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QUALIFICATION OF EQUIPMENT

ANNEX 3: QUALIFICATION OF UV-VISIBLE SPECTROPHOTOMETERS

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**ANNEX 3 OF THE OMCL NETWORK GUIDELINE
“QUALIFICATION OF EQUIPMENT”**

QUALIFICATION OF UV-VISIBLE SPECTROPHOTOMETERS

Introduction

The present document is the third Annex of the core document “Qualification of Equipment”, and it should be used in combination with it when planning, performing and documenting the UV-Visible spectrophotometer qualification process.

The core document contains the Introduction and general forms for Level I and II of qualification, which are common to all type of instruments.

For UV-Visible spectrometers, an example has been added to give instrument-specific proposals that may be used in combination with the general requirements presented in the core document “Qualification of Equipment”, when drawing up a Level I checklist.

The present annex contains instrument-related recommendations on parameters to be checked at Level III and IV of qualification and the corresponding typical acceptance limits, as well as practical examples on the methodology that can be used to carry out these checks.

TABLE I

Level I. Selection of instruments and suppliers**Example of check-list (non-exhaustive)**

Manufacturer:

Provider/Distributor:

Name of instrument and type:

Attribute (This list may be adapted if necessary)	Specifications	Benefits (Instrument/ supplier)	Assessment	
			Pass	Fails
Spectrophotometer				
Single beam and double beam mode	Allow both modes			
Wavelength range	190nm - 900nm			
Photometric range	Minimum 2.0 Absorbance (Abs.) units			
Lamp switching	Allow both modes manual or automatic			
Band width	0.2 nm - 4.0 nm or better, with 0.1 nm of increments			
Automatic baseline corrections	Must			
Wavelength accuracy	Minimum of ± 0.2 nm			
Wavelength reproducibility	0.05 nm or better			
Wavelength resolution	0.2 nm or better			
Photometric accuracy	± 0.003 Abs. units or better for 1.0 Abs. units			
Photometric stability	after 2 hour should not be more than 0.0005 Abs. units/h			
Photometric reproducibility	should not be more than 0.0005 Abs. units at 0.5 Abs. units			
Photometric noise	should not be more than 0.0003 Abs. units at 1.0 Abs. units			
Scan speed	should be between 0.25 nm/sec. and 8 nm/sec. or better			

Attribute (This list may be adapted if necessary)	Specifications	Benefits (Instrument/ supplier)	Assessment	
			Pass	Fails
Monochromator slew rate	should be 1500 nm/min. or better			
Data acquisition and processing system				
Acquisition at more than one wavelength	Minimum of two			
Photometric scaling in Abs. units, %T, log Abs. units and concentration				
Abscise scaling in nm, min., deg. and mm.				
Calibration at one or more levels and one or more wavelengths				
Calculate and give factor for linear regression and other				
Build and memorise in file form: data, method and report				
Accessories				
Flow-through cuvette; holder for n cuvette(s)				
Thermostated cell holder (temperature range and stability)				
Filters for qualification checks				

Notes:

- This check-list, containing examples of attributes that can be taken into account in the selection of an instrument and supplier, can be used in combination with the general check-list presented in Level I in the core document "Qualification of Equipment".
- For Table II (Level II of Equipment Qualification: Installation and release for use) please refer to the core document.

TABLE III**Level III. Periodic and motivated instrument checks****Examples of requirements for UV-Visible spectrophotometers**

Parameter to be checked	Typical tolerance limits
1. Spectral slit-width (if applicable)	$\pm 10 \%$
2. Wavelength accuracy	$\pm 1 \text{ nm}$ for the UV range $\pm 3 \text{ nm}$ for the Visible range
3. Wavelength precision (for mechanically set wavelengths)	See manufacturer's specifications
4. Photometric accuracy (Control of absorbance)	See Annex I
5. Photometric linearity	$r^2 \geq 0.999$
6. Limit of stray light	$A > 2.0$ at 198 nm
7. Baseline noise	± 0.002 Absorbance units (500 nm) or ± 0.01 Absorbance units (200, 300, 400 nm)
8. Photometric drift	± 0.001 Absorbance units/h (250 nm) or ± 0.002 Absorbance units/h (500 nm)

TABLE IV**Level IV. In-use instrument checks****Examples of requirements for UV-Visible spectrophotometers**

Parameter to be checked	Typical tolerance limits
1. System suitability check of the method - e.g. Repeatability - e.g. Resolution (if required for qualitative analysis)	According to Ph. Eur. or MAH dossier or validated in-house method
2. Absorption cells	Absorbance difference ≤ 0.005

ANNEX I

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 a UV-Visible spectrophotometer.

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".

GENERAL CONSIDERATIONS

- Measurements made by comparing samples against external standards should be made under conditions during which temperature is held constant. This is particularly relevant where the carrier solvent is organic and measurements may be distorted by expansion or evaporation of the solvent.
- It is recommended to perform the qualification within the spectral range corresponding to the region of analytical interest.
- Ensure that the spectrophotometer has stabilized, according to the manufacturer's recommendations, before starting the qualification tests.
- When references are made to the European Pharmacopoeia, e.g. reagents R, then the reagent quality complies with the EP specifications.
- When using commercial filters as alternative to the proposed tests, a set of filters covering the entire range of interest should be used. They should be calibrated with traceability to national/international standards, preferable through a national metrology laboratory or NIST.

1. SPECTRAL SLIT WIDTH (if applicable)

When using an instrument on which the slit-width is variable at the selected wavelength, the slit-width must be small compared with the half-width of the absorption band but it must be as large as possible to obtain a high value of I_0 . Therefore, a slit-width is chosen such that further reduction does not result in a change in absorbance reading.

Method and Limits:

1. Switch the system on and start the **Scan** module.
2. Select **SETUP** and set the following parameters:
 - X Mode = Nanometres
 - Start wavelength = 660.0 nm
 - Stop wavelength = 650.0 nm
 - Y Mode = % T
 - Scan rate = 10 nm/min
 - Gain = (100) see 4 below

3. Select **Options** tab and set the following parameters:

SWB = 4 nm

Beam mode = Single front

Lamps on = Deuterium

Source change = 700.0 nm

4. Start a scan and examine the trace for a spectral peak around 656.1 nm.

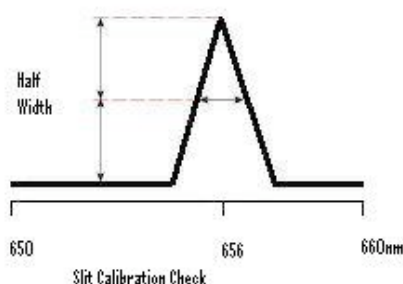
If no peak is seen or it is less than 50% T, increase the gain.

If the signal exceeds 100% T, reduce the gain.

5. Measure the width of the peak (in nanometres) at half the height of the peak.

This represents the spectral bandwidth and should be within $\pm 10\%$ of that selected via the computer.

6. Check the calibration at a slit width of 0.2 nm. If the measured slits are too small then, for a selected width, the instrument will have more photometric noise than normal. If the slit width is unacceptable, then reset the slit calibration.



Effect of spectral slit width on absorbance fluctuation (performed with pure solvents).

Slit width 5.0 nm	Slit width 2.0 nm	Slit width 1.0 nm	Slit width 0.05 nm	Difference in Abs.	Theoretical Values	Test status
Cyclohexane						
0.016	0.015	0.015	0.015	< 0.001	< 0.010	Passed
Ethanol						
0.020	0.019	0.019	0.019	< 0.001	< 0.010	Passed
Methanol						
0.005	0.005	0.005	0.005	0.00	< 0.010	Passed

2. WAVELENGTH ACCURACY

Note: if the determination is performed by using the specific absorbance ($A_{1\%}^{1\text{ cm}}$), the frequency of this check should be higher.

Materials:

Verify the wavelength accuracy using the absorption maxima of holmium perchlorate solution R prepared, for example, with a 40 g/l solution of holmium oxide R in a solution of perchloric acid R containing 141 g/l of HClO₄ (Ph. Eur. Chapter 4.1.1, ref 1043101), or the line of a hydrogen or deuterium discharge lamp.

If available, the built-in mercury lamp of the instrument may be used for this test.

Alternatively, use suitable commercial certified filters¹ typically made incorporating rare earth metal oxides. Ideally the calibrant should demonstrate traceability to national or international standards and should carry a statement about the associated uncertainty.

Method:

Compare the measured absorbance maxima with the absorbance maxima shown in the table below:

241.15 nm (Ho)	404.66 nm (Hg)
253.7 nm (Hg)	435.83 nm (Hg)
287.15 nm (Ho)	486.0 nm (D β)
302.25 nm (Hg)	486.1 nm (H β)
313.16 nm (Hg)	536.3 nm (Ho)
334.15 nm (Hg)	546.07 nm (Hg)
361.5 nm (Ho)	576.96 nm (Hg)
365.48 nm (Hg)	579.07 nm (Hg)

When using commercial filters, compare the measured absorbance with the certified values given by the manufacturer. The measurement is made against air.

Limits:

± 1 nm in the UV range

± 3 nm in the Visible range

¹ Commercial certified filters, e.g. Hellma or NIST (National Institute of Standards and Technology).

3. WAVELENGTH PRECISION (for mechanically set wavelengths)

Materials:

For this test, the same materials of the previous test can be used:
Holmium perchlorate solution R prepared, for example, with a 40 g/l solution of holmium oxide R in a solution of perchloric acid R containing 141 g/l of HClO₄ (Ph. Eur. Chapter 4.1.1, ref 1043101).

If available, the built-in mercury lamp of the instrument may be used for this test.
Alternatively, suitable commercial certified filters may be used¹.

Method:

Carry out 6 measurements of the absorbance maxima.

Limits:

Repeatability: the relative standard deviation of the absorbance maxima should satisfy the manufacturer's specifications.

The difference between the 6 individual peaks should comply with the manufacturer's specifications (e.g. < 0.5 nm).

Where old instruments are involved, the wavelength may be set by turning a wheel until the selected wavelength is reached. Where this is the case, mechanical wear may result in a different wavelength being selected depending on whether starting above or below the target wavelength. Examine the variation by setting a wavelength from above and below and seeing if there is a difference.

4. PHOTOMETRIC ACCURACY (CONTROL OF ABSORBANCE)

Note: if the determination is performed by using the specific absorbance ($A_{1\text{ cm}}^{1\text{ per cent}}$), the frequency of this check should be higher.

Materials:

Solution of potassium dichromate R, previously dried to constant mass at 130 °C.
For the control of absorbance at 235 nm, 257 nm, 313 nm and 350 nm, dissolve 57.0-63.0 mg of potassium dichromate R in 0.005 M sulphuric acid and dilute to 1000.0 ml with the same acid. For the control of absorbance at 430 nm, dissolve 57.0-63.0 mg of potassium dichromate R in 0.005 M sulphuric acid and dilute to 100.0 ml with the same acid.

Alternatively, suitable commercial certified filters may be used².

Note: If commercial filters are used, the wavelengths and the exact absorption values with the corresponding tolerance limits will depend on the type of filters. The selection of the filters should be adequate in order to cover the range used in routine work.

Method:

Check the absorbance using the solution of potassium dichromate R or the certified filters at the wavelengths indicated in the table below, which gives for each wavelength the exact values and the permitted limits of the specific absorbance.

² Commercial certified filters, e.g. Hellma or NIST (e.g. NIST Standard Reference Material 930e).

Limits:

Wavelength (nm)	Specific absorbance A_1^1 per cent cm	Maximum tolerance
235	124.5	122.9 to 126.2
257	144.5	142.8 to 146.2
313	48.6	47.0 to 50.3
350	107.3	105.6 to 109.0
430	15.9	15.7 to 16.1

Example of certified commercial filters:

Filter	Wavelength (nm)	Maximum tolerance
Potassium dichromate in sulphuric acid ³ , against sulphuric acid (blank)	235	0.750 ± 0.01
	257	0.868 ± 0.01
	313	0.292 ± 0.01
	350	0.644 ± 0.01

Filter	Wavelength (nm)	Maximum tolerance
Sulphuric acid (blank), against air	235	0.043 ± 0.01
	257	0.040 ± 0.01
	313	0.035 ± 0.01
	350	0.034 ± 0.01

Filter	Wavelength (nm)	Maximum tolerance
Potassium dichromate in sulphuric acid, against sulphuric acid (blank)	430	0.960 ± 0.01
Sulphuric acid (blank), against air	430	0.033 ± 0.01

5. PHOTOMETRIC LINEARITY

Materials:

A series of solutions of a suitable absorbing compound (e.g. potassium dichromate) spanning the concentration range of interest. The absorbing solutions need to be stable. The concentrations should be regularly spaced (1, 2, 3, 4, 5) rather than doubling (1, 2, 4, 8, 16) in order to reduce the leverage effects when fitting the line.

³ A potassium dichromate in perchloric acid cuvette is also available from Hellma.

Method:

Measure the net absorbance of the solutions against a blank at 235, 313, 257 and 350 nm. Make 3 measurements for each solution. Calculate the mean value and the linearity of the concentration of each solution against the measured absorbance at the control wavelengths.

Limits:

At each wavelength: $r^2 \geq 0.999$

Note: It is recommended to use these results to regularly monitor the trend of the instrument (control chart of r^2).

6. LIMIT OF STRAY LIGHT

Materials:

Stray light may be measured at a given wavelength with suitable certified filters or different solutions. For example, a 12 g/l solution of potassium chloride R in water.

Method:

The absorbance of a 12 g/l solution of potassium chloride R in a 1 cm cell increases steeply between 220 nm and 200 nm.

When available, use the in-built software of the instrument.

Limits:

$A > 2.0$ at 198 nm, when compared with water as compensation liquid.

Alternatively:

Method: Check stray light at 220 nm by using a 20 g/l NaI solution.

Limits: according to manufacture's specifications (e.g. Transmittance ≤ 0.1 %).

Note:

A range of tests using other salts are described in the literature to cover different wavelength ranges⁴.

7. BASELINE NOISE

For this test, 2 alternative methods are proposed.

TEST 1

Method:

Make 61 absorbance measurements with an integration time of 1 second at a wavelength of 500 nm, with no sample in the sample chamber, and calculate the mean.

Limits:

Mean ± 0.002 Absorbance units

⁴ e.g. ASTM E 387-2004: Standard test method for estimating stray radiant power ratio of dispersive spectrophotometers by the opaque filter method.

TEST 2

Method:

Record the absorbance for 60 seconds at 200, 300 and 400 nm with a highly pure, synthetic Quartz block⁵. The measurement is made against air.

Note: If commercial filters are used, the wavelengths and the exact absorption values with the corresponding tolerance limits will depend on the type of filters.

Example of limits:

Filter	Wavelength (nm)	Maximum tolerance
Quartz block (against air)	200	0.049 ± 0.01
	300	0.033 ± 0.01
	400	0.031 ± 0.01

8. PHOTOMETRIC DRIFT

Notes:

- Photometric drift should be checked at both the Visible and UV region, at appropriate wavelengths.
- The limits are in accordance with the user's requirements (as defined in Level I) and with manufacturer's specifications.

For this test, 2 alternative examples are proposed.

TEST 1

Method:

As routine test, the drift is measured at 250 nm over a period of 2 hours by using the Time Scan mode of the instrument, with no sample in the sample chamber.

Limits: ± 0.001 Absorbance units/h

Note: In certain cases, for example when several samples are measured over a long period of time (or when using auto sampler) the drift can also be determined at the wavelength used for the analytical method, in the same way, before the measurement of the samples.

TEST 2

Method:

Record the baseline for 60 minutes at 500 nm and compare the absorbance with the initial value.

Limits: ± 0.002 Absorbance units/h

⁵ e.g. Hellma filter 667-UV 0.

ANNEX II

Level IV. In-use instrument checks

This Annex contains practical examples of tests and their associated tolerance limits for several parameters related to the performance of an UV-Visible spectrophotometer. These examples can be considered by the OMCLs as possible approaches to perform the Level IV of the equipment qualification process: “In-use instrument checks”.

1. SYSTEM SUITABILITY TEST OF THE METHOD

- REPEATABILITY
 - RESOLUTION
- } (for quantitative analysis)

Method:

Criteria such as repeatability or resolution are usually given when test methods are performed according to Ph. Eur., MAH dossier or a suitably validated in-house method.

- RESOLUTION (if required for qualitative analysis)

Materials:

0.02 per cent V/V solution of toluene R in hexane R.
Alternatively, suitable certified reference materials may be used.

Method:

When prescribed in a monograph, measure the resolution of the apparatus as follows: record the spectrum of a 0.02 per cent V/V solution of toluene R in hexane R. Alternatively, record the spectrum of the reference material.

Limits:

The minimum ratio of the absorbance at the maximum at 269 nm to that at the minimum at 266 nm is stated in the monograph or in the test method description.

2. ABSORPTION CELLS

Method:

The following method for checking cleanliness and gross differences in thickness or parallelism of the windows of optical cells is described in ASTM E275-01 (American society of Testing and Materials), “Standard Practice for Describing and Measuring Performance of UV, Visible, and Near Infrared Spectrophotometers”:

Fill the cell with distilled water and measure its apparent absorbance against air at 240 nm for quartz cells and at 650 nm for glass cells. With recording instruments it is desirable to scan over the optical region of interest. The apparent absorbance should be not greater than 0.093 for 1 cm quartz cells (UV region) and 0.035 for 1 cm glass cells (Visible region). Rotate the cell in its holder (180°) and measure the apparent absorbance again.

Limits:

Rotating the cells should give an absorbance difference not greater than 0.005.

REFERENCES

(For all references, the latest version applies)

- 1) Ph Eur. 2.2.25, Absorption spectrophotometry, Ultraviolet and Visible.
- 2) Guidance on Equipment Qualification of Analytical Instruments: UV-Visible Spectro(photo)meters (UV-Vis). Valid Analytical Measurement (VAM) Programme.