not exceed 2 % the peak area of the caffeine peak in the chromatogram obtained with reference solution (b) injected after the blank injection 2, this corresponds to 0.2 %.

AUTOSAMPLER

Thermostating accuracy can be tested in the frame of the periodic and motivated check of the autosampler.

THERMOSTATING ACCURACY

Materials:
Calibrated (electronic) thermometer with a suitable probe.

Procedure:
Select a temperature along the operational or required temperature range of the equipment (e.g. the lowest point can be checked, for example + 5º C, or typical temperature used). Wait until the system is equilibrated.
By means of the calibrated thermometer, measure the actual temperature in the autosampler and compare it to the selected temperature. As an alternative procedure, fill a vial with water, wait until equilibration and measure the temperature of the water by using an appropriate probe. If applicable, repeat the measure from several points of the autosampler (mapping) following the instructions provided by the manufacturer.

Limits:
The actual temperature should be within ± 3°C with respect the selected temperature.

OVEN/COOLING DEVICE

Thermostating accuracy and stability are the parameters tested in this example of periodic and motivated check of the oven/cooling device.

THERMOSTATING ACCURACY AND STABILITY

Materials:
Calibrated thermometer.

Procedure:
1) Set the column oven temperature to 40 °C, wait about 30 minutes to equilibrate the system, put a calibrated thermometer into the oven and take 6 temperature readings at 4 minutes intervals.

2) If applicable, repeat this procedure setting the oven/cooler at other operational temperatures based on the working range of temperature of the equipment.

The accuracy is calculated as average of 6 temperature readings.

Limits: ± 2 °C

The stability is verified using the results obtained in the accuracy test. The stability is calculated for each temperature as the difference between the highest and the lowest temperature of the six readings.

Limits: ≤ 1 °C

MULTIWAVELENGTH DETECTOR

The periodic and motivated check of the LC UV/visible and DAD detectors can be performed by testing the linearity, wavelength accuracy and drift.

LINEARITY

Solutions:
Std. 1: 0.5 µg/mL caffeine
Std. 2: 1.0 µg/mL caffeine
Std. 3: 5.0 µg /mL caffeine
Std. 4: 25.0 µg/mL caffeine
Std. 5: 50.0 µg/mL caffeine
Std. 6: water for chromatography R (blank)

For example, prepare a stock solution (Std. 0) by weighing about 10.0 mg of caffeine and fill up to 20.0 mL with water for chromatography R (dissolve using sonication or mix). Dilute this solution, for example following the subsequent scheme:

Std. 5: dilute 10.0 mL of stock solution to 100.0 mL
Std. 4: dilute 5.0 mL of stock solution to 100.0 mL
Std. 3: dilute 1.0 mL of stock solution to 100.0 mL
Std. 2: dilute 10.0 mL of Std. 3 to 50.0 mL
Std. 1: dilute 10.0 mL of Std. 3 to 100.0 mL

Use water for chromatography R to dilute.

Settings:
Column: suitable column or capillary (e.g. RP 18)
Mobile phase: acetonitrile R: water for chromatography R (15:85 V/V)
Oven temperature: 40 °C

Flow rate:
- HPLC 1.0 mL/min
- UHPLC 0.5 mL/min (or appropriate flow rate based on instrument specifications/operational use)

Detection: 273 nm
Injection volume:

- HPLC ("short pathway" flow-cell, for example 5 or 10 mm): 20 µL
- UHPLC ("short pathway" flow-cell, for example 5 or 10 mm): 10 µL
- HPLC/UPLC ("long pathway" flow-cell, for example 60 or 85 mm): 2 □L (or appropriate volume based on instrument specifications/operational use)

Procedure:
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: $r^2 \geq 0.9990$

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

WAVELENGTH ACCURACY

Wavelength accuracy (and related adjustment) can be carried out by built-in test procedures, following instructions of the instrument manual/manufacturer.
In all other cases use the procedure described below.

Solutions:
Caffeine Std. 5 from the linearity testing

Settings:
Use the operational conditions described under linearity testing except for detection.

Procedure:
Inject the caffeine solution and record the spectrum by scanning from 190 to 290 nm (1 nm incremental steps, whenever possible). The maxima are at 205 nm and 273 nm, the minimum at 245 nm.

Limits: ± 2 nm

DRIFT

Typically drift verification is carried out by built-in test procedures. Follow the instructions of the instrument manual/manufacturer.

Limits: according to manufacturer.

FLUORESCENCE DETECTOR

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

WAVELENGTH ACCURACY FOR EXCITATION

Method:
Rinse and fill the measuring cell with de-ionized water
Adjust the excitation wavelength to 350 nm and emission wavelength to 397 nm.
Measure the excitation spectrum.

Limits for excitation maximum: 350 ± 3 nm

WAVELENGTH ACCURACY FOR EMISSION

Method:
Rinse and fill the measuring cell with de-ionized water
Adjust the excitation wavelength to 350 nm and emission wavelength to 397 nm.
Measure the emission spectrum.

Limits for emission maximum: 397 ± 3 nm

SIGNAL TO NOISE RATIO

Method:
Rinse and fill the measuring cell with de-ionized water.
Set-up the excitation wavelength at 350 nm and the emission wavelength at 397 nm.
The signal is acquired over a total of 23 minutes. At 20.30 minutes the emission wavelength is switched to 450 nm.
The signal is measured at the maximum wavelength of Raman band (i.e. 397 nm for 350 nm excitation) and the noise in a region (450 nm) where no Raman signal is present.
Signal-to-noise is calculated dividing the height of the Raman band divided by the noise.

Limits: Signal to noise ≥ 400

ELECTROCHEMICAL DETECTOR

There are several types of electrochemical detectors available from different manufacturers, based on amperometry, coulometry or conductivity method of detection.

Amperometric detector

DRIFT AND NOISE

Settings:
Cell potential of a dummy cell: 800 mV
Rise time filter: 0.1 s
Range: 0.1 nA
Temperature: 30 °C

Procedure:
Drift: Measure the electric current and subtract 2.67 nA (theoretical value)
Noise: Measure the noise over a period of 5 minutes

Limits:
Drift (cell current): ≤ 8 pA/h
Noise of the signal: ≤ 2 pA or 20 mV

Integrated amperometric detector

DRIFT AND NOISE

Noise: Read the signal every minute.

Settings:
Pressure: ≥1000 psi
Flow: 1.0 mL/min
Restriction capillary: PEEK, 150 cm x 0.13 mm
Column: CarboPac PA1 Guard
Eluent: 50 mM NaOH, filtered through 0.2 µm Nylon, degassed
Detector settings: Waveform "Amino Acids" (pH, Ag, AgCl Reference)
Run time: 20 min

Limits:
Drift: ≤ 1250 pC/20 min
Noise: $MW_{20\text{read outs}} < 160$ pC

LINEARITY

Solutions:
1, 2, 4, 8, and 10 µM of Threonine in water for chromatography R

Settings:
Pressure: ≥1500 psi
Flow: 0.25 mL/min
Restriction capillary: PEEK, 150 cm x 0.13 mm
Column: CarboPac PA1 Guard 2 mm
Eluent: 50 mM NaOH, filtered through 0.2 µm Nylon, degassed
Detector settings: Waveform "Amino Acids" (pH, Ag, AgCl Reference)
Duration/run time: 3 min
Injection volume 25µL

Limits:
$r^2 \geq 0.999$

Conductivity detector

DRIFT AND NOISE

Pure water is pumped through the detector cell at 1.0 mL/min. Measure the baseline noise over an appropriate period.

Limits:
Noise: ≤ 2 nS
Drift: ≤ 20 nS/h

LINEARITY

Solutions:
5, 10, 25, 50 and 100 ppm solutions of Nitrate in water for chromatography R.

Settings:
Column: Backpressure loop
Eluent: Deionized water
Flow rate: 1.0 mL/min

Limits: $r^2 \geq 0.999$

Coulometric detection

Follow the manufacturer instructions.

REFRACTIVE INDEX DETECTOR

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

SIGNAL TO NOISE RATIO

Method 1

Solutions:
D-fructose solution at 4.0 mg/mL (e.g. dilute 200.0 mg fructose in 20 mL with water for chromatography R, add 25 mL acetonitrile R and dilute to 50.0 mL with water for chromatography R)

Settings:
Column: Spherisorb NH_2, 5 µm, 250 x 4.6 mm or equivalent
Oven temperature: 38 °C
Cell temperature: 35 °C
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Mobile phase: 0.253g sodium dihydrogen phosphate R in 220 mL of water for chromatography R and 780 mL of acetonitrile R

Procedure:
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 µV.

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

Method 2

Solutions:
Saccharose solution at 0.1 mg/mL in water for chromatography R.
Water for chromatography R (blank)

Settings:
Column: Atlantis dC18, 5 µm, 150 x 4.6 mm or equivalent
Mobile phase: water for chromatography R
Oven temperature: 40 °C
Flow rate: 1.0 mL/min
Injection volume: 10 µL

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

To calculate the signal to noise ratio, inject three times standard solution and calculate the mean of the two replicates.

Limits: S/N ≥ 10

DRIFT OVER TIME

Procedure:
Calculate the slope of the signal within the specified range within a defined time range (e.g. 60 minutes).

Limits: ± 0.1 mV/min

Alternatively, the limits can be expressed in ΔRI/min or in % of full scale of the selected range, following manufacturer methods/specifications.

LINEARITY

Method 1

Solutions:
Appropriately dilute the stock solution of D-fructose at concentration of 4.0 mg/mL to obtain minimum 5 diluted solutions in a range from 0.4 to 2.0 mg/mL, for example:

Std. 1: 2.0 mg/mL D-fructose
Std. 2: 1.6 mg/mL D-fructose
Std. 3: 1.2 mg/mL D-fructose
Std. 4: 0.8 mg/mL D-fructose
Std. 5: 0.4 mg/mL D-fructose

Use water for chromatography R to dilute.

Settings:
Apply the same conditions as described for signal-to-noise ratio testing.

Procedure:
After equilibration, inject each diluted solution once (starting from the more diluted) and the blank. Integrate the peak corresponding to D-fructose and calculate the linearity of response by linear regression mode.

Limits: $r^2 \geq 0.9950$

Method 2

Solutions:
Appropriately dilute a stock solution of saccharose to obtain for example:

Std. 1: 50.0 mg/mL saccharose
Std. 2: 20.0 mg/mL saccharose
Std. 3: 10.0 mg/mL saccharose
Std. 4: 5.0 mg/mL saccharose
Std. 5: 1.0 mg/mL saccharose

Use water for chromatography R to dilute.

Settings:
Follow conditions given in method 2 for signal-to-noise ratio testing.

Procedure:
Follow instructions given in procedure for method 1. Integrate the peak corresponding to saccharose and calculate linearity of response by linear regression mode.

Limits: $r^2 \geq 0.9950$

CIRCULAR DICHROISM DETECTOR

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

LINEARITY AND SIGNAL TO NOISE RATIO

Solutions:
Reference solution (a): dissolve 25.0 mg D (-) pantolactone in 50.0 mL of water
Reference solution (b): dilute 2.0 mL of reference solution (a) to 10.0 mL
Reference solution (c): dilute 4.0 mL of reference solution (a) to 10.0 mL
Reference solution (d): dilute 6.0 mL of reference solution (a) to 10.0 mL
Reference solution (e): dilute 8.0 mL of reference solution (a) to 10.0 mL
Reference solution (f): dilute 0.5 mL of reference solution (b) to 25.0 mL

Use water for chromatography R to dilute.

Settings:
Column: C18, 5 µm, 150 x 4.0 mm
Mobile phase: acetonitrile R : water for chromatography R (10:90 V/V)
Flow: 1.0 mL/min
Detection: 225 nm
Injection volume: 20 µL

Procedure:
Check the linearity of 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 amount (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 ($0.01 \times 2 / S/N_{calculated}$)

Limits:
Linearity: The linearity of the calibration line obtained with reference solution (a), (b), (c), (d), (e) should be $r^2 \geq 0.9950$
Signal-to-noise ratio: > 1.0

DRIFT OVER TIME

Settings:
Column: C18, 5 µm, 150 x 4.0 mm
Mobile phase: acetonitrile R: water for chromatography R (10:90 V/V)
Flow: 1.0 mL/min
Detection: 290 nm
Injection volume: 20 µL

Procedure:
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:
≤ 0.1 mdeg/h

SPECTRA COMPARISON

Solutions:
Reference solution (a): dissolve 5.0 mg dexamethasone in 10.0 mL of mobile phase

Settings:
Column: C18, 5 µm, 150 x 4.0 mm
Mobile phase: acetonitrile R : water for chromatography R (40:60 V/V)
Flow: 1.0 mL/min
Detection: 230 nm
Injection volume: 20 µL

Procedure:
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.

CHARGED AEROSOL DETECTOR

The following tests are proposed to perform the periodic and motivated check of the Charged Aerosol Detector (CAD) coupled with liquid chromatography instrument.

BASELINE NOISE, LARGEST RANDOM SPIKE AND BASELINE DRIFT

Settings:
Column: Suitable column or capillary (e.g. RP18)
Mobile phase: methanol R1: water for chromatography R (20:80 V/V)
Flow rate: 1.0 mL/min
Nebulizer temperature: 25 °C
Gas pressure: 35 psi

Procedure:
Record the baseline during a run time of 30 minutes. Measure the peak-to-peak noise observed in a 5 minutes window taken in a region of the chromatogram free from random spikes and taken from the last 15 minutes of the chromatogram. Determine the baseline drift observed in the last 15 minutes of the chromatogram.

Limits:
Baseline noise ≤ 0.04 pA (or 0.4 mV)
Largest random spike ≤ 0.2 pA (or 2 mV)

Baseline drift ≤ 0.04 pA/min (or 0.4 mV/min)

REPEATABILITY AND SIGNAL TO NOISE RATIO

Solution:
Caffeine solution at 25.0 µg/mL in water for chromatography R

Settings:
Column: Suitable column or capillary (e.g. RP18)
Mobile phase: methanol R1: water for chromatography R (20:80 V/V)
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Nebulizer temperature: 25 °C
Gas pressure: 35 psi

Procedure:
Inject 6 times 25.0 µg/mL caffeine solution.

Limits:
Repeatability of peak areas: The relative standard deviation of the peak areas of caffeine on the chromatograms obtained with the reference solution should be ≤ 10 %.

S/N ≥ 10

SIGNAL CALIBRATION

Solutions:
Std 1. 25.0 µg/mL of caffeine
Std 2. 125.0 µg/mL of caffeine
Std 3. 250.0 µg µg/mL of caffeine
Std 4. 500.0 µg/mL of caffeine
Std 5. water for chromatography R

Use water for chromatography R to dilute

Settings:
Follow conditions given in repeatability and S/N testing.

Procedure:
Injection scheme:
1 x blank
1 x Std. 1
1 x Std. 2
1 x Std. 3
1 x Std. 4
1 x blank

Limits: $r^2 \geq 0.9990$ (quadratic regression)

EVAPORATIVE LIGHT SCATTERING DETECTOR

The following tests are proposed to perform the periodic and motivated check of the Evaporative Light Scattering Detector (ELSD).

NOISE AND BASELINE DRIFT

Settings:
Noise or baseline drift are depending on the characteristics of the instrument, stability of the electronics, light source energy and photomultiplier tube sensitivity (gain and filter used).
See manufacturer procedure as reference for those parameters.

Limits:
Mean ASTM noise ≤ 2 mV
Baseline drift ≤ 2.0 mV/h

REPEATABILITY

Solutions:
250.0 µg/mL of caffeine in water for chromatography R

Settings:
Inlet tube: 1/16" tubing loop, 0.005" I.D. x 200 cm (for backpressure)
Mobile phase: water for chromatography R
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Temperature of the drift tube: 40 °C
Gas pressure: 3.5 bar (50 psi)

Procedure:
Inject 6 times 250.0 µg/mL caffeine solution.

Limits:
Repeatability of peak areas: The relative standard deviation of the peak areas of caffeine on the chromatograms obtained with caffeine solution should be ≤ 3.0 %.