

United States and European Pharmacopeia

What Every UV/Vis Lab Should Know

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1. Introduction and Glossary

Pharmacopeias are regulatory bodies which define guidelines on the procedures and analytical instrumentation for the preparation and analytical measurement of pharmaceutical products. Both the United State Pharmacopeia (USP) and European Pharmacopoeia (Ph. Eur.) have dedicated chapters in their respective pharmacopeias on the requirements of UV/Vis spectrophotometers and the verification of their performance. Pharmaceutical companies are facing the challenge of adapting their workflow to pharmacopeia regulation revisions that became mandatory at the beginning of 2020. To ensure customers can comply with the changes, METTLER TOLEDO has adapted its automated performance verification accessories. This paper provides an overview of the important UV/Vis spectroscopy-relevant changes in both the USP and Ph. Eur., taking a closer look at their impact and the solutions available to ensure compliance.

The verification of photometric linearity has not been mandatory until this latest revision. This test is now explicitly defined in both USP 42-NF37 2nd suppl. and Ph. Eur. 10. Other parameters that can be tested but are not required by regulation are photometric noise, photometric drift, and baseline flatness. These parameters are assessed by performing spectroscopic measurements using different liquid or solid filters, depending on the test. As the procedure for comprehensive performance verification is intricate and time-consuming, there are great benefits in integrating it into the analytical workflow so that it is executed automatically as a matter of course. In the second section of this paper, the positive impact of automating these important tests on workflow efficiency and security will be assessed against manual execution. Furthermore, the importance of automatic data-integrity-compliant management of certification data will be emphasized as an important parameter for regulation-compliant documentation.

In the third chapter, useful tips and hints are provided for correct cuvette handling. Insights into environmental aspects that might negatively influence the accuracy of UV/Vis measurements are covered as well.

Glossary of Abbreviations

A: Absorbance

C: Certified

CRM: Certified Reference Material

M: Measured

MT: METTLER TOLEDO

NA: Nicotinic Acid

ND: Neutral Density

OQ: Operational Qualification

PA: Photometric Accuracy

PDC: Potassium Dichromate

Ph. Eur.: European Pharmacopoeia

PL: Photometric Linearity

R2: Coefficient of Determination

REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals

Res: Resolution

SD: Standard Deviation

SL: Stray Light

SOP: Standard Operating Procedure

T: Transmittance

USP: United States Pharmacopeia

UV: Ultraviolet

Vis: Visible

WA: Wavelength Accuracy

WR: Wavelength Repeatability (Precision)

Xe: Xenon

2. Optical Chapters of Ph. Eur. 10 and USP 42 NF37 2nd suppl.

Recent changes in the optical chapters of the latest versions of the pharmacopeias Ph. Eur. 10 and USP 42 NF37 2nd suppl. require adaptation of the qualification process. Here, the respective pharmacopeia requirements for performing operational qualification (OQ) tests are presented in detail on a comparison basis, with particular attention paid to changes from the previous editions (Ph. Eur. 9, USP 41) to the most recent (Ph. Eur. 10, USP 42). These changes are explained in light of the respective impacts they have on the operational qualification of a UV/Vis spectrophotometer.

2.1 The importance of performance verification

What needs to be considered to verify the performance of a UV/Vis spectrophotometer? Increasingly, laboratories must be accredited to internationally recognized standards of Good Laboratory Practice (GLP), ISO/IEC 17025 or ISO 9001. This accreditation requirement brings with it the need to provide evidence of control of the instrument performance with certified reference materials. The certified reference materials used must adhere to the concept of traceability, which requires a defined traceable path to recognized primary materials. This ensures that compatible measurements are made across national borders and results in unambiguous and reliable communication of specifications.

Why is monitoring of the optical performance of a UV/Vis spectrophotometer important? It is important because instrument performance directly affects measurement accuracy and repeatability. For critical UV/Vis measurements, especially in clinical, pharmaceutical or industrial quality-control, it is imperative that the instrument is performing according to its specification. In laboratories working according to pharmacopeia regulations (e.g. USP or Ph. Eur.), the instrument performance should be monitored regularly and documented accordingly. Monitoring must be based on a defined schedule where appropriate; error-free reporting is especially critical can be facilitated with automation. Aspects of verification including automated scheduling and reporting are discussed in the second chapter of this paper.

2.2 A brief introduction: Operational Qualification (OQ)

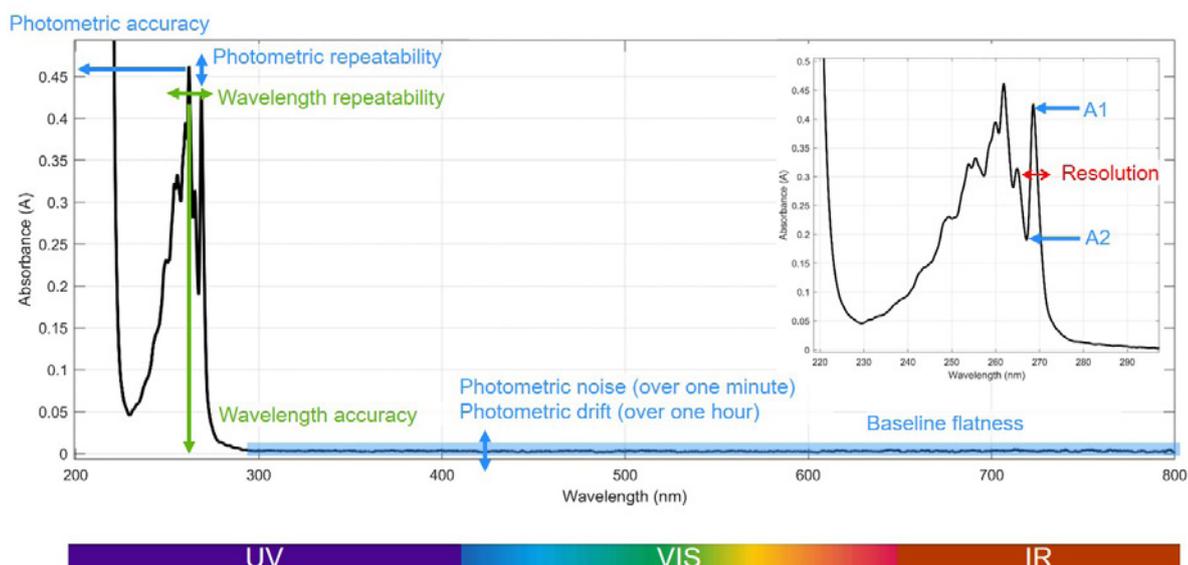


Figure 1: UV/Vis spectrum of 0.02% toluene in hexane including visualization of optical performance parameters which are used for operational qualification of a UV/Vis spectrophotometer.

Figure 1 illustrates the basic performance parameters of a UV/Vis spectrophotometer. The spectrum of a 0.02% toluene in n-hexane solution shall serve as an example.

Photometric accuracy defines how accurately the UV/Vis instrument measures absorbance at a specific wavelength over a specified absorption range. An example where this parameter is important is obtaining reliable quantification measurements. Photometric repeatability is a parameter that defines the uncertainty of the

photometric accuracy. This means the lower the uncertainty the more reliable is the absorption measurement. The significant influence of stray light on photometric accuracy requires measurement of this parameter for the operational qualification of a UV/Vis spectrophotometer. It is not illustrated in this slide but will be discussed in chapter 2.3.4.

Wavelength accuracy and repeatability describe how distinct positions on the wavelength scale are measured accurately and repeatably. Wavelength accuracy is needed for accurate peak maxima and minima position determination, which is required for reliable analyte identification, purity determination and quantification. Resolution determines the capability of the instrument to resolve absorption maxima and minima. For example, the higher the resolution, the better adjacent, partially overlapping peak structures can be resolved. This is nicely illustrated in the spectrum excerpt, where the respective peak minima and maxima of the well-resolved peak are used to determine the instrument's spectral resolution.

Parameters such as photometric noise or photometric drift as well as baseline flatness have an important impact on the photometric accuracy and thus the specific measurement of the UV/Vis spectrophotometer. These parameters are usually part of the instrument specification but are not mandated by either European or United States pharmacopeias. Therefore, these parameters will not be discussed further in this paper.

2.3 Ph. Eur. and USP optical chapters revisions and their impact on OQ

Now that the fundamentals of optical performance qualification have been discussed, it is time to review changes in the optical chapters from the previous versions to the latest versions of the European and United States pharmacopeias. The following tables offer high-level overviews of the changes to the Ph. Eur. and USP, respectively.

Table 1: Optical chapter 2.2.25 revisions based on the comparison of the 9th and 10th editions of the European Pharmacopoeia (Ph. Eur.). Changes have been mandatory since January 1st 2020.

Ph. Eur. 9: Mandatory tests	Ph. Eur. 10: Mandatory tests
Photometric accuracy	Photometric accuracy (UV/Vis)
	Photometric linearity (UV/Vis)
Wavelength accuracy	Wavelength accuracy
Stray light – procedure B	Stray light – procedure B
Resolution	Resolution

Table 2: Optical chapter 857 revisions based on the comparison of the 41st to 42nd edition of the United States Pharmacopoeia (USP). Changes have been mandatory since January 1st 2020.

USP 41: Mandatory tests	USP 42 NF 37 2 nd suppl.: Mandatory tests
Photometric accuracy	Photometric accuracy (UV/Vis)
Photometric repeatability	Photometric repeatability (UV/Vis)
	Photometric linearity (UV/Vis)
Wavelength accuracy	Wavelength accuracy
Stray light – procedure A	Stray light – procedure A or B
Resolution	Resolution

January 1st 2020, the following changes in operational qualification became mandatory:

- The determination of the photometric linearity became mandatory in both pharmacopeias.
- In the newest edition of the USP, the stray light test according to procedure B, which is the specific wavelength method, is accepted in addition to procedure A, the solution filter ratio method. Both methods will be explained in chapter 2.3.4.
- In the newest issues of both pharmacopeias, the use of nicotinic acid for photometric qualification in the UV region in addition to the hitherto used potassium dichromate is permitted.
- Both pharmacopeias demand qualification of the same optical parameters. The only exception is the test of photometric repeatability, which is required by the USP.

In the following chapter, each of the optical parameters demanded by the newest edition of both pharmacopeias are presented in the form of a comparison table for better overview. The respective materials, acceptance criteria and standard operating procedure are presented.

2.3.1 Wavelength accuracy

Table 3: Comparison of wavelength accuracy and repeatability requirements of the USP42 NF37 and Ph. Eur. 10 in their respective optical chapters.

Optical Test	Parameter	USP 42 NF37, chapter 857	Ph. Eur. 10.0, chapter 2.2.25
Wavelength accuracy	Materials	Rare earth oxide solutions/glasses (Ce, Ho) Hg/D ₂ emission lines	Ho(ClO ₄) ₃ , Ce ₂ (SO ₄) ₃ solutions, Ho/Didymium glasses, Hg, D ₂ , Ne, Xe lines
	Acceptance criteria	UV (200 – 400 nm): ± 1 nm Vis (400 – 780 nm): ± 2 nm	UV (< 400 nm): ± 1 nm Vis (> 400 nm): ± 3 nm
	SOP	1 measurement (no replicates) for diode array instruments in UV and Vis region. 6 replicate measurements for scanning instruments over the operational range	2 wavelengths that bracket the operational range
Wavelength precision	Materials	See above	Not specified
	Acceptance criteria	< 0.5 nm for scanning instruments	
	SOP	Not required for diode array instruments Standard deviation of 6 replicate measurements	

The recommended materials for wavelength accuracy qualification in both pharmacopeias are the same. The Ph. Eur. recommends the usage of atomic emission lines for the first time. Acceptance criteria are identical in UV but different in the Vis range: The Ph. Eur. has a less strict acceptance criterion in the VIS range than the USP. The SOP in the USP differentiates between scanning and diode array instrument types. For the latter, only one wavelength without measurement repetition shall be measured in each range. Scanning instruments require a six-fold repetition of wavelength measurement over the operational range. The Ph. Eur. does not differentiate between instrument types and requires measurement of two wavelengths bracketing the operational range without specification of replicates.

The determination of wavelength precision is only demanded by the USP and only for scanning instruments, not for diode array instruments. This parameter is not specified in the Ph. Eur. Usually the wavelength precision is very well defined in diode array UV/Vis spectrophotometers due to the non-mechanical design of the monochromator.

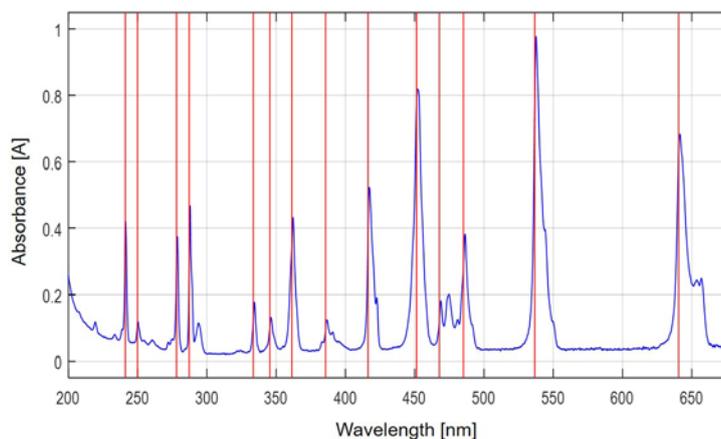


Figure 2: UV/Vis spectrum of 4% H₂O₂ in 10% v/v HClO₄ highlighting specific wavelengths, which are used for the qualification of the wavelength accuracy of METTLER TOLEDO UV/VIS Excellence spectrophotometers.

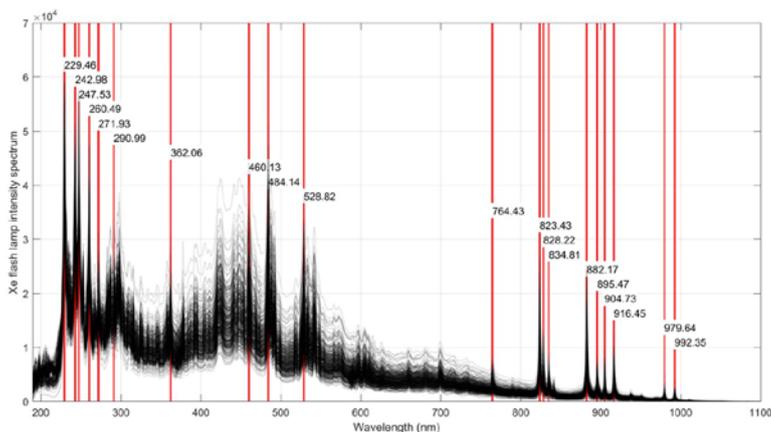


Figure 3: Overlaid UV/Vis spectra of hundreds of Xe flash lamp integrated into METTLER TOLEDO UV/VIS Excellence spectrophotometers. The wavelengths which can be used for qualification are highlighted.

Figures 2 and 3 illustrate the spectra of two sources that are recommended by both pharmacopeias for wavelength accuracy qualification.

Figure 2 shows the spectrum of a holmium oxide in perchloric acid solution, which is used to measure and verify the wavelength accuracy of a UV/Vis spectrophotometer against acceptance criteria. In METTLER TOLEDO array-based UV/Vis spectrophotometers, 14 well-resolved wavelengths are used for the calibration of wavelength accuracy, which are displayed as red lines. This material provides a useable wavelength range from 240 – 650 nm, which covers most applications described in the relevant monographs of both pharmacopeias.

An elegant method for wavelength accuracy qualification can be performed by using emission lines from lamp sources built into the instrument. In METTLER TOLEDO UV/VIS spectrophotometers, the built-in Xe lamp can be used for this purpose, which provides well-resolved emission lines of sufficient intensity in order to qualify the wavelength accuracy (Fig. 3). Two wavelengths (at 260.6 and 528.6 nm) are used.

2.3.2 Photometric accuracy

Table 4: Comparison of photometric accuracy requirements of the USP42 NF37 and Ph. Eur. 10 in the respective optical chapters.

Optical Test	Parameter	USP 42 NF37, chapter 857	Ph. Eur. 10.0, chapter 2.2.25
Photometric accuracy	Materials	Nicotinic acid in HCl (6 – 60 mg/L, up to 2.5 A, 213 and 261 nm) K ₂ Cr ₂ O ₇ in HClO ₄ (6 – 200 mg/L, up to 3.0 A, 235, 257, 313, 350 nm) Neutral density glass filters (up to 3.5 A, 440, 465, 546.1, 590, and 635 nm)	Suitable solid and liquid filters such as certified PDC or neutral density filters Nicotinic acid in HCl stock solution: dissolve 57.0 - 63.0 mg of NA in 0.1 M HCl and dilute to 200.0 mL (up to 40 mg/L, up to 2 A, 213 and 261 nm)
	Acceptance criteria	Liquid solutions: ± 0.010 A (for absorbance ≤ 1A); ± 1% (for absorbance > 1A) Solid (neutral density glass) filters: ± 0.008 A (for absorbance ≤ 1A); ± 0.8% (for absorbance > 1A)	± 0.010 A or ± 1%, whichever is greater (for absorbances < 2A)
	SOP	Compare measured absorbance with reference absorbance values. Test at least one absorption value at each wavelength range (UV, Vis) in the 0 – 2.00 A region.	Test at selected wavelengths at approx. the 2 limits of the expected absorbance range (< 2A) using one or more suitable solid or liquid filters

The qualification of photometric accuracy determination is important, as the performance of the UV/Vis spectrophotometer with regards to accurate quantification and thus absorption measurement needs to be verified. Nicotinic acid is now an approved filter for photometric accuracy determination in the UV range. Ph. Eur. no

longer approves the manual preparation of potassium dichromate (PDC) since it is listed in Annex XIV of the REACH regulation. Instead, it defines the preparation procedure of a nicotinic acid solution. However, certified PDC solutions in sealed quartz cuvettes remain fully permissible.

USP maintains the suitability of PDC as an acceptable filter for the determination of photometric accuracy and repeatability, and like Ph. Eur., also approves nicotinic acid. Nicotinic acid provides two wavelengths in the far UV, whereas PDC provides up to five wavelengths that can be used for qualification in the UV range.

For the qualification of the Vis range, the USP specifies among other materials neutral density glass filters, which allow qualification of the photometric precision using various wavelengths in the range from 440 nm up to 635 nm. The Ph. Eur. does not explicitly specify the qualification of the photometric accuracy in the Vis range.

The acceptance criteria in the Vis range are comparable in both pharmacopeias. The USP specifies 20% tighter acceptance criteria in the Vis than in the UV range. The SOP for qualification of photometric accuracy is comparable in both pharmacopeias, however the USP procedure description appears to be more precise. In both pharmacopeias, the measurements for photometric precision should be performed at both the upper and lower absorbance values of the operational range.

The Ph. Eur. explicitly states that absorption measurements above 2 A used for quantitative measurements should be avoided. The USP states the qualification shall be tested within the 2.00 A region but also lists concentration ranges or transmission values for liquid and solid filters which allow testing above 3 A.

It should be emphasized that the most suitable certified reference material in the application-relevant, appropriate concentration for the absorption range shall be selected for qualification. METTLER TOLEDO recommends the use of certified PDC solutions in perchloric acid in sealed quartz cuvettes in a concentration range that allows qualification from the 0.2 A to the 2 A operational range.

Table 5: Comparison of photometric repeatability (precision) requirements

Optical Test	Parameter	USP 42 NF37, chapter 857	Ph. Eur. 10.0, chapter 2.2.25
Photometric precision	Materials	See above	Not specified
	Acceptance criteria	Repeatability acceptance criteria: SD ≤ 0.005 A (for absorbance ≤ 1A); SD ≤ 0.5% (for absorbance > 1A)	

Only the USP specifies qualification of photometric precision based on the measurement of at least six replicates and the calculated standard deviation which shall not exceed 0.005 A below 1 A or 0.5% above 1 A. In the Ph. Eur. 10 edition, nothing is mentioned concerning qualification of this parameter.

2.3.3 Photometric linearity

Table 6: Comparison of photometric linearity requirements

Optical Test	Parameter	USP 42 NF37, chapter 857	Ph. Eur. 10.0, chapter 2.2.25
Photometric linearity	Materials	Use the same materials used for PA determination in UV and VIS range	Use the same materials used for PA determination in UV and VIS range
	Acceptance criteria	All measured filters fulfill photometric accuracy acceptance criteria	The coefficient of determination R2 is not less than 0.999
	SOP	Test the photometric response (linearity) using a standard type appropriate for the wavelength(s) required. Measure at least 3 different absorbance levels spanning the required operational range.	Test the photometric linearity in the UV and VIS range. Ensure that the CRM absorbance levels are compatible with the intended linear range.

For the first time, Ph. Eur. and USP now explicitly state that the photometric linearity (photometric response) of UV/Vis spectrophotometers must be checked. The same filters used for photometric accuracy can be used to check the linearity. Ph. Eur. clearly states that multiple concentrations/filters should be measured in accordance with the "intended linear range", depending on the operational range of the customer. For most pharmaceutical applications, the operational range is between 0.1 A -1.5 A. The upper end corresponds to a concentration of 36 mg/L for nicotinic acid solutions and 100 mg/L for potassium dichromate solutions.

In the USP 42 NF37, the calculation of the coefficient of determination R square to assess the quality of the linearity is not required, whereas the Ph. Eur. explicitly demands that this parameter be assessed. USP requires that the same acceptance criteria for each individual filter be applied for the photometric accuracy determination. The USP acceptance criteria are therefore stricter than those of the Ph. Eur.

The USP now states that the photometric response (linearity) needs to be tested at three absorbance levels, whereas, in previous editions, it only defined 'two or more' absorbance levels. The filters and concentrations used also depend on the operational range of the customer, as in the Ph. Eur.

In contrast to photometric accuracy determination, the Ph. Eur. explicitly requires the testing of photometric linearity in the UV and Vis regions; however, it does not specify the number of CRM absorbance levels, which should be at least three.

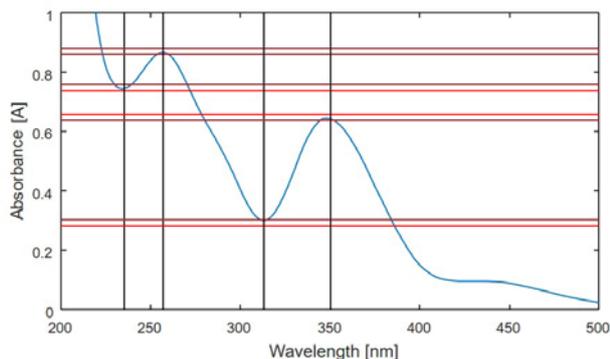


Figure 4: UV/Vis spectrum of 60 mg/L PDC in 0.001 M HClO₄ including the +/- 0.010 A acceptance criteria per wavelength (235, 257, 313, 350 nm).

2.3.3.1 In the UV region

For qualification of the photometric accuracy in the UV region, potassium dichromate solution in perchloric acid is a useful reference material and therefore recommended in the pharmacopeia monographs. The extract of the spectrum in figure 4 shows the five absorption maxima. The absorption ranges, which are the acceptance limits used for calibration, are marked with red lines.

Figure 5 shows the nice linearity response of a METTLER TOLEDO UV7 spectrophotometer in the UV range using the absorption minima at 235 (blue) and 313 (grey) nm and the absorption maxima at 257 (orange) and 350 (yellow) nm of the PDC spectrum, respectively. Eleven certified PDC concentrations from 20 mg/L to 220 mg/L are used spanning an absorption range from 0.2 A up to 3.5 A and 2.5 A, respectively. In all cases, the photometric accuracy acceptance criteria for each PDC concentration (+/- 10 mA) demanded by USP/Ph. Eur. and the coefficient of determination ($R^2 = 0.999$) demanded by Ph. Eur. are fulfilled.

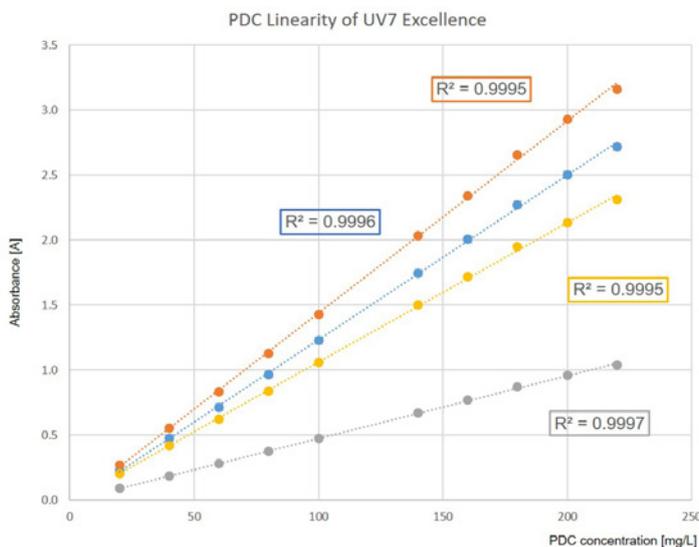


Figure 5: Photometric linearity of a UV7 Excellence spectrophotometer evaluated at the absorption minima (235 (blue) and 313 (grey) nm) and the absorption maxima (257 (orange) and 350 (yellow) nm) of PDC concentrations ranging from 20 mg/L to 220 mg/L.

Nicotinic acid in hydrochloric acid can be used as an alternative to PDC to qualify the UV/Vis spectrophotometer in the far UV region by using two wavelengths at 213 and 261 nm, respectively. Figure 6 shows the respective UV/Vis spectrum of 6 mg/L nicotinic acid in 0.1 M hydrochloric acid. Figures 7 and 8 show the excellent linear response of a METTLER TOLEDO UV7 Excellence spectrophotometer determined with nicotinic acid concentrations of 6, 18 and 36 mg/L which allow the qualification of this parameter at 213 and 261 nm ranging from 0.26 A to 1.6 A: the values of the coefficient of determination (R^2) are excellent in both cases. It should be noted that the wavelength range provided by PDC is significantly broader and thus more representative of the majority of measurements in the UV region than can be obtained using nicotinic acid. METTLER TOLEDO therefore recommends the use of certified PDC for OQ of photometric accuracy and linearity.

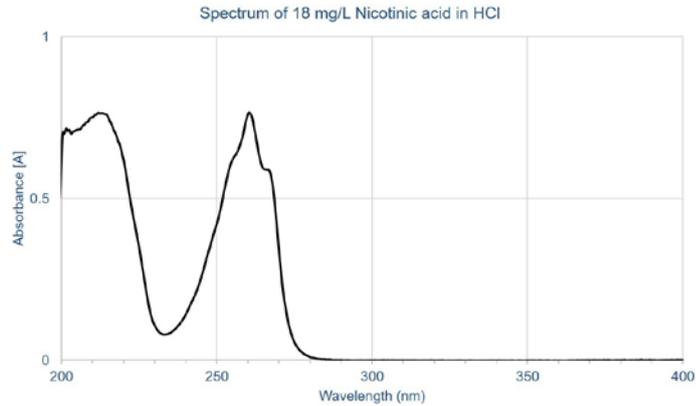


Figure 6: UV spectrum of 18 mg/L nicotinic acid in HCl showing the pronounced peak maxima at 213 and 261 nm.

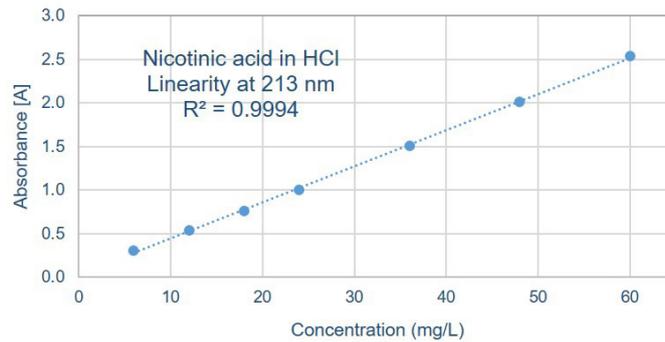


Figure 7: Photometric linearity of a UV7 Excellence spectrophotometer evaluated at the 213 nm absorption maximum of nicotinic acid concentrations ranging from 6 mg/L to 60 mg/L.

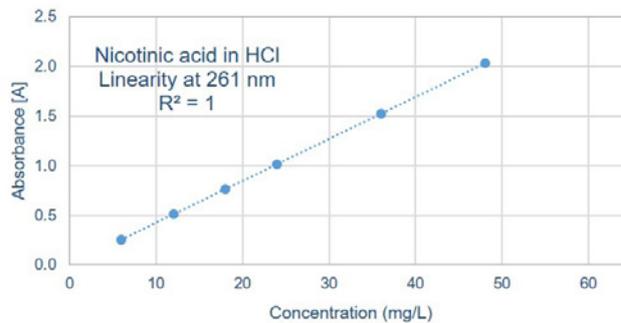


Figure 8: Photometric linearity of a UV7 Excellence spectrophotometer evaluated at the 261 nm absorption maximum of nicotinic acid concentrations ranging from 6 mg/L to 60 mg/L.

2.3.3.2 In the Vis region

The absorption maximum at 430 nm of PDC can be used to qualify the UV/Vis spectrophotometer in the Vis region. It requires a PDC concentration of 600 mg/L in order to yield a pronounced peak maximum that can be used for qualification reliably. Considering that PDC concentrations higher than 100 mg/L are less stable than lower concentrations and solid neutral density filters provide up to five wavelengths in the Vis region (and thus a comparable broader wavelength coverage), the PDC CRM can be replaced by preferable neutral density filters.

Figures 9a - e show the linear response of a METTLER TOLEDO UV7 spectrophotometer in the Vis region obtained at five wavelengths (440, 465, 546.1, 590, 635 nm) with certified ND filters covering an absorption range from 0.5 to 2.3 A. At each wavelength, an excellent linear response is achieved represented by the closeness to 1.000 value of the coefficient of determination. A certified glass blank was used for each measurement.

Figures 9a - e: Individual absorption values of a METTLER TOLEDO UV7 Excellence spectrophotometer in the Vis region measured at five wavelengths 440 (a), 465 (b), 546.1 (c), 590 (d), 635 (e) nm in the absorption measurement range from 0.5 A to 2.3 A. All measured values are within the acceptance criteria of ± 0.008 A for absorbance values < 1 A and $\pm 0.8\%$ for absorbance values > 1 A. Each chart shows the certified (C) against the measured value (M) and includes the respective coefficient of determination R^2 , which is in all cases very close or equal to the ideal value 1.

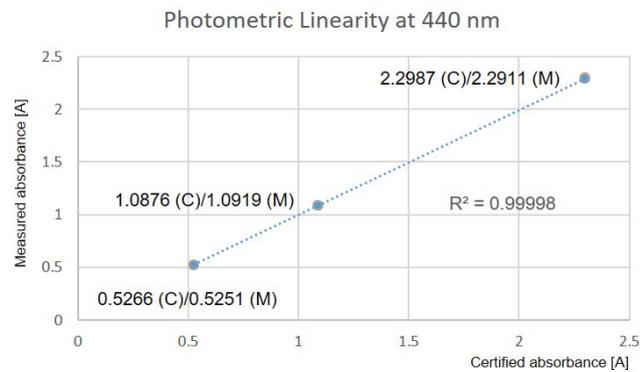


Figure 9a: Photometric linearity of a UV7 Excellence spectrophotometer at 440 nm.

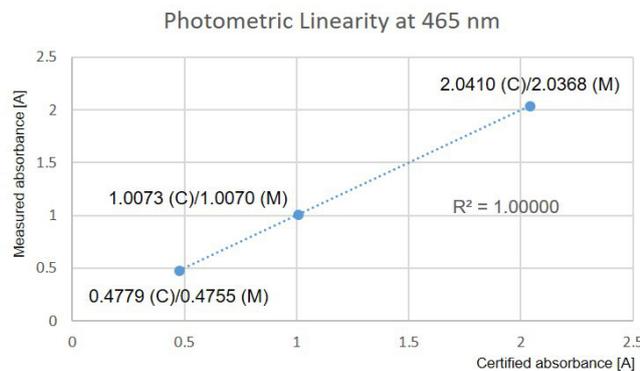


Figure 9b: Photometric linearity of a UV7 Excellence spectrophotometer at 465 nm.

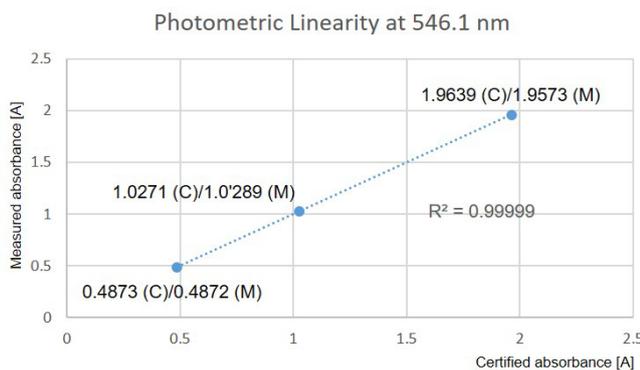


Figure 9c: Photometric linearity of a UV7 Excellence spectrophotometer at 546.1 nm.

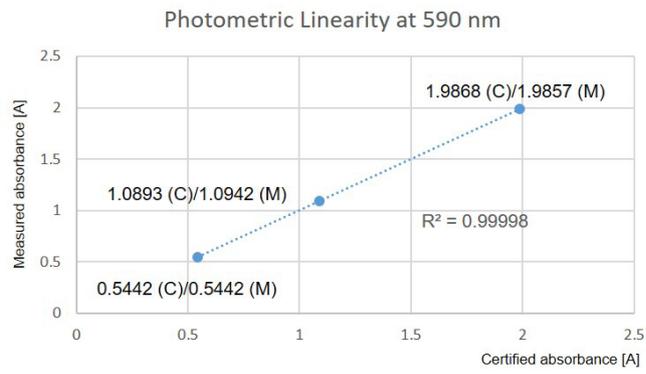


Figure 9d: Photometric linearity of a UV7 Excellence spectrophotometer at 590 nm.

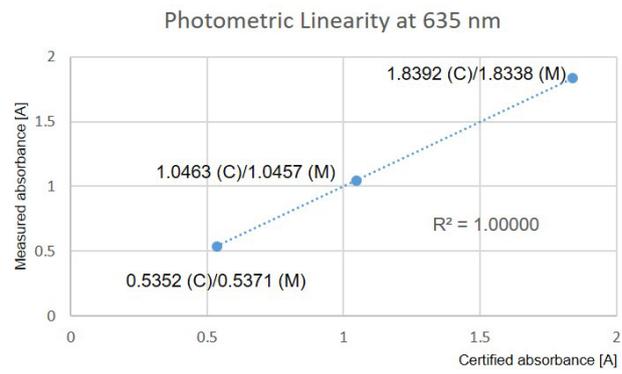


Figure 9e: Photometric linearity of a UV7 Excellence spectrophotometer at 635 nm.

2.3.4 Stray light OQ

Stray light is an important optical parameter that has a significant influence on the accuracy, i.e. the “true” value of the absorption measurement. It therefore needs to be tested accordingly with appropriate certified test materials, the so-called cut-off filters.

Table 7: Comparison of stray light requirements

Optical Test	Parameter	USP 42 NF37, chapter 857	Ph. Eur. 10.0, chapter 2.2.25
Stray light	Materials	Aqueous solutions of <ul style="list-style-type: none"> • 10 g/L NaI or KI • 50 g/L NaNO₂ • 12 g/L KCl, Acetone 	Aqueous solutions of <ul style="list-style-type: none"> • 10 g/L NaI or KI • 50 g/L NaNO₂ • 12 g/L KCl
	Acceptance criteria	$A_{\max} \geq 0.7 A$ (SFRM) $A_{\max} \geq 2.0 A$ (SWM)	$A_{\max} \geq 3.0 A$ NaI (220 nm) $A_{\max} \geq 3.0 A$ KI (250 nm) $A_{\max} \geq 3.0 A$ NaNO ₂ (340, 370 nm) $A_{\max} \geq 2.0 A$ KCl (198 nm)
	SOP	Evaluate one or more UV wavelengths by selection of appropriate material(s) to span the UV operational range required. Procedure A (SFRM) Measure a cut-off solution filter in a 10 mm cell against the same cut-off solution in a 5 mm cell. Procedure B (SWM) Measure a cut-off solution filter in a 10 mm cell against an appropriate reference in a 10 mm cell.	Procedure B (SWM) Measure a cut-off solution filter in a 10 mm cell against an appropriate reference in a 10 mm cell. Use the same measurement parameters as for the actual measurement.

Table 7 reveals that both pharmacopeias describe the same cut-off CRM filters which can be used to qualify the stray light behavior of a UV/Vis spectrophotometer. Neither of the pharmacopeias state that all listed cut-off filters should be used to qualify stray light, so one cut-off filter is sufficient. METTLER TOLEDO recommends the use of aqueous potassium chloride for stray light qualification in the UV range, as the influence of stray light is more predominant in the UV range than it is in the Vis range.

USP states only two acceptance criteria for the two different methods of determination used, which are the:

- Solution Filter Ratio Method abbreviated as SFRM (procedure A)
- Specific Wavelength Method abbreviated as SWM (procedure B)

How these methods differ from each other is explained in the following. Like the Ph. Eur., USP now accepts procedure B, the specific wavelength method, as well.

Ph. Eur. only specifies the specific wavelength method for the qualification of stray light. In contrast to the USP, the absorption maxima limits of various filters are tighter than those specified in the USP (cf. table 7).

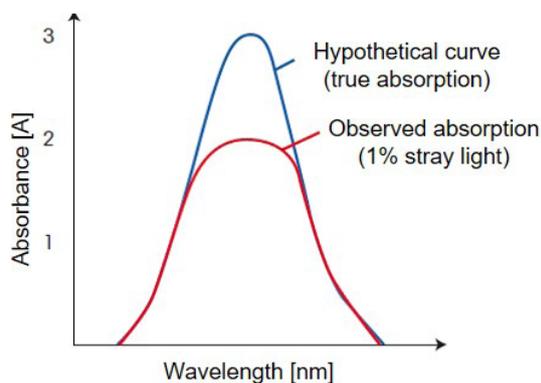


Figure 10: The effect of stray light on the absorption accuracy schematically demonstrated at an absorption maximum: The measured absorption differs significantly from the true absorption.

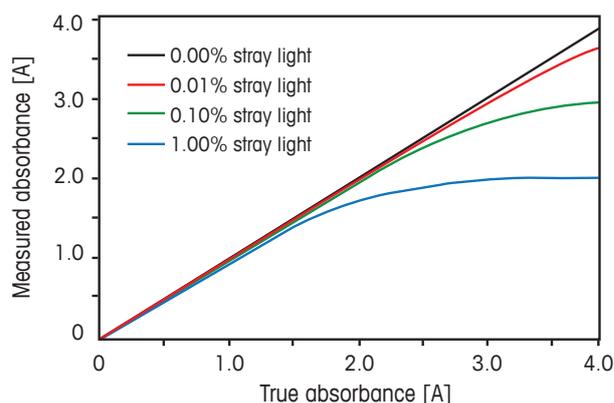


Figure 11: The effect of stray light on the linear optical response of a UV/Vis spectrophotometer: The higher the stray light influence at a given wavelength of the optical spectrum, the more pronounced the deviation from linearity is.

What is the cause and effect of stray light? Generally, stray light effects the linearity of a UV/Vis spectrophotometer, which determines the spectrophotometer's ability to determine correct concentrations. Specifically:

- Stray light is light reaching the detector that is not part of the analysis.
- It can be caused by optical imperfections, stray reflections within the monochromator itself, or light leaks in the optical system.
- Therefore, the absorbance of the sample is altered by stray light and the sample results include a systematic error. This is illustrated in the absorbance maximum in figure 10, which shows the deviation of the true and the observed absorptions due to stray light influence. Precisely, the linear relationship between concentration and absorbance (Beer-Lambert law) is negatively deviated.
- Stray light primarily defines the upper limit of the linear dynamic range. This is nicely illustrated in figure 11, which shows larger linearity deviations at higher amounts of stray radiant energy.

2.3.4.1 Specific Wavelength Method (SWM)

The traditional technique used to measure stray light is the specific wavelength method or SWM, which is based on the following optical aspects:

- The deeply ascending absorbance at a substance specific cut-off wavelength is measured against water as the compensation liquid (blank).
- At wavelengths lower than the cut-off wavelength, all light is absorbed, which would lead to an infinite absorbance.
- At wavelengths higher than the cut-off wavelength, all light passes, which would lead to zero absorbance.

Each substance shows a specific cut-off-wavelength (cf. table 8), which can be used to determine the amount of stray light within the wavelength region that is actually used for measurement. Stray light occurs as the background radiant energy in the spectrograph and therefore causes a non-infinite absorbance at wavelengths lower than the cut-off wavelength (cf. fig. 12). In order to obtain comparable measured values, the absorbance is measured as close as possible to the cut-off wavelength. The higher the absorbance value at the cut-off wavelength is, the more resistant the spectrograph of the UV/Vis spectrophotometer is to stray-light radiant energy.

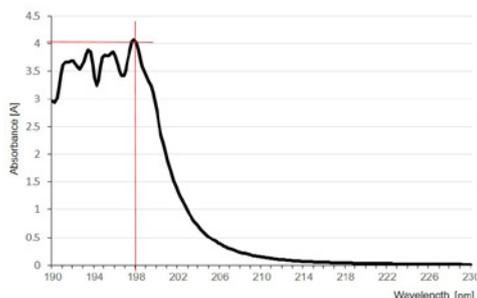


Figure 12: The UV/Vis spectrum of aqueous potassium chloride solution of a METTLER TOLEDO UV7 spectrophotometer which shows a steep absorption increase towards the cut-off wavelength at 198 nm. The crossed lines show how the absorption value at the cut-off wavelength of 198 nm is determined.

Table 8: Overview of substances including their specific cut-off wavelengths that can be used for stray light OQ of UV/Vis spectrophotometers.

Material	Concentration	Cut-off wavelength [nm]	Useable range [nm]
Sodium nitrite	5% aqueous	391	300 - 385
Acetone	Spectroscopy grade	326	250 - 320
Potassium iodide	1% aqueous	260	210 - 259
Sodium iodide	1% aqueous	260	210 - 259
Lithium carbonate	Saturated aqueous	227	210 - 225
Sodium chloride	1% aqueous	201	175 - 200
Potassium chloride	1.2% aqueous	201	175 - 200

2.3.4.2 Solution Filter Ratio Method (SFRM)

The 42nd issue of the USP that in addition to the SWM method, the solution filter ratio method abbreviated as SFRM can also be used. In the SFRM, the same cut-off filter material as in the SWM method is used, which means, for example, an aqueous potassium chloride solution. Compensation liquid (blank) and filter material are identical. However, they are measured in cuvettes of different path length (1 cm and 0.5 cm). Figure 13 illustrates the individual spectra of both blank and filter material and the resulting difference spectrum: Towards the cut-off wavelength, the absorption of the 1 cm pathlength cuvette (blue) increases at lower wavelengths than that of the 0.5 cm pathlength cuvette (red). The resulting difference (black) of both spectra yields a distinct absorption maximum. The peak height serves as the criteria for the amount of stray light. The USP acceptance criteria for the SFRM determined stray light are given in table 8.

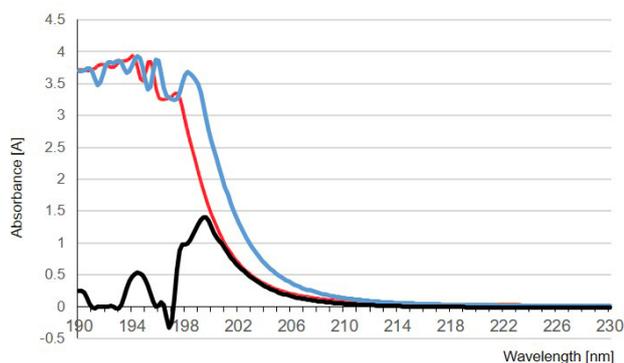


Figure 13: The individual spectra of both blank and filter material measured in individual cuvettes of different optical pathlengths and the resulting difference spectrum of aqueous potassium chloride solution.

2.3.5 Resolution

Table 9: Comparison of resolution requirements

Optical Test	Parameter	USP 42 NF37, chapter 857	Ph. Eur. 10.0, chapter 2.2.25
Resolution	Materials	0.02% v/v toluene in n-hexane (Blank: n-hexane)	0.02% v/v toluene in n-hexane or n-heptane (Blank: n-hexane or n-heptane)
	Acceptance criteria	$A_{\max,269}/A_{\min,267} > 1.3$	$A_{\max,269}/A_{\min,267}$ levels are stated in the respective monograph
	SOP	Measure $A_{\max,269}$ and $A_{\min,267}$ Calculate the ratio of A_{\max}/A_{\min} .	Measure $A_{\max,269}$ and $A_{\min,267}$ Calculate the ratio of A_{\max}/A_{\min} .

The last optical parameter included in a comprehensive operational qualification is the resolution. It defines the resolving power of a UV/Vis spectrophotometer: for example, the capability of resolving adjacent peak structures. Both pharmacopeias specify the same test material, which is a diluted solution of an aromatic compound (toluene) in a saturated hydrocarbon chain liquid such as n-hexane. The tenth edition of the Ph. Eur. now allows the usage of n-heptane as the alternative of n-hexane because of REACH regulations: n-heptane is less harmful than n-hexane.

In both pharmacopeias, the ratio between the minimum and maximum absorbance at respective wavelengths of 267 and 269 nm shall be calculated. In the USP, the ratio must exceed 1.3 to provide sufficient resolution, whereas the Ph. Eur. acceptance criteria are stated in the individual monograph. Figure 14 illustrates the typical spectrum obtained with the test solution recommended by both pharmacopeias. The absorption minima and maxima of the sharp, well resolved peak are used for the assessment of the resolution.

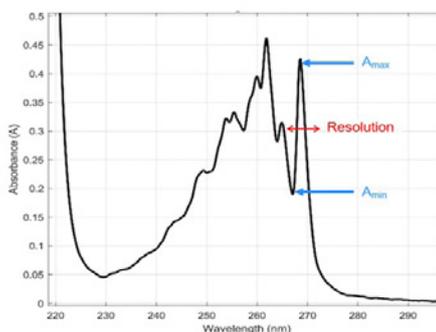


Figure 14: The UV/Vis spectrum of 0.02% toluene in n-hexane or n-heptane.

2.4 Pharmacopeia-compliant METTLER TOLEDO recommendations

After this thorough consideration of the changes in both pharmacopeias, the following chapters explain USP42-NF37 (chapter 2.4.1) and Ph. Eur. 10 (chapter 2.4.2) compliant recommendations for the operational qualification of METTLER TOLEDO UV7 Excellence UV/VIS spectrophotometers.

2.4.1 For USP 42 NF37

Table 10: USP 42 NF37 compliant operational qualification recommendations from METTLER TOLEDO

Parameter	Described materials	METTLER TOLEDO solutions
Wavelength accuracy	Liquid CRMs Solid filters Lamps	Ho(ClO₄)₃ : 4% Ho ₂ O ₃ in 10% v/v HClO ₄ , blank: air 14 wavelengths (240 nm – 650 nm) Xe: (built-in) 2 wavelengths (260.6, 528.6 nm)
Photometric accuracy Photometric precision Photometric linearity	Liquid CRMs Solid filters Self-made solutions	PDC (UV) : K ₂ Cr ₂ O ₇ in 0.001 M HClO ₄ , blank: 0.001 M HClO ₄ 40, 60, 80, 140 mg/L, 0.20 A – 2 A, 235, 257, 313, 350 nm (60 mg/L PDC is used for PA qualification) NA (UV) : Nicotinic acid in 0.1 M HCl, blank: 0.1 M HCl 6, 12, 18, 36 mg/L, 0.26 A – 1.6 A, 213, 261 nm (12 mg/L NA is used for PA qualification) ND filters (Vis) : certified ND, blank: certified glass 0.5, 1, 2 A, 440.0, 465.0, 546.1, 590.0, 635.0 nm (1 A ND filter is used for PA qualification)
Stray light	Liquid CRMs Self-made solutions	Procedure A (SFRM) : 1.2% m/v KCl/H ₂ O; 10 mm pathlength Blank: 1.2% m/v KCl/H ₂ O, 5 mm pathlength
Resolution	Liquid CRMs Self-made solutions	0.02% v/v toluene in n-hexane, blank: n-hexane

For wavelength accuracy qualification, METTLER TOLEDO recommends the use of certified holmium perchlorate solution, as it provides the large wavelength range from UV to Vis which is typically used in UV/Vis measurements according to USP monographs. Fourteen wavelengths are used to qualify both wavelength accuracy and precision, which is far beyond USP requirements. In addition, the built-in Xe lamp can be used as an alternative for wavelength qualification; however, only two wavelengths are used for this purpose.

For photometric accuracy, precision and linearity in the UV range, METTLER TOLEDO offers two choices, which are PDC in perchloric acid and nicotinic acid in hydrochloric acid. The latter provides compliance with both updated USP and Ph. Eur. optical chapter regulations. In both cases, the concentrations used, i.e. 40 – 140 mg/L PDC and 6 – 36 mg/L NA, provide qualification of both photometric accuracy and linearity in the typical absorption range used when testing active product ingredients as described in the respective monographs.

Each of the wavelengths provided by the respective material is used for qualification. For photometric accuracy qualification in the UV region, either the 60 mg/L PDC or 12 mg/L NA CRM is used.

For the visible range, METTLER TOLEDO recommends the usage of neutral density filters measured against a certified optical glass blank covering an absorption range from 0.5 to 2 A at five different wavelengths which are all used for qualification. For photometric accuracy qualification in the Vis region, the 1 A neutral density filter is used.

For stray light qualification according to USP42, METTLER TOLEDO recommends procedure A, the SFRM method. Aqueous potassium chloride solution is used as the cut-off material, providing a strict stray light qualification in the UV region. The SFRM method provides clear results thanks to its unambiguous absorption-maximum evaluation.

Last but not least, resolution is measured using the proven and widely accepted toluene in n-hexane solution.

All materials used for the METTLER TOLEDO solutions described in table 10 are commercially available, traceability-certified reference materials from accredited suppliers that provide long and reliable manufacturing histories.

2.4.2 For Ph. Eur. 10

Table 11: Ph. Eur. 10 compliant operational qualification recommendations from METTLER TOLEDO

Parameter	Described materials	METTLER TOLEDO solutions
Wavelength accuracy	Liquid CRMs Solid filters Lamps	Ho(ClO₄)₃ : 4% Ho ₂ O ₃ in 10% v/v HClO ₄ , blank: air 14 wavelengths (240 nm – 650 nm) Xe: (built-in) 2 wavelengths (260.6, 528.6 nm)
Photometric accuracy Photometric linearity	Liquid CRMs Solid filters Self-made solutions	PDC (UV) : K ₂ Cr ₂ O ₇ in 0.001 M HClO ₄ , blank: 0.001 M HClO ₄ 40, 60 , 80, 140 mg/L, 0.20 A – 2 A, 235, 257, 313, 350 nm (60 mg/L PDC is used for PA qualification) NA (UV) : Nicotinic acid in 0.1 M HCl, blank: 0.1 M HCl 6, 12 , 18, 36 mg/L, 0.26 A – 1.6 A, 213, 261 nm (12 mg/L NA is used for PA qualification) ND filters (Vis) : certified ND, blank: certified glass 0.5, 1 , 2 A, 440.0, 465.0, 546.1, 590.0, 635.0 nm (1 A ND filter is used for PA qualification)
Stray light	Liquid CRMs Self-made solutions	Procedure B : 1.2% m/v KCl/H ₂ O; 10 mm pathlength Blank: H ₂ O, 10 mm pathlength
Resolution	Liquid CRMs	0.02% v/v toluene in n-hexane, blank: n-hexane

In order to comply with Ph. Eur. 10 regulations, the respective METTLER TOLEDO materials are substantially the same as those shown in table 11. The Ph. Eur. 10 chart only differs in that:

- Photometric repeatability does not need to be determined
- Stray light qualification is performed according to procedure B, the specific wavelength method (SWM).

All materials used for the METTLER TOLEDO solutions described in table 11 are commercially available, traceability-certified reference materials from accredited suppliers that provide long and reliable manufacturing histories.

3. Automated Performance Verification

Before diving deeper into the advantages of automated performance verification, aspects verifying UV/Vis spectrophotometer optical performance including traceability, the measurement itself, the benefits of automation, and data integrity will be discussed. All these topics are relevant in regulated industries such as the pharmaceutical industry. However, they are of general fundamental importance when verifying the correct function of UV/Vis spectrophotometers in any application.

3.1 Traceability

In order to comply with metrological and regulation requirements, the usage of reliable, traceability-certified reference materials is highly recommended due to the following:

- Commercially available, traceability-certified reference materials, which are manufactured and certified by an accredited company.
- Liquid CRMs are contained in permanently heat-fusion-sealed quartz cuvettes which protect them from environmental influences and damage, allowing long-term storage and use.
- Solid CRMs such as neutral density filters contain the absorbing material immobilized in glass.
- Recertification of CRMs on regular basis shall be considered as part of the maintenance plan to establish trustworthiness.
- CRMs are prepared in accordance with ASTM E387 and USP general chapter 857 or Ph. Eur. chapter 2.2.25
- If the respective regulatory monograph and the wavelength region allows it, it is highly recommended that the optical performance of the UV/Vis instrument be verified with CRM solutions, which represent the physical state of the great majority of UV/Vis samples.

3.2 Measurement

After discussing the selection of appropriate reference materials, the measurement itself should be considered. This involves the correct execution of a strict protocol including repetitive measurements, measured value acquisition, mean value calculation and result validation against predefined acceptance criteria. These workflows are time-consuming and error prone. Automation of the complete workflow can provide productivity gains and enhance measurement security in the following steps:

- Preparatory work: Instrument warm-up and preparing CRM cuvettes
- Running of tests: Changing CRM and blank cuvettes
- After-run handling: Cleaning and storing of CRM cuvettes
- Calculation: Calculation and validation of results
- Reporting: Compiling data and creating formal reports

3.3 Automation

If the required CRMs are integrated into cuvette-changing devices that can be operated automatically using predefined method-based measuring sequences, the following benefits can be achieved:

- Automatic, unattended verification of up to 10 optical parameters according to USP and Ph. Eur. regulations with NIST-traceable certified reference materials within 27 minutes.
- Time to result, including traceable documentation and result validation, is approximately three times faster than manually executed performance measurements.
- Workflow execution and data transcription errors can be avoided so tests do not need to be repeated.
- Comparing automated with manual checks using single CRMs in sealed cuvettes, return on investment is achieved within a reasonable time frame of 1.5 – 2 years.
- As the units can reside on the UV/Vis spectrophotometer without interfering with sample measurements, performance checks prior to each measurement can be considered. This helps to create significantly higher quality measurements.

3.3.1 METTLER TOLEDO CertiRef™ automated qualification solutions

The METTLER TOLEDO CertiRef equipment qualification accessory family consists of five modules. Each module is a certified CRM-containing cuvette changing device, which enables fully automated qualification of UV/VIS Excellence spectrophotometers UV7, UV5 and UV5Bio according to the latest USP and Ph. Eur. regulations. Table 12 shows the respective CertiRef module and the individual pharmacopeia compliance validity.

Table 12: METTLER TOLEDO CertiRef modules and their individual pharmacopeia compliance.

Module	USP 42 – NF 37	Ph. Eur. Ed. 10
CertiRef EUP v1	✓	✓
CertiRef EUP v2	✓	✓
CertiRef USP	✓	
CertiRef LinSet PDC	✓	✓
CertiRef LinSet Niacin	✓	✓

Each module is automatically recognized by the instrument during installation and enables unattended optical performance qualification according to a predefined workflow. This includes data-integrity-compliant measured value acquisition (cf. chapter 3.4) and automatic result assessment against stored certified reference values of the respective CRMs. Figure 15 shows how a CertiRef USP/EUP and a LinSet PDC/Niacin module is mounted on a UV7 spectrophotometer.

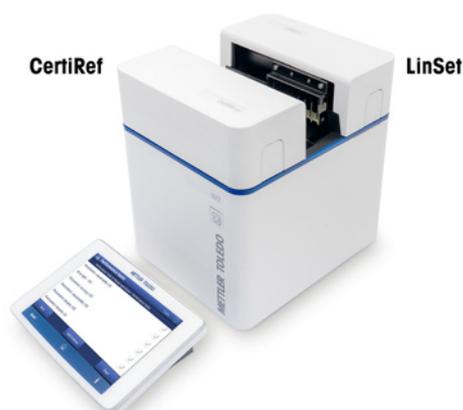


Figure 15: Opposite installation of a CertiRef and a LinSet module on top of a UV7 Excellence spectrophotometer.

Supported by comprehensive documentation, the CertiRef-based optical performance qualification of UV/VIS Excellence spectrophotometers is a state-of-the-art solution that fulfills the high demands of regulated industries. Tables 13 - 17 detail the contents of each CertiRef module and their compliance with the latest US and European pharmacopeias.

Table 13: The optical performance tests provided by the CertiRef USP module.

Performance test	CRM	Test parameters	Acceptance criteria USP 42
Wavelength accuracy UV - VIS	Ho(ClO ₄) ₃ : 4% Ho ₂ O ₃ in 10% v/v HClO ₄ Blank: air	14 wavelengths (240 nm – 650 nm)	UV (200 – 400 nm): ± 1 nm Vis (400 – 780 nm): ± 2 nm
Photometric accuracy/precision UV	PDC (UV): K ₂ Cr ₂ O ₇ in 0.001 M HClO ₄ Blank: 0.001 M HClO ₄	60 mg/L 235, 257, 313, 350 nm	± 0.010 A
Stray light according to procedure A (Solution Filter Ratio Method)	1.2% m/v KCl/H ₂ O; 10 mm path length Blank: 1.2% m/v KCl/H ₂ O, 5 mm path length	A _{max}	A _{max} ≥ 0.7 A
Resolution	0.02% v/v toluene in n-hexane Blank: n-hexane	A _{max,269} /A _{min,267}	A _{max,269} /A _{min,267} > 1.3

Table 14: The optical performance tests provided by the CertiRef EUP v1 module. This module provides potassium dichromate (PDC) for the qualification of the photometric accuracy.

Performance test	CRM	Test parameters	Acceptance criteria USP 42	Acceptance criteria Ph. Eur. 10
Wavelength accuracy UV - VIS	Ho(ClO ₄) ₃ : 4% Ho ₂ O ₃ in 10% v/v HClO ₄ Blank: air	14 wavelengths (240 nm – 650 nm)	UV (200 – 400 nm): ± 1 nm Vis (400 – 780 nm): ± 2 nm	UV (< 400 nm): ± 1 nm Vis (> 400 nm): ± 3 nm
Photometric accuracy/ precision UV	PDC (UV): K ₂ Cr ₂ O ₇ in 0.001 M HClO ₄ Blank: 0.001 M HClO ₄	60 mg/L 235, 257, 313, 350 nm	± 0.010 A	± 0.010 A
Stray light according to procedure B (Specific Wavelength Method)	1.2% m/v KCl/H ₂ O; 10 mm pathlength Blank: H ₂ O, 10 mm path- length	A _{max} at 198 nm	A _{max} ≥ 2.0	A _{max} ≥ 2.0
Resolution	0.02% v/v toluene in n-hexane Blank: n-hexane	A _{max,269} /A _{min,267}	A _{max,269} /A _{min,267} > 1.3	A _{max,269} /A _{min,267} levels are stated in the respec- tive monograph

Table 15: The optical performance tests provided by the CertiRef EUP v2 module. This module provide nicotinic acid (NA) for the qualification of photometric accuracy.

Performance test	CRM	Test parameters	Acceptance criteria USP 42	Acceptance criteria Ph. Eur. 10
Wavelength accuracy UV - VIS	Ho(ClO ₄) ₃ : 4% Ho ₂ O ₃ in 10% v/v HClO ₄ Blank: air	14 wavelengths (240 nm – 650 nm)	UV (200 – 400 nm): ± 1 nm Vis (400 – 780 nm): ± 2 nm	UV (< 400 nm): ± 1 nm Vis (> 400 nm): ± 3 nm
Photometric accuracy/ precision UV	NA (UV): Nicotinic acid in 0.1 M HCl Blank: 0.1 M HCl	12 mg/L 213, 261 nm	± 0.010 A	± 0.010 A
Stray light according to procedure B (Specific Wavelength Method)	1.2% m/v KCl/H ₂ O; 10 mm pathlength Blank: H ₂ O, 10 mm path- length	A _{max} at 198 nm	A _{max} ≥ 2.0	A _{max} ≥ 2.0
Resolution	0.02% v/v toluene in n-hexane Blank: n-hexane	A _{max,269} /A _{min,267}	A _{max,269} /A _{min,267} > 1.3	A _{max,269} /A _{min,267} levels are stated in the respec- tive monograph

Table 16: The optical performance tests provided by the CertiRef LinSet PDC module. This module provides automatic linearity qualification for both UV (PDC) and Vis spectral region (ND filters).

Performance test	CRM	Test parameters	Acceptance criteria USP 42	Acceptance criteria Ph. Eur. 10
Photometric linearity UV	PDC (UV): K ₂ Cr ₂ O ₇ in 0.001 M HClO ₄ Blank: 0.001 M HClO ₄	40, 80, 140 mg/L 0.20 A – 2 A, 235, 257, 313, 350 nm	± 0.010 A (for absor- bance ≤ 1A); ± 1% (for absorbance > 1A)	± 0.010 A or ± 1%, whichever is greater
Photometric linearity Vis	ND filters (Vis): certi- fied ND Blank: certified glass	0.5, 1, 2 A 440.0, 465.0, 546.1, 590.0, 635.0 nm	± 0.008 A (for absor- bance ≤ 1A); ± 0.8% (for absorbance > 1A)	± 0.010 A or ± 1%, whichever is greater
Photometric accuracy/ precision Vis	ND filter (Vis): certified ND Blank: certified glass	0.5 A 440.0, 465.0, 546.1, 590.0, 635.0 nm	± 0.008 A	± 0.010 A

Table 17: The optical performance tests provided by the CertiRef LinSet Niacin module. This module provides automatic linearity qualification for both UV (NA) and Vis spectral region (ND filters).

Performance test	CRM	Test parameters	Acceptance criteria USP 42	Acceptance criteria Ph. Eur. 10
Photometric linearity UV	NA (UV): Nicotinic acid in 0.1 M HCl, blank: 0.1 M HCl	6, 18, 36 mg/L 0.26 A – 1.6 A 213, 261 nm	± 0.010 A (for absorbance ≤ 1 A); $\pm 1\%$ (for absorbance > 1 A)	± 0.010 A or $\pm 1\%$, whichever is greater
Photometric linearity Vis	ND filters (Vis): certified ND Blank: certified glass	0.5, 1, 2 A 440.0, 465.0, 546.1, 590.0, 635.0 nm	± 0.008 A (for absorbance ≤ 1 A); $\pm 0.8\%$ (for absorbance > 1 A)	± 0.010 A or $\pm 1\%$, whichever is greater
Photometric accuracy/precision Vis	ND filter (Vis): certified ND Blank: certified glass	0.5 A 440.0, 465.0, 546.1, 590.0, 635.0 nm	± 0.008 A	± 0.010 A

3.4 Data-integrity compliance

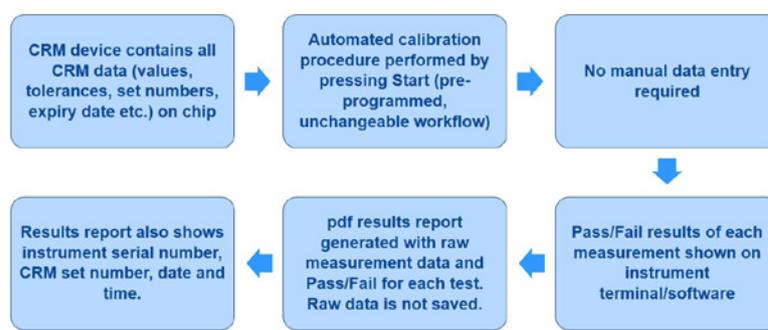


Figure 16: Schematic illustration of the automated test calibration routine workflow provided by METTLER TOLEDO CRM device CertiRef.

In taking a closer look at the detail of the workflow steps which were previously described and what the automated performance verification CRM tool CertiRef pursues during a calibration sequence, it can clearly be seen in the workflow illustration in figure 16 that the workflow is completely controlled by the analytical system. This means that the standalone UV/Vis spectrophotometer or an attached software completely controls the entire sequence. This does not allow any alteration or falsification, which enhances security and compliance. In detail:

- The CRM device connected to the UV/Vis spectrophotometer contains all CRM data (values, tolerances, set numbers, expiry date etc.) on the integrated data chip.
- The automated calibration procedure is performed by pressing 'start', which initiates a pre-programmed, unchangeable workflow.
- No manual data entry is required.
- Pass/fail result of each measurement is shown on the instrument terminal or attached software.
- An original PDF report is automatically generated, which includes pass/fail results for each test and the respective measurement data.
- The standalone instrument does not store any data. If a PC software is attached to the instrument, the respective data is stored in the database strictly following ALCOA principles.
- The result report includes all relevant metadata such as time stamp and CRM/instrument identification figures.

If the automatic performance verification against ALCOA principles is assessed, it can be shown that the complete workflow is compliant with data-integrity standards. This is a significant benefit with respect to FDA-compliant data management, which is of particular importance to regulated industries such as pharma. As such, following ALCOA principles, the automated performance-verification sequence is:

- Attributable. The complete qualification process is unambiguously attributable as each user is identified and all actions are tracked.
- Legible. It is legible as all data are in pdf and printed reports or are stored in the software database.
- Contemporaneous. As each measurement is documented automatically at the point of origin, the process is completely contemporaneous.
- Original. Records are also original as the original data is transferred/printed at the point of measurement.
- Accurate. As all results are printed automatically, the process does not allow any changes or falsification.

4. Best Practice OQ Guidelines

The following chapters discuss important parameters which are relevant for both routine operation as well as performance checks of UV/Vis spectrophotometers.

4.1 Temperature influence on operational qualification

The following optical performance parameters which are verified by measurement with liquid CRM are NOT influenced by temperature within a range from 10 – 40 °C. They are:

- Wavelength accuracy/repeatability
- Photometric repeatability
- Stray light

Photometric resolution (toluene/hexane ratio) and photometric accuracy wavelengths (USP and Ph. Eur.) ($K_2Cr_2O_7$ in $HClO_4$) show a relatively significant temperature dependency ranging from -0.014 to -0.034 absorption units within 10 to 40 °C. This is due to the temperature expansion coefficient of the respective solvent. The spectrum excerpt of a toluene/hexane solution in figure 17 shows the temperature effect on the absorption measurement.

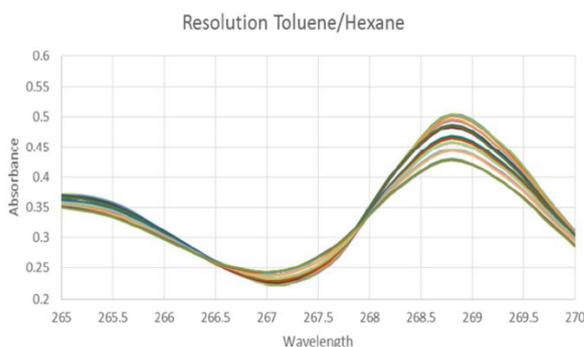


Figure 17: Temperature variations influence the absorption values of both the valley and the peak of the absorption curve of the CRM toluene in hexane differently, which causes an alteration of the absorption coefficient and thus the resolution value.

In general, expansion of the solvent, mainly when working with organic solvents, may change the apparent absorbance causing temperature dependency in quantification determination. This is especially relevant in long-term kinetic studies which require appropriate thermostating. For further information concerning thermostating accessories for METTLER TOLEDO UV/VIS Excellence spectrophotometers please refer to www.mt.com/cuvei-peltier-uvvis.

4.2 Cuvette Selection

The right cuvette choice is fundamental for accurate UV/Vis measurements.

- Ideally, cuvettes should be completely transparent at all wavelengths in the whole UV/Vis range.
- The transmission/wavelength graph in figure 18 illustrates the usability of cuvette material for the respective application.

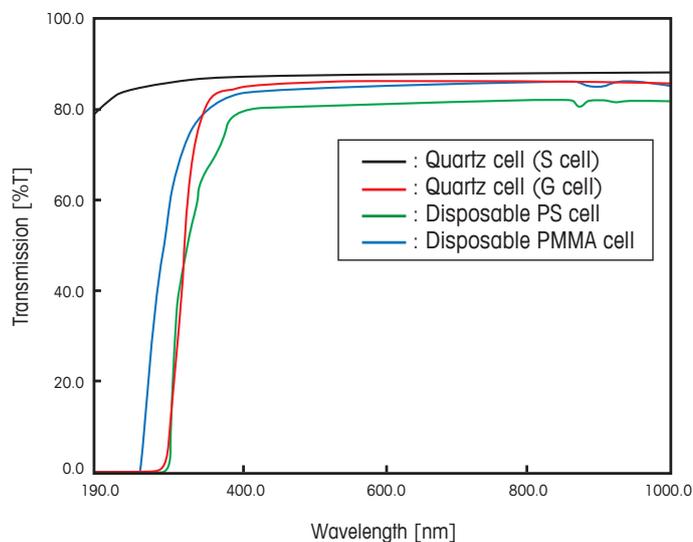


Figure 18: Wavelength-dependent transmission of various cuvette material

- Cuvettes made of other materials have cutoff = 230 nm and are not suitable for analysis in the UV range.
- For the visible range, disposable polymethylmetacrylate or polystyrene cuvettes are frequently used.

4.3 Cuvette Handling

Cleanliness of cuvettes has a major effect on results, so this important factor must be considered. This chapter provides useful tips for correct cuvette handling as follows:

- Avoid touching the blank optical surfaces, as fingerprints can cause significant absorbance changes and thus impact the accuracy of results!
- A simple and effective measure to avoid contamination of cuvettes is to store them in ethanol in closed compartments as shown in figure 19.
- For thorough inner and outer cleaning, use a 60% isopropanol/water solution, then rinse with water and wipe with a lint-free or microfabric tissue.
- If necessary, clean the cuvette with suitable solvent to dissolve any remaining samples, then rinse with water.
- Avoid using glass pasteur pipettes to fill the cuvette; they could scratch the optical surface. Pipettes with disposable plastic tips are the preferred option.

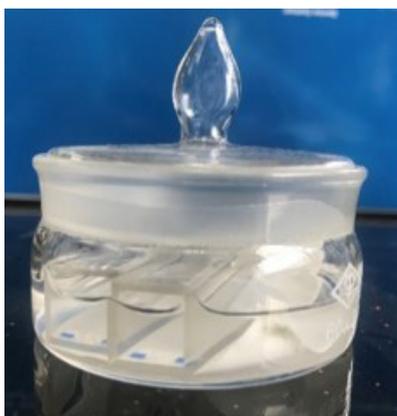


Figure 19: Storage of cuvettes in ethanol in a closed compartment.

4.4 Cuvette performance check

The updated chapter 2.2.25 of the Ph. Eur. describes a procedure which allows assessment of the mechanical integrity (parallelism) and cleanliness of an optical cuvette either made of quartz or optical glass. METTLER TOLEDO recommends that this procedure be applied on a monthly basis or even less frequently, depending on usage. If the cuvette is manufactured according to previously mentioned standards and is handled appropriately, integrity concerning dimension or cleanliness should be maintained.

The procedure is as follows:

- Fill the cuvette with deionized, degassed water and measure absorbance against air at 240 nm for quartz cuvettes and 650 nm for glass cuvettes.
- After this measurement, rotate the cuvette by 180 degrees in its holder and measure the absorbance again at the same wavelength.
- The acceptance criteria are less than 93 mA absorption at 240 nm and less than 35 mA at 650 nm. The deviation of absorption measurement obtained after 180 degree rotation shall not exceed 5 mA.
- For well-maintained cuvettes, the following typical values can be obtained:
 - A (240 nm) < 0.01 for quartz cuvettes
 - A (650 nm) < 0.002 for glass cuvettes
 - A (240/650 nm) < 0.001 after 180° rotation

5. Conclusions

USP 42 NF37 and Ph. Eur. 10 updated their optical chapters 857 and 2.2.25. The respective contents for optical OQ became better aligned. The updates emphasize that UV/Vis instruments must show the required performance during OQ in order to function according to measurement parameters given in an analytical workflow.

In both pharmacopeias, nicotinic acid is allowed as alternative to PDC. The test of photometric linearity becomes mandatory for optical OQ. Automated optical performance verification contributes to measurement quality, keeps protocols up-to-date, and can be easily integrated in UV/Vis workflows supported by UV/VIS Excellence spectrophotometers from METTLER TOLEDO. These spectrophotometers provide security in operational qualification processes, help to ensure compliance with data-integrity requirements and support laboratories in transitioning securely to the updated UV/Vis spectroscopy pharmacopeia requirements.

6. References

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