Validating Your Spectrometer

From Good Practice to Regulatory Requirements

Using Certified Reference Material (CRM) to show and prove confidence in your results

A
Helping-Hand
Guide

Hellma UK Ltd Cumberland House,
Baxter Avenue, Southend on Sea, SS2 6HZ

Call: 01702 335266
Email: info.uk@hellma.com
Visit: www.hellma.com

Contents:

Why is validation needed?

A simple absorbance measurement on a UV/Vis spectrometer, at a specified wavelength, can be made following a single blank or background correction. But that basic measurement, and all the more complex ones too, rely on a number of pre-set conditions being maintained. Many instruments rely on significant mechanical and electro-mechanical assemblies for wavelength selection and blocking filter activation; while those designs that have limited moving parts still have opto-mechanical assemblies where components are locked in critical positions but are still subject to movement from shock, vibration and cycles of heating and cooling.

Many spectrometers have multiple uses and multiple users, making their current condition and history of 'events' very difficult to determine before any critical measurements are made. **How would you justify, defend or prove the accuracy of your results?**

Types of validation:

Validating the performance of your spectrometer with certified reference materials (filters) will give you confidence in the results you are reporting; and used correctly they will give you traceable proof to national standards.

Depending on individual circumstances you may choose from a number of different types of validation, three in common use are:

1. Validate its performance against the manufacturer's specification.
2. Validate its performance is suitable for defined applications.
3. Validate its performance against specific regulatory requirements.

You may in-fact use one or more of the above procedures, for instance, during an annual service or preventative maintenance visit the manufacturer or contractor may check its performance against the original specification; while more routine checks of its suitability for specific applications may be carried out by the users; and a quality or compliance engineer may do periodic tests to ensure it meets any regulatory requirements for your area of operation.

If you are following Good Laboratory Practice (GLP) or another organisational quality programme it is important that all your validation procedures are approved and recorded in your quality manual and reviewed with time and changing circumstances.

What to check and when:

These are two of the most asked questions, but unfortunately neither has a definitive answer. The five parameters that are generally regarded as giving a comprehensive indication of a spectrometer's perfrormance are:

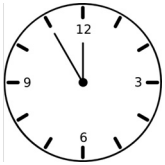
1. Wavelength accuracy
2. Absorbance accuracy
3. Photometric linearity
4. Limits of stray light
5. Optical resolution (Spectral Bandwidth SBW)

If you are not sure what the specifications for these are for your spectrometer, then now would be a good time to find out and record them in the table at the end of this guide for future reference; along with how they relate to your key applications.

All five tests would certainly be carried out for a full validation against manufacturer's specifications as in an Operational Qualification (OQ) procedure. In routine use maybe only wavelength accuracy and absorbance accuracy would be checked, while adding photometric linearity would make this a more thorough test. The latest version of the European Pharmacopoeia makes the following recommendations based on the type of measurements being made:

Test	Wavelength Accuracy	Absorbance Accuracy	Photometric Linearity	Stray Light	Optical Resolution
Measurement of absorbance at one or more wavelength	✓	✓	✓	✓	If required in the monograph
Identification of a wavelength of an absorbance peak or valley	✓	–	–	✓	–
Identification of a wavelength peak and measurement of its absorbance	✓	✓	–	✓	–
Comparison of a sample spectrum with that of a reference substance (acquired on the same spectrometer)	✓	✓	–	–	–

When to validate your spectrometer is another difficult question, certainly it should be more than once in a blue moon, but is monthly OK, or perhaps daily, or maybe at the begining of each shift, or every time you use it? Of course the answer will depend entirely on how critical the results are, on how it it is used, on what type of work or work-flow you have, the environment you are working in, how you care for it and of course any regulatory requirements that define your testing regimen too.



More frequent checking to start with is certainly a good idea, to build up some data and identify any changes and trends, but like the detail of 'what to check', the 'when to check' must be part of your recorded quality procedures too.

Reference Material Selection:

The two main types of reference material for spectrometer validation are glass filters and liquid filters. Glass filters are made by adding various substances to molten glass, then grinding and polishing the glass to controlled dimensions to get the required optical or spectral response. A strip of this material is then mounted in a metal or plastic frame suitable for a 10mm cell holder.

Liquid filters are made by dissolving various substances, at different concentrations, in a range of solvents to bring their signature optical or spectral response into the required range. The resulting solutions are then sealed inside quartz cuvettes for use in the spectrometer.

Glass filters are more physically robust than liquid filters but offer a limited range of options, especially in the UV wavelength range. Changes in the melt may cause batch to batch variation in glass filters, but for liquid filters careful control in production can reduce this type of variation. Although as we will see it is not the nominal value of the filters that is important - but it is the certified value that is critical in enabling you to compare your spectrometer directly to NIST traceable standards.

Certification and Recertification:

Every filter is supplied with a certificate detailing the actual values recorded during measurement on one of the reference instruments in the controlled conditions of our ISO17025 certified laboratory. These in-turn are traceable to NIST standards, so the filters are a vector that enables you to compare your spectrometer directly to NIST reference materials.

However there are a number of uncertainties to be considered when comparing your results to the values on the certificate. Firstly the uncertainty of our measurement which will be given on the certificate. Then there will be the uncertainty of your measurement, defined primarily by the tolerance of the measurement shown in your spectrometer's specification. These are cumulative uncertainties and should be added together to give you the specific limits of the acceptance window around the certified value for your spectrometer and operating conditions.

If you would like a useful tool for recording these uncertainties and calculating a suitable acceptance window, as well as recording your validation results and plotting trends then contact info.uk@hellma.com

We do not specify a period after which your filters require recertification as this will be dependent on your usage, operating conditions, storage conditions and care routines.

However we do offer a thirty-year warranty for all filters routinely returned for recertification; this means after the first year for all types; and then annualy for liquid filters and bi-annualy for glass filters.

Whether you take advantage of this offer or choose your own schedule for recertification this must also be recorded in your quality documentation. For details of how to return your filters for recertification please contact sales.uk@hellma.com.

Regulatory requirements:

Various industry bodies specify methods and materials for validating spectrometers for general use, or for specific applications relevant to their processes. Probably the most common and rigorous of these are the international pharmacopoeia. Of these the European Pharmacopoeia (EP) and the United States Pharmacopoeia (USP) are the most common.

Working to the requirements of the pharmacopoeia is mandatory for all manufactures of pharmaceutical and medicinal products, as well as certain categories of cosmetics; they also cover the suppliers of raw materials and ingredients to these industries too, so have a very wide-ranging impact.

The latest version of the European Pharmacopoeia (EP10) has moved from validating spectrometers against the manufacturers specification to validating them as suitable for the prescribed test. This means more focused testing in the ranges of interest, and to enable this a wider selection of filter materials have been adopted. However selecting the optimum validation now becomes a personalised solution depending on the usage of your spectrometer. If you need help or would like to discuss the various possible options please contact us on info.uk@hellma.com, you can also request a copy of our brochure and user guide here too.

A detailed discussion of the different requirements is not possible in this short reveiw but the tables below will give an indication of the materials available; which will be expanded on in the next section.

Wavelength	
Material	Range
Holmium Glass	UV/Vis
Didymium Glass	UV/Vis
Holmium Liquid	UV/Vis
Didymium Liquid	UV/Vis
HoDi Liquid	UV/Vis
Cerium Liquid	Deep UV

Absorbance	
Material	Range
ND Glass	Vis
Potassium Dichromate	UV/Vis
Niacin Liquid	UV

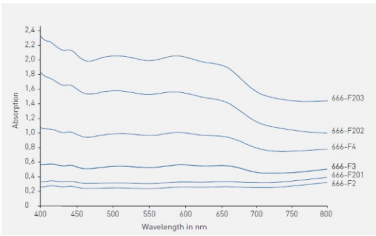
Stray Light	
Material	Range
KCl	200nm
NaI	259nm
$NaNO_3$	385nm
Acetone	325nm
Optical Resolution	
Toluene in Hexane	0.5 to 4.0nm

Filters and filter sets:

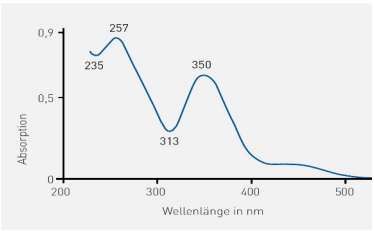
Filters for absorbance accuracy and photometric linearity:

For EP10 simply select filters with absorbance values that bracket the range for your application and that are certified at wavelengths that also bracket the wavelength(s) of interest. Then add one or two more in-between to enable photometric linearity to be checked too. *Note that EP10 requires a risk assesment to be carried out on all measurements made above 2.0A

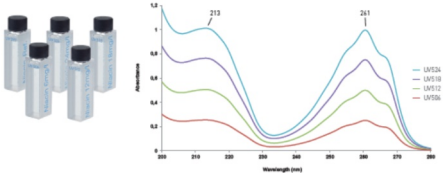
Neutral Density (ND) glass filters can be used over the visible wavelength range from 400 to over 800nm. Their relatively flat response over this range enables many alternative certifications to be acheived. As standard, absorbance values are certified at wavelengths of 440, 465, 546.1, 590 and 635nm, but any others are possible within the useable range. Filters with nominal absorbances at **0.04A** (F309), **0.25A** (F2), **0.3A** (F201), **0.5A** (F3), **0.7A** (F204), **1.0A** (F4), **1.5A** (F202), **2.0A** (F203), **2.5A*** (F301) and **3.0A*** (F303) are available.



Potassium Dichromate liquid filters have a characteristic absorbance spectrum with peaks at 257 and 350nm and valleys at 235 and 313nm which are directly proportional to concentration, enabling a series of standards to be produced giving certified absorbance levels between 0.1 and 3.0A. Filter UV20 gives values from **0.1 to 0.3A**, UV40 from **0.2 to 0.6A**, UV60 from **0.3 to 0.9A**, UV80 from **0.4 to 1.2A**, UV100 from **0.5 to 1.45A**, UV120 from **0.6 to 1.7A**, UV140 from **0.7 to 2.0A**, UV160 from **0.8 to 2.3A***, UV180 from **0.9 to 2.6A*** and UV200 from **1.0 to 3.0A***; while at a higher concentration our UV600 filter has an absorbance of **1.0A at 430nm**



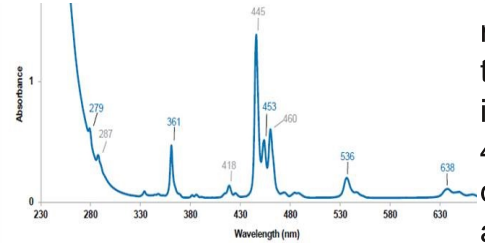
Niacin liquid filters are ideal for validating spectrometers used for life science applications with their absorbance peaks at 213 and 261nm in the UV range. At different concentrations filters are available up to 2.0A. Filter UV506 is **0.25A**, UV512 is **0.5A**, UV518 is **0.75A**, UV524 is **1.0A**, UV536 is **1.5A**, UV548 is **2.0A** and UV599 is the blank for use with all the above. Three or more of these filters can also be used for checking the photometric linearity in the UV region.



Filters for wavelength accuracy:

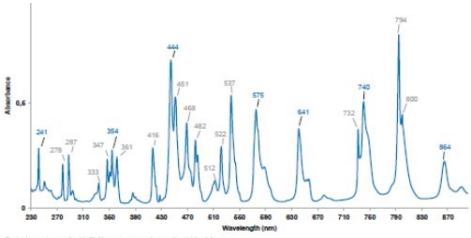
For EP10 select filters with wavelength peaks that bracket the range for your application, if this is a broad range then more than one filter type may be required, and checking at other peaks close to your analytical wavelength should also be considered.

The Holmium glass filter is the most robust and popular wavelength reference offering up to nine wavelength peaks in the UV and visible wavelength ranges. Standard certification of the F1 Holmium Oxide filter includes the peaks at 279, 361, 453, 536 and 638nm, with optional peaks at 287, 418, 445 and 460nm. The holmium filter is also available in a number of other filter sets.



The Didymium glass filter offers wavelength peaks from 329 to 875nm, with standard certification to 329, 472, 512, 681 and 875nm and options for 302, 430 and 582nm as our F7W filter. It can also be certified as an absorbance filter at wavelengths between 270 and 321nm as our F7A filter, with the possibility to have both as an F7AW filter, offering wavelength peaks at 329, 472, 512, 681 and 875nm and absorbance values typically between 0.5 and 1.0A at 270, 280, 297, 321 and 342nm. A very flexible filter!

The Holmium liquid filter (UV5) offers a wider selection of wavelength peaks, as standard 241, 287, 361, 451, 485, 536 and 640nm are certified; other options are for 250, 278, 333, 345, 386, 416 and 468nm.



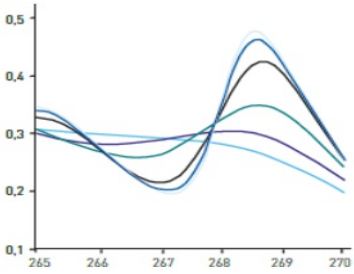
The Didymium liquid filter (UV25) is certified at 329, 469, 575, 740 and 864nm as standard with options for 354, 444, 482, 512, 522, 732, 794 and 801nm.

The HoDi liquid filter (UV45) is made from a mixture of these two liquids and offers up to 22 wavelength peaks! As standard 241, 354, 444, 575, 641, 740 and 864nm are certifiied, with options for 278, 287, 333, 347, 361, 416, 451, 468, 482, 512, 522, 537, 732, 794 and 801nm; a single filter that can be tailored to meet most UV/vis wavelength validation requirements.

The Rare Earth liquid filter (UV35) is suitable for wavelength validation in the deep UV wavelength range, and complements the Niacin filters used for absorbance validation in this region. With five certified peaks at 201, 211, 222, 239 and 252nm there are no other options for this filter.

Stray Light filters check the integrity of the optics from the source to the detector. Their function is to block all the wavelengths of light above a defined cut-off wavelength, so should give a very high absorbance reading for all wavelengths below that point. This helps to identify any internal or external light that is reaching the detector without first passing through the sample. A number of different solutions can be used for this; each with unique cut-off wavelengths. Our UV1 filter is Potassium Chloride and has a cut-off at 200nm, UV10 is Sodium Iodide with a cut-off at 259nm, UV11 is Sodium Nitrite with a cut-off at 385nm; these should all be measured against UV12 as the blank. UV19 is an Acetone stray-light filter with a cut-off at 325nm that can be measured against an air blank.

The Optical Resolution or Spectral Bandwidth (SBW) of your spectrometer will be set in design and manufacture and will be primarily dependent on the slit-width and grating quality. For resolving complex or adjacent peaks having the correct SBW will be vital, and where an application defines a required SBW your spectrometer's performance should be validated using our Toluene in Hexane (UV6) and Hexane Blank (UV9) filters. These are also available together as our UV200 filter set. The SBW is calculated using the peak to valley ratio of the scan between 265 and 270nm.



We supply a wide range of filter sets made up of different combinations of the filters described here to enable you to carry out a full validation of your spectrometer. Some of these sets (or combinations of sets) are designed to meet the requirements of the European and US Pharmacopoeia. Where any sets do not meet your specific requirements we can modify them or put together filters that are suitable for the level of validation you are undertaking. Please contact Hellma UK Ltd, as below, if you have any questions or to discuss the performance validation of your spectrometer(s).

Hellma UK Ltd Cumberland House,
Baxter Avenue, Southend on Sea, SS2 6HZ

Call: 01702 335266
Email: info.uk@hellma.com
Visit: www.hellma.com

Your Spectrometer	
Manufacturer & model	
Wavelength range & accuracy	
Absorbance range & accuracy	
Photometric linearity	
Stray light limits	
Optical Resolution (SBW)	
Other information:	

Your Application #1	
Application name	
Wavelengths measured (range)	
Absorbance limits expected	
Spectral resolution required	
Other information:	

Your Application #2	
Application name	
Wavelengths measured (range)	
Absorbance limits expected	
Spectral resolution required	
Other information:	