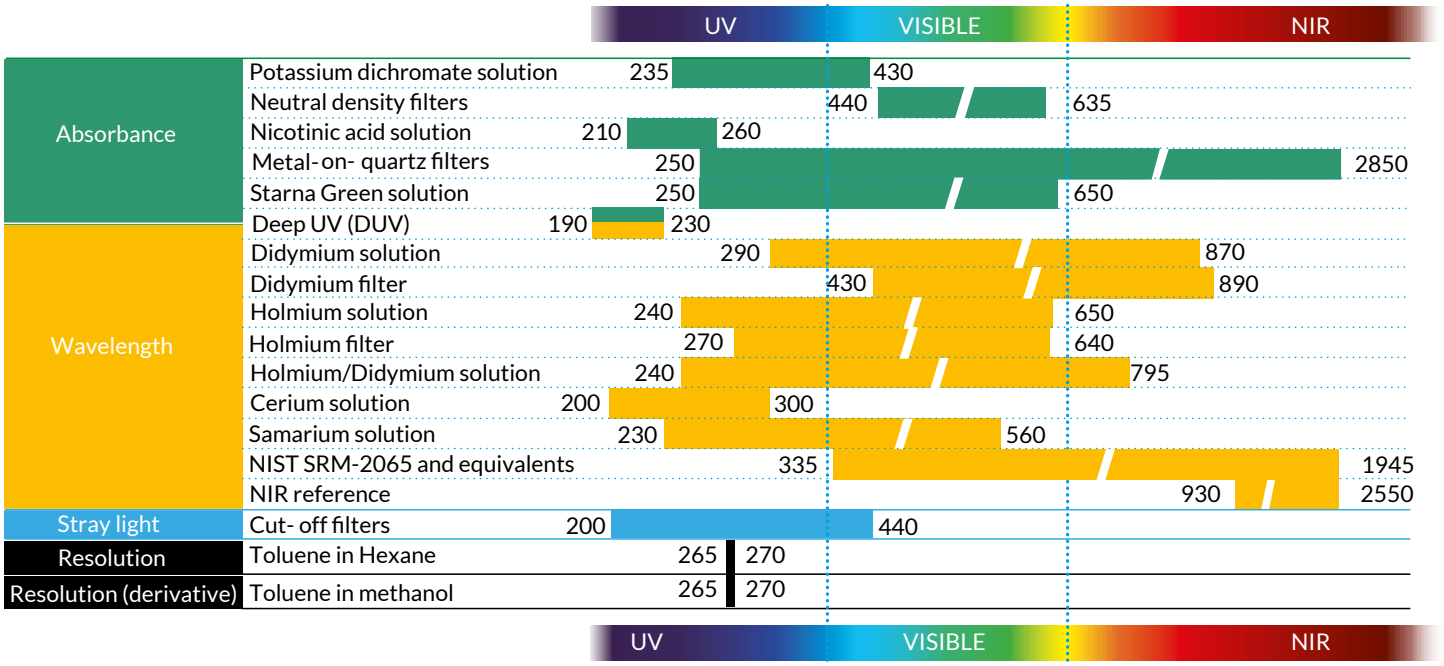
Selecting References for Compliance
a User Guide

The Regulations

The regulations for ultraviolet and visible spectroscopy were recently updated by the US and European pharmacopoeias. The revised **US Pharmacopeia (USP) Chapter <857>** became mandatory on 1st December 2019. **European Pharmacopoeia 10.0 (EP) Chapter 2.2.25** became mandatory on 1st January 2020. Earlier versions of the pharmacopoeias described a limited set of generic tests to qualify an instrument for wavelength, absorbance, stray light and resolution (spectral bandwidth). If the instrument passed these tests, in could be described as 'pharmacopoeia compliant'. This is no longer the case: these limited tests no longer qualify an instrument for the variety of measurements encountered in the modern pharmaceutical laboratory. Users must now demonstrate '**fitness for purpose**', i.e. that the instrument has the capability to perform the actual analysis to the required accuracy and precision. The qualification measurements must therefore be made at parameter values that match or '**bracket**' those used in the analysis. This means that it is no longer possible to specify 'kits' that can qualify instruments for all applications. Both standards now require absorbance linearity to be qualified. The Basic Qualification Kits described below will meet the routine needs of many laboratories and can be supplemented with additional references if required. Starna CRM kits offer cost savings when compared to the purchase of individual references.

Five steps to compliance

1. Identify the **Standard(s)** that you need to comply with (e.g. European Pharmacopoeia, US Pharmacopeia)
2. Select the **monographs** describing the analyses you wish to perform.
3. Identify the **wavelength** and **photometric (absorbance)** ranges over which you will be working.
4. Identify the **instrument qualification requirements** at these wavelengths from the monographs or, if no specific requirements are given, from the pharmacopoeia.
5. Select appropriate reference materials for the required parameters. Starna has the widest Certified Reference Materials (CRMs) range covering wavelengths from deep UV to near Infrared:



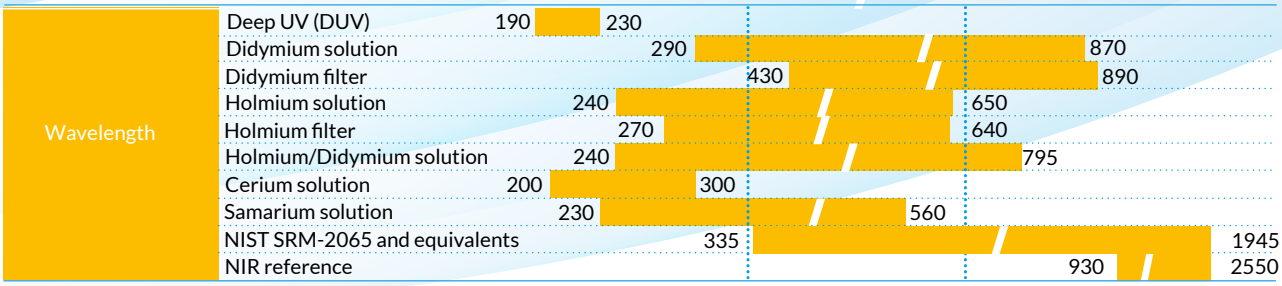
As Certified Reference Materials, they are all recognised for instrument qualification. Many of these materials are cited specifically in the pharmacopoeias for this purpose.

The first step in selecting references is to identify the wavelength(s) to be used for analysis.

Selecting References for Compliance - a User Guide

Selecting Wavelength References

Starna wavelength CRMs cover a range from 190 nm in the deep UV to 2550 nm (2.55µ) in the near infrared.



Most pharmaceutical analyses are conducted at wavelengths between 210 nm and 450 nm, with some colorimetric measurements in the visible region up to about 700nm. Holmium Oxide solution has been the wavelength reference of choice for many years but does not cover this whole range. Starna's cerium oxide and holmium/didymium solutions will cover most situations, with 19 well-spaced certified peaks between them from 201 nm to 794 nm. These materials are cited in both US and European Pharmacopoeias. holmium and didymium glasses are also cited in the pharmacopoeias and are very convenient as a routine check of wavelength accuracy but do not cover the important region around 250 nm where many aromatic compounds are measured. At the extremes of the wavelength scale, Starna's Deep UV reference covers wavelengths from 190 to 230 nm and Wide Range and NIR references extend the range up to 1945 nm and 2550 nm respectively.

Instrument bandwidth (resolution) can affect the measured peak wavelength so it is recommended that the instrument bandwidth is confirmed (see test below) even if the monograph in use does not require it. Starna wavelength references are calibrated at several different bandwidths.

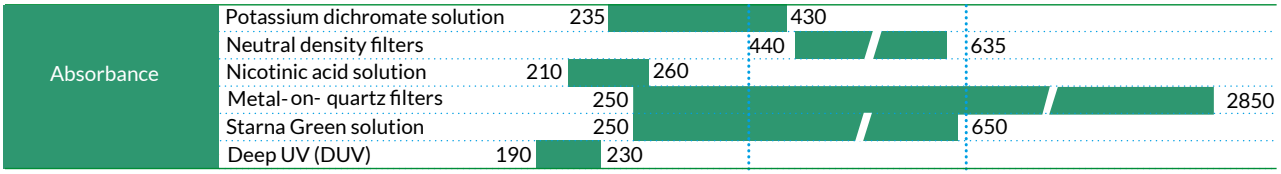
Summary

- Select wavelength references with peaks that match or 'bracket' your analytical wavelengths.
- To achieve this any wavelength CRM may be used for instrument qualification, not just those cited in the pharmacopoeias.
- Be mindful of instrument bandwidth when making qualification measurements.

Selecting Absorbance References

The latest regulations require that both absorbance (photometric) accuracy and photometric linearity is qualified. The type(s) of reference chosen will depend on the analytical wavelength(s):

The certified absorbance values should ideally match or 'bracket' the expected analytical values.



Potassium dichromate has been used for many years and is recommended by the USP. It is no longer specifically listed by the EP but it remains a perfectly valid reference material to meet the EP requirements. For the far UV, nicotinic acid is now listed by both USP and EP for absorbance values up to 1A. For higher wavelengths, Starna has developed Starna Green, a very stable CRM with peaks at 258, 416 and 630 nm, a range that would previously have required two different references.

Neutral density filters can be used at wavelengths from 440 nm to 635 nm. For the visible region, the USP now lists metal-on-quartz filters; these filters can be used over a wide wavelength range (250 to 850 nm) but are not compatible with all instruments – users should check with their instrument supplier.

Absorbance references can be certified up to 3, 4 or even 5 A, but such values are unusual in routine analysis. Indeed, the optimum absorbance range for analysis is normally from 0.2 A to 1.5 A. Above 2 A, instrument stray light can be an issue, and the EP recommends that at these levels a risk assessment is applied to instrument qualification.

The same references used to test absorbance accuracy can be used to test linearity. The USP requires that three absorbance references covering the range of values encountered in the analyses. If all three meet the accuracy requirement then the linearity requirement is also met. The same measurement process applies for the EP. Again, at higher absorbances, stray light can affect the results.

Summary

- Select absorbance references with peaks that lie within the instrument's qualified wavelength range and have certified absorbance values that 'bracket' those expected in the analysis.
- Any absorbance CRM meeting these criteria may be used for instrument qualification, not just those cited in the pharmacopoeias.
- Be mindful of stray light when making qualification measurements

Selecting References for Compliance - a User Guide

Selecting Stray Light References

For stray light qualification USP and EP both allow the "specified wavelength" method. A range of solutions allows stray light to be estimated from 200 nm to 400 nm – the USP does not require stray light qualification above 400 nm. Again, solutions should be chosen whose cut-off wavelengths (where absorbance ≥ 2A) 'bracket' the analytical wavelengths. The two pharmacopoeias have different requirements, see table below:

MATERIAL	CONCENTRATION	CUT-OFF WAVELENGTH	
		USP <857>	EP 2.2.25
Potassium Chloride	12 g/l	198 nm	198 nm
Sodium Iodide	10 g/l	220 nm	220 nm
Potassium Iodide	10 g/l	220 nm	250 nm
Acetone	Spectroscopy grade	300 nm	Not specified
Sodium Nitrite	50 g/l	340 nm	340 nm 370 nm

When working at high absorbance levels – above 2 A on most instruments - the USP recommends the "filter ratio" method. This method can be used to calculate the level of Stray Light in a system.

Summary

- Select stray light references with cut-off wavelengths that 'bracket' those expected in the analyses.
- Consider the 'filter ratio' method when working at high absorbances

Resolution (Bandwidth) References

The only reference usually recommended is Toluene in Hexane. It is recommended that the bandwidth is always verified even where this test is not specified in monographs. The previous edition of EP 2.2.25 contained a test for resolution in 2nd derivative mode; whilst this is no longer specified the reference (Toluene in Methanol) is still available should it be required.

Basic Qualification Kits

The kits below will allow instrument qualification over the stated wavelength and absorbance ranges for compliance with EP 2.2.25 and USP <857>. References are provided to qualify wavelength accuracy, absorbance accuracy and 3-point linearity, stray light and resolution/ bandwidth. The stray light references for USP compliance are for Chapter <857> Method B.

Note: Potassium Chloride solution is included in all the basic kits as even if the instrument is not being used in the far UV; increasing stray light at this wavelength (200nm) is a sensitive indication of deterioration of the instrument optics. It also often features in the stray light specification quoted by instrument manufacturers. Performance to specification is an important part of instrument qualification (IQ and OQ).

These kits have been designed to qualify UV-visible spectrophotometers over the wavelength and absorbance ranges commonly used in pharmaceutical analysis. If your operating parameters fall outside these ranges, Starna offers the world's biggest range of spectroscopy CRMs as illustrated elsewhere in this catalogue and will be pleased to recommend additional or alternative references to meet individual requirements. Please contact sales@starna.com for advice and information.

Basic Kit for EP2.2.25 compliance

Catalogue number: RM-EP20
[Kit Identity: RM-020610HLKISISNTX]

REFERENCE	TO QUALIFY	WAVELENGTH RANGE
Holmium cell	Wavelength accuracy	240 - 650 nm
Potassium dichromate 20, 60 & 100 mg/l & blank cell	Absorbance accuracy and linearity, 0-1 A	235 – 350 nm
Potassium chloride	Stray light, 200nm	200 -370 nm
Sodium iodide	Stray light, 220 nm	
Potassium iodide	Stray light, 250 nm	
Sodium nitrite	Stray light, 340, 370 nm	
Toluene in Hexane	Resolution (bandwidth), 265-270nm	265-270 nm

Selecting References for Compliance - a User Guide

Basic Kit for USP Chapter <857> compliance

Catalogue number: RM-USP20
[Kit Identity: RM-02061060HLKCKIACSNTX]

REFERENCE	TO QUALIFY	WAVELENGTH RANGE
Holmium cell	Wavelength accuracy	240 - 650 nm
Potassium Dichromate 20, 60 & 100 mg/l & blank cell.	Absorbance accuracy and linearity, 0-1 A	235 – 350 nm
Potassium dichromate 600 mg/l	Absorbance accuracy, 1 A	430 nm
Potassium chloride	Stray Light, 200 nm	240 -370 nm
Potassium iodide	Stray light, 220 nm	
Acetone	Stray light, 300 nm	
Sodium nitrite	Stray light, 340 nm	
Toluene in Hexane	Resolution (bandwidth), 265-270nm	265-270 nm

Basic Combination kit for EP and USP compliance

Catalogue number: RM-USPEP20
[Kit Identity: RM-02061060HLKCSIKIACSNTX]

REFERENCE	TO QUALIFY	WAVELENGTH RANGE
Holmium cell	Wavelength accuracy	240 - 650 nm
Potassium Dichromate 20, 60 & 100 mg/l & blank cell.	Absorbance accuracy and linearity, 0-1 A	235 – 350 nm
Potassium dichromate 600 mg/l	Absorbance accuracy, 1 A (USP)	430 nm
Potassium chloride	Stray light, 200 nm (EP &USP)	200 -370 nm
Sodium Iodide	Stray light, 220 nm (EP)	
Potassium Iodide	Stray light, 220 nm (USP), 250nm (EP)	
Acetone	Stray light, 300 nm (USP)	
Sodium Nitrite	Stray light, 340 nm (EP & USP), 370 nm (EP)	
Toluene in Hexane	Resolution (bandwidth), 265-270nm	265-270 nm

Far UV extension kit – EP and USP compliance

Catalogue number: RM-06A12A24AREKC

REFERENCE	TO QUALIFY	WAVELENGTH RANGE
Cerium cell	Wavelength accuracy	200 -270 nm
Nicotinic acid 6, 12 & 24 mg/l	Absorbance accuracy and linearity, 0-1 A	210 - 270 nm

Visible Absorbance and Wavelength kit

Catalogue number: RM-1N3N5DHG

REFERENCE	TO QUALIFY	WAVELENGTH RANGE
Holmium Filter	Wavelength Accuracy	240 – 640 nm
Neutral Density Filters, 0.25, 0.50 & 1.00A	Absorbance Accuracy and Linearity	440 – 635 nm

Note: the Pharmacopoeias do not require stray light qualification above 400 nm.

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